Exploring the Neural Mechanisms Underlying Pain Modulation by Positive and Negative Expectations

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1 Synopsis

1.1 Introduction

Pain may be considered as one of the most fundamental conscious experiences (Baron & Devor, 2022). While it serves as an important warning signal to avoid bodily damage, it has also emerged as a significant public health issue (Melzack, 1961; Tracey, 2022). About 19% of the adult population in Europe and one in five adults in the United States experiences chronic pain, which adversely impacts many facets of everyday life (Atlas, 2023; Breivik et al., 2006). Therefore, studying pain is of high importance, as it is essential to understand the mechanisms underlying its experience and how it can be modulated.

Placebo analgesia and nocebo hyperalgesia, in which pain perception is shaped by positive or negative expectations, are powerful examples of the inherent subjectivity and constructed nature of pain (Colloca & Barsky, 2020). For instance, a study demonstrated that a placebo fake knee operation provided pain relief for osteoarthritis patients equivalent to that of an actual surgery over a period of two years (Moseley et al., 2002). Conversely, expectations can also negatively impact well-being. A recent study illustrated this by showing that following increased media coverage of cerebral venous sinus thrombosis cases related to Covid vaccinations in 2021, there was a notable increase in patients seeking emergency room consultations for headaches (Asan et al., 2024). Given their far-reaching consequences, placebo and nocebo effects have been heavily investigated over the last years with a special emphasis on understanding the neural mechanisms underlying these effects (e.g., Atlas & Wager, 2014; Colloca & Barsky, 2020; Fields, 2018; Geuter et al., 2017).

Despite important insights into how expectations affect brain activity during pain processing (Atlas & Wager, 2014; Colloca, 2024; Zunhammer et al., 2021), several key questions remain. While expectations must, per definition, form prior to the painful stimulus, it remains elusive how they are represented in anticipatory neural activity and how anticipatory processes differ from those during pain processing. Research often investigated placebo and nocebo effects separately, hindering an evaluation of their similarities and differences on a neural level. Additionally, only few studies focused on the stability of placebo and nocebo effects. One might expect effects to diminish over time when positive or negative expectations are not reinforced after their induction, but there are reports of highly stable placebo effects in clinical trials (Khan et al., 2008; Perlis et al., 2005; Previtali et al., 2021). This further prompts the question of whether

any characteristics of neural processing can serve as predictors of the stability of these effects. Lastly, the extent to which expectations can be consciously generated remains unclear.

This dissertation encompasses three studies designed to shed light on these questions. The first study aimed to characterize how expectations affect neural processing from the anticipation of pain to its perception, and to identify similarities and differences between positive and negative expectations using combined EEG-fMRI measurements. The second study, using the same sample of participants, focused on the temporal stability of expectation effects on pain, and on the identification of neural markers that could predict this stability. The third study examined whether expectations could be consciously generated, rather than through deceptive information, and whether these consciously generated expectations would still affect pain perception.

Before detailing the research questions, methods, results, and discussing the findings of the three studies encompassed in this cumulative dissertation, the next pages will give an overview of pain processing, including ascending and descending pathways, introduce expectations as a specific factor that can modulate pain, and provide current evidence that addresses how the brain may convert sham treatments into measurable outcomes.

1.2 Background

1.2.1 The experience of pain and its underlying pathways

According to a rather outdated understanding of pain based on René Descartes, pain perception relies on a hard-wired system, in which sufficient tissue damage leads to a passive transmission of information through physical tubes to the brain (see Bingel et al., 2007; Stilwell & Harman, 2019). This perspective frames pain as a mere bottom-up processing of sensory information and implies a proportional relationship between physical inputs and pain perception, a concept mirrored in the more recent biomedical model of diseases (Ongaro & Kaptchuk, 2019). However, both models fail to account for phenomena such as phantom limb pain, where symptoms are perceived without tissue damage, and placebo treatments, which alleviate symptoms without active medication. This leads to the conclusion that the experience of pain encompasses more than just sensory transmission (Ongaro & Kaptchuk, 2019).

Consequently, in the contemporary biopsychosocial model, the perception of pain is defined as an “unpleasant sensory and emotional experience associated with, or

resembling that associated with, actual or potential tissue damage" (Raja et al., 2020) and seen as subjective outcome of a complex interaction of multiple processes in the brain that can be modulated by different factors (Reddan & Wager, 2018). Previous research has identified ascending pathways leading from the tissue damage to the perception of pain and the descending neural pathways underlying the modulation of pain.

Ascending pathways

To prevent further harm, it is crucial that signals indicative of actual or potential damage reach the brain, thereby generating the perception of pain in order to motivate behavior that protects us from additional damage (Melzack, 1961; Nikolenko et al., 2022). The process of encoding noxious stimuli is referred to as nociception and generally begins with the activation of free nerve endings in the periphery by intense, potentially tissue-damaging stimuli such as mechanical, chemical and thermal stimulation (Fields, 2004; Nikolenko et al., 2022). The fibers of these free nerve endings terminate in the dorsal horn of the spinal cord, where they activate second-order neurons. Axons of dorsal horn neurons cross over to the contralateral quadrant and ascend to the brainstem and thalamus (Fields, 2004; Institute of Medicine (US) Committee on Pain et al., 1987). From there, higher-order neurons project to multiple cortical regions, including the somatosensory, anterior cingulate and insular cortices (Fields, 2004).

In contrast to all other sensory modalities, there is no single dedicated primary cortex for the processing of incoming pain information. Instead, pain perception relies on a dynamic network of brain regions that receive and process pain with the involved brain areas also being part of other cortical networks (Tracey, 2011). The so-called *pain matrix* comprises of a multitude of regions that are responsive to noxious stimuli, such as the thalamus, primary somatosensory (S1), secondary somatosensory (S2), periaqueductal grey (PAG), insula, orbitofrontal, prefrontal, motor, inferior parietal, and anterior cingulate cortex (ACC; Derbyshire, 2000; Reddan & Wager, 2018).

The three key components that characterize pain are sensory, affective and cognitive aspects, each linked to distinct neural substrates (Apkarian et al., 2005; Peyron et al., 2000). Sensory-discriminative information, which reflect aspects such as the intensity, location, and modality of pain sensations, are related to activity in the lateral thalamus, S1, S2, and the posterior insula (Apkarian et al., 2005; Peyron et al., 2000). Affective aspects, such as fear-related aspects or the unpleasantness of pain,

are primarily encoded in the anterior insula and the ventral posterior ACC (Apkarian et al., 2005; Lu et al., 2016; Peyron et al., 2000). Cognitive-attentional aspects, including attention and memory, are linked to activity in the posterior parietal cortex, prefrontal cortex, and more anterior parts of the ACC (Apkarian et al., 2005; Lu et al., 2016; Peyron et al., 2000). Responses in other areas may be related to pain inhibition (e.g., PAG), or motor control (e.g., basal ganglia, supplementary motor area, cerebellum; Apkarian et al., 2005).

Pain is also associated with complex changes in neuronal oscillations measured using electroencephalography (EEG), reflecting the synchronous activity of large neuronal populations that are related to dynamic excitation and inhibition processes of neurons (Ploner et al., 2017). When applying phasic pain stimuli, low frequency (below 10 Hz) and gamma oscillations are typically increased shortly after the stimulus is administered, while oscillations at alpha and beta frequencies are suppressed (Ploner et al., 2017; Zis et al., 2022).

The modulation of pain perception through descending pathways

Pain perception is much more complex, as evidenced by phenomena in which pain occurs without nociceptive input, such as chronic pain (Reddan & Wager, 2018). Conversely, there are examples in which extreme pain can be endured under certain circumstances. For instance, Olympic gymnast Kerri Strug successfully landed a perfect dismount on her second try on the vault and won her team the gold medal in 1996, despite sustaining a severe injury to her ankle during her first attempt (Penner, 1996). The motivational and biological implications of pain perception may aid in understanding why it is largely context-dependent. In certain situations, the biological cost of tending to the pain outweighs the necessity of doing so. For instance, while it is generally advisable to avoid putting weight on a broken leg, doing so may still be the better option for escaping dangerous situations, such as when a predator is present (Fields, 2004). However, not only extreme external circumstances may shape pain perception, but it is also influenced by psychological, biological, and social factors in everyday life (Atlas & Wager, 2012; Raja et al., 2020). These factors, among others, include attention (versus distraction), saliency, emotions, former experiences, beliefs, and expectations (Atlas & Wager, 2012; Bingel et al., 2007).

On the neural level, one major pathway of endogenous pain modulation is the so-called *descending pain modulatory system* (DPMS), a top-down pathway that can be

activated by exteroceptive stimuli and motivational states (Bingel et al., 2007; Fields, 2004; Geuter et al., 2017). The DPMS can exert both pro- and anti-nociceptive influences through a signaling cascade originating from forebrain structures and extending down to nociceptive neurons in the spinal cord. Areas such as the ACC, ventromedial prefrontal cortex (vmPFC), the hypothalamus, and amygdala, transmit signals through direct and indirect pathways to the PAG. The PAG in turn can either inhibit or excite pain signals by activating neurons in the rostral ventromedial medulla (RVM). Within the RVM, two reciprocally active neuronal subpopulations modulate nociceptive transmission in the dorsal horn of the spinal cord: ON-Cells facilitate the transmission of pain signals, while OFF-Cells inhibit it. At every level of this pain-modulation circuit, μ-opioid receptors (MOR) are present. The activation of these receptors by MOR agonists inhibits pain by inducing an OFF-cell state (Bingel et al., 2007; Fields, 2004; Geuter et al., 2017). Typically, this occurs through the release of endogenous opioids, but externally administered opioids which act as analgesic drugs utilize the same inhibitory descending pathway to alleviate pain (Fields, 2004). In contrast, negative emotional states such as anxiety may produce pro-nociceptive effects using the same network (Colloca, 2024). As a caveat, top-down and bottom-up pathways may not be entirely separated as many areas play a role in both the perception and modulation of pain. Rather, it was proposed that there is only one single recurrent system with interconnected pathways (Büchel et al., 2014).

1.2.2 Placebo and nocebo: The interaction of expectations and pain

So far, pain perception has been presented as a dynamic interplay of bottom-up nociceptive and top-down processes. In addition to other top-down processes such as attention and memory, expectations are a major influencing factor for pain (Schwarz et al., 2016). Expectations shape perception and learning in many domains, from sensory to affective processes (Atlas, 2023; Atlas & Wager, 2012). The most prominent example for the interaction between expectations and treatment outcomes are placebo and nocebo effects (Schwarz et al., 2016). As early as 1955, Beecher (1955) published a meta-analysis assessing the efficacy of placebo treatments for various conditions, including seasickness or the common cold, and noted their impact on objective health outcomes. Since then, significant attention has been directed towards researching the analgesic effectiveness and (neural) mechanisms underlying placebo and nocebo effects (Benedetti et al., 2022; Fields, 2018).

Placebo and nocebo effects

Per definition, a placebo - derived from the Latin term “placeo”, meaning “I shall please” - is an inactive treatment that does not possess any specific therapeutic effects (Benedetti et al., 2022). Thus, its efficacy is not due to any active ingredients, but rather processes such as beliefs, expectations, contextual factors, and past experiences (Benedetti et al., 2022; Geuter et al., 2017). Placebo effects have been shown to affect various medical conditions and procedures, including anxiety and depression (e.g., Kirsch, 2019), pain (e.g., Colloca, 2019), Parkinson’s disease (e.g., T. B. Freeman et al., 1999), or surgeries (e.g., Wartolowska et al., 2014). Notably, Benedetti (2018) showed that even fundamental life functions may be affected by placebo treatments, as “placebo oxygen” reduced bodily reactions to hypoxia at high altitude levels.

In contrast, the nocebo effect (from Latin “nocere” – “to harm”) is often referred to as the “evil twin” of the placebo effect and involves negative responses to active or inert treatments that cannot be explained by their pharmacological mechanisms (Barsky et al., 2002; Colloca, 2024). While nocebo effects have historically received less attention than placebo effects, they are increasingly being studied (Sweeney et al., 2022). These effects can diminish the success of real treatments by reducing the efficacy of active substances or even inducing symptoms (Bingel et al., 2011; Colloca, 2024; Colloca & Barsky, 2020). For example, in clinical trials for antimigraine treatments, control groups frequently exhibit comparable rates of side effects and similar symptoms to those of the treatment group, despite not receiving any active medication (Amanzio et al., 2009). In general, informing individuals about potential adverse side effects significantly increases their likelihood of experiencing these symptoms (Barsky et al., 2002; Faasse & Petrie, 2013).

Placebo and nocebo effects on pain perception have been reported to be particularly strong (Atlas, 2023; Fields, 2018; Petersen et al., 2014; Zunhammer et al., 2021). *Placebo hypoalgesia* refers to a decrease in pain perception due to a placebo, while *nocebo hyperalgesia* describes the heightened perception of pain as a result of nocebo treatments (Büchel et al., 2014).

What contributes to placebo and nocebo effects?

While various processes, such as caregiver instructions, the social context, and emotional states, may shape placebo and nocebo effects, previous experiences and

expectations are considered the most significant factors (Benedetti et al., 2022; Colloca, 2024; Geuter et al., 2017).

Expectations related to future outcomes are widely acknowledged as a major factor underlying placebo and nocebo effects (Benedetti et al., 2022; Fields, 2004) and specifically conscious expectations have been proposed to be critical for many forms of placebo effects (Geuter et al., 2017). A typical way to induce placebo and nocebo effects by changing conscious expectations is through verbal suggestions, which have been shown to suffice alone to modulate pain perception (Colloca, Sigaudo, et al., 2008; Fields, 2004).

Prior experiences and learning also play a crucial role in placebo and nocebo effects on pain (Atlas, 2023). Associative learning in particular has been proposed as a key mechanism underlying placebo and nocebo effects. Correspondingly, classical conditioning is often used to induce effects in experimental research, which can be implemented by associating reduced (for placebo effects) or increased (for nocebo effects) pain intensities with a specific “treatment” cue (Atlas, 2023). Conditioning alone may induce placebo and nocebo effects, with factors such as the number of conditioning trials modulating the magnitude of effects (Bąbel et al., 2017; Colloca et al., 2010; Geuter et al., 2017).

As learning can reinforce and shape conscious expectations and causal attributions may influence learning processes, the distinction between expectations and conditioning processes may not be clear-cut (Atlas, 2023; Atlas & Wager, 2012; Benedetti et al., 2022; Büchel et al., 2014; Geuter et al., 2017). It was rather proposed that a conceptual representation of a treatment is formed by integrating all available information, subsequently affecting its efficacy (Geuter et al., 2017).

Importantly, although overall similar mechanisms appear to be involved in placebo and nocebo effects, there is also evidence of asymmetries in their respective learning processes (Colagiuri et al., 2015). For instance, placebo effects are larger when a combination of conditioning and verbal instructions is employed, while nocebo effects were shown to be similarly high regardless of whether they were elicited through verbal instructions or conditioning (Atlas, 2023; Colloca, 2024; Colloca, Sigaudo, et al., 2008; Colloca, Tinazzi, et al., 2008; Petersen et al., 2014). Moreover, hyperalgesic responses could be shown to be induced without conscious awareness, unlike hypoalgesic responses (Jensen et al., 2012; Jensen, Kirsch, et al., 2015; C. Liu et al., 2020). These observations have also led to the question of how similar placebo and nocebo effects

are on a neural level (S. Freeman et al., 2015), which will be further elaborated in chapter 1.2.3.

Predictive coding as a mechanism to explain placebo and nocebo effects on pain

An explanation for how and why placebo and nocebo effects emerge may come from predictive coding, a computational theory that has recently been proposed as a general framework for understanding brain function (Büchel et al., 2014; Friston, 2010; Geuter et al., 2017; Ongaro & Kaptchuk, 2019). On a computational level, predictive coding is grounded in Bayes' Theorem, which describes how incoming signals are integrated with existing knowledge and expectations. Predictive coding posits that the brain has an internal model of the world, from which it generates predictions (*prior*) about sensory inputs (*observations*). The actual perception (*posterior*) results from integrating top-down predictions with sensory data throughout every stage of neural processing in a hierarchical recurrent system. A mismatch between internal predictions and sensory inputs leads to *prediction errors* which are sent to higher levels to optimize the internal model and thereby reduce prediction errors in the future (Büchel et al., 2014; Geuter et al., 2017). Importantly, prediction errors can also be minimized by aligning sensory evidence with the prior expectations (*active inference*), such as engaging in a modulation of incoming sensory signals (Poublan-Couzardot & Talmi, 2024). The uncertainty about sensory signals (*precision*) is also encoded and determines the balance between sensory evidence and prior beliefs (Büchel et al., 2014).

In the context of placebo hypoalgesia, the prior may entail a highly precise expectation that pain will be alleviated due to a treatment. Upon administering the treatment, the perception of pain is integrated from the prior and sensory evidence, leading to a shifted perception of pain towards lower pain levels (Büchel et al., 2014; Geuter et al., 2017). The more precise the expectation, the more incoming sensory signals will be adjusted (e.g., Brown et al., 2008). Using the framework of predictive coding, placebo hypoalgesia and nocebo hyperalgesia could be viewed as byproducts of the brain's general mechanisms for processing and perceiving the world.

The stability of placebo and nocebo effects

The stability of placebo and nocebo effects has mainly been investigated with the aim to identify stable "placebo responders", i.e., to find out what makes individuals more likely to respond to placebo effects across contexts (Atlas & Wager, 2012). However,

another important open question is how stable placebo hypoalgesia and nocebo hyperalgesia can be after their induction. Any stability might be regarded as surprising within the framework of predictive coding, as a sufficient mismatch between expected and perceived pain should prompt an update of the internal model, especially when expectations are not reinforced over longer periods of time (Büchel et al., 2014).

However, placebo effects have been shown to be remarkably stable over weeks and months in clinical trials (e.g., Khan et al., 2008; Perlis et al., 2005; Quessy & Rowbotham, 2008). In controlled experiments, it has been shown that placebo effects may not extinguish over one week, but may decrease in magnitude (Colloca & Benedetti, 2006; Whalley et al., 2008), while comparable research on nocebo hyperalgesia is lacking. Within one session, several studies have demonstrated that both placebo and nocebo effects remain stable (e.g., Camerone et al., 2021; Colagiuri et al., 2015; Colloca, Sigaudo, et al., 2008; Jepma et al., 2018), or even that effects may increase over time (Camerone et al., 2021; Montgomery & Kirsch, 1997).

These findings cannot be explained by simple learning models and suggest that more elaborate mechanisms take place (Jepma et al., 2018). A confirmation bias in learning, in which expectations are more strongly updated when pain is in line with those expectation, may be a key mechanism and result in expectations becoming self-fulfilling prophecies (Jepma et al., 2018). On the neural level, this mechanism might depend on the prefrontal cortex suppressing learning from prediction errors (Schenk et al., 2017).

Similar to the previously reported differences in learning, placebo and nocebo effects might also differ in terms of their stability. Some studies have shown that nocebo effects might demonstrate a higher stability within a single session and are less dependent on the type of conditioning scheme used to induce them, in contrast to placebo effects (Au Yeung et al., 2014; Camerone et al., 2021; Colagiuri et al., 2015; Colagiuri & Quinn, 2018). This has been related to higher autonomic arousal in nocebo trials that interferes with learning (Colagiuri & Quinn, 2018). However, evidence regarding the stability of placebo and nocebo effects over sessions is lacking.

1.2.3 How and when are pain-related expectations reflected in the brain?

While placebo and nocebo effects were once dismissed as mere response biases, it is now widely recognized that positive and negative expectations can fundamentally alter pain perception on a neurobiological basis (Atlas, 2023; Wager et al., 2004). Despite

these important insights, the question of whether positive and negative expectations rely on common or distinct neural pathways is a topic that remains the subject of an ongoing debate, particularly given their reported differences in terms of learning and stability (S. Freeman et al., 2015; Fu et al., 2021; Petrovic, 2008).

One central focus of the research on the neurobiological basis of placebo and nocebo effects is on how positive and negative expectations interfere with the neural processing of incoming sensory information during pain perception (*pain phase*). Crucially, since expectations relate to future events, they must be established during the anticipation of these events (Büchel et al., 2014). The significance of anticipatory activity for subsequent processing has been demonstrated across multiple fields, e.g., in memory performance (Schneider & Rose, 2016; Scholz et al., 2017), visual processing (Salari et al., 2012), crossmodal associations (Ostrowski & Rose, 2024), and in general pain processing (Taesler & Rose, 2016; Tu et al., 2016). In the context of placebo and nocebo effects, it is likely that positive and negative expectations are reflected in anticipatory activity, thereby enabling subsequent hyperalgesic and hypoalgesic responses (Büchel et al., 2014). Investigating anticipatory activity also allows for an investigation of the neural representation of expectations without being contaminated by the actual nociceptive input (Fields, 2004; Wager et al., 2004, 2011). Therefore, another focal topic is on how expectations are reflected in anticipatory activity preceding the actual pain stimulation (*anticipation phase*), which has received considerably less attention up until today.

Activity during pain processing

In contrast to studies on EEG activity, in which expectations appear to generally not modulate oscillatory responses to pain stimuli (Bott et al., 2022; Nickel et al., 2022; Ploner et al., 2017; Strube et al., 2021), functional magnetic resonance imaging (fMRI) studies have revealed complex patterns of brain activity that underlie placebo and nocebo effects during pain processing.

Under placebo manipulations, reduced activity in a subset of the areas of the pain matrix such as the thalamus, parts of the ACC, and the insula has reliably been shown, indicating that placebos lead to actual changes in nociception (Amanzio et al., 2013; Atlas & Wager, 2012, 2014; Bingel et al., 2007; Vase & Wartolowska, 2019; Zunhammer et al., 2021). However, activity in other areas classically related to the processing of sensory-discriminative pain information, such as S2 and the posterior

insula, has not consistently been shown to decrease, suggesting that placebo effects may alter instead of simply reduce pain processing (Atlas & Wager, 2014; Wager et al., 2011).

The reductions are accompanied by increases in other brain regions, including parts of the cingulate cortex, the vmPFC, dorsolateral prefrontal cortex (DLPFC), anterior insula, PAG, and RVM (Amanzio et al., 2013; Atlas & Wager, 2012; Fu et al., 2021; Geuter et al., 2017; Wager et al., 2004; Zunhammer et al., 2021). The prefrontal cortex, ACC, PAG, and RVM are key areas of the descending pain modulatory system, pointing towards placebos reducing pain through the opioid-mediated pathways of the DPMS (Benedetti et al., 2022; Fields, 2004; Geuter et al., 2017). Thus, placebos and drugs may operate through the same neural network to influence the perception of pain (Benedetti et al., 2022; Bingel et al., 2007).

The prefrontal cortex has been proposed as a crucial structure for placebo effects, more specifically by generating, upholding, and integrating expectations of pain relief (Benedetti et al., 2022; Bingel et al., 2007). The DLPFC in particular is suggested to represent and update goals and expectations, which can top-down modulate activity in downstream pain processing regions (Geuter et al., 2017; Wager et al., 2004). Another important hub within the prefrontal cortex is the vmPFC (Geuter et al., 2017). It was suggested that information from different sources, including inputs from the DLPFC, are integrated with conceptual knowledge in the vmPFC to maintain expectations and to generate affective meaning (Geuter et al., 2017). The vmPFC is also connected to descending pathways, consistent with reports of enhanced functional coupling between the vmPFC and PAG during placebo (Bingel et al., 2006; Geuter et al., 2017; Petrovic et al., 2002; Wager et al., 2007).

Other neural processes that have been linked to placebos are not specific to pain, including cognitive reappraisal, a reduction in anxiety and negative emotions, and general reward processing (Atlas & Wager, 2012; Fu et al., 2021; Wager et al., 2004). This suggests that placebos might depend on multiple interconnected mechanisms (Zunhammer et al., 2021).

Concerning nocebo effects, increased activity across multiple brain regions of which many receive afferent nociceptive sensory signals has consistently been reported, including the posterior insula, ACC, hippocampus, S1, motor cortex, and PAG (Atlas & Wager, 2012; Colloca, 2024; Crawford et al., 2021; Fu et al., 2021; Kong et al., 2008; Tinnermann et al., 2017). Moreover, ascending signals at the level of the

spinal cord have also been shown to increase during pain processing in nocebo conditions (Tinnermann et al., 2017). In contrast, activity in the DLPFC, orbitofrontal cortex (OFC), and RVM has been demonstrated to decrease (Atlas & Wager, 2012; Crawford et al., 2021). Many of the areas modulated by nocebo conditions are part of the DPMS, suggesting the involvement of this system in generating nocebo hyperalgesia through augmented pain transmission in ascending pathways (Colloca, 2024; Colloca & Barsky, 2020).

Nocebo effects may also operate through fear and anxiety (Benedetti et al., 2007). The release of cholecystokinin (CCK), a neurotransmitter involved in anxiety and panic that may act within the DPMS to increase pain transmission, has been documented in nocebo effects (Atlas & Wager, 2012; Benedetti et al., 2022; Büchel et al., 2014; Colloca, 2024). Moreover, reduced activity of the reward system may also mediate nocebo effects (Scott et al., 2008).

To sum up, although some areas and processes may be specifically relevant for either placebo or nocebo effects, there may also be common pathways through which they exert control over pain perception. The DPMS seems to at least partially mediate the up- vs. downregulation of pain perception in both placebo and nocebo effects during pain perception, with opposite responses for positive and negative expectations down to the level of the spinal cord (Benedetti et al., 2020; Crawford et al., 2021; Koyama et al., 2005; Scott et al., 2008). However, it is unlikely that this is the only mechanism through which placebo and nocebo effects operate; rather, other mechanisms by which expectations shape perception in general are also likely to be involved (Atlas, 2023; Geers et al., 2021).

Anticipatory activity

EEG studies have shown that pain-related expectations alter activity in alpha and beta frequency bands during pain anticipation (Babiloni et al., 2004, 2006; Nickel et al., 2022; Strube et al., 2021). While Strube et al. (2021) observed spatially broad increases in alpha activity for higher expectations of pain, other studies found decreased alpha-to-beta activity to reflect higher expectations of pain, particularly over the contralateral sensorimotor cortex (Babiloni et al., 2004, 2006; Nickel et al., 2022). Although the direction of the anticipatory alpha-to-beta modulation is inconsistent over studies, they still generally fit with the notion that activity within these frequency bands reflects the top-down signaling of expectations (Ploner et al., 2017).

The first study to show how placebo manipulations influence anticipatory processes using fMRI was conducted by Wager et al. (2004). In this pioneer study, it was found that anticipatory activity in the DLPFC, OFC, and PAG was increased for placebo compared to control. Moreover, reported behavioral placebo effects correlated with anticipatory changes in prefrontal areas, as well as with placebo-induced reductions in several pain-responsive areas during pain processing, including the thalamus, insula, and rostral ACC (Wager et al., 2004). These important findings pointed towards an anticipatory preparation for descending spinal inhibition of incoming pain signaling via the DPMS (Wager et al., 2004).

Further studies have corroborated these findings and demonstrated that multiple brain regions are activated during the anticipation phase under placebo conditions. Especially the DLPFC, ventrolateral prefrontal cortex, and OFC have frequently been reported (Amanzio et al., 2013; Frisaldi et al., 2015; Rossettini et al., 2023; Vase & Wartolowska, 2019; Wager et al., 2011; Watson et al., 2009). In addition to its proposed function within the DPMS, the activation of the DLPFC has been interpreted as a representation of the expected value of placebo treatments and aspects of cognitive control (Atlas & Wager, 2012). Alongside prefrontal areas, other areas of the DPMS such as the ACC and PAG have also frequently been observed to be activated during the anticipation phase (Amanzio et al., 2013; Geuter et al., 2013; Rossettini et al., 2023; Vase & Wartolowska, 2019).

There are also reports of higher activity in the anterior insula, cerebellum, and striatum during the anticipation phase by placebo (Geuter et al., 2013; Wager et al., 2011). Further, it has also been demonstrated that anticipatory activity in emotion-related networks, rather than in regions associated to pain and cognitive control, predicted the magnitude of placebo analgesia, potentially reflecting affective appraisal processes (Wager et al., 2011). This indicates that a complex interplay of mechanisms during the anticipation phase may shape placebo effects.

Although evidence is sparse, nocebo manipulations have been shown to result in increases across a broad range of brain areas during the anticipation phase. These regions include major components of the pain matrix, such as the ACC, insular cortex, S1, S2, and thalamus (Colloca & Benedetti, 2007; Manaï et al., 2019; Rossettini et al., 2023; Schmid et al., 2015). The heightened preparatory activity of the pain matrix may subsequently lead to increased cortical processing of incoming sensory information. Other regions that have demonstrated anticipatory activations in nocebo conditions

include the amygdala, cerebellum, and, as in placebo conditions, the DLPFC (Colloca & Benedetti, 2007; Manaï et al., 2019; Rossettini et al., 2023; Schmid et al., 2015).

Comparing placebo and nocebo, initial evidence suggests some overlap in anticipatory activity within areas of the DPMS, including the ACC and DLPFC, as well as the insula and cerebellum (Amanzio et al., 2013; Rossettini et al., 2023; Schmid et al., 2015). However, this assumption should be approached with caution, as anticipatory activity associated with positive and negative expectations has not been directly compared within the same subjects. An overlap may indicate shared mechanisms for both positive and negative expectations in the generation and maintenance of expectations, for example the pre-activation of the DPMS to either facilitate or reduce pain transmission, or other processes that might not be specific to pain processing.

1.3 Research goals

Previous research has demonstrated that positive and negative pain-related expectations have a real, lasting, and measurable impact on pain perception. However, the neural basis of placebo and nocebo effects, especially during the anticipation phase, as well as general mechanisms are still not fully understood. The three empirical studies that were conducted for this dissertation in order to address these open questions will be outlined in the following.

The anticipation phase appears to be crucial for the initiation of expectations. To investigate this time frame in detail, a combination of high spatial and temporal resolution is necessary to not only detect which brain regions are active, but also to detect temporal sequences. Moreover, the relationship between positive and negative expectations remains unclear. Thus, *Study 1* was conducted as a combined EEG-fMRI study with the aim to delineate between processes in the anticipation and pain phase that reflect expectations and to identify common and distinct mechanisms of positive and negative expectations. For this aim, both the anticipation and pain phase were tested for *common effects*, i.e., activation that is similar for positive and negative expectations, but different from a control condition, and for *distinct effects*, i.e. activation that differs between positive and negative expectations, using fMRI. Further, the high spatial resolution of the fMRI in combination with the high temporal resolution of the EEG was exploited for an in-depth investigation of processes within the anticipation phase.

The temporal stability of placebo effects in clinical settings has been documented, but there has not been much research on the stability of placebo and nocebo effects in experimental settings over sessions. *Study 2* was designed to test for the stability of expectation effects induced in one session until a second session one week later. Another aim was to see if neural processing during the anticipation and pain phase on the initial day of expectation acquisition, measured by fMRI, could predict the strength of subsequent effects. Moreover, it was investigated whether EEG oscillatory activity would be affected by expectations in the second session, as prior research has shown that expectations may be reflected in anticipatory alpha-to-beta activity.

In most experimental studies, placebo and nocebo effects are induced via deceptive verbal instructions and/or conditioning procedures, which is not applicable in the clinical practice due to the apparent ethical constraints. However, recent developments in the field of open-label placebos (OLPs) show that deceptive information might not be necessary, as placebo treatments have also shown to be effective when participants were given honest information that they would only receive a sham treatment (Blease et al., 2020). Thus, in *Study 3* it was investigated whether subjects would be able to consciously change their expectations regarding upcoming pain stimuli. As a neurobiological readout, it was tested whether the conscious modulation would lead to changes in oscillatory activity during the pain phase.

1.4 Methods

All three studies shared the same pain stimulation and calibration and used the same basic paradigm, the so-called *test phase*, to investigate mechanisms underlying pain-related expectations in healthy subjects. In short, in all experiments, participants' expectations regarding an upcoming thermal heat stimulus were manipulated on a trial-by-trial basis using a visual cue during the test phase while neurobiological data was recorded. The studies differed in terms of expectation manipulation and the instructed meaning of the visual cues presented in the test phase, contingent upon the specific focus of each study. In Study 1, the main objective was to analyze neural dynamics of positive and negative expectations during the anticipation and pain phase. Study 2 investigated the temporal stability of pain-related expectations in the same sample by re-inviting the participants from Study 1 into the lab one week later and tested whether characteristics of the neural processing on the initial day of expectation induction could predict this stability. Thus, Study 1 and Study 2 used the same (deceptive) expectation

manipulation. In Study 3, a separate sample was asked to consciously modulate their expectations.

1.4.1 General paradigm and procedure

Pain stimulation and calibration

Throughout all experiments, heat stimuli were used to induce pain using a Pathway CHEPS (Contact Heat-Evoked Potential Stimulator) thermode (https://www.medoc-web.com/pathway-model-cheps). In each session, a pain calibration was performed to individually calibrate target intensities for each participant following a procedure implemented by Horing et al. (2019) to ensure a similar level of perceived pain. Individual temperatures that corresponded to specific ratings on a visual analogue scale (VAS; see Katz & Melzack, 1999) from 0 ("no pain") to 100 ("unbearable pain") were calculated by employing either linear or sigmoidal regression to participants' pain ratings during the calibration process. Temperature values corresponding to ratings of VAS30, VAS60, and VAS70 were extracted and used in the following parts of the experiment.

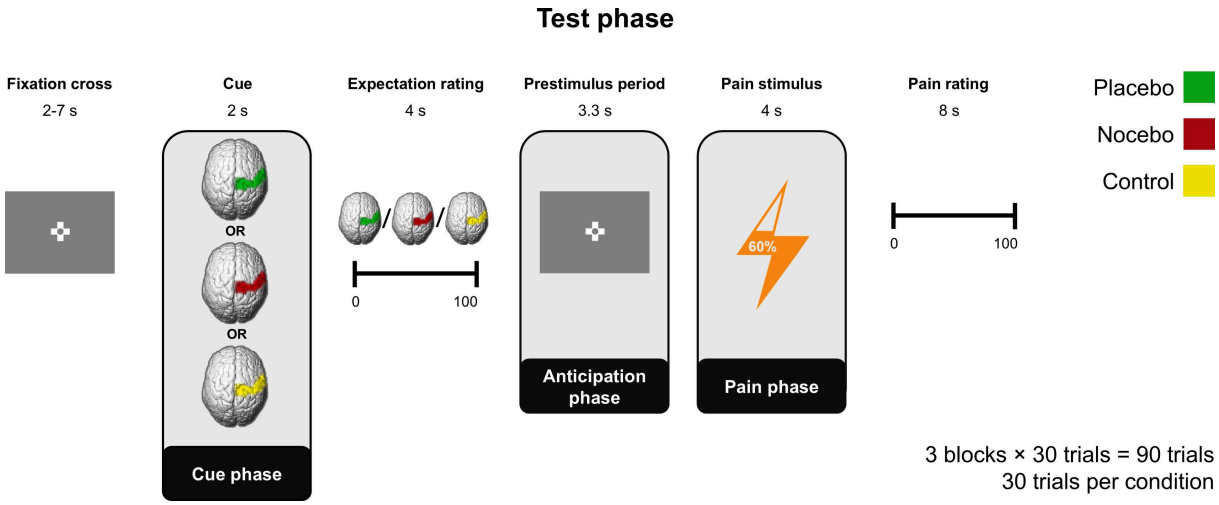

Figure 1. The trial structure of the test phase was the same in all three studies. After being presented with a cue, participants had to indicate how painful they expected the next stimulus to be. Following an anticipation phase, a brief heat pain stimulus was applied to the skin, which participants then had to rate. Figure adapted from Wittkamp & Wolf et al. (2024) and Wolf & Wittkamp et al. (2024).

Test phase

All experiments contained a test phase in which participants' expectations were manipulated on a trial-by-trial basis, that only differed based on the instructions given for the used visual cues. To foreshadow, the visual cues supposedly represented

feedback on the participant's current pain sensitivity in Studies 1 and 2, while it indicated how participants should consciously modulate their expectations in Study 3 (*see Table 1*). A trial started with the presentation of one of the three different cues (*see Figure 1*). The cues consisted of an image of a brain with the right primary somatosensory cortex highlighted in either green (placebo condition; positive expectation), red (nocebo condition; negative expectation), or yellow (control condition; neutral expectation). Participants were then asked to rate how painful they expected to perceive the next stimulus on a VAS ranging from 0 to 100 (expectation rating). Next, there was a fixed pre-stimulus period of 3.3 s (anticipation phase), in which a fixation cross was presented. This anticipation phase was crucial for the experimental design, as it was reasoned that expectations would have to be represented in neural activity during this period. Lastly, the pain stimulus was administered for 4 s (pain phase) and participants had to rate how painful they perceived the stimulus. The stimulation temperature was always the same (VAS60), independent of the cue color. Participants were not aware that the temperature remained constant; they were only told that they would receive different pain stimuli of medium intensity. There were 30 trials per condition, divided into 3 measurement blocks with 30 trials each in a pseudo-randomized order.

1.4.2 Study-specific procedures

In the following, study-specific procedures mainly regarding the specific expectation manipulation and recording of neurobiological data will be introduced (*see Table 1*).

Table 1. *Differences in the procedures between the three studies*

	Study 1	**Study 2**	**Study 3**
Expectation manipulation	Conditioning + Deceptive verbal instructions	Only conducted one week prior: Conditioning + Deceptive verbal instructions	Non-deceptive verbal instructions
Instructed meaning of the cues	Brain state of pain sensitivity		Direction into which pain expectation should be modulated
Neurobiological data	fMRI, EEG, SCR	EEG	EEG

Study 1

The first step of the expectation induction involved deceptive verbal instructions. Participants were told that they would receive real-time visual feedback on their current pain sensitivity by means of a brain-computer interface (BCI) during the test phase. This BCI was only part of the cover story and served as “sham” treatment signal by supposedly classifying participants’ brain activation into “brain states” of high or low pain sensitivity. The cues shown in the test phase would signal the current brain state: A green color would indicate a state of low pain sensitivity (placebo condition), a red color would indicate a state of high pain sensitivity (nocebo condition), and a yellow color would indicate that the BCI algorithm was not able to detect a clear-cut brain state and would thus not make any prediction (control condition).

In the subsequent conditioning phase, participants were told that the BCI algorithm would be calibrated to their individual brain activity. In truth, this phase was used to strengthen the credibility of the BCI algorithm and to establish connections between green cues and less intense pain stimuli, while associating red cues with more intense pain stimuli. Each trial started with the presentation of either red or green visual cue, followed by the application of the heat pain stimulus. Without the participants’ knowledge, green cues were always followed by stimuli corresponding to VAS30, and red cues by stimuli corresponding to VAS70. There were 10 trials per condition.

During the test phase, participants were informed that the individually calibrated BCI algorithm would now give them real-time feedback on their current pain sensitivity. Additional to expectation and pain ratings, EEG, fMRI, and electrodermal activity (EDA) data were collected during the test phase (*see section 4.1 for specifics regarding the recording of EDA data and subsequent analysis of skin conductance responses*). FMRI data were recorded using a 3 T Siemens PRISMA Scanner using EPI blood-oxygenation-level-dependent (BOLD) sequences for each block of the test phase (TE: 29 ms, TR: 1679 ms). EEG data were recorded from 64 passive sintered Ag/AgCl electrodes using a custom BrainCap MR for 3 T and the BrainVision Recorder software (Version 1.10, BrainProducts, Gilching, Germany). All necessary measures to minimize scanner-related artifacts and to synchronize the clock of the EEG recording to the MR recording were taken. As Study 1 and Study 2 used the same sample of participants, behavioral and fMRI data collected in Study 1 were also used for the analyses conducted in Study 2.

Study 2

All participants of Study 1 were re-invited to a second session (Day 8) approximately one week after the first session (Day 1). Crucially, no further verbal instructions or conditioning were performed, as the aim was to test the stability of the effects induced on Day 1 one week later. Participants were only informed that the same BCI algorithm as on Day 1 would be used to give them live feedback on their current pain sensitivity during the test phase. The test phase was performed in an electrically shielded EEG lab. Data from 64 active Ag/AgCl electrodes (actiCAP, BrainProducts) were recorded using the BrainVision Recorder.

Study 3

A separate sample was used for Study 3. Before taking part in the test phase, participants received non-deceptive verbal instructions. They were told that pain perception is highly variable and may be modulated by various factors, including expectations, and that the aim of the current study was to examine how consciously generated expectations could influence the perception of pain stimuli. The visual cue presented in the test phase would indicate how they should adjust their expectation of the upcoming pain stimulus. Following a red cue, participants were instructed to actively build up an expectation of a highly intense pain stimulus. In contrast, after a green cue, they should try to expect a mild pain stimulus. No specific strategies were given. When presented with a yellow cue, participants should not attempt to modify their expectation and the perception of the subsequent pain stimulus.

During the test phase, EEG data were recorded with similar settings compared to Study 2. Upon completion of the test phase, participants were asked whether they felt if their expectations had affected their pain perception, if they noticed any differences in pain intensity, and if they used any specific strategy in order to modulate their pain perception.

1.4.3 Data preprocessing and analysis

Modulation of pain and expectation ratings (all studies)

Pain and expectation ratings were collected on each trial throughout all three studies and were the main behavioral readout. Ratings were averaged within conditions for subsequent analyses. For an evaluation of whether placebo and nocebo effects were induced, repeated measures ANOVAs were used in Studies 1 and 3 to test whether

the condition cue (placebo, nocebo, control) affected pain and expectation ratings, separately. To test whether effects were stable over time, repeated-measures ANOVAs were performed including the factors time point (Day 1, Day 8) and condition (placebo, nocebo, control) on pain and expectation ratings for the aims of Study 2.

Further analyses in all studies focused on the magnitudes of *placebo effects* and *nocebo effects*, which were quantified as the absolute difference between placebo or nocebo condition and the control condition (placebo effect: control – placebo; nocebo effect: nocebo – control). A focal analysis of Study 3 involved comparing whether consciously generated expectations (Study 3) would lead to similarly strong placebo and nocebo effects as deceptively generated expectations (Study 1). For this aim, a mixed-effects ANOVA was conducted with the induction method (between-subjects; deceptively generated vs. consciously generated expectations) and the effect type (within-subjects; placebo effect vs. nocebo effect) as predictors.

EEG oscillatory data (all studies)

Data collected inside the MRI scanner was corrected for cardioballistic and MR artifacts using BrainVision Analyzer 2.2 (BrainProducts). All further steps of preprocessing and the analyses were performed using the Fieldtrip toolbox (Oostenveld et al., 2011) for MATLAB (Version R2021b; The MathWorks). Preprocessing steps included a visual screening for artifacts and the rejection of compromised trials, and an independent component analysis including the rejection of artifactual components. To enhance sensitivity to detect and remove artifacts, a recent preprocessing approach was implemented in which data is split into high- and low-frequency data and processed in parallel (Hipp et al., 2011; Strube et al., 2023). EEG data was time-frequency transformed using the multi-taper method based on discrete prolate spheroid sequences (DPSS). Power from 21 logarithmically spaced frequencies from 4 to 128 Hz (0.25-octave increments) in time steps of 0.1 s was extracted.

Time-frequency decomposed data were used for the combined EEG-fMRI analysis in Study 1 as specified below. In Study 2, the effect of the condition cues on neural processing was evaluated by comparing EEG power between the three conditions, separately for the anticipation and pain phase and for low- (4-25 Hz) and high-frequency data (25-128 Hz). Nonparametric cluster-based permutation tests were used to correct for multiple comparisons.

In Study 3, EEG power was compared between the three conditions during the pain phase using rmANOVAs at each time-frequency-electrode point. The *F*-statistics obtained from the rmANOVAs were subsequently tested using nonparametric cluster-based permutation tests as implemented in Fieldtrip (nonparametric individual cluster threshold: p = .05, cluster value: weighted cluster mean, number of randomizations: 2000, α = .05). Statistics were calculated separately for low and high frequencies.

FMRI data (Studies 1 and 2)

The Statistical Parameter Mapping toolbox (SPM 12, Wellcome Department of Imaging Neuroscience, London, UK, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB was used for the preprocessing and analysis of fMRI data. Images were realigned and unwarped, registered onto Montreal Neurological Institute (MNI) coordinate space and spatially smoothed (6 mm Gaussian kernel). To remove cardioballistic and respiratory artifacts, they were modelled as nuisance regressors and entered in the analyses using the RETROICOR algorithm included in the PhysIO toolbox (Frässle et al., 2021; Kasper et al., 2017).

A central focus of the analyses was the anticipation phase, in which the emergence of dynamic processes that would reflect expectations were expected. As these processes would not necessarily be time-locked to the onset of the anticipation phase and might not conform to the shape of the canonical hemodynamic response function, this model was opted against, which would assume a similar response over all brain regions and that the onset of the BOLD response would be locked to a stimulus (Henson et al., 2001; Penny et al., 2011). A finite impulse response (FIR) model was implemented as a more flexible solution that can capture any shape of responses. FIR models consist of successive boxcar functions within a given time window and yield parameter estimates for each time bin (Henson et al., 2001; Penny et al., 2011). The FIR model was set up to encompass a time window of 18.4 s, starting from the cue onset, divided into 11 bins with a duration of 1.675 s each.

Common and distinct effects of positive and negative expectations (Study 1)

Study 1 focused on the evaluation of common and distinct effects of positive and negative expectations. For this aim, a flexible factorial design was used at the group level to test for these effects separately during the anticipation and pain phase. Common effects were analyzed by computing directed *t*-contrasts comparing placebo

and nocebo (both reflecting directed expectations) against the control condition (reflecting neutral expectations) of the regressors covering the respective time period, exclusively masked with the *F*-contrast of placebo vs. nocebo to identify only areas that showed similar responses for both placebo and nocebo. Distinct effects were assessed through directed *t*-contrasts comparing placebo and nocebo. Multiple comparisons were corrected for controlling the family-wise error (FWE) rate ($p < .05$).

Temporal localization of effects within the anticipation phase (Study 1)

In Study 1, a combined EEG-fMRI analysis was performed to further assess the time-course of activity within areas that were found to represent expectations during the anticipation phase. For this aim, single-trial BOLD activation within several regions of interest (ROIs) was extracted and correlated with the time-frequency EEG data (*see Figure 2*).

From the preprocessed fMRI data, single-trial hemodynamic responses during the anticipation phase were extracted by implementing a boxcar function with a length of 1.679 s starting at anticipation onset using the GLMsingle toolbox for MATLAB (Prince et al., 2022). As implemented in the toolbox, GLMdenoise was used to enhance the accuracy of single-trial betas, while additional noise reduction was achieved through the application of fractional ridge regression (Prince et al., 2022). This procedure yielded beta values for every voxel in each trial. Around the significant peak voxels identified in the MR analysis of common effects during the anticipation phase, 10 mm spheres were centered to define ROIs, and beta values were averaged within each ROI for every trial.

On the participant level, the correlation between the ROI beta estimates and EEG data was then computed on each time-electrode-frequency point over trials, resulting in one time-frequency-decomposed correlation pattern per participant and ROI. As power values are non-normally distributed, Spearman rank correlation was used as a nonparametric measure of the correlation between beta ROI estimates and oscillatory power (M. X. Cohen, 2014). For subsequent statistical testing on the group level, Spearman correlation values were Fisher-z-transformed in order to obtain normally distributed values (M. X. Cohen, 2014). These values were tested against zero using nonparametric cluster-based permutation tests as implemented in Fieldtrip from cue onset until pain offset.

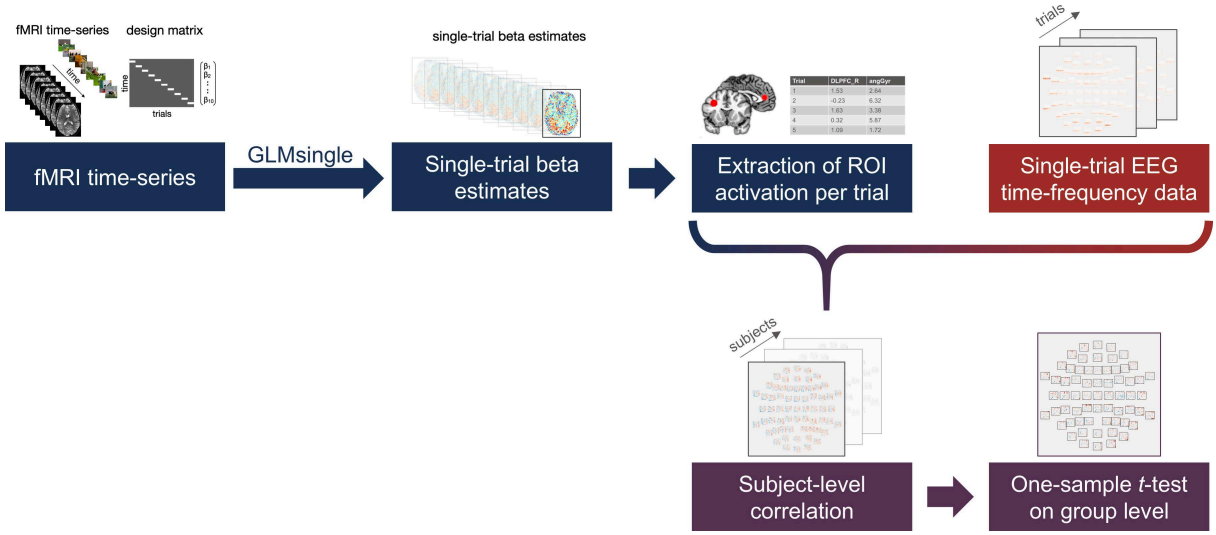

Figure 2. Procedure for the combined EEG-fMRI analysis. From the fMRI time series, single-trial beta estimates were extracted using GLMsingle, of which ROI activity values were extracted. These were correlated with single-trial EEG time-frequency data on a subject level which were then tested on the group level. Parts of the figure adapted from Prince et al. (2022).

Neural activity related to the stability of expectation effects (Study 2)

A primary focus of Study 2 was to examine whether characteristics of the neural processing during placebo and nocebo trials on Day 1 affected the magnitude of behavioral effects observed on Day 8. For this aim, separate one-sample *t*-tests of placebo (placebo vs. control) and nocebo-related fMRI activity (nocebo vs. control) on Day 1 were conducted at the second level, including the corresponding behavioral effect on Day 8 (placebo or nocebo effect on pain ratings) as covariate in the design matrix. To control for the strength of the behavioral effect on Day 1, this effect was also included as a covariate in the first position of the design matrix. Multiple comparisons were corrected for controlling the FWE rate ($p < .05$).

1.5 Results summary

1.5.1 Study 1: The neural dynamics of positive and negative expectations of pain

This study focused on the neural dynamics of positive and negative expectations of pain from the anticipation phase before the onset of the pain stimulus to the actual pain phase (*see Figure 1 for details regarding the trial structure*). To recap, it was of interest to assess both similarities in neural processing between positive and negative expectations compared to the control condition (*common effects*) and where and when

positive and negative expectations were processed differently compared to each other (*distinct effects*). For this aim, combined EEG-fMRI data was analyzed from a sample of n = 50 healthy participants (n_{fMRI} = 45, $n_{EEG\text{-}fMRI}$ = 41, n_{SCR} = 26).

As a first step, it was validated that the novel paradigm successfully induced expectations and modulated pain perception (*see Figure 3*). This was the case, as indicated by higher expectation and pain ratings in the nocebo compared to the control condition and in turn for the placebo condition. Moreover, skin conductance responses (SCRs) to pain stimuli as a measure of autonomic arousal were larger in the nocebo compared to the placebo condition.

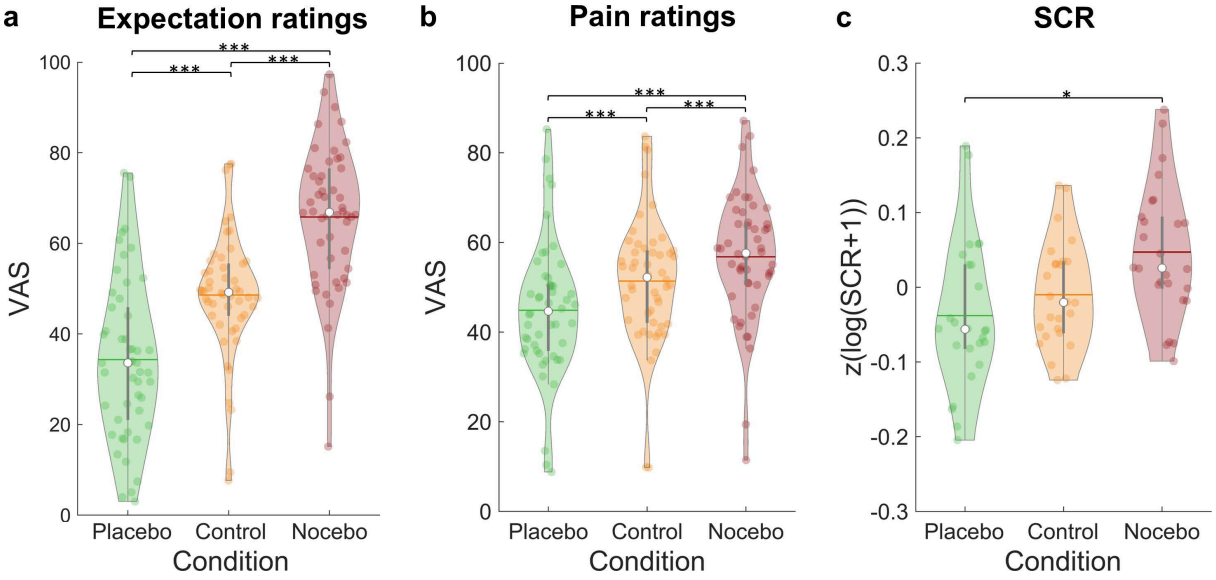

Figure 3. Expectation **(a)** and pain ratings **(b)** on a visual analogue scale show clear rating differences between the three conditions. **(c)** Mean phasic skin conductance responses to pain stimuli were higher for the nocebo compared to the placebo condition. White dots = mean, horizontal lines = median, thick grey vertical lines = upper and lower quartile, colored dots = mean ratings/SCRs of individual participants per condition. Figure adapted from Wittkamp & Wolf et al. (2024).

Further, responses of the stimulus-intensity independent pain signature (SIIPS-1), a marker for subjective pain perception of similarly intense stimuli that has been shown to be affected by psychological interventions such as placebo and nocebo effects (Botvinik-Nezer et al., 2024; Woo et al., 2017), were higher for nocebo compared to placebo and control during the pain phase, but not during the anticipation phase. These analyses demonstrated that reliable placebo and nocebo effects were induced using the sham-BCI intervention, allowing an investigation of expectation effects on the neural level.

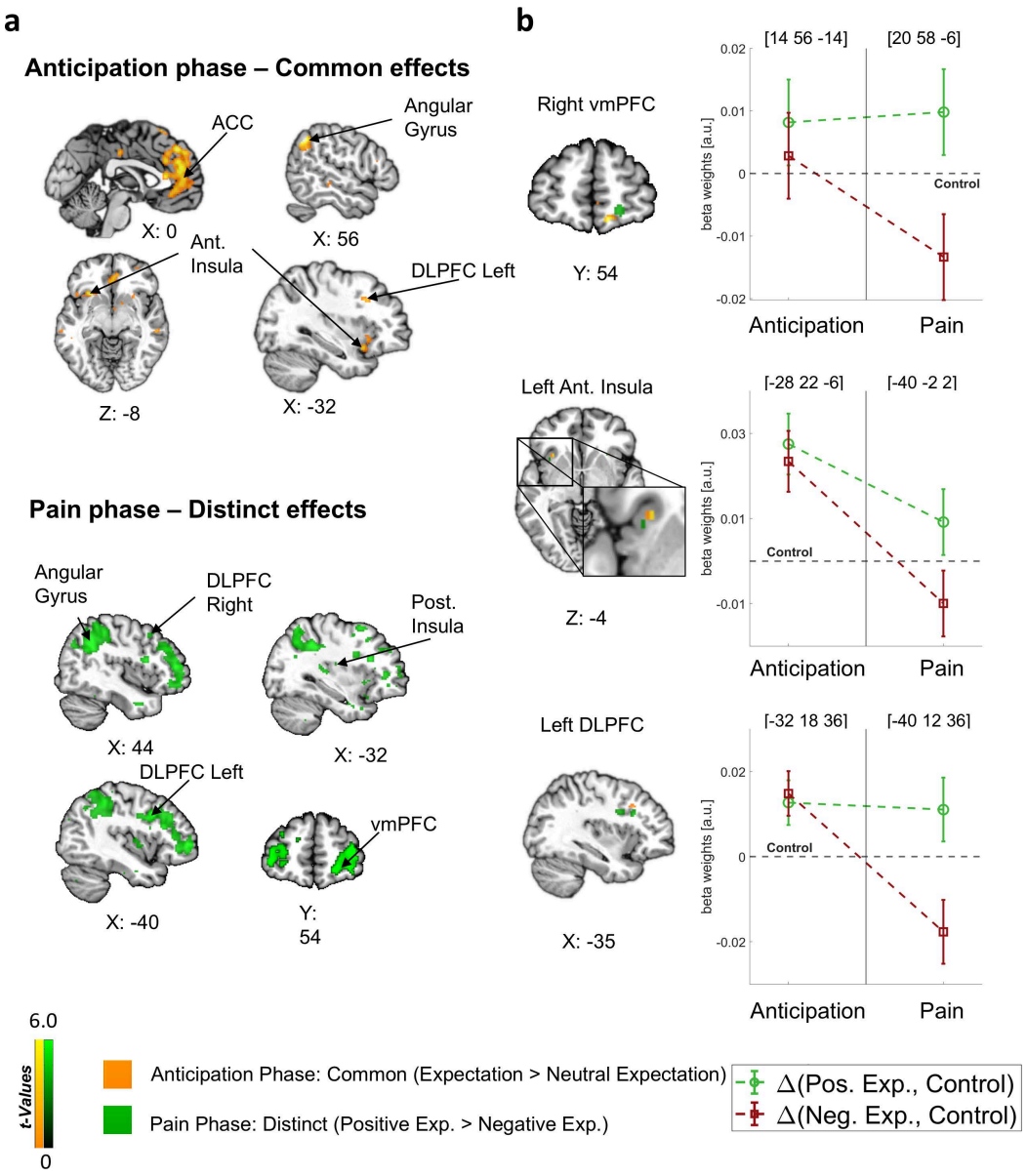

Figure 4. (a) Common effects (directed expectations > neutral expectation) dominated during the anticipation phase, as shown on top. During the actual pain processing, activity differentiated, as evidenced by largely distinct effects (positive > negative). Images are thresholded at $p < .001$ for display purposes. **(b)** On the left, the spatial proximity of common effects during the anticipation phase (yellow) and distinct effects during the pain phase (green) is shown within selected areas. On the right, the evolution from common towards distinct effects within these areas is highlighted. Activation levels for positive and negative expectations (i.e. beta weights from the finite impulse response model) baselined by the control condition at the respective peaks for each phase (coordinates in parentheses) are shown. Figure adapted from Wittkamp & Wolf et al. (2024).

The analysis of common and distinct effects of positive and negative expectations during the anticipation and pain phase revealed a general pattern over the entire brain: First, expectations were represented in a rather general way, as indicated by dominantly common effects of positive and negative expectations compared to the

control condition during the anticipation phase (*see Figure 4*). This included areas commonly associated with the DPMS such as the bilateral DLPFC, bilateral ACC, and right vmPFC. Only after the onset of the pain stimulus, the picture shifted towards largely distinct effects with widespread larger activity for placebo compared to nocebo, e.g. in the amygdala and hippocampus.

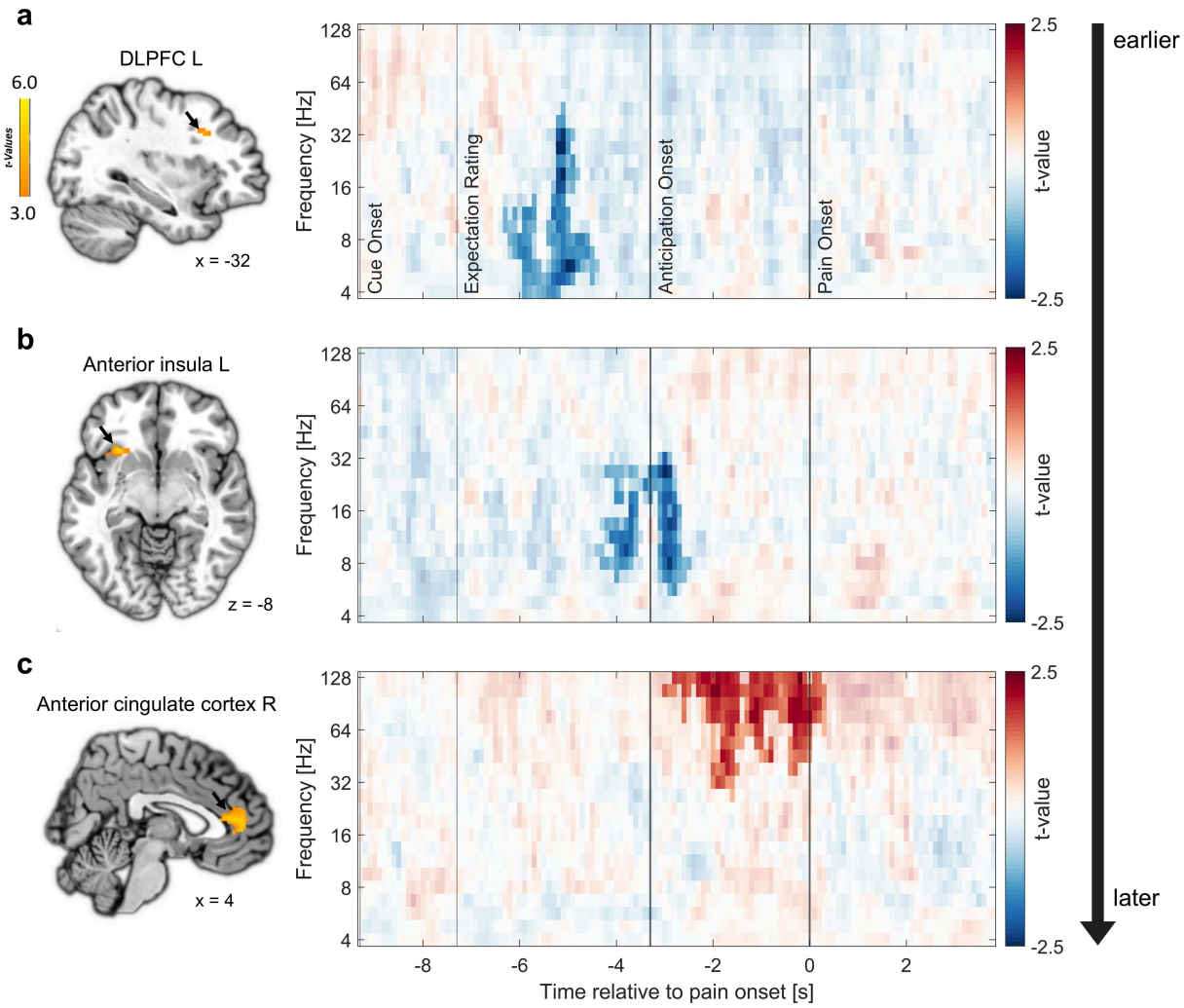

Figure 5. Anticipatory hemodynamic responses in the left DLPFC **(a)**, left anterior insula **(b)**, and right ACC **(c)** were significantly correlated with EEG oscillatory activity on a single-trial level and showed a clear temporal sequence of effects. On the left, common effects of expectations (directed expectations > neutral expectation) during the anticipation phase are shown. Displayed on the right is the cluster-corrected correlation of fMRI activity with EEG power, averaged across all cluster electrodes. Non-significant time-frequency points are masked. Figure from Wittkamp & Wolf et al. (2024).

Most strikingly, some areas were involved in the representation of expectations in both anticipation and pain phase and in themselves showed the same temporal pattern of first common and then distinct effects. The analysis revealed that the bilateral

DLPFC, right vmPFC, left anterior insula, and angular gyrus all first showed higher activity for positive and negative expectations compared to control during the anticipation phase, and then dissociated towards higher activity for positive compared to negative expectations in the pain phase. The thalamus also first showed common effects during pain anticipation, but then shifted towards larger activity for negative compared to positive expectations in the pain phase.

To achieve a deeper understanding of the temporal sequence of neural processes during the anticipation phase, a combined EEG-fMRI analysis was conducted on a single-trial level (*see Figure 5*). This allowed for a temporal and spectral localization of effects observed in the fMRI analysis. Activity within the left DLPFC during the anticipation phase was correlated with an early decrease in theta to low gamma frequencies, indicating an early role of this region in the initiation of expectations. Anticipatory activity in the left anterior insula could be pinpointed to a reduction of power only a little later. At the transition from the anticipation to the pain phase, anticipatory activity in the right and left ACC were associated with an increase in gamma activity. The results demonstrated a clear sequence of effects during the anticipation phase. Prefrontal areas and the anterior insula were more associated in early processes, potentially related to top-down control, while the ACC might have acted as direct link to pain modulation at the transition from anticipation to pain.

1.5.2 Study 2: Differential neural activity predicts the long-term stability of the effects of positive and negative expectations on pain

All participants that took part in both sessions of Study 2, i.e., Day 1 and Day 8, were included in this study (n = 41). The main objective of Study 2 was to determine the stability of expectation effects on a behavioral level. Another aim was to determine if neural activity on Day 1 might mediate the stability of expectation effects. Two important findings from Study 1 informed the procedure: First, it was found that there were both similarities and differences in the neural representation of positive and negative expectations, leading to the question whether the same or different regions would determine the stability of positive versus negative expectations. Second, there was a temporal dissociation in engaged regions and associated processes between the anticipation and pain phase, sparking interest in which of these processes would predict the stability.

Over both sessions, raw expectation and pain ratings were found to be similarly modulated by the condition cue with the highest ratings for the nocebo compared to the control and in turn for the placebo condition. Most importantly, there was no interaction between the factors condition and session, which suggests that the experimental manipulation was still similarly effective on Day 8.

Placebo and nocebo *effects* on expectation ratings, i.e., the absolute difference in expectation ratings between these conditions and the control condition, remained stable over both sessions. In contrast, the magnitude of placebo and nocebo effects on pain ratings slightly decreased from Day 1 to Day 8. The decrease was significant, but small in size ($M_{\Delta(\text{Day 1, Day 8})}$ = 1.42), which could also explain the differing results of this analysis from the rmANOVA on raw pain ratings. Although placebo and nocebo effects on pain ratings decreased in magnitude, they were still highly evident on Day 8 over all blocks and, most importantly, in the last block. This was also true for placebo and nocebo effects on expectation ratings. Further exploratory analyses showed that placebo and nocebo effects on both pain and expectation ratings even increased from the last trials of Day 1 to the first trials of Day 8.

The evaluation of EEG activity on Day 8 revealed that the nocebo cue led to larger theta-to-alpha activity during the anticipation phase compared to the placebo cue (-2.1 until -0.1 s, 4 to 9.5 Hz, *see Figure 6*). This neural signature showed that expectations had a measurable effect on anticipatory processes on Day 8.

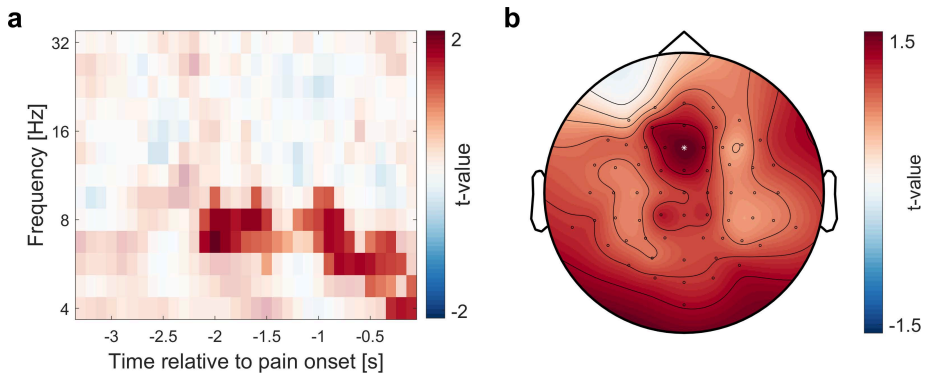

Figure 6. **(a)** Time-frequency plot of *t*-values for placebo vs. nocebo during the anticipation phase, averaged over all cluster electrodes. Non-significant time-frequency points are masked. **(b)** The corresponding topography of the cluster. Figure from Wolf & Wittkamp et al. (2024).

The remaining analyses were focused on identifying predictors for the magnitude of placebo and nocebo effects on Day 8. Perhaps not surprisingly, for both pain and expectation ratings, subjects that reported higher placebo and nocebo effects on Day 1

also experienced larger corresponding effects on Day 8. On a neural level, subjects that reported larger placebo effects on pain ratings on Day 8 had lower amygdala activity during the anticipation of pain on Day 1 and enhanced activity of the bilateral anterior insula and right DLPFC during pain processing on Day 1 in placebo compared to control trials (*see Figure 7*). Nocebo effects on Day 8 were found to be more pronounced for subjects that had larger thalamus activity during pain processing on Day 1 in nocebo compared to control trials. These findings suggest that activations and deactivations in these regions may not only directly affect placebo and nocebo effects, but also their longer-term stability.

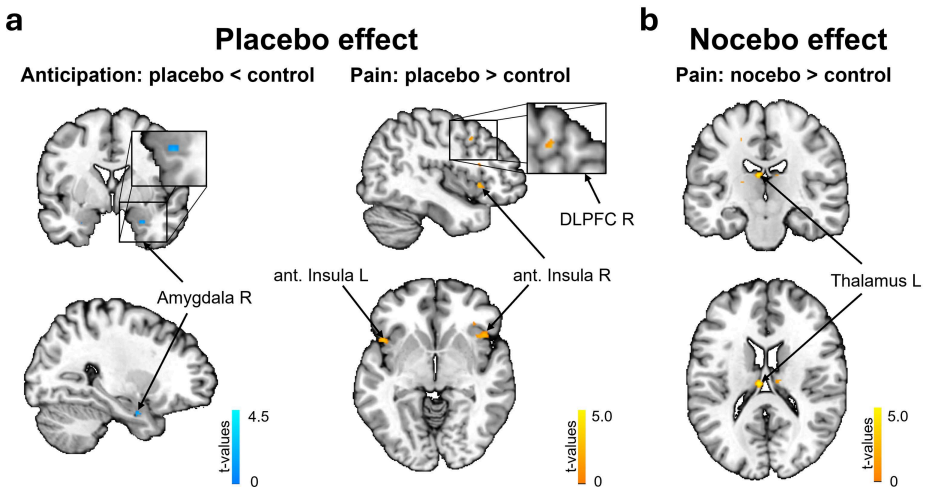

Figure 7. (a) Individual placebo effects on Day 8 were predicted by lower activation in the amygdala for placebo compared to control during the anticipation phase, and by enhanced activity of the anterior insula and DLPFC during the pain phase. **(b)** Individual nocebo effects on Day 8 were related to larger thalamus activation for nocebo versus control during the pain phase. Figure from Wolf & Wittkamp et al. (2024).

1.5.3 Study 3: The influence of consciously generated expectations on pain perception

The aim of Study 3 was to evaluate whether consciously generating the expectation of a highly or weakly painful stimulus, compared to no expectation, would affect pain perception and EEG oscillatory activity. For the sake of simplicity and comparability to the other studies, the conditions were labelled as placebo, nocebo and control, because although the underlying mechanisms may differ, this intervention as well as classical placebo and nocebo interventions focused on changing the expectations

regarding an upcoming stimulus (*see section 1.6.1*). Data from *n* = 42 subjects were analyzed.

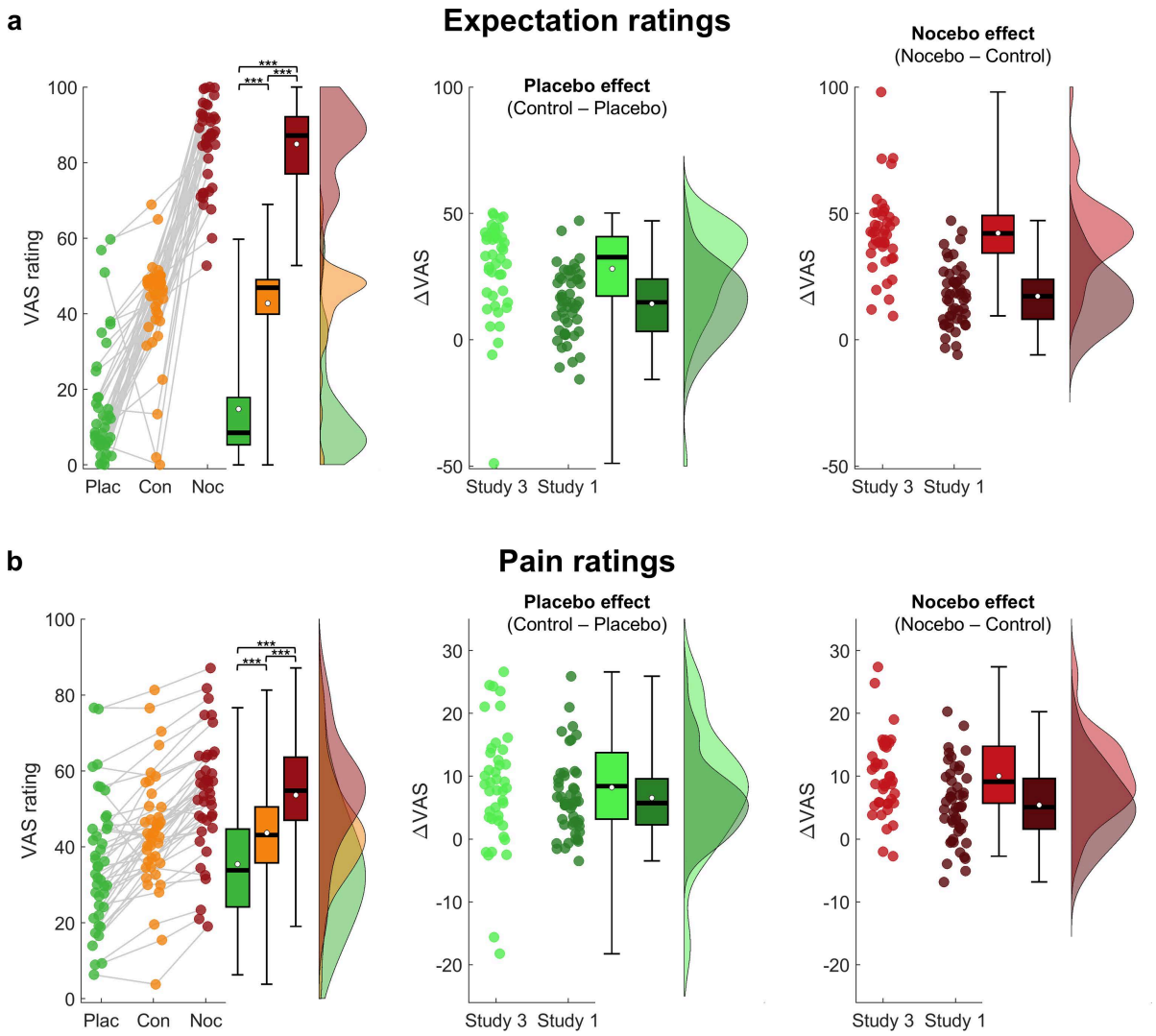

Figure 8. (a) *Left*: Raincloud plots of expectation ratings per condition. Individual dots denote the average rating of a subject per condition, with grey lines connecting the ratings of the same subject over conditions. *Middle*: Placebo effects (control minus placebo) in expectation ratings in Study 3 (consciously generated expectations) compared to Study 1 (deceptively generated expectations). *Right*: Same for nocebo effects (nocebo minus control). **(b)** Same for pain ratings. Black thick line = Median. White dot = Mean. Plac = Placebo. Con = Control. Noc = Nocebo.
*p < .05. **p < .01. ***p < .001.

A repeated measures ANOVA revealed significant effects of the condition cue on pain ratings ($F(2,82) = 79.78$, $p < .001$, $\eta^2_p = 0.66$; *see Figure 8a*). Bonferroni-Holm corrected post-hoc tests showed that participants rated pain stimuli in the nocebo condition ($M = 53.64$, $SD = 15.43$) higher than in the control condition ($M = 43.64$, $SD = 14.91$) and again higher in the control condition than in the placebo condition

(M = 35.45, SD = 16.77; all p_{holm} < .001). Similarly, expectation ratings were significantly modulated by the condition cue, as evidenced by a rmANOVA (F(2,82) = 283.74, p < .001, η^2_p = 0.87; *see Figure 8b*). Expectation ratings were higher in the nocebo condition (M = 84.93, SD = 11.23) compared to the control condition (M = 42.79, SD = 13.22) and in the control condition than in the placebo condition (M = 14.76, SD = 15.21; all p_{holm} < .001). Following Cohen's conventions, these analyses indicated that consciously generated expectations had large effects on the expectation and perception of painful stimuli (J. Cohen, 1988; Lakens, 2013).

These findings motivated an additional analysis in comparing the consciously generated placebo and nocebo *effects* (i.e., the difference between the respective condition and the control condition) observed in this study to those deceptively generated in Study 1. For pain ratings, a mixed ANOVA (induction type x effect type) revealed a significant effect of the induction type (F(1,90) = 7.95, p = .006, η^2_p = 0.08), driven by a larger pain rating modulation by consciously generated (pooled over both effect types: M = 9.10, SD = 5.76) compared to deceptively generated expectations (pooled over both effect types: M = 5.96, SD = 4.90), while there was no difference between placebo and nocebo effects (p = .724), and no interaction effect (p = .133). Similarly, the modulation of expectation ratings was larger for consciously generated (pooled over both effect types: M = 35.90, SD = 10.90) compared to deceptively generated expectations (pooled over both effect types: M = 15.73, SD = 11.55; F(1,90) = 67.46, p < .001, η^2_p = 0.43).

However, for expectation ratings, the effect type was also significant (F(1,90) = 15.63, p < .001, η^2_p = 0.15). Post-hoc tests indicated generally higher nocebo effects (pooled over both induction types: M = 28.60, SD = 18.86) compared to placebo effects (pooled over both induction types: M = 20.54, SD = 17.58). Moreover, the interaction term was significant (F(1,90) = 6.64, p = .012, η^2_p = 0.07), which was driven by larger differences between the strength of placebo and nocebo effects in consciously generated expectations ($M_{\Delta(\text{nocebo effect, placebo effect})}$ = 14.10, SD = 28.14) compared to deceptively generated expectations ($M_{\Delta(\text{nocebo effect, placebo effect})}$ = 2.97, SD = 10.94). Thus, reported placebo and nocebo effects were not only comparable to those deceptively generated, but even larger.

Next, it was assessed whether consciously generated expectations led to differences in EEG oscillatory signaling during the processing of the pain stimuli. Cluster-based permutation tests revealed significant differences in gamma band

activity between the three conditions, mainly in posterior electrodes (p = .017, 0 - 2.7 s, 26.89 - 128 Hz; *see Figure 9*). To further explore this effect, the mean power within the cluster per condition was extracted for each participant and a rmANOVA was conducted comparing the values between the three conditions. There was a significant effect of condition (F(1.37,56.35) = 16.84, p < .001, η^2_p = 0.29). Power within the cluster was higher in the nocebo (M = 0.096, SD = 0.082) compared to the placebo condition (M = 0.092, SD = 0.078; p_{holm} = .003) and control condition (M = 0.088, SD = 0.075; p_{holm} < .001), and in the placebo condition higher compared to the control condition (p_{holm} = .016). This suggests that the expectation modulation indeed led to differences in the neural processing of the pain stimuli.

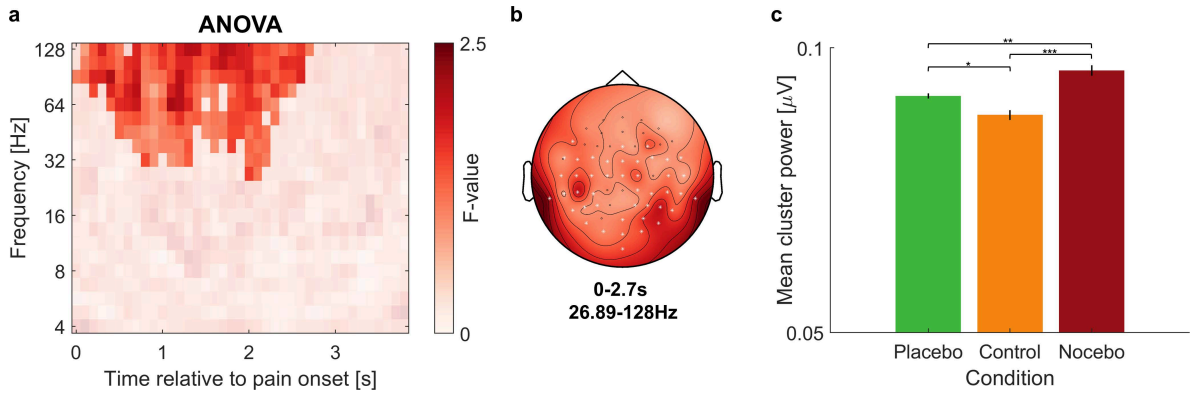

Figure 9. (a) Time-frequency plot of *F*-values comparing EEG oscillatory power between the three conditions during the pain phase, averaged over all electrodes within the cluster. Non-significant time-frequency points are masked. **(b)** The topography of gamma effects over all frequencies and time points included in the cluster. Cluster channels are highlighted with a white star. **(c)** Raw power within the cluster was extracted and averaged per condition for each subject. A rmANOVA showed that gamma activity was highest in the nocebo condition, followed by the placebo condition, and gamma power was lowest in the control condition. Error bars represent the corrected standard error of the mean using the Cousineau-Morey method (Cousineau, 2005; Morey, 2008).
*p < .05. **p < .01. ***p < .001.

Regarding the subjective efficacy of the procedure, most participants stated that their expectations had at least partly modulated their pain perception (71.43%) and that they perceived the pain stimuli as being of different intensities (68.63%). Only a small proportion of participants felt no differences between stimuli (11.76%). As no specific strategy was instructed, it was also of interest which strategies were used. A variety of strategies to maintain and implement expectations was reported, e.g., internal verbalization (29.63%), imagination (11.11%), and distraction (only for the green cue; 11.11%), while some subjects stated using no specific strategy (22.22%).

1.6 Discussion

The goal of this dissertation was to shed light on the neural basis and overarching mechanisms underlying the effects of positive and negative expectations on pain. For this aim, three studies were conducted, focusing on (1) the neural dynamics of positive and negative expectations from the anticipation phase to the pain phase, (2) the stability of these expectations and the predictors of their persistence, and (3) the extent to which expectations can be consciously modulated. The findings revealed that positive and negative expectations were similarly represented in neural activity during the anticipation phase and only upon the onset of pain stimulation activity began to differentiate between positive and negative expectations. Furthermore, both positive and negative expectations were relatively stable over two sessions one week apart. Neural activity from the first session was predictive of the strength of effects observed during the second session, with distinct neural areas identified as being relevant for either placebo or nocebo effects. Lastly, participants were able to consciously modulate their pain expectations and experiences, with their ratings showing an even greater degree of modulation compared to when placebo and nocebo effects were induced deceptively. The next sections will put these findings into context, discuss limitations and introduce future research directions.

1.6.1 The strength and stability of positive and negative expectations

In contrast to most previous studies that researched either positive or negative expectations, compared the effects of positive and negative expectations between subjects, or used block designs (Bąbel et al., 2017; Bingel et al., 2011; Colloca, Sigaudo, et al., 2008; Crawford et al., 2021; Shi et al., 2021), a within-subjects design with a trial-by-trial manipulation of expectations was successfully implemented in the three studies of this dissertation. This allowed for a good comparison between the strength and stability of positive and negative expectation effects, as previous studies have indicated differences in terms of learning and stability (Colloca et al., 2010; Colloca, Sigaudo, et al., 2008). In addition, the use of the same test phase in all three experiments allowed for a comparison of effects between studies.

Overall, pain and expectation ratings indicated large placebo and nocebo effects in all three studies. Following the deceptive expectation induction in Studies 1 and 2, these findings can be attributed to the combined influence of conditioning and verbal instructions that were used to induce effects. The novel cover story centered on a BCI

algorithm that supposedly classified brain activity into different states of pain sensitivity presents a promising method for modulating participants' expectations on a trial-by-trial basis, similar to manipulations using sham transcutaneous electrical nerve stimulation (e.g., Grahl et al., 2018). Both conscious expectations and learning, two primary mechanisms proposed to give rise to placebo and nocebo effects, were targeted through this approach (Benedetti et al., 2022; Colloca, 2024; Geuter et al., 2017).

Using a conscious modulation of expectations, pain and expectation ratings could also be modulated, which fits findings from a previous study showing that a self-regulation of pain perception can affect reported pain (Woo et al., 2015). Surprisingly, the effects of the conscious modulation were even larger than those induced through deceptive information and conditioning. This may be partly due to a response bias, as participants were aware of which ratings would "help" the researchers in proving that the modulation is working. A similar challenge is faced in studies on open-label placebos, where participants know that they are receiving an inert treatment (Kaptchuk, 2018). However, the observed differences in EEG gamma band activity between conditions during the pain phase may suggest actual perceptual differences, as gamma activity is associated with the signaling of pain information and prediction errors (Ploner et al., 2017; Strube et al., 2021). Moreover, self-reports of subjects indicated that they subjectively perceived their conscious modulation to be effective, pointing towards at least partly real changes in pain perception.

Identifying mechanisms that explain how a conscious modulation of expectations may affect pain perception is challenging. According to predictive coding, even when individuals are aware that a treatment signal is inert, automatic processes related to the treatment per se may still contribute to pain modulation, as the prior may consist of multiple conscious and non-conscious facets (Geuter et al., 2017; Kaptchuk, 2018). Moreover, the inherently variable nature of pain perception could result in small differences in perceived intensity, and changes that aligned with the cued direction might have been overinterpreted due to a confirmation bias (Jepma et al., 2018). Generally, it has been shown that more unprecise sensory signals amplify the relative influence of the prior (Kuperman et al., 2020). This could give the impression that the expectation modulation is working, a belief that may become a self-fulfilling prophecy over time (Jepma et al., 2018). These mechanisms equally apply to the deceptive expectation manipulation.

However, specific to this modulation, the effects may also be tied to participants' feelings of control over their own expectations and perceptions, after being informed that they were capable to exert modulatory influence on their own expectations in the verbal instructions. Past research has demonstrated that feelings of agency and control over treatments can enhance placebo effects and overall treatment success (Strube et al., 2023; Tang et al., 2022). Moreover, the processing of value and emotional appraisal may be particularly relevant for this type of modulation, parallel to findings showing that these processes mediated the effects of self-regulation of pain, more than a modulation of nociceptive signaling (Woo et al., 2015).

Interestingly, following both deceptive and non-deceptive expectation induction, the modulation of pain ratings was equally strong for placebo and nocebo effects. Given prior findings indicating greater nocebo compared to placebo effects, as well as a relatively easier induction of nocebo effects, it might have been expected that a similar procedure for the induction of positive and negative expectations would result in larger nocebo effects (Jensen et al., 2012; Jensen, Kaptchuk, et al., 2015; Jensen, Kirsch, et al., 2015; Tu et al., 2019). This would be in line with the generally assumed high significance of negative information for survival and findings showing that associations between cues and aversive states are more readily formed than those between cues and appetitive states (Colloca, Sigaudo, et al., 2008; van der Schaaf et al., 2022).

A possible explanation for the absence of this pattern in the present findings could be the shared cover story for both effects. It may not make sense for participants to only follow half of the narrative, leading to the possibility that experiencing effects in one direction could influence their beliefs about the other effect. In the studies using the BCI cover story, experiencing heightened pain after receiving the feedback of a state of high pain sensitivity may have reinforced participants' beliefs in the entire treatment, extending to the belief in the validity of the feedback indicating a state of low pain sensitivity. In a similar vein, experiencing mild side effects was shown to enhance treatment efficacy by signaling to the patient that the treatment is working, thus increasing treatment expectations (Berna et al., 2017; Schenk et al., 2024).

In terms of stability, both placebo and nocebo effects slightly decreased over one week. However, they did not go extinct over a total of 180 test trials conducted across two sessions, despite the absence of further reinforcement of the initially induced expectations during the second session. This finding of relatively stable effects is comparable to findings from clinical studies (e.g., Khan et al., 2008; Perlis et al., 2005,

2005; Quessy & Rowbotham, 2008), experimental research with multiple sessions (Colloca & Benedetti, 2006; Whalley et al., 2008) and within one session (e.g., Camerone et al., 2021; Colagiuri et al., 2015; Colloca, Sigaudo, et al., 2008; Jepma et al., 2018). The present findings expand this body of knowledge by demonstrating that nocebo effects can also persist over sessions.

When participants are continuously presented with sensory evidence that contrast their expectations, most learning models would predict that they would learn from those experiences and update their expectations accordingly (Jepma et al., 2018; Schenk et al., 2017). A potential mechanism underlying the observed stability could be self-reinforcing feedback loops, wherein participants learn more from experiences that match their expectations (Jepma et al., 2018; Schenk et al., 2017). This process could render expectations resistant to contradicting sensory inputs. Another reason for the relatively persistent placebo and nocebo effects may be active inference, in the way that incoming sensory information was aligned with internal predictions through descending modulation, which diminished prediction errors and effectively removed the necessity to update expectations (Büchel et al., 2014; Poublan-Couzardot & Talmi, 2024). This is supported by the observed engagement of the DPMS during the first session, in line with previous research (Benedetti et al., 2022; Fields, 2004; Geuter et al., 2017; *see section 1.6.2*).

Similar to the finding of equal strength, it was also rather unexpected that placebo and nocebo effects demonstrated a comparable level of stability. Previous research suggested that nocebo effects may be more robust against extinction (Au Yeung et al., 2014; Camerone et al., 2021; Colagiuri et al., 2015; Colagiuri & Quinn, 2018), which has been related to higher autonomic arousal in nocebo trials leading to a reduced learning rate from prediction errors (Colagiuri & Quinn, 2018). For instance, a recent study found that the placebo effect completely extinguished over the course of one week, although the initial placebo effect was considerably lower than in the present studies (Kunkel et al., 2024). The discrepancy to previous findings might be related to the trial-by-trial manipulation used in the present studies. As conditions changed on every trial and participants never received safety signals (i.e., placebo cues) for a prolonged period of time, it is possible that participants experienced comparable and high levels of arousal across all three conditions. This might have led to a stabilization of both placebo and nocebo effects.

1.6.2 Positive and negative expectations in neural activity from anticipation to pain

A key objective of these studies was to elucidate the neural basis of expectation processing and the stability of expectations during both the anticipation and pain phase. Similarities and differences between positive and negative expectations were of particular interest, given the ongoing debate surrounding this topic (S. Freeman et al., 2015; Fu et al., 2021). During the anticipation phase, there was a similar representation of positive and negative expectations in large parts of the brain, suggesting that valence-neutral processes dominated during this phase. In contrast, a distinct representation was observed during the pain phase, indicating valence-dependent processes occurring during pain processing. These findings align with previous research that highlighted the importance of anticipatory activity for subsequent pain processing (Wager et al., 2004), and support the idea that anticipatory activity might be a neural correlate of the prior in terms of predictive coding (Büchel et al., 2014). Furthermore, the differentiation from the anticipation phase to the pain phase suggests that separate processes may take place during these phases, from common mechanisms for expectation generation to distinct activity reflecting the actual modulation of pain.

Concerning stability, the findings indicated that the longer-term stabilization of placebo and nocebo effects was depending on activity in different brain regions, potentially related to a varying importance of neuronal subprocesses for the stability of effects. Discovering that characteristics of neuronal processing during the initial expression of expectations can determine the persistence of effects one week later represents a novel contribution to the field, as previous research has predominantly focused on identifying neural predictors for a higher placebo and nocebo responsiveness, but not on predictors of their stability (Atlas & Wager, 2012; Colloca, 2024).

Several areas were identified to be crucial for expectation effects and their persistence. Among these, key areas of the descending pain modulatory system (DLPFC, vmPFC, and ACC) mirrored the generally observed neural pattern of common effects during the anticipation phase, followed by distinct effects during the pain phase. This implies that regions within the DPMS were engaged throughout the anticipation and pain phase, potentially reflecting subprocesses related to the generation, maintenance, and integration of expectations with the pain percept. The DPMS has

been suggested to be at least partially responsible for the neural implementation of placebo and nocebo effects, with higher cortical structures modulating pain-related activity down to the level of the brainstem and spinal cord (Benedetti et al., 2022; Eippert et al., 2009; Fields, 2004; Geuter et al., 2017). Some areas of the DPMS have shown to exhibit opposite responses during pain perception, which aligns with the current findings (Benedetti et al., 2020; Crawford et al., 2021; Koyama et al., 2005; Scott et al., 2008). Additionally, the findings presented here contribute to the limited understanding of DPMS activity during the anticipation phase, where there have been some hints for shared activity for both placebo and nocebo (Amanzio et al., 2013; Rossettini et al., 2023; Schmid et al., 2015).

The prefrontal cortex has been suggested to play a crucial role in the modulation of pain within the DPMS by exerting top-down control over pain-sensitive areas (Benedetti et al., 2022; Bingel et al., 2007; Geuter et al., 2017; Wager & Atlas, 2015). Specifically, the DLPFC has been proposed to represent and update expectations and goals, while the vmPFC integrates signals from the DLPFC to maintain expectations and attach affective meaning to them (Geuter et al., 2017; Wager et al., 2004). The current findings corroborate and extend this suggested role by demonstrating a valence-neutral representation of directed expectations during the anticipation phase. Furthermore, the relatively early onset of DLPFC effects within the anticipation phase strongly supports the significant role of the prefrontal cortex in initiating expectation effects and that it occupies a leading position within the signaling cascade of the DPMS (Geuter et al., 2017; Koban et al., 2017; Wager et al., 2004; Wager & Atlas, 2015).

Only during pain processing, prefrontal activity differentiated between positive and negative expectations, in line with previous findings (Amanzio et al., 2013; Atlas & Wager, 2012; Crawford et al., 2021; Fu et al., 2021; Geuter et al., 2017; Wager et al., 2004; Zunhammer et al., 2021). This may reflect the exertion of descending control over pain transmission. Moreover, greater DLPFC activation during the pain phase was predictive of the persistence of placebo effects over one week. This suggests that the strength of top-down control exerted during the pain phase may also affect the persistence of placebo effects, and is not only related to the individual strength of placebo responses (Wager et al., 2011). A possible explanation may be the role of the prefrontal cortex in suppressing learning from violated expectations in placebo hypoalgesia, resulting in a reduced updating of expectations (Schenk et al., 2017). However, it remains unclear why this mechanism should exclusively apply to placebo

effects, especially as structural changes of the DLPFC have been observed in chronic pain conditions (Seminowicz & Moayedi, 2017).

Another pivotal area within the DPMS is the ACC (Fields, 2004). The ACC may exert direct control over the activity of the PAG, thereby enabling the modulation of incoming pain signals by either facilitating or inhibiting transmission (Fields, 2004; Geuter et al., 2017; Livrizzi et al., 2022). The present findings support this proposed role and are consistent with the few previous reports of common ACC activation in response to both positive and negative expectations during the anticipation phase and differential activation during the pain phase (Amanzio et al., 2013; Kong et al., 2008; Rossettini et al., 2023). Moreover, the combined EEG-fMRI analysis temporally localized the ACC activity to the transition from the anticipation phase into the pain phase, suggesting that the observed ACC activity may reflect a preparation for the subsequent modulation of downstream areas and its execution (Geuter et al., 2013, 2017; Kong et al., 2008).

In addition to the areas of the DPMS, the anterior insula was found to reflect expectations throughout both the anticipation and pain phase, with a relatively early onset of activity. As part of the pain matrix, the anterior insula is crucial for pain processing, especially regarding the affective aspects of pain perception (Apkarian et al., 2005; Lu et al., 2016; Peyron et al., 2000). It is also involved in the evaluation of prediction errors (Horing & Büchel, 2022) and has been implicated as important area for placebo analgesia (Amanzio et al., 2013; Atlas & Wager, 2014; Zunhammer et al., 2021). Beyond its role in pain, the anterior insula serves as a key area in multiple networks and neural processes. It functions as a multimodal network hub (Adler-Neal et al., 2019; Dionisio et al., 2019; Lu et al., 2016), is a central area of the salience network (Menon & Uddin, 2010), and participates in the evaluation of threat (Fullana et al., 2016; Taesler & Rose, 2016; Wiech et al., 2010). Given its complex connections within multiple networks, the anterior insula may integrate signals from various sources into an expectancy signal, including information about the expected saliency and threat of upcoming stimuli during the anticipation phase (Taesler & Rose, 2016; Wiech et al., 2010). This aligns with the early timing of activity, suggesting that the anterior insula plays a pivotal role in the initiation of pain-related expectations.

During the pain phase, the role of the anterior insula may shift towards integrating expectations with the incoming nociceptive information. In this context, it may evaluate prediction errors and the saliency of pain stimuli (Fazeli & Büchel, 2018; Geuter et al.,

2017; Horing & Büchel, 2022). The responses of the anterior insula during the pain phase were found to predict the individual stability of placebo effects, indicating that the evaluation of prediction errors, saliency, and affective processes triggered by pain stimulation might influence the persistence of placebo effects. This could reflect enhanced learning processes from expectation-consistent information, resulting in self-reinforcing feedback loops (Jepma et al., 2018; Schenk et al., 2017). Although a similar relationship was not established for nocebo in the present study, further research is warranted on this matter, as abnormal learning signals from the insula have been associated with the chronification of pain (Ferraro et al., 2022; Horing & Büchel, 2022).

The stability of placebo effects was also predicted by a reduction of amygdala activity during the anticipation phase. The amygdala is connected to several areas implicated in placebo effects, such as the vmPFC and PAG (Geuter et al., 2017). Decreased amygdala activity has been associated with increased placebo effects (Wager & Atlas, 2015). Given that the amygdala is thought to play a role in assessing threat levels (Johansen et al., 2010; Wager & Atlas, 2015) and in associative learning (Büchel et al., 1998; Geuter et al., 2017; Milad & Quirk, 2012), the reduced activation may be linked to diminished learning from subsequent corrective experiences or a reduced perception of threat. When integrating this finding with the other observed effects, anticipatory processes in the amygdala and anterior insula related to affect and learning appear to be crucial determinants of the stability of placebo effects, while the DLPFC activity during the pain phase may reflect an inhibition of expectation updating.

Another key area that emerged from the present findings is the thalamus. The observed increased activation for nocebo during the pain phase may be attributed to the thalamus' role in the processing of sensory-discriminative aspects of pain, as it is a critical component of the pain matrix (Apkarian et al., 2005; Peyron et al., 2000). Enhanced thalamus activity may reflect heightened processing of incoming sensory information in response to the nocebo cue. Moreover, the individual stability of nocebo effects was predicted by thalamic activity during the pain phase. Differences in the strength of sensory modulation cannot fully account for this relationship, as the individual strength of behavioral nocebo effects during the first session was controlled for. It is more likely that, as demonstrated in previous research showing a link between conditioning processes and thalamus activity (Jensen, Kaptchuk, et al., 2015), subjects with more persistent nocebo effects may have experienced a stronger influence from

non-consciously learned associations between the cue and pain intensities during the first session, which contributed to the stabilization of the nocebo effect.

The present studies also provided valuable insights into how expectations are represented in EEG oscillatory activity. An important role for anticipatory low-frequency oscillations for expectation processing emerged, as they were found to be correlated with activity in neural regions that are suggested to generate expectations, and anticipatory theta-to-alpha activity was modulated by the valence of expectations one week following the induction of expectations. These findings align with previous studies indicating that low-frequency activity may be modulated by expectations and that it may affect the subsequent processing of pain stimuli (Babiloni et al., 2004; Nickel et al., 2022; Strube et al., 2021; Taesler & Rose, 2016, 2021; Tu et al., 2016). The functional relevance of low-frequency oscillations has been explained within the framework of predictive coding as reflecting the top-down signaling of expectations (Ploner et al., 2017; Strube et al., 2021). While the present findings indicated larger theta-to-alpha activity for negative expectations, the direction of modulation has been inconsistent across previous studies, leaving it unclear why these discrepancies exist (Babiloni et al., 2004, 2006; Nickel et al., 2022; Strube et al., 2021).

Additionally, a correlation between ACC activity and high frequency oscillatory activity was observed at the transition from the anticipation phase into the pain phase. High-frequency oscillatory activity has been linked to the bottom-up processing of sensory information, signaling higher perceived intensities or higher stimulation temperatures during the pain phase or reflecting prediction errors (Nickel et al., 2022; Ploner et al., 2017; Strube et al., 2021; Tu et al., 2016). Considering the present findings, high-frequency oscillations might also be essential in the transfer of positive and negative expectations into the pain phase.

1.6.3 Practical implications

The three studies presented in this dissertation have demonstrated that both positive and negative expectations have real, stable, and neurobiologically measurable effects on pain perception, and that these expectations may even be consciously induced. Especially in the light of previous research showing that both types of effects may interact with real treatments, it is essential to discuss the clinical implications of the findings (Bingel et al., 2011; Colloca, 2024; Colloca & Barsky, 2020). On the one hand, treatment efficacy may be enhanced by reinforcing the effects of positive expectations,

while on the other hand, negative expectations also pose risks for treatment success (Evers et al., 2018).

One goal for the clinical practice is to minimize negative expectations and the emergence of nocebo effects (Evers et al., 2018). The potential negative consequences have become evident through a recently proposed integrative psychobiological model of chronic pain, which suggests that persisting negative expectations may lead to a vicious cycle of chronic negative modulation of pain (Büchel, 2023). Therefore, it appears crucial to break this cycle by addressing and reducing negative expectations before they become permanent and adversely impact future pain perception and treatments. However, adherence to the principle of autonomy in healthcare, which mandates that patients need to be informed about possible negative treatment consequences, presents an ethical dilemma regarding how to achieve this (Colloca, 2024). Possible strategies to reduce nocebo effects may focus on how negative information is conveyed. One approach is authorized concealment, which can be applied when expected side effects are mild. In such cases, patients may be informed about nocebo effects and the impact of negative expectations, and are subsequently allowed to choose whether they want to be informed about side effects (Colloca, 2024). Another strategy is to frame side effects as an indicator that the treatment is effective, which has been shown to affect patients' belief in the treatment and modulate DPMS activity (Berna et al., 2017; Schenk et al., 2024). Similarly, the experience of a functioning nocebo condition in the present studies may have also boosted the efficacy of the placebo condition, as this could have enhanced the legitimacy of the entire BCI feedback for the participants. The findings regarding the conscious modulation of expectations also indicate that it may be possible to consciously reduce expectations regarding side effects and negative treatment outcomes. This underscores the importance of not only carefully considering and addressing pre-existing beliefs and experiences, but also the patients' strategies regarding expectation management.

The second goal is to maximize positive expectations and thereby harness the therapeutic potential placebos offer without the necessity of administering actual drugs (Enck et al., 2013; Evers et al., 2018; Rief & Wilhelm, 2024; Thompson et al., 2009). Positive expectations had large effects on pain perception and remained relatively stable over one week, in line with previous reports from clinical studies (e.g., Khan et al., 2008; Perlis et al., 2005, 2005; Quessy & Rowbotham, 2008). It can be

hypothesized that they may even foster a beneficial cycle, serving as positive counterpart of the previously discussed vicious cycle (Büchel, 2023). However, similar to the ethical concerns associated with reducing negative treatment expectations, promoting positive expectations is also challenging. Practitioners are obligated to provide a truthful description of treatments and prescribed medication (Blease et al., 2020; Colloca & Barsky, 2020). In addition to providing more positive descriptions of treatments within the ethical boundaries, the present studies offered evidence for alternative strategies, such as neurofeedback or a conscious modulation of expectations, to effectively harness the effects of positive expectations (Colloca & Barsky, 2020).

While only sham neurofeedback was employed in Studies 1 and 2, there have been attempts at using genuine EEG- or fMRI-based neurofeedback to train participants to up- or downregulate their brain activity (Roy et al., 2020; Salari et al., 2014). For instance, it was demonstrated that participants can learn to increase or decrease theta power in the insula, leading to changes in pain discrimination (Taesler & Rose, 2021), and that learned control over rostral ACC activation and SIIPS-1 expression changes pain perception (Berman et al., 2024; deCharms et al., 2005). Neurofeedback could also be employed as noninvasive technique to directly modulate activity in areas related to positive expectations. The DLPFC and anterior insula have already been proposed as therapeutic targets for the treatment of chronic pain (D. Liu et al., 2024; Poublan-Couzardot & Talmi, 2024) and emerge as promising targets from the present findings, given their role in generating expectations and their association with the stability of placebo effects. However, as the neural pathways during pain anticipation appear to be shared between positive and negative expectations, further studies should explore the potential to specifically enhance positive expectations through neurofeedback, ensuring that negative expectations are not inadvertently substantiated. Although this method presents an exciting opportunity, it may currently be impracticable for the clinical practice due to the high time and cost demands. Nonetheless, in clinical research, neurofeedback holds promise for verifying pathways and mechanisms by assessing the functional relevance of activity in areas related to expectations.

Moreover, despite the simplicity of this procedure, a conscious modulation of expectations also led to changes in pain perception. While these findings appear promising, this represents only a preliminary step towards actual clinical applications.

The intervention shared some characteristics with open-label placebos, which refer to the non-deceptive, transparent prescription of placebos (Blease et al., 2020; Colloca & Barsky, 2020). In both conscious expectation modulation and OLPs, individuals are aware that they will not receive any active treatment, thereby addressing the ethical concerns related to prescribing placebos. First studies have indicated that OLPs may be effective in many conditions, including pain, stress, and well-being, and among patients experiencing back pain, cancer-related fatigue or major depression (Blease et al., 2020; Charlesworth et al., 2017; Kaptchuk, 2018; Spille et al., 2023; von Wernsdorff et al., 2021). The intervention of consciously modulating expectations goes beyond OLPs by illustrating that participants may voluntarily enhance the efficacy of inert treatments, despite being aware that the condition cues did not indicate actual changes in stimulation. Another important difference is that participants who benefit from OLPs often do not report positive expectations concerning the procedure, suggesting that more unconscious and embodied processes may mediate those effects (Kaptchuk, 2018). In contrast, the participants of the present study rated their expectations consistent with the condition cues, indicating more conscious processes.

Practical implications of these findings include the potential to educate patients about the influence they may have over their own expectations related to treatments and perceptions. This may also enhance the perceived control that patients have over their own treatment, which could facilitate positive expectations and the placebo effect (Strube et al., 2023; Tang et al., 2022). However, to prevent discouragement and disengagement, it may be crucial to ensure that patients are not burdened with excessive responsibility concerning their symptoms. Moreover, participants reported various strategies to modulate their expectations, including internal verbalization and the imagination of positive consequences. Practitioners could guide patients in employing these techniques to leverage the beneficial effects associated with positive expectations.

1.6.4 Limitations and future directions

One limitation across all three studies of this dissertation concerns the control condition. Although the aim was to induce no expectations regarding the subsequent pain stimulation in the control condition, participants rated their pain and expectation levels in the control condition at an average of about the midpoint of the scale and in between the nocebo and placebo conditions, suggesting that they developed

expectations of medium pain intensities. Previous studies reported similar ratings for the control condition relative to placebo and nocebo conditions (Bingel et al., 2011; Colloca et al., 2010; Shih et al., 2019), and it may be hypothesized that automatic learning processes throughout the course of the experiment make it virtually impossible to avoid the development of any expectations following the control cue. The conclusions drawn in this dissertation focus on a comparison between directed expectations, i.e., conditions where pain perception was modulated and shifted away from the actual stimulus input, to a condition where perception aligned with the stimulated intensity. Whether the observed ratings in the control condition resulted from valid expectations of medium intensity or solely from sensory inputs driving pain perception is of secondary importance. Future studies should carefully consider how to design optimal control conditions for the effects in question and interpret findings according to the specific comparisons being made.

It is also important to note that a short-term modulation of pain perception in cue-based paradigms on a trial-by-trial basis may involve different mechanisms compared to block-wise or between-subjects designs. Both opioid and CCK release are thought to have lasting effects that may not fluctuate enough between stimuli to fully account for placebo and nocebo effects (Atlas & Wager, 2012). Therefore, future research should compare trial-by-trial to block-wise designs within participants to disentangle the contributions of short- and long-term mechanisms that may underlie placebo and nocebo effects.

Lastly, besides EEG, there was no physiological readout measure to validate the reported placebo and nocebo effects in Studies 2 and 3. This limitation is particularly relevant in Study 3, where participants were aware of how they were intended to rate the stimuli, potentially inducing effects of social desirability. To enhance the rigor of future investigations into the promising approach of conscious expectation modulations, it is essential to employ other objective outcome measures, such as skin conductance responses.

1.6.5 Conclusion

The present dissertation demonstrated that both positive and negative expectations can lead to equally large effects on pain perception and could even be consciously induced. Both types of expectations remained relatively stable over one week, possibly due to biases in learning from corrective experiences. There were both shared and

distinct patterns of brain activity representing positive and negative expectations, while the stability of effects was predicted by different correlates. During the anticipation phase, positive and negative expectations were similarly represented, possibly reflecting the generation and maintenance of expectations, while activity differentiated during the pain phase, which may reflect the integration of expectations with the incoming sensory signals. The stability of placebo and nocebo effects was supported by distinct neural correlates observed during this process, indicating a different relevance of subprocesses for the persistence of effects. Especially the descending pain modulatory system and the anterior insula were found to play a significant role in the expectation-based modulation of pain and in maintaining the stability of placebo and nocebo effects. Additionally, the findings support the proposed relevance of anticipatory activity for subsequent perception and are compatible with a special role of low-frequency oscillations in the signaling of expectations. Regarding the ongoing debate on whether the neural representations of positive and negative expectations reflect two sides of the same coin, these studies provide evidence for both shared and distinct characteristics, highly contingent upon the investigated process and time point. Beyond enhancing the fundamental understanding of the processes related to positive and negative expectations, the findings may pave the way for practical applications in the future. This includes the development of new targets for neurofeedback aimed at enhancing placebo effects, as well as interventions focused on the conscious modulation of pain expectations.

2 Abkürzungsverzeichnis / List of Abbreviations

ACC ..... Anterior cingulate cortex
BCI ..... Brain-computer interface
BOLD ..... Blood-oxygenation-level-dependent
CCK ..... Cholecystokinin
DLPFC ..... Dorsolateral prefrontal cortex
DPMS ..... Descending pain modulatory system
DPSS ..... Discrete prolate spheroid sequences
EDA ..... Electrodermal activity
EEG ..... Electroencephalography
FIR ..... Finite impulse response
fMRI ..... Functional magnetic resonance imaging
FWE ..... Family-wise error
MNI ..... Montreal Neurological Institute
MOR ..... μ-opioid receptors
OFC ..... Orbitofrontal cortex
OLP ..... Open-label placebo
PAG ..... Periaqueductal grey
ROI ..... Regions of interest
RVM ..... Rostral ventromedial medulla
S1 ..... Primary somatosensory cortex
S2 ..... Secondary somatosensory cortex
SCR ..... Skin conductance response
SIIPS-1 ..... Stimulus-intensity independent pain signature
VAS ..... Visual analogue scale
vmPFC ..... Ventromedial prefrontal cortex

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4 Reprints

4.1 Study 1: The neural dynamics of positive and negative expectations of pain

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RESEARCH ARTICLE

The neural dynamics of positive and negative expectations of pain

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eLife Assessment

Wittkamp et al. investigated the spatiotemporal dynamics of expectation of pain using an original fMRI-EEG approach. The methods are solid and the evidence for a substantially different neural representation between the anticipatory and the actual pain period is **convincing**. These **important** findings are discussed within a general framework that encompasses their research questions, hypotheses, and analysis of results. Although the choice of conditions and their influence on the results might accept different interpretations, the manuscript is strong and contributes beneficial insights to the field.

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Abstract Pain is heavily modulated by expectations. Whereas the integration of expectations with sensory information has been examined in some detail, little is known about how positive and negative expectations are generated and their neural dynamics from generation over anticipation to the integration with sensory information. The present preregistered study employed a novel paradigm to induce positive and negative expectations on a trial-by-trial basis and examined the neural mechanisms using combined EEG-fMRI measurements (n=50). We observed substantially different neural representations between the anticipatory and the actual pain period. In the anticipation phase i.e., before the nociceptive input, the insular cortex, dorsolateral prefrontal cortex (DLPFC), and anterior cingulate cortex (ACC) showed increased activity for directed expectations regardless of their valence. Interestingly, a differentiation between positive and negative expectations within the majority of areas only occurred after the arrival of nociceptive information. FMRI-informed EEG analyses could reliably track the temporal sequence of processing showing an early effect in the DLPFC, followed by the anterior insula and late effects in the ACC. The observed effects indicate the involvement of different expectation-related subprocesses, including the transformation of visual information into a value signal that is maintained and differentiated according to its valence only during stimulus processing.

Introduction

Nociceptive input can result in highly variable sensations of pain with expectations playing a crucial role in pain modulation (***Atlas and Wager, 2012***). Positive expectations can lead to hypoalgesia (placebo effect), while negative expectations can increase the perceived intensity of pain (nocebo effect; ***Kong and Benedetti, 2014***). Although many studies have shown that expectations can influ- ence pain perception, the neuronal processes underlying the generation of expectations prior to the appearance of the pain stimulus are not yet fully understood (***Benedetti, 2014***; ***Büchel et al., 2014***; ***Koyama et al., 2005***; ***Wager and Atlas, 2015***). Several studies have demonstrated that placebo and nocebo effects influence brain activity during pain perception (***Egorova et al., 2015***; ***Wager and Atlas, 2015***; ***Zunhammer et al., 2021***). This may occur through multiple pathways that are involved

in integrating expectations and sensory information (*Geuter et al., 2017*). Especially the descending pain modulatory system (DPMS; *Geuter et al., 2017*; *Tu et al., 2022*) is associated with placebo and nocebo effects and is thought to consist of areas like the periaqueductal gray (PAG), dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), and amygdala (*Eippert et al., 2009*), as well as frontal areas like the vmPFC (*Geuter et al., 2017*; *Tu et al., 2022*). Furthermore, placebo and nocebo effects modulate activity in areas classically associated with pain processing like the thalamus and the insula during noxious stimulation (*Atlas and Wager, 2014*; *Wager and Atlas, 2015*; *Wager et al., 2004*; *Zunhammer et al., 2021*).

A central feature of expectations is that they are generated prior to the appearance of the stimulus and should, therefore, be reflected in anticipatory neural activity (*Kong et al., 2007*; *Wager et al., 2004*). The mere expectation of the appearance of a painful stimulus has been shown to activate regions relevant to subsequent pain processing, such as the insula, DLPFC, and thalamus (*Palermo et al., 2015*; *Ploghaus et al., 1999*). Similarly, there is evidence that expecting reduced pain (e.g. via a placebo) modulates activity in parts of the DPMS and the insula already during pain anticipation (*Wager et al., 2011*). This includes the DLPFC (*Amanzio et al., 2013*; *Geuter et al., 2013*; *Wager et al., 2004*; *Watson et al., 2009*) and the ACC (*Amanzio et al., 2013*; *Geuter et al., 2013*) and takes place prior to the widespread modulation of neural activity in the DPMS during pain processing (*Amanzio et al., 2013*; *Atlas and Wager, 2014*). The activation of brain areas prior to the adminis- tration of a painful stimulus aligns with the general framework of placebo effects and nocebo effects proposed by *Büchel et al., 2014*. This framework posits that placebo hypoalgesia and nocebo hyper- algesia can be attributed to predictive coding, suggesting that perception is the result of a constant matching of incoming sensory data with the top-down predictions of an internal or generative model (*Büchel et al., 2014*). These top-down predictions should be reflected in the expectation generation happening before the stimulus. However, the neural mechanisms of this process remain unclear, with little information about where and how the expectations relevant to these top-down predictions are generated. Furthermore, it is uncertain whether the anticipatory modulation reported in the literature reflects only a pre-activation of later relevant networks or indicates functionally distinct processes in expectation generation. Comparing neural processing during both the anticipatory and pain periods prior to the stimulus is crucial for better dissociating the formation and maintenance of pain-related expectations from their integration with nociceptive input (*Wager et al., 2004*).

Another important factor when examining the representation of expectations is their valence, i.e., whether they are positive (as in placebo effects) or negative (as in nocebo effects). It remains elusive whether positive and negative expectations share a common neural basis or depend on different networks (*Freeman et al., 2015*). On the basis of behavioral differences between positive and negative expectations, such as their varying correlations with the amount of prior experience and differences in learning (*Colloca et al., 2010*; *Colloca et al., 2008*), it seems reasonable to assume at least partially dissimilar neural representations of positive and negative expectations. Especially during pain perception, there is evidence of distinct neural processes for positive and negative expectations, as well as valence-dependent modulation of similar systems. More specifically, some findings suggest differential modulation of activity in key areas of the DPMS and reward system by the valence of expectations (*Benedetti et al., 2020*; *Crawford et al., 2021*; *Koyama et al., 2005*; *Scott et al., 2008*). Alternatively, some studies have reported the absence of shared brain activations during the perception of pain (*Bingel et al., 2011*; *Freeman et al., 2015*; *Fu et al., 2021*; *Shi et al., 2021*; *Shih et al., 2019*). Similar findings have been reported during pain anticipation, as some areas have been reported to be specifically activated for either placebo or nocebo (*Fu et al., 2021*; *Rossettini et al., 2023*) or show opposing valence-dependent responses (*Amanzio et al., 2013*; *Kong et al., 2008*; *Palermo et al., 2015*). However, there is conflicting evidence of shared anticipatory activation for both placebo and nocebo in some areas of the DPMS like the DLPFC and ACC (*Amanzio et al., 2013*; *Amanzio and Palermo, 2019*; *Colloca and Benedetti, 2007*; *Frisaldi et al., 2015*; *Manaï et al., 2019*; *Palermo et al., 2015*; *Rossettini et al., 2023*; *Schmid et al., 2015*). This illustrates an ongoing debate about whether there is a common neural basis for positive and negative expectations instead of entirely separated representations (*Freeman et al., 2015*; *Fu et al., 2021*). Taken together, the neuronal representations of positive and negative expectations may consist of shared valence-neutral processes (common effects) and different or differentially modulated valence-dependent processes (distinct effects), and this relationship may change over time from the formation of expectations until

their integration with the sensory information. To adequately examine common and distinct neuronal representations, it is imperative to compare positive and negative expectations against each other, as well as to an appropriate control condition without any directed expectation, meaning that perception is not biased in any direction in this condition. Hence, in this study, we implemented a within-subjects design in which participants were subjected to positive, negative, or neutral expectations on a trial-by-trial basis, enabling an exploration of common and distinct processes.

Even during the anticipation phase, it is reasonable to presume the involvement of distinct processes unfolding in a temporal sequence. For instance, the visual cue must first be encoded and transformed into an expectation signal that can be interpreted by a 'pain system'. A potential candidate for this integration process is the insula, which is recognized for its function as a multimodal network hub (***Adler-Neal et al., 2019***; ***Dionisio et al., 2019***; ***Lu et al., 2016***). The multimodal role of the insula is further reflected in its role in fear conditioning, with the insula being associated with threat (***Fullana et al., 2016***), while other areas that are important for placebo modulations like the vmPFC are closely connected to the default mode network, potentially reflecting a safety signal (***Fullana et al., 2016***). Within the DPMS, prefrontal areas have been suggested to provide predictions for downstream pain-sensitive systems, implying an early role within this system (***Geuter et al., 2017***; ***Koban et al., 2017***; ***Wager and Atlas, 2015***). Subsequently, it appears plausible that other areas of the DPMS would need to be 'informed' and activated in close temporal proximity to the pain stimulus in order to exert their influence on pain perception (***Amanzio et al., 2013***). The assessment of the temporal profile of expectancy generation is beyond the possibility of fMRI. We therefore combined fMRI with simultaneous EEG measurements, allowing us to temporally localize neural activity by utilizing the temporal and spatial advantages of both techniques at the single-trial level.

In this study, we investigated the neural basis of the common and distinct processes underlying positive and negative expectations, and the formation and integration of expectations into pain perception, using a novel paradigm that allowed the manipulation of expectations on a trial-by-trial basis, while EEG and fMRI measures were recorded. We presented cues to induce expectations (positive, negative, or neutral expectations) followed by an anticipatory period in which different expectations emerged. This allowed us to examine the distinct and common effects of placebo and nocebo in the anticipation and pain phase by comparing the different expectation conditions. We focused on the evaluation of the different neuronal processes that contribute to the generation of directed expectations (i.e. positive and negative) in the anticipatory period and the effects during pain perception, using combined EEG and fMRI.

Based on the literature and the assumed theoretical approach of predictive coding, proposing an expectation formation before a stimulus, we expected that representations of pain-related expectations undergo dynamic changes during the anticipation phase and pain phase, reflecting different processes during these phases such as expectation formation, expectation integration, and pain modulation. These processes could involve either separate networks during the anticipation and pain phase or they could take place in the same networks, with the anticipatory activity having preparatory qualities for the later perception. Furthermore, different patterns of activity for positive and negative expectations could arise. If the valences do not differ in their activation patterns either during pain anticipation or pain processing, this would mark similar processes for both positive and negative expectations. On the other hand, different effects would either indicate distinct processes or a different modulation of the same process. We would mainly expect a distinct nature of the valences, but that similar areas would be engaged throughout anticipation and pain processing. Therefore, we hypothesized to see neural representations of expectations in similar areas during the anticipation and pain phase, albeit that those positive and negative valences would be differentially represented, marking dissociable dynamics of positive and negative expectations.

Results

In total, we investigated 50 participants (32 female) in a combined EEG-fMRI paradigm. In short, participants were told that they would be given real-time visual feedback on their current pain sensitivity based on their EEG activity using a Brain-Computer Interface (BCI). The feedback indicated one of three different brain states: either a state of high pain sensitivity (red cue/nocebo condition/negative expectation), low pain sensitivity (green cue/placebo condition/positive expectation), or that the BCI algorithm would not make any prediction (yellow cue/control condition/neutral expectation). In fact, the visual

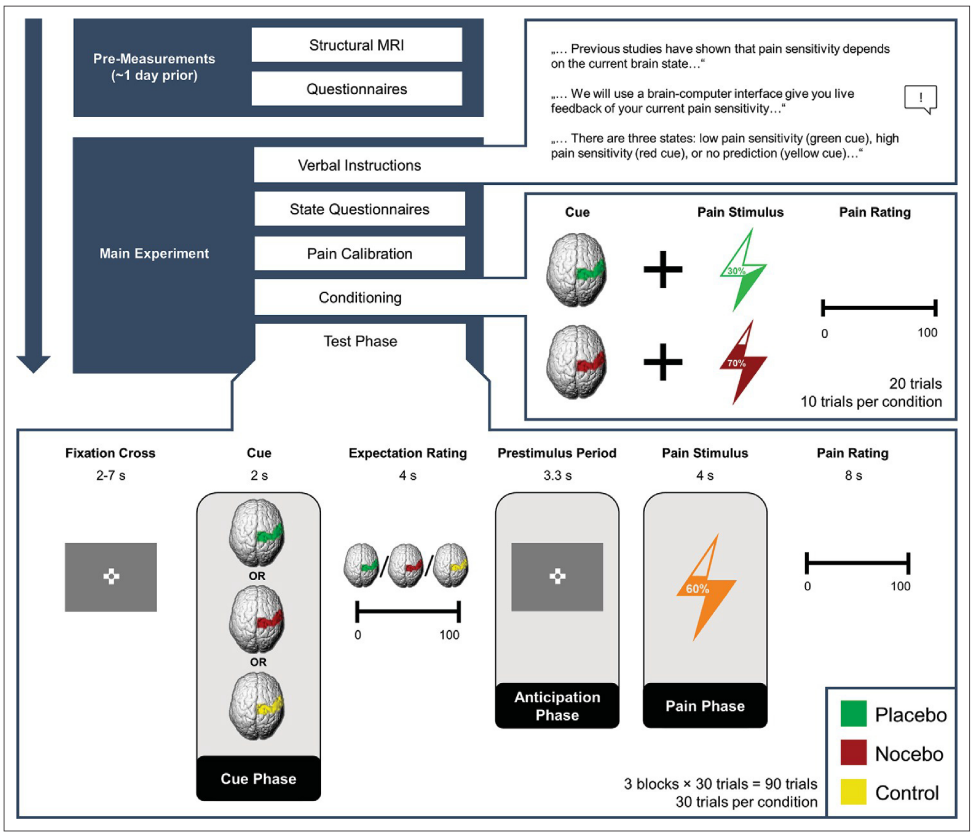

Figure 1. Experimental Procedure. Structure of the experiment including pre-measurements and the main experiment. Expectations were generated using a sham brain-computer interface (BCI), i.e., participants were told that they would receive real-time feedback regarding their pain sensitivity (verbal instructions) and experienced the validity of this feedback (conditioning). In the conditioning phase, green cues were paired with lower pain intensities compared to red cues unbeknownst to the participants. In the test phase, the stimulation temperature was always the same, regardless of the cue. The presentation of the condition cue varied from trial to trial.

cues were not related to any brain activity but were only used to produce the corresponding expectations. To reinforce expectations, we also performed a learning (i.e. conditioning) phase. Here, red cues were paired with higher pain intensity (VAS level 70), while green cues were paired with lower pain intensity (VAS level 30). In the ensuing test phase (see *Figure 1*), temperatures were kept constant (VAS level 60). Participants were informed that they would receive different pain stimuli of medium intensity and were unaware that the stimulation temperature was always exactly the same. In each trial, participants were given a BCI-based feedback supposedly related to their current brain state (cue phase) and subsequently rated their pain expectation for the next stimulus (expectation rating). After a fixed anticipation phase, they were presented with a brief heat pain stimulus with a constant target temperature irrespective of condition (pain phase), and lastly had to rate how intensely they perceived the stimulus (pain rating). Apart from EEG and fMRI we also continuously recorded electrodermal activity.

Successful induction of placebo and nocebo effects in behavioral ratings and skin conductance responses

Our data showed successfully induced expectations in line with the cued sham brain states as evidenced by a significant main effect of condition in a repeated-measures ANOVA for mean expectation ratings

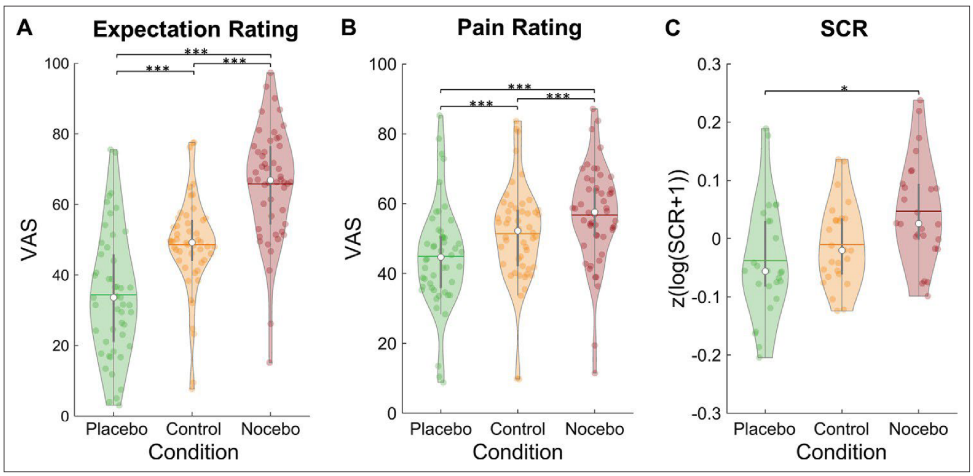

Figure 2. Expectation ratings, pain ratings, and skin conductance response by condition. Mean expectation (**A**) and pain ratings (**B**) on a visual analogue scale separately for each condition (n = 50). (**C**) Mean skin conductance responses in the three conditions (n = 26). White dots = mean, horizontal lines = median, thick gray vertical lines = upper and lower quartile, coloured dots = pain ratings of individual participants per condition.*p<0.05. **p<0.01. ***p<0.001.

($F_{(2,98)}$ = 86.51, p<0.001, η_P^2=0.64; see ***Figure 2A***). Expectation ratings were higher in the nocebo (M=65.80, SD = 15.80) compared to the control condition (M=48.58, SD = 13.79, p<0.001), which in turn were higher than in the placebo condition (M=34.33, SD=17.71, p<0.001). Similarly, mean pain ratings were affected by our manipulation (rmANOVA: $F_{(2,98)}$ = 63.00, p<0.001, η_P^2=0.56; see ***Figure 2B***). Post-hoc Tukey tests revealed higher pain ratings in the nocebo (M=56.80, SD=14.21) compared to the control condition (M=51.40, SD=14.31, p<0.001), which in turn led to higher pain ratings than the placebo condition (M=44.88, SD=15.06, p<0.001). Moreover, placebo (control - placebo) and nocebo (nocebo - control) effects were significantly correlated across subjects for both expectation (r=0.64, p<0.001) and pain ratings (r=0.30, p=0.033), indicating that subjects who expe- rienced stronger placebo effects also experienced larger nocebo effects.

To assess whether ratings within the three conditions were stable or varied over time, we compared the relative variability index (***Mestdagh et al., 2018***), a measure that quantifies intra-subject variation over multiple ratings, between the three conditions and over the three measurement blocks. We observed differences in relative variance indices between conditions for both expectation (F(2,96) = 8.14, p<0.001) and pain ratings (F(2,96) = 3.41, p=0.037). For both measures, post-hoc tests revealed that there was significantly more variance in the placebo compared to the control condition (both p_{holm}<0.05), but no difference between control and nocebo. Variance in expectation ratings decreased from the first block compared to the other two blocks (F(1.35,64.64)=5.69, p=0.012; both p_{holm}<0.05), which was not the case for pain ratings. There was no interaction effect of block and condition for neither expectation (F(2.65,127.06)=0.40, p=0.728) nor pain ratings (F(4,192) = 0.48, p=0.748), which implies that expectations were similarly dynamically updated in all conditions over the course of the experiment.

The expectation manipulation not only affected behavioral ratings but also the skin conductance responses (SCRs) to the pain stimuli (rmANOVA: F(2,50) = 4.33, p=0.018, η_P^2=0.15; see ***Figure 2C***). A post-hoc Tukey test showed larger SCRs in the nocebo (M=0.05, SD=0.09) compared to the placebo condition (M=-0.04, SD=0.10, p=0.049). SCRs in the control condition (M=-0.01, SD=0.07) did not significantly differ from neither the nocebo (p=0.072) nor placebo condition (p=0.607).

Successful induction of expectation effects in fMRI pattern

Induction of expectation effects was also tested in functional imaging data. For all fMRI analyses, a finite impulse response (FIR) model was used to characterize BOLD fluctuations over time from cue

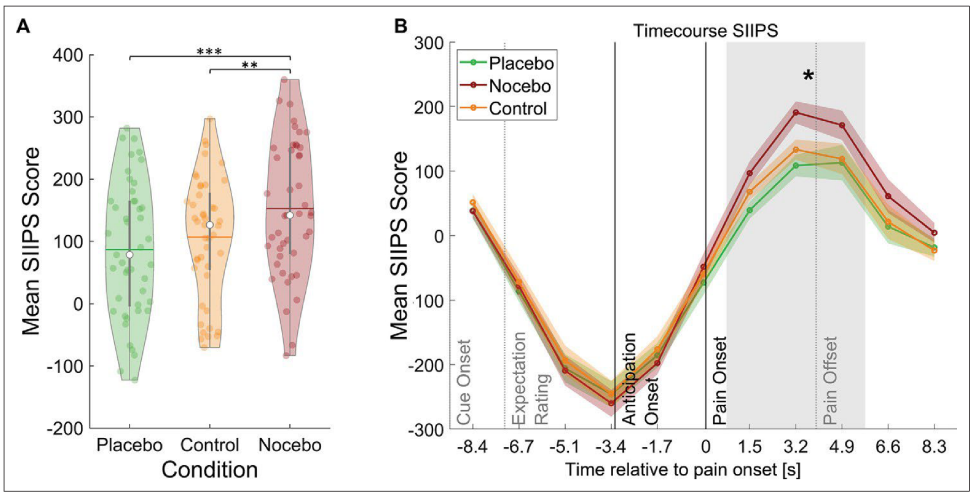

Figure 3. Stimulus intensity independent pain signature (SIIPS) scores by condition. (**A**) Mean SIIPS score per condition for all time-points during pain perception. White dots = mean, horizontal lines = median, thick gray vertical lines = upper and lower quartile, coloured dots = pain ratings of individual participants per condition. *p<0.05. **p<0.01. ***p<0.001. (**B**) Mean SIIPS score per condition plotted over the duration of the whole trial. The mean SIIPS scores shown in A were extracted from the gray-marked period. n = 45.

onset to the pain rating (see Methods for details). The stimulus intensity independent pain signature (SIIPS) has been introduced as a marker for subjective pain perception going beyond intensity differences as it has been reported to be affected by psychological factors such as expectations (***Botvinik-Nezer et al., 2023***; ***Woo et al., 2017***). To further validate our experimental design, we estimated the SIIPS score for each condition during the pain phase as a marker for differences in pain perception between the three conditions (see Methods). A rmANOVA revealed significant differences between the three conditions within the pain period ($F(2,88) = 11.59$, $p<0.001$, $\eta_p^2=0.21$), with Bonferroni-corrected paired *t*-tests showing significant differences between the placebo and nocebo condition ($t_{(44)} = 4.79$, $p<0.001$) and between the nocebo condition and the control condition ($t_{(44)} = 3.36$, $p=0.002$) but not between the control and the placebo condition ($t_{(44)} = 1.35$, $p=0.184$, see ***Figure 3***). We therefore conclude that the manipulation of expectations led to significant perceptual differences. Contrastingly, the SIIPS signature failed to discern between conditions during the anticipation period, suggesting fundamentally distinct processes in the two phases ($F(2,88) = 0.79$, $p=0.455$, $\eta_p^2=0.02$).

Neuronal representation of expectations over time

In our main analysis, we found a clear dissociation between the anticipation and pain phases with a predominantly common representation of positive and negative expectations during the anticipation phase and a later shift towards distinct effects during the pain phase (see ***Figure 4A***). In order to investigate how the representations of directed expectations changed over time from the anticipation to the pain phase, we identified common (i.e. positive and negative vs. control; constrained to areas with no statistical difference between positive and negative) and distinct (positive vs. negative) effects of directed expectations in each phase (see ***Supplementary file 1a-d*** for all comparisons). During the anticipation phase, common effects of directed expectations were found in several important areas of the DPMS, e.g., in the bilateral DLPFC, bilateral ACC, and right vmPFC, indicating that directed expectations were represented in a rather general and nonspecific way during this period. With the stimulus onset, activity in these areas showed differential activation between positive and negative expectations. Further differential activity was observed in e.g., the left insula, amygdala, thalamus, and hippocampus during the pain phase.

Crucially, the bilateral DLPFC, right vmPFC, left anterior insula, and thalamus were engaged during both the anticipation and pain phases (see ***Figure 4B***). In all these areas, directed expectations shifted

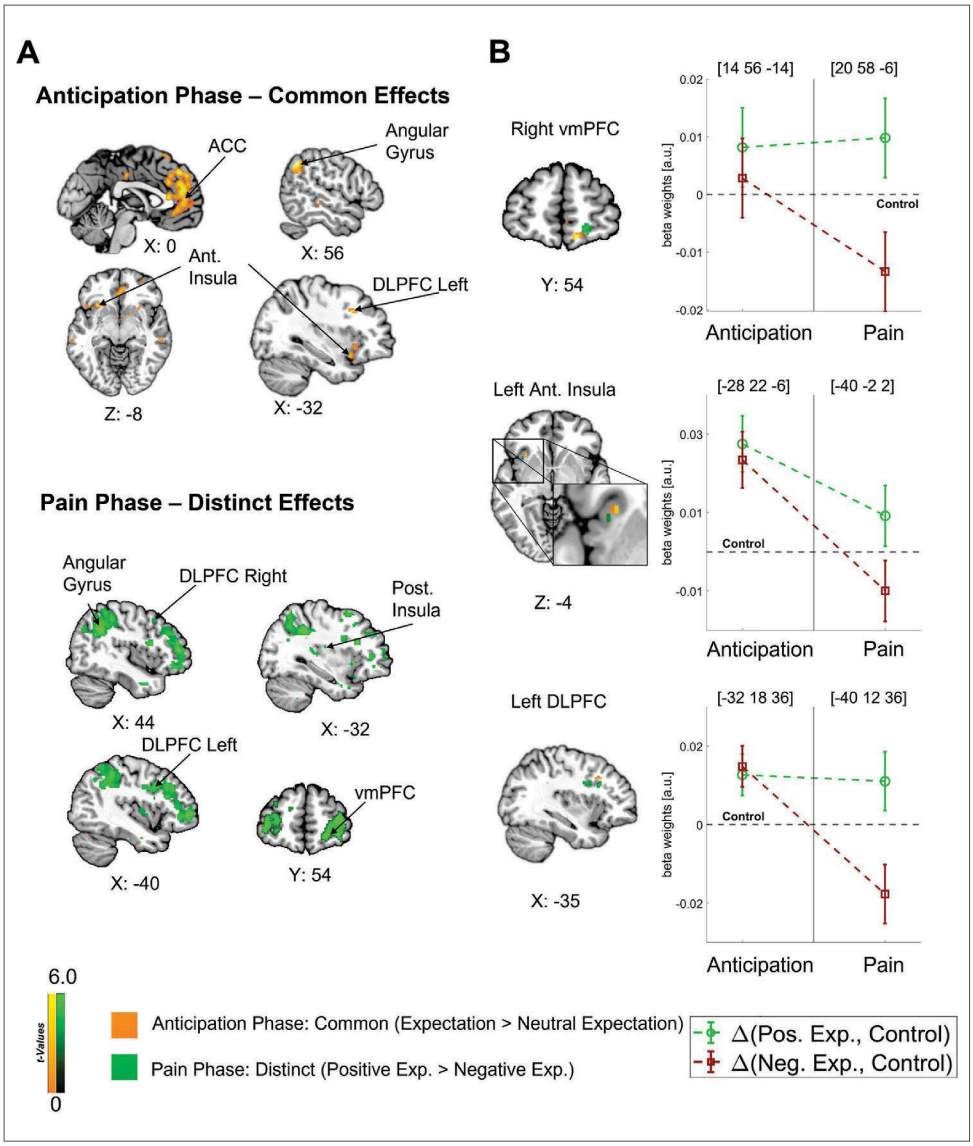

Figure 4. Differentiation of effects during the anticipation and pain phase. (**A**) Top: Common effects during pain anticipation (expectation > neutral expectation) at p<0.001 (uncorrected for display purposes) show widespread higher activity for both positive and negative expectations compared to the control condition. Bottom: Distinct effects (positive > negative) during pain perception are shown, indicating broadly higher activity for positive compared to negative expectations. (**B**) Left: For selected areas, the overlap between common effects of expectations during the anticipation phase (yellow) and distinct effects of positive and negative expectations during the pain phase (green) in the respective area is shown. Right: The corresponding activation levels of positive and negative expectations (i.e. beta weights from the finite impulse response (FIR) model) baselined by the control condition are plotted for each phase at the respective peaks (peak coordinates in parentheses). The visualization highlights the differentiation of effects following the onset of pain. n = 45.

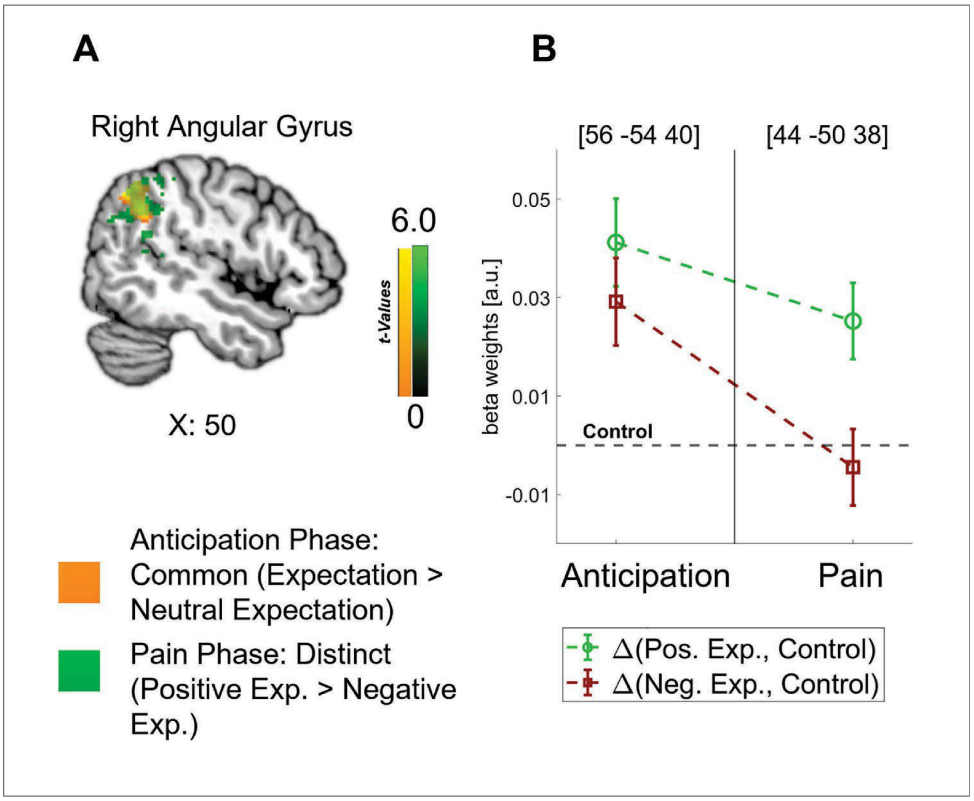

Figure 5. Representation of expectations in the angular gyrus. (**A**) Overlap between common effects of expectations during the anticipation phase (expectation > neutral expectation; yellow) and distinct effects of positive and negative expectations during the pain phase (positive > negative; green) is shown for the angular gyrus at p<0.001 (uncorrected for display purposes). (**B**) The corresponding activation levels of positive and negative expectations (i.e. beta weights from the finite impulse response (FIR) model) baselined by the control condition are plotted for each phase at the respective peaks (peak coordinates in parentheses). n = 45.

from a common (positive and negative > control) towards a distinct representation (positive > negative: bilateral DLPFC, right vmPFC, and left anterior insula; negative > positive: thalamus) over time. In addition to these areas that are frequently related to expectation effects, the right angular gyrus was also engaged throughout the time course, similarly initially showing a common representation of positive and negative expectations during the anticipation phase and differentiating only during pain perception (p<0.05 whole-brain FWE-corrected; see *Figure 5*).

The differences between the anticipation and the pain phase demonstrate that specific expectations were mediated by different processes during these phases and arise from a dynamic interplay of brain regions such as the DLPFC, vmPFC, anterior insula, and thalamus over time.

Timing of effects during the anticipation phase

To obtain detailed information on the temporal characteristics of the expectation effects during pain anticipation, we performed fMRI-informed EEG analyses. Specifically, we were interested in the temporal sequence of the areas involved. Single-trial estimates of fMRI activity during the anticipation phase were correlated with time-frequency decomposed EEG measures for each participant and then statistically tested at the group level. This analysis was conducted separately for the identified regions of interest that represented directed expectations during both the anticipation and pain phase (left

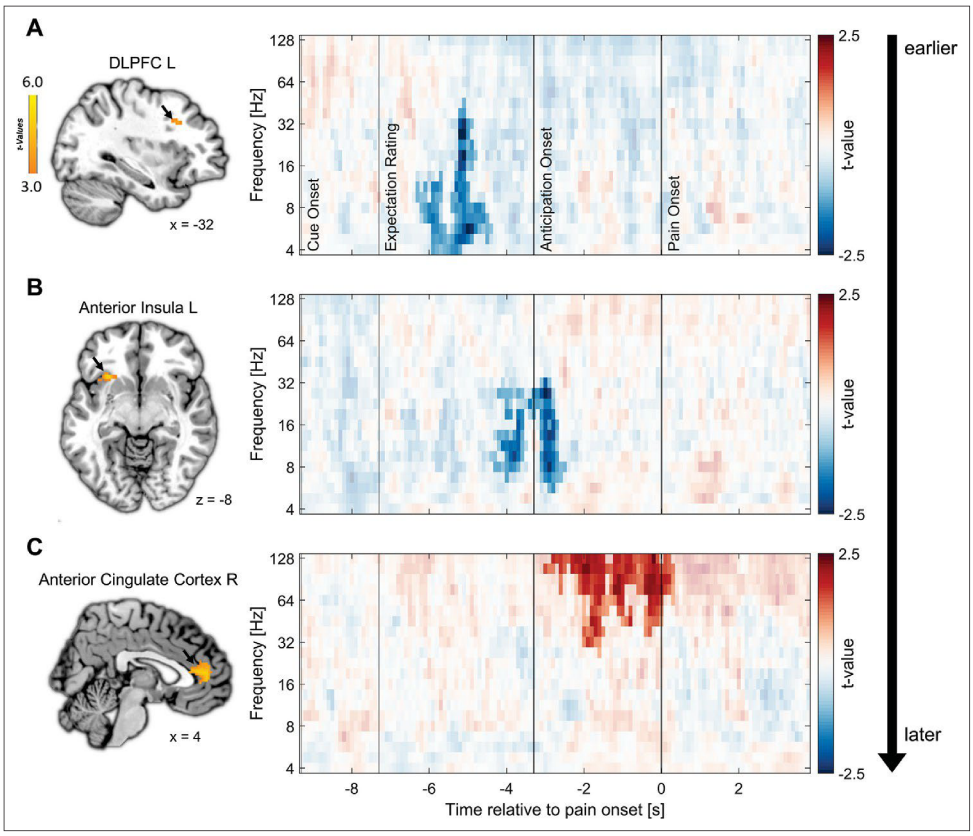

Figure 6. Relation of fMRI activity with EEG oscillatory power. Correlation of single-trial hemodynamic responses with time-frequency resolved EEG activity in the left dorsolateral prefrontal cortex (DLPFC) (**A**), left anterior insula (**B**), and right anterior cingulate cortex (ACC) (**C**) during the anticipation phase, ordered by the timing of observed correlations as indicated by the arrow on the right. Single-trial beta weights were extracted from spherical ROIs (10 mm radius) centered around the peak voxels based on the comparison of beta weights from the finite impulse response (FIR) model between expectation and neutral expectation during the anticipation phase, as shown on the left ($p<0.001$ uncorrected for display purposes). On the right, the cluster-corrected correlation of oscillatory power with fMRI activity averaged over all cluster electrodes is depicted. Non-significant time-frequency points are masked (n = 41).

anterior insula, right vmPFC, bilateral DLPFC, and thalamus). We further included the bilateral ACC, as we were interested in all areas that reflected directed expectations during the anticipation phase.

Clear temporal differences between areas were observed. The earliest correlation of fMRI activity with EEG oscillatory power was found in the left DLPFC already during the expectation rating at theta to low gamma frequencies in a negative direction (-6.3 until -4.4 s, 4-45.28 Hz; p=0.007; see ***Figure 6A***; additional information for all regions can be found in ***Supplementary file 1e***). Next, antic- ipatory activity in the left anterior insula was associated with decreased EEG oscillatory power during the late expectation rating and anticipation phase (−4.4 to -2.5 s) spanning from theta to low gamma frequencies (5.67-32 Hz; p<0.001; see ***Figure 6B***). Lastly, we found a significant positive association of EEG activity with fMRI activity in the right ACC during the anticipation and early pain phase in the gamma frequency range (−3 to 0.3 s, 26.89-128 Hz; p=0.003; see ***Figure 6C***). As expected due to the close spatial proximity, activity in the left ACC was similarly positively correlated with EEG activity in the anticipation and pain phase (cluster 1: -2.3 to -1.3 s, 32-128 Hz; p=0.019; cluster 2: -0.9 to 0.3 s,

32–128 Hz; p=0.021). We did not observe significant correlations of EEG power with fMRI activity in the other ROIs.

In summary, the analyses indicated that areas exhibiting similar fMRI effects for positive and negative expectations during the anticipation phase are linked to distinct temporal and oscillatory patterns. Notably, the left DLPFC and the left anterior insula displayed an early negative correlation between anticipatory activity and EEG power, primarily in the lower frequency range. Conversely, the subsequent effects in the bilateral ACC were associated with an increase in gamma oscillations and were observed at later time points during the anticipation phase indicating different processes.

Discussion

The manipulation of positive, negative, or neutral expectations on a trial-by-trial basis allowed for a detailed analysis of their neural representations during the generation and integration of expectations with the nociceptive input. Our results revealed fundamental differences between the anticipation and pain phases, indicating the involvement of divergent processes. During the anticipation phase, valence-neutral representations were observed in areas of the DPMS and the anterior insula. After the onset of the nociceptive stimulus, these areas showed differentiated representations indicating separate processes for the formation of expectations and their integration within stimulus processing. The excellent temporal resolution of our fMRI-informed EEG measures further revealed a temporal sequence of expectation processing during the anticipation phase, with an early effect within the DLPFC, followed by activation in the anterior insula, and late effects within the ACC, in line with the occurrence of different time-sensitive sub-processes of expectation generation in this phase.

Our novel within-subjects paradigm was highly effective, as expressed by 47 out of 50 subjects consistently expecting and perceiving the intensity of pain stimuli in line with the cues that induced positive, negative, or neutral expectations. The trial-by-trial manipulation of expectations did not only affect self-reports, but also objective markers such as skin conductance responses and scores of the SIIPS (***Woo et al., 2017***). The SIIPS is an indicator of neural activity that tracks differences in pain perception that go beyond pain intensity and is classically affected by placebo and nocebo interventions (***Botvinik-Nezer et al., 2023***; ***Woo et al., 2017***), thus serving as an excellent manipulation check for our paradigm. In line with our expectations, the SIIPS scores discriminated between positive and negative expectations only during the pain phase and not during the anticipation phase. This is a first hint at substantially different processes during the two phases.

The neural representations of directed expectations differed depending on the valence of the expectation, i.e., if they were positive or negative, and the time period. A shift from common (valence-neutral) to distinct (valence-dependent) effects was evident in areas classically involved in placebo analgesia during pain processing, including the bilateral DLPFC, right vmPFC, left anterior insula, and thalamus. The observation of this pattern in key areas of the DPMS indicates that the activation during the anticipation phase is not just mere pre-activation for later pain modulation, but that prepa- ratory processes that are distinguishable from those that occur during pain perception take place. These anticipatory processes may include expectation generation and maintenance, while the focus shifts towards the integration of these expectations into the sensory stream and the evaluation of the percept during pain processing. Importantly, the spatial resolution of fMRI is limited when it comes to discriminating whether the same pattern of activity is due to identical activation or to activation in different sub-circuits within the same area. Nonetheless, the overlap of areas is an indicator of similar processes involved in a more general preparation process. The observed differentiation during pain is in agreement with the few studies that looked into the neuronal representations of positive and negative expectations and mainly found differential effects, e.g., opposite responses or differentially modulated areas during pain (***Benedetti et al., 2020***; ***Crawford et al., 2021***; ***Koyama et al., 2005***; ***Scott et al., 2008***).

The network of the DPMS around the DLPFC, ACC, and vmPFC appears to play a pivotal role in expectation processes and in the valence-dependent modulation of pain perception. Following the framework of predictive coding, our results would suggest that the DPMS is the network responsible for integrating ascending signals with descending signals in the pain domain and that this process is similar for positive and negative valences during anticipation of pain but differentiates during pain processing. One important node of the DPMS is the DLPFC. The outstanding role of the DLPFC in placebo effects is well-established and supported by numerous reports of increased DLPFC activity

during pain anticipation (*Watson et al., 2009*) as well as during pain processing (*Zunhammer et al., 2021*). This area has been classically associated with top-down-regulation and expectation modula- tion (*Geuter et al., 2017*), as when its neuronal excitability is experimentally manipulated, placebo effects can be diminished or enhanced (*Egorova et al., 2015*; *Krummenacher et al., 2010*; *Tu et al., 2021*). The early onset of DLPFC activity implied by the EEG analysis now suggests that the DLPFC plays an important role in the initiation of both positive and negative expectation effects within the DPMS prior to the noxious stimulation (*Frisaldi et al., 2015*; *Geuter et al., 2017*; *Rossettini et al., 2023*; *Wager and Atlas, 2015*; *Wager et al., 2011*). The vmPFC is another important hub within the DPMS which is suggested to integrate the input from the DLPFC and to generate affective meaning in order to maintain expectations (*Geuter et al., 2017*). It further modulates pain perception by directly affecting brainstem systems (*Koban et al., 2017*), e.g., by influencing PAG activity (*Geuter et al., 2017*). The notion that prefrontal areas exert top-down control over other areas of the DPMS is further supported by the earlier timing of DLPFC effects compared to the ACC (*Craggs et al., 2014*; *Watson et al., 2009*). The ACC also has a direct influence on the activity of the PAG, suggesting that the ACC is another crucial area for pain modulation (*Geuter et al., 2017*; *Livrizzi et al., 2022*). The effect of the bilateral ACC at the transition from the anticipation to the pain phase as indicated by our combined EEG-fMRI analysis could be interpreted as a preparatory mechanism for the modulation of incoming sensory information in downstream areas, consistent with its supposed role in the DPMS (*Geuter et al., 2013*; *Geuter et al., 2017*; *Kong et al., 2008*). Using this framework, reports of ACC activations for both positive and negative expectations during pain anticipation could be understood as a pre-activation of the ACC for pain modulation in either direction (*Rossettini et al., 2023*). The link between anticipatory activity in the ACC and EEG oscillatory activity was observed in the high gamma band, which is consistent with findings that demonstrate a connection between increased fMRI BOLD signals and a relative shift from lower to higher frequencies (*Kilner et al., 2005*). Gamma oscillations have been repeatedly reported in the context of pain and expectations and have been interpreted as reflecting feedforward signals of noxious information (e.g. *Ploner et al., 2017*; *Strube et al., 2021*). In combination with our findings, this might imply that high-frequency oscillations may not only signal higher actual or perceived pain intensity during pain processing (*Nickel et al., 2022*; *Ploner et al., 2017*; *Strube et al., 2021*; *Tu et al., 2016*), but might also be instrumental in the transfer of directed expectations from anticipation into pain processing.

Similarly, a shift from valence-neutral towards valence-dependent processing over time was demonstrated in the anterior insula. The insula is a key brain region involved in the network responsible for pain processing (e.g. *Atlas and Wager, 2014*; *Kober et al., 2008*) but is also part of several non-pain-related networks and works as a multimodal network hub (*Horing and Büchel, 2022*). Results of the fMRI-informed EEG analysis indicated early effects in the anticipatory period consistent with an early role of the anterior insula in expectation generation and initiation. Due to the multimodal nature of the insula, it may be speculated that multiple networks interact to integrate relevant information from different domains (e.g. from the visual cue and interoceptive information) into an expectancy signal in the anterior insula. Due to its connections and function within the salience network, this may involve encoding the expected threat level and salience of subsequent pain stimuli, leading to an anticipatory activation prior to the actual perceptual modulation (*Taesler and Rose, 2016*; *Wiech et al., 2010*). During pain perception, the anterior insula may then be more engaged in a network responsible for stimulus processing and evaluating actual salience and prediction errors in this perceptual process (*Horing and Büchel, 2022*), which may imply that the insula is involved in multiple tasks over time.

Similar to the anterior insula, the activation of the angular gyrus during pain processing and pain anticipation could be understood as an indicator for processes related to the formation and integration of directed expectations. The angular gyrus has been connected to expectation-related pain modulation only a few times (e.g. *Atlas and Wager, 2014*; *Tu et al., 2019*) but its involvement is prominent in our results. Based on its presumed function in maintaining recollected multimodal representations (*Jablonowski and Rose, 2022*; *Vilberg and Rugg, 2012*), the angular gyrus may be engaged in transforming sensory information from the visual cues into expectancy signals that can be processed by e.g., the DPMS and salience network.

With our rather unconventional and new paradigm, we were able to manipulate participants expectations on a trial-by-trial level and derive insights into the neural dynamics of positive and nega- tive expectations. While this may give rise to questions regarding the comparability of our study to

previous paradigms and the manner in which our control condition was employed, we would argue that our expectation manipulation falls in line with a manipulation of treatment expectancies, a typical method of expectation manipulation in placebo paradigms (see ***Atlas and Wager, 2014***). Accordingly, it is comparable to previous studies on placebo and nocebo effects. In our study, participants were presented with a cue that induced expectations regarding a 'treatment,' although in this case the 'treatment' originated from changes in their own brain activity. This is, in a broader sense, comparable to studies utilizing sham TENS-devices that are supposedly altering peripheral pain transmission (***Skvortsova et al., 2020***).

Moreover, implementing a proper control condition in expectation modulation paradigms is an inherently difficult task as forming expectations about our environment is a natural process. Therefore, we recognize that participants most likely did form expectations of medium pain intensities in the control condition over the course of the experiment. This is in line with previous research on placebo and nocebo effects, in which participants also typically rated control stimuli in between placebo and nocebo conditions (***Bingel et al., 2011***; ***Colloca et al., 2010***; ***Shih et al., 2019***). However, we would still argue that we can meaningfully compare the placebo and nocebo condition to the control conditions to investigate the neuronal underpinnings of expectation effects. Independently of whether participants build up an expectation of 'medium' intensities in the control condition, which caused them to perceive stimuli in line with this, or if they simply perceived the stimuli as they were (of medium intensity) with limited effects of expectations, the crucial difference to the placebo and nocebo conditions is that there was no alteration of perception due to previous experiences or verbal information and no shift of perception from the actual stimulus intensity towards any direction in the control condition. Thus, we were able to identify the effects of directed expectations by comparing positive and negative expectations to neutral expectations as a baseline. Our analysis of within-condition variability further showed that ratings indeed varied within conditions and that the amount of variation was comparable between nocebo and control. Over time, expectations were dynamically updated in all three conditions, speaking against alternative explanations of the rating differences between conditions such as a regression to the mean of ratings in the control condition.

Based on the present results, understanding the processing of expectations requires an examination of its temporal and spatial dynamics from anticipation to pain processing, while comparing positive and negative valences to each other and to a control condition. We found largely comparable activation for positive and negative expectations during the anticipation phase, including regions outside of those classically observed in pain processing and modulation. Based on the observed temporal profiles, the DLPFC and anterior insula may be related to the top-down initiation and generation of expectations, while the ACC is activated in close proximity to pain onset as a direct link to pain modulation. During the pain phase, the focus shifts from expectation generation and maintenance towards pain modulation in either positive or negative direction, leading to distinct effects of positive and negative expectations in many areas that initially encoded expectations independently of their valence. It is not surprising that expectations are not a static process but involve different time-dependent components, as pain perception was also recently described as a complex process of interactions among multiple brain systems that are reconfigured over time (***Lee et al., 2022***). Expectation generation, integration, and pain perception all appear to be dynamic processes, with both common and distinct routes for positive and negative expectations, depending on the time point of examination.

Materials and methods

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
software, algorithm	Matlab (2021b)	mathworks.com	RRID:SCR_001622	
software, algorithm	SPM 12 (7771)	https://www.fil.ion.ucl.ac.uk/spm/	RRID:SCR_007037	
software, algorithm	Ledalab (V3.4.9)	http://ledalab.de/		
software, algorithm	JASP (0.18.3)	https://jasp-stats.org/	RRID:SCR_015823	

Table 1. Characteristics of study participants.

	Mean	SD	Range	Number (%)
Gender				
Male				18 (36%)
Female				32 (64%)
Age (years)	25.4	3.5	18–34	
FOP				
Severe Pain	36.5	5.4	22–47	
Minor Pain	18.9	4.9	10–33	
Medical Pain	25.8	6.4	12–42	
STADI				
Anxiety	15.2	4.1	10–28	
Depression	17.0	3.2	11–25	
Global Score	32.2	5.1	23–43	
BDI-II Global Score	6.0	3.8	0–16	

Note. STADI = State-Trait Anxiety Depression Inventory. FOP = Fear of Pain Questionnaire. BDI-II=Beck Depression Inventory-II.

Participants

In total, 55 volunteers were recruited via an online job platform and participated in our preregistered study (German Clinical Trials Register; ID: DRKS00025872). All participants were right-handed, had normal or corrected-to-normal vision, reported no neurological or psychiatric diseases, pain conditions, current medication, substance abuse, or pregnancy, and were non-smokers. They gave written informed consent and were compensated with 15 Euros per hour of participation. Of these 55 participants, five had to be excluded from all analyses (four due to technical issues leading to the abortion of the measurement, one due to a severe BDI score), leading to a final sample size of n=50 (see ***Table 1***). As preregistered, three participants who rated expectation and pain averaged over the entire experiment higher for placebo compared to nocebo and/or stated that they did not believe in the BCI method were excluded from the analysis of neural data, as we reasoned that the analysis of expectation-related neural activity requires a successful induction of expectations. Analyses for fMRI data were performed additionally excluding two participants with bad MRI data (leading to n=45 for fMRI analyses), and for combined EEG-fMRI analyses additionally excluding four participants with excessive artifacts and/or recording equipment malfunction (leading to n=41 for combined EEG-fMRI analyses). The study was approved by the local ethics committee (PV7170).

Procedure

Pre-measurements (Collaborative Research Centre recordings)

One day before the actual study, we recorded fMRI data (T1, functional EPI, DW EPI) and asked the participants to complete a comprehensive psychosocial questionnaire battery that will be analyzed by other projects under the structure of the overarching collaborative research center and are beyond the scope of this manuscript. Participants were pseudonymized using ALIIAS (***Englert et al., 2023***).

Main experiment

The experiment consisted of four phases: a verbal instruction phase, a pain calibration phase, a conditioning phase, and a test phase. The experiment was programmed using Psychtoolbox3 (http://psychtoolbox.org/) for Matlab (Version R2021b; The MathWorks). Rating responses were given by the participants using a Button Box MR. Instructions and ratings were presented on an MR-compat- ible monitor with a resolution of 3840×2160, placed at one end of the scanner. Participants saw the monitor through a mirror that was placed approximately 12 cm away from the participant's eyes and

had a distance of approximately 151 cm from the monitor. Two researchers and one radiographer guided the participants through the instructions and preparations.

Verbal instruction phase

After being prepared for the EEG and fMRI recording, participants were verbally informed that their current oscillatory state of the primary somatosensory cortex would be measured in real-time using a BCI (Brain Computer Interface) and that this state would reflect their pain sensitivity. They were further told that the measured brain state would be visualized in the form of visual stimuli consisting of a brain image with the right primary somatosensory cortex highlighted in one of three different colors (green, red, or yellow). A green stimulus represented a state in which their brain would be less susceptible to pain, a red stimulus represented a state in which their brain would be highly susceptible to pain, and a yellow stimulus represented a state in which the algorithm was not able to detect a clear-cut state and would thus make no prediction (e.g. due to high fluctuations in brain activity or intermediate activity levels). With this procedure, a positive expectation was induced by the green cue, and a negative expectation was induced by the red cue. After the verbal instructions, participants were asked to fill out state questionnaires, including the State-Trait Anxiety Depression Inventory (***Laux et al., 2013***) and the Fear of Pain Questionnaire (***McNeil and Rainwater, 1998***).

Pain calibration phase

Heat stimuli were delivered with a PATHWAY CHEPS (Contact Heat-Evoked Potential Stimulator) thermode (https://www.medoc-web.com/pathway-model-cheps), which has a rapid heating rate of 70 °C/s and a cooling rate of 40 °C/s and can deliver pain stimuli in the range of 30 to 55°C in less than 300 ms. For all phases, the baseline temperature was set to 32 °C, and the rise and fall rates were set to 70 °C/s. The thermode head was attached to a location directly proximal to the volar mid-forearm. Using a stepwise procedure, we determined individual temperatures for each participant corresponding to values of VAS30, VAS60, and VAS70 on a visual analog scale (VAS) from 0 (‘no pain’) to 100 (‘unbearable pain’). Target temperatures were calculated using linear regression.

Conditioning phase

For the conditioning phase, the location of the thermode head was changed to a location directly distal to the volar mid-forearm to avoid unnecessary sensitization of one location and skin irritations. Participants were instructed that the next phase would serve as the calibration of the BCI algorithm introduced in the verbal instruction phase. They were informed that in this phase only green and red cues would appear because the pain stimulation would only occur once a clear-cut state of their brain has been detected. Each trial began with the presentation of either a red or green visual cue for 2 s, then the painful stimulus was administered for 4 s, and lastly, participants were asked to rate their pain experience for 8 s. Between trials, there was a fully randomized inter-trial interval (ITI) of between 2 and 7 s. During the painful stimulation and ITIs, a fixation cross was presented in the middle of the screen. Perceived pain intensity was again rated on a VAS from 0 (‘no pain’) to 100 (‘unbearable pain’). Unbeknownst to the participants, green cues were always followed by less painful stimuli (VAS30), and red cues were always followed by more painful stimuli (VAS70). They received 10 stimuli of each condition, leading to 20 trials in total. The order of stimuli was pseudo-randomized with the restrictions of no more than two direct repetitions of the same condition and the last two trials of this phase belonged to the less painful condition.

Test phase

For the test phase, the thermode head was once again attached to the location directly proximal to the volar mid-forearm. Participants were informed that the BCI algorithm has now been calibrated and would be tested in the next phase. They were told that the painful stimulation would occur at random predetermined points in time, so that either highly pain-sensitive (red; nocebo condition) or less pain-sensitive states (green; placebo condition) could be detected and reported back to the participant, or that they would receive feedback that the algorithm was not able to detect a clear-cut state (yellow; control condition). The trial structure was similar to the conditioning phase, with the change that after cue presentation, participants were asked to rate how painful they expected the next stimulus to be

on a VAS ranging from 0 to 100 while the cue was still presented on the screen (4 s). After this expectation rating, a fixation cross was presented for 3.3 s (anticipation phase) before the pain stimulus was administered for 4 s (pain phase). Independently of the cue color, participants always received painful stimuli corresponding to values calibrated to VAS60. Importantly, participants were only informed that they would receive different stimuli of medium intensity and were thus not aware that the stimulation temperature remained constant. There were 30 cues of each condition followed by pain stimulation divided into three blocks, summing up to a total of 90 stimuli. Similarly to the conditioning phase, the ITI was fully randomized between 2 and 7 s. The order of cues was pseudo-randomized with no more than two direct repetitions of the same condition. Before each block, we applied one pain stimulus of VAS60 without a cue to desensitize the new skin area.

Follow-Up

One week after the main experiment, participants were invited for a follow-up measurement which is beyond the scope of this manuscript. They were asked to fill out questionnaires, including the Beck Depression Inventory-II (***Beck et al., 1996***; ***Hautzinger et al., 2006***). Lastly, participants were debriefed and paid.

Data acquisition

Electrodermal data

Electrodermal activity was measured with MRI-compatible electrodes on the thenar and hypothenar. Electrodes were connected to Lead108 carbon leads (BIOPAC Systems, Goleta, CA). The signal was amplified with an MP150 analogue amplifier (also BIOPAC Systems) and sampled at 5000 Hz using a CED 1401 analogue-digital converter (Cambridge Electronic Design, Cambridge, UK).

fMRI data

MRI was performed with a 3T Siemens PRISMA Scanner, and a 64-channel head coil was used. On the day of the pre-measurements, a T1 image with the following parameters was acquired: T1 FLASH 3D: TE 2.98 ms, TR: 2300 ms, matrix flip angle: 9°, FOV 25.6 * 25.6 cm, TA: 7:22 min. Two sequences on the day of the main experiment were acquired: An EPI BOLD sequence and a field map sequence. Participants were prepared with a 64-channel standard BrainCap MR for 3Tesla (2020 Version) and the EPI BOLD sequence had therefore to be adjusted to meet the necessary safety criteria. The following parameters were used: 2 D EPI BOLD: TE: 29.0 ms, TR: 1679.00 ms, FOV: 22.4 * 22.4 cm, flip angle: 70°, s1: 2 mm, TA: 20:17 min, fat saturation, 715 volumes in total; 2 D field map sequence: TR: 594 ms, TE1: 5.51 ms, TE2: 7.79 ms, FOV: 22.4 * 22.4 cm, flip angle: 40°, s1: 2 mm, TA: 1:31 min.

EEG data

Continuous EEG data was recorded inside the MRI scanner using a custom 64-channel BrainCap-MR for 3 Tesla using BrainVision Recorder (Version 1.10, BrainProducts, Gilching, Germany). The cap contained 64 passive sintered Ag/AgCl electrodes arranged according to the 10/20 System, as well as one ECG electrode. FCz served as the reference and Pz served as the ground electrode. The cap was connected to two Brain Amp MR plus amplifier systems with 32 channels each (BrainProducts, Gilching, Germany), powered by one rechargeable battery unit. Amplifiers and the battery unit were positioned on foam cushions directly behind the head coil inside the scanner. Electrode skin impedance was kept below 10 kΩ. EEG data was recorded with a sampling rate of 5000 Hz and an amplitude resolution of 0.5 µV for EEG channels and 10 µV for the ECG channel. The EEG system was synchronized with the clock of the MRI system using a SyncBox (BrainProducts, Gilching, Germany). The helium pump of the MRI system was switched off during data recording. Data was transmitted from the amplifiers to the recording computer outside of the scanner room via a fiber-optic cable.

Preprocessing

Electrodermal data

Preprocessing and analysis of electrodermal data were performed using the Ledalab toolbox for MATLAB (***Benedek and Kaernbach, 2010***). Single-subject data was downsampled to 100 Hz and visually screened. In total, 21 subjects were excluded from the electrodermal analysis (18 due to

physiological non-responsiveness, three due to equipment malfunction). From the remaining 26 subjects, all data segments around pain stimulation were screened for excessive artifacts, resulting in the exclusion of 55 of the 2340 segments (2.35%). Using a deconvolution method implemented in Ledalab, raw electrodermal data were decomposed into continuous phasic (driver) and tonic components. Subsequent analyses were performed on the extracted phasic skin conductance responses (SCRs). The response window for pain was determined by visual inspection of the curve to cover the peak and set between 2 and 7.5 s. SCR segments within the response window were log- and z-transformed within participants. For the log-transform, a constant (minimum of the driver plus 1) was added to the data to shift it to positive values. Lastly, segments were averaged per subject for each of the three conditions.

fMRI data

Preprocessing of fMRI data was done using the Statistical Parameter Mapping software (SPM 12, Wellcome Department of Imaging Neuroscience, London, UK, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The first two volumes of each block were dropped to get full MRI saturation effects. Furthermore, realignment and unwarping, registration to standard space (Montreal Neurological Institute), and spatial smoothing with a 6 mm Gaussian kernel were used on the data.

EEG data

MR and cardioballistic artifacts were corrected using BrainVision Analyzer 2.2 (BrainProducts, Gilching, Germany) for each block separately. Continuous MR artifacts were corrected with sliding baseline corrected average templates. Data was then downsampled to 500 Hz. Cardioballistic artifact correction was done by semi-automatically detecting a pulse template, marking it in the electrocardiogram channel, and then subtracting it from recordings.

For the remaining preprocessing and analysis, we used the Fieldtrip toolbox for Matlab (***Oostenveld et al., 2011***). Data were cut into trials including all relevant time intervals from 1000 ms before cue onset to the end of pain 15,800 ms after cue onset. The resulting segmented data were low-pass filtered at 150 Hz and high-pass filtered at 0.5 Hz. We adapted a recent preprocessing approach introduced by ***Hipp et al., 2011***. The data was split into low- and high-frequency data (34 Hz low-pass filter and 16 Hz high-pass filter, respectively) and processed in parallel. This approach leads to high sensitivity in detecting and removing artifacts from the data as e.g., heartbeats cause more artifacts at lower frequencies and muscle activity affects higher frequencies more strongly. All trials were visually inspected and removed for both subsets when containing large artifacts. Then, both high- and low-frequency data were subjected to an independent component analysis (ICA) using a logistic infomax algorithm. Components reflecting residual cardioballistic and MR artifacts, blinks, eye- and head movement, and muscle activity were identified by visual inspection of the time course, spectrum, and topography of each component and discarded. Both subsets were re-referenced to the average of all channels and the original reference electrode was regained. Lastly, we subjected all data to another full visual scan and shifted the time axis so that the onset of pain stimulation occurred at t=0 s. In total, the visual artifact screening led to the exclusion of 228 of the 3944 recorded trials (5.78%).

Time-frequency decomposition

Our procedure was again adapted from ***Hipp et al., 2011***. Time-frequency decomposition was conducted for 21 logarithmically spaced frequencies ranging from 4 to 128 Hz (0.25-octave incre- ments) in 0.1 s steps using the multi-taper method based on discrete prolate spheroid sequences (DPSS). The high-frequency data were used for the frequency transformation of frequencies above 25 Hz, and the low-frequency data for frequencies below 25 Hz. Temporal and spectral smoothing were adjusted to match 250 ms and 3/4 octave, respectively. This was achieved by fixing the time window to 250 ms and adjusting the number of Slepian tapers for frequencies larger than 16 Hz, while for frequencies up to 16 Hz, a single taper was used, and the time window was adjusted. We extracted single-trial time-frequency resolved data for each participant.

Data analysis

Behavioral data

We compared differences in pain and expectation ratings for the different cue conditions by computing two repeated-measures ANOVAs with cue type (placebo vs. nocebo vs. control) as predictor and pain and expectation ratings as outcome, respectively. Partial eta-squared was used to describe effect sizes.

Furthermore, we analyzed variability within conditions indicated by the relative variability index (***Mestdagh et al., 2018***) by computing two repeated-measures ANOVA with cue type (placebo vs. nocebo vs. control) and measurement block (block 1, block 2, block 3) for the relative variability index of expectation and pain ratings, respectively.

Electrodermal data

We compared differences in SCRs in the pain phase by conducting a repeated-measures ANOVA with the factor cue type (placebo vs. nocebo vs. control) as predictor and SCR as outcome.

fMRI data

Statistical inference

For each subject, a finite impulse response model (FIR model) was set up on a time course of 18.4 s starting at the onset of the cue, divided into 11 bins, with a bin roughly covering the duration of one TR (1.679 s compared to 1.675 s). The FIR model was implemented separately for each condition. Data was also corrected for cardioballistic and respiratory artifacts by including them as regressors built with the RETROICOR algorithm of the PhysIO toolbox (***Frässle et al., 2021***; ***Kasper et al., 2017***).

On the group level in a flexible factorial design, directed *t*-contrasts were set up for common effects (placebo and nocebo vs. control), exclusively masked with the *F*-contrast between placebo and nocebo (thresholded at $p<0.05$ uncorrected) to identify areas that showed a similar response for placebo and nocebo but different to the control condition in the anticipation phase. For the comparison between placebo and nocebo, directed *t*-contrasts were set up to identify areas that showed distinct modulation by placebo and nocebo in both the anticipation and pain phases. Analyses in the anticipation phase were performed by including the FIR regressors covering the time period from -4.275 s until -0.925 s relative to pain onset (bins 4 and 5), analyses in the pain phase by including the FIR regressors covering the time from 0.75 s until 5.8 s relative to pain onset (bins 7, 8, and 9). All analyses were corrected for multiple comparisons using FWE ($p<0.05$) correction.

ROI analyses

Additionally, ROI analyses were conducted regarding a priori hypotheses in the following areas defined by the anatomy based on the Harvard-Oxford atlas: insular cortex, thalamus, ACC, hippocampus, and amygdala. Furthermore, an ROI analysis was conducted on the DLPFC based on the clusters identified in the meta-analysis conducted by ***Zunhammer et al., 2021*** by applying a 15 mm-radius sphere around the two reported peak coordinates (xyz_{MNI}: 42, 11, 33, and xyz_{MNI}: -30, 13, 54) bilaterally.

Combined EEG-fMRI analysis

Single-trial fMRI BOLD response amplitudes were estimated based on the preprocessed MR data using GLMsingle (***Prince et al., 2022***). The hemodynamic response during the anticipation phase was estimated by fitting a boxcar function with a length of 1.679 s to the anticipation onset. The accuracy of beta estimates was improved by an adaptation of GLMdenoise for single-trial beta estimation. Furthermore, the noise was reduced by using fractional ridge regression as integrated into the GLMsingle toolbox. For each trial, we extracted the mean beta within several regions of interest centered around the significant peak voxels derived from the MR analyses of common effects of expectations during the anticipation phase. These included the left anterior insula (xyz_{MNI}: -28, 22,-6), left (xyz_{MNI}: -2, 40,-4) and right ACC (xyz_{MNI}: 4, 42, 12), right vmPFC (xyz_{MNI}: 14, 56,-14), left (xyz_{MNI}: -32, 18, 36) and right DLPFC (xyz_{MNI}: 40, 24, 36), and left thalamus (−6,-12, 4; all with 10 mm sphere). For each participant on a single-trial level, Spearman's rank correlation coefficients between beta ROI estimates and time-frequency EEG data were computed, resulting in one time-frequency-resolved correlation pattern per participant and ROI. For the group-level analysis, correlations were Fisher-z-transformed

and tested against zero using nonparametric cluster-based permutation tests as implemented in the Fieldtrip toolbox (cluster threshold: p=0.05, minimum neighbors: 2, number of randomizations: 2000). Statistics were calculated from cue onset until pain offset (-9.3 until 3.9 s relative to pain onset).

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Author contributions

Christoph Arne Wittkamp, Maren-Isabel Wolf, Conceptualization, Data curation, Software, Formal analysis, Validation, Investigation, Visualization, Methodology, Writing - original draft, Project administration, Writing - review and editing; Michael Rose, Conceptualization, Resources, Software, Supervision, Funding acquisition, Validation, Methodology, Writing - original draft, Project administration, Writing - review and editing

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Ethics

The study was approved by the local ethics committee (Ethikkomission der deutschen Arztekammer Hamburg) (PV7170). Infomed consent and consent to publish was obtained.

Peer review material

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Additional files

Supplementary files

- Supplementary file 1. Tables containing all fMRI contrasts and results from the combined EEG-fMRI analysis. (a) Common effects of positive and negative expectations compared to control in the anticipation phase. (b) Differential activation for expectation compared to neutral expectation in the pain phase. (c) Differential activation between placebo and nocebo in the anticipation phase. (d) Activation for positive expectations compared to negative expectations in the pain phase. (e) Combined EEG-fMRI analysis.

- MDAR checklist

Data availability

Derived data that support the findings of this study are available at https://osf.io/3g49v/. Due to data privacy restrictions, further data is only available on request.The only data not publicly available are the unprocessed raw data, which could potentially be used to re-identify participants; this measure is in place to safeguard participant privacy. Researchers interested in accessing the raw data should contact the lead investigator Michael Rose (rose@uke.de), providing a rationale for their request. Upon review, they will be granted access to the raw data.

The following dataset was generated:

Author(s)	Year	Dataset title	Dataset URL	Database and Identifier
Wittkamp CA, Wolf M, Rose M	2024	The neural dynamics of positive and negative expectations of pain	https://osf.io/3g49v/	Open Science Framework, 10.17605/OSF.IO/3G49V

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4.2 Study 2: Differential neural activity predicts the long-term stability of the effects of positive and negative expectations on pain

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Differential neural activity predicts the long-term stability of the effects of positive and negative expectations on pain

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Expectations modulating pain perception is a well-researched phenomenon, but less is known about the persistence of expectation effects over longer time-courses. In this preregistered study, we examined the persistence of positive (placebo) and negative (nocebo) expectation effects over one week and investigated whether neural activity on day I (fMRI) can predict the stability of these effects one week later (n = 41). We tested whether expectations were reflected in EEG oscillatory activity at the second measurement. Both positive and negative pain modulation effects persisted over the tested time-period and did not undergo extinction. Expectations of higher compared to lower pain led to larger theta-to-alpha EEG activity. Most interestingly, differential neural activity in fMRI was correlated with persistent expectations. Individual differences in the persistence of positive expectation effects were related to reduced amygdala activity and enhanced activity in the anterior insula and dorsolateral prefrontal cortex (DLPFC) during the first session. In contrast, persistence of negative expectation effects was predicted by enhanced thalamus activity. Our findings indicate relatively stable placebo and nocebo effects over longer time courses, but this persistence is based on different neural areas for positive and negative expectations.

Expectations can influence the perception of pain and thereby lead to hypoalgesia (placebo effect) or hyperalgesia (nocebo effect)[1]. These perceptual modulations are thought to be instantiated through activation and deactivation of the so-called descending pain modulatory system on the neural level (DPMS)[2,3], leading to measurable differences in the processing of painful stimuli[1,4]. However, few studies so far investigated the stability of these expectation effects across longer time periods[5–7].

In a standard Bayesian or non-Bayesian learning model, placebo and nocebo effects would be expected to decrease after the offset of reinforcement and disappear over time, as participants would update their beliefs when continuously receiving stimuli that contradict their expectations[8,9]. In spite of this, recent behavioral and neural evidence suggests that expectations can work via self-reinforcing feedback loops that prevent them from extinction[8,9]. This fits with evidence of highly stable placebo effects in clinical studies[10–14], while controlled experimental research on the stability of expectation effects over multiple days is sparse but much needed to gain mechanistic insights into these effects[15]. Within one session, both placebo hypoalgesia and nocebo hyperalgesia have shown to be relatively stable over multiple test trials[8,9,16–22], depending e.g. on the number of conditioning stimuli[23] and the valence of expectations[18,21]. Regarding the valence of expectations, there is some evidence for a higher persistence of nocebo compared to placebo effects, possibly due to higher arousal that impedes learning from experiences in nocebo groups[18,21]. Additionally, it has been demonstrated that partial reinforcement during conditioning is more effective in creating stable placebo effects over a test phase than consistent reinforcement[24]. Few studies examined effects over longer time-courses. For example, Whalley et al. induced placebo effects using only verbal instructions and observed similarly high effects in two sessions between one and eight days apart[5]. In contrast, Colloca et al. found a slight decrease of placebo effects after four to seven days, although importantly, effects were still evident in the second session[19]. Regarding negative expectations, a nocebo effect in tactile perception was still detectable one week later[25], while to our knowledge there has not been any investigation on the consistency of nocebo responses in pain perception after multiple days.

The conceivable persistence of placebo and nocebo effects in pain perception further raises the crucial question of which neuronal areas predict the persistence of expectations over a longer period. Considering that the prefrontal cortex might be responsible for the neural suppression of learning from prediction errors in

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placebo hypoalgesia[8], the persistence of expectations might be connected to this area. Beyond this, activity in other areas that give rise to the expectation-related modulation of pain might mediate the stability of placebo and nocebo effects, such as areas of the DPMS like the dorsolateral prefrontal cortex (DLPFC), ventromedial prefrontal cortex (vmPFC), or the anterior cingulate cortex (ACC)[2], as well as the insula[26,27]. Interestingly, we have previously demonstrated that many of these areas were similarly activated for both positive and negative expectations during pain anticipation, while activation in the same regions differentiated during pain perception[28]. Likewise, predictive activity for persistent expectation effects might differ between pain anticipation and pain perception. Moreover, it is unclear whether predictive activity for upholding positive and negative expectations can be found in similar or different areas, when considering findings on the nocebo effects being more easily induced and more enduring compared to placebo effects[18,21].

With this study, we aimed to induce both positive and negative expectations to elicit placebo hypoalgesia and nocebo hyperalgesia in healthy participants, with the main objective to test the stability of the effects after one week. Further, we investigated neural predictors of the stability of effects. Lastly, we aimed to assess whether there were any differences in neural activity during the anticipation and pain phase between positive and negative expectations on day 8, measured by EEG oscillatory activity. We examined participants that underwent a conditioning procedure and verbal instructions inducing positive and negative expectations in one session (day 1), while being subjected to a combined fMRI and EEG measurement. Participants were then re-invited to the lab roughly one week later for a second test session (day 8), undergoing EEG measurement only. In this second session, we performed an identical procedure, but without a conditioning phase or verbal instructions to reinstate expectations (see Fig. 1). Due to the change in the external environment from day 1 (MR lab) to day 8 (EEG lab), we were also able to evaluate whether expectation effects would remain stable despite a change in the "treatment" context. Previously, we have shown that expectations were reliably induced on day 1 and were reflected in common neural activity for positive and negative expectations before the painful stimulus was applied but differentiated during pain stimulation[28]. Here, we focused on the stability of the behavioral effects

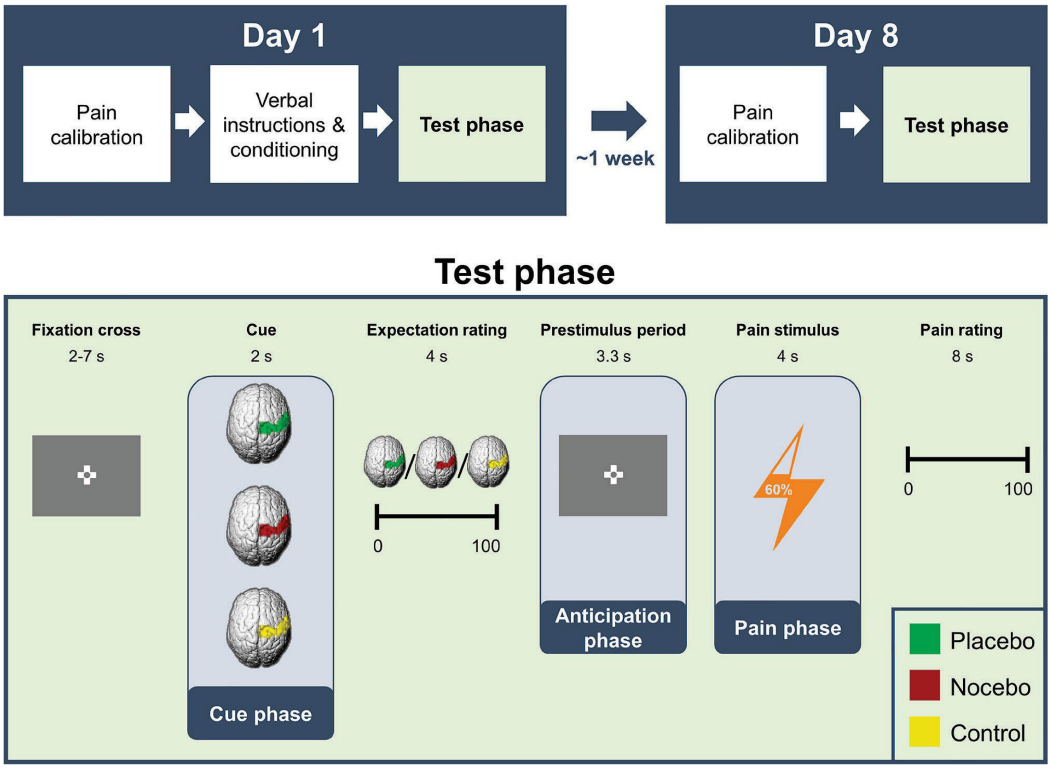

Fig. 1. Experimental structure. The experiment consisted of two sessions approximately one week apart (day 1 and day 8). On day 1 only, positive and negative pain-related expectations regarding visual cues were induced using verbal instructions and a conditioning procedure. On both day 1 and day 8, a similar test phase was performed, in which participants always received the same pain intensity, but condition cues varied from trial to trial. Pain intensities were individually calibrated on both days.

over one week, more specifically, how stable placebo and nocebo effects were over the time course of one week, and how the stability could be predicted using behavioral and neuronal markers.

Based on previous findings, we expected to find persisting placebo and nocebo effects after one week on the behavioral level[5,8,9,19]. As positive and negative expectations have shown to affect EEG oscillatory activity, especially when comparing expectations of high vs. low pain, we also expected to see differences in EEG activity between placebo and nocebo on day 8[29–31]. Lastly, we expected that the strength of behavioral effects on day 8 could be predicted by the magnitude of placebo- and nocebo-related fMRI activity on day 1, more specifically, in areas commonly connected to expectation effects such as parts of the DPMS. As previous research has demonstrated different patterns of activity in the anticipation phase preceding the application of the stimulus compared to the actual pain phase, we aimed to identify predictive effects within both of these periods.

Results

To test for the stability of expectation effects, 41 participants (26 female) came to the lab twice, approximately one week apart (see Fig. 1). On day 1, participants received sham feedback regarding their current pain sensitivity in the form of a colored cue, that was supposedly calculated by a brain-computer interface algorithm, to induce either placebo hypoalgesia, nocebo hyperalgesia or no directed expectation (control) while being subjected to EEG and fMRI measurements (for more information see Methods). Participants also underwent a conditioning procedure in which the placebo cue was paired with 10 lowly painful stimuli (VAS 30) and the nocebo cue was paired with 10 highly painful stimuli (VAS 70) on day 1. In the subsequent test phase, participants received feedback regarding their supposed pain sensitivity on each trial, had to indicate how painful they would expect to perceive the next stimulus, and after being presented with the same pain stimulus in every trial (VAS60) had to rate the actual perceived intensity. An identical test phase was conducted on day 8 while undergoing EEG measurement with the difference that no conditioning or verbal suggestion took place. Further details regarding this procedure and findings regarding the combined EEG-fMRI measurement on day 1 have been published elsewhere[28].

Induction and stability of effects

The successful induction of effects on day 1 has already been reported elsewhere[28]. In brief, expectation and pain ratings were significantly modulated by the three condition cues on day 1, leading to higher ratings for the nocebo compared to the control and in turn for the control condition compared to the placebo condition.

To investigate whether the induced effects were still evident one week later, we performed repeated measures ANOVAs with pain and expectation ratings as outcomes and included the time point (day 1, day 8) additionally to the condition (placebo, nocebo, control) as factor (descriptive time courses of expectation and pain ratings per condition and day are shown in Figs. 2a and 3a).

Expectation ratings were significantly affected by the condition, but there was no significant effect of time point and no interaction effect, indicating that there were no differences between day 1 and day 8 (see Table 1; Fig. 2b). Over both days, expectation ratings were higher in the nocebo ($M = 68.66$, $SD = 11.25$) compared to the control condition ($M = 50.23$, $SD = 10.91$, $p_{holm} < 0.001$), which in turn were higher than in the placebo condition ($M = 34.60$, $SD = 15.91$, $p_{holm} < 0.001$).

Similarly, pain ratings were significantly affected by the condition, but not by the time point nor their interaction (see Table 1; Fig. 3b). Pain ratings were higher in the nocebo ($M = 58.19$, $SD = 11.46$) compared to the control condition ($M = 52.63$, $SD = 10.70$, $p_{holm} < 0.001$), which in turn led to higher ratings than the placebo condition ($M = 46.75$, $SD = 12.47$, $p_{holm} < 0.001$). Thus, both pain and expectation ratings were significantly modulated by the condition cue in line with our manipulation and this effect appeared to be stable between day 1 and day 8. Moreover, the non-significant effect of time point hints that pain perception over all three conditions was stable over both measurement days, indicating that participants subjectively perceived pain stimuli on both days as equally painful.

We further compared the amount of variability in individual ratings between conditions and days. For expectation ratings, we found significant differences in the relative variability index (see Methods section for details) between conditions ($F(2,80) = 9.99$, $p < .001$), but no differences between days. Post-hoc tests revealed that there was less variability in the control condition compared to both placebo and nocebo condition (both $p_{holm} < 0.05$). In contrast, variability in pain ratings did not significantly differ between conditions, but increased from day 1 to day 8 ($F(1,40) = 13.45$, $p < .001$; post-hoc test day 1 vs. day 8: $p_{holm} < 0.001$). These results indicate that expectation ratings in the control condition might have been more driven by the cue compared to the other conditions, which was not the case in the pain ratings. Further, the similar level of variability in pain ratings across all three conditions indicated a dynamic updating of expectations.

Next, we assessed the stability of placebo and nocebo effects separately. *Placebo effects* (control minus placebo) and *nocebo effects* (nocebo minus control) were calculated as absolute differences in a manner that ensured that a higher rating modulation in the intended direction was always indicated by higher scores.

For expectation ratings, a rmANOVA revealed no differences in rating modulation between day 1 and day 8, no differences between placebo and nocebo effects and no interaction, implying no reduction in neither placebo nor nocebo effect between day 1 and day 8 (see Fig. 2c; Table 2). In contrast, pain rating modulations significantly differed between day 1 and day 8 (see Fig. 3c; Table 2). Post-hoc tests showed a significant decrease in pain rating modulation from day 1 ($M = 6.43$, $SD = 4.30$) compared to day 8 ($M = 5.01$, $SD = 5.25$, $p_{holm} = 0.036$), indicating that both placebo and nocebo effects were stronger on day 1 compared to day 8.

Although placebo and nocebo effects on pain ratings appeared to decrease over time, they were still highly evident on day 8 (placebo effect: $t(40) = 4.65$, $p < .001$, $d = 0.73$; nocebo effect: $t(40) = 4.68$, $p < .001$, $d = 0.73$), just like expectation rating effects were (placebo effect: $t(40) = 9.00$, $p < .001$, $d = 1.41$; nocebo effect: $t(40) = 8.95$, $p < .001$, $d = 1.40$). Most strikingly, this was still true at the final block of day 8 for both expectation (placebo

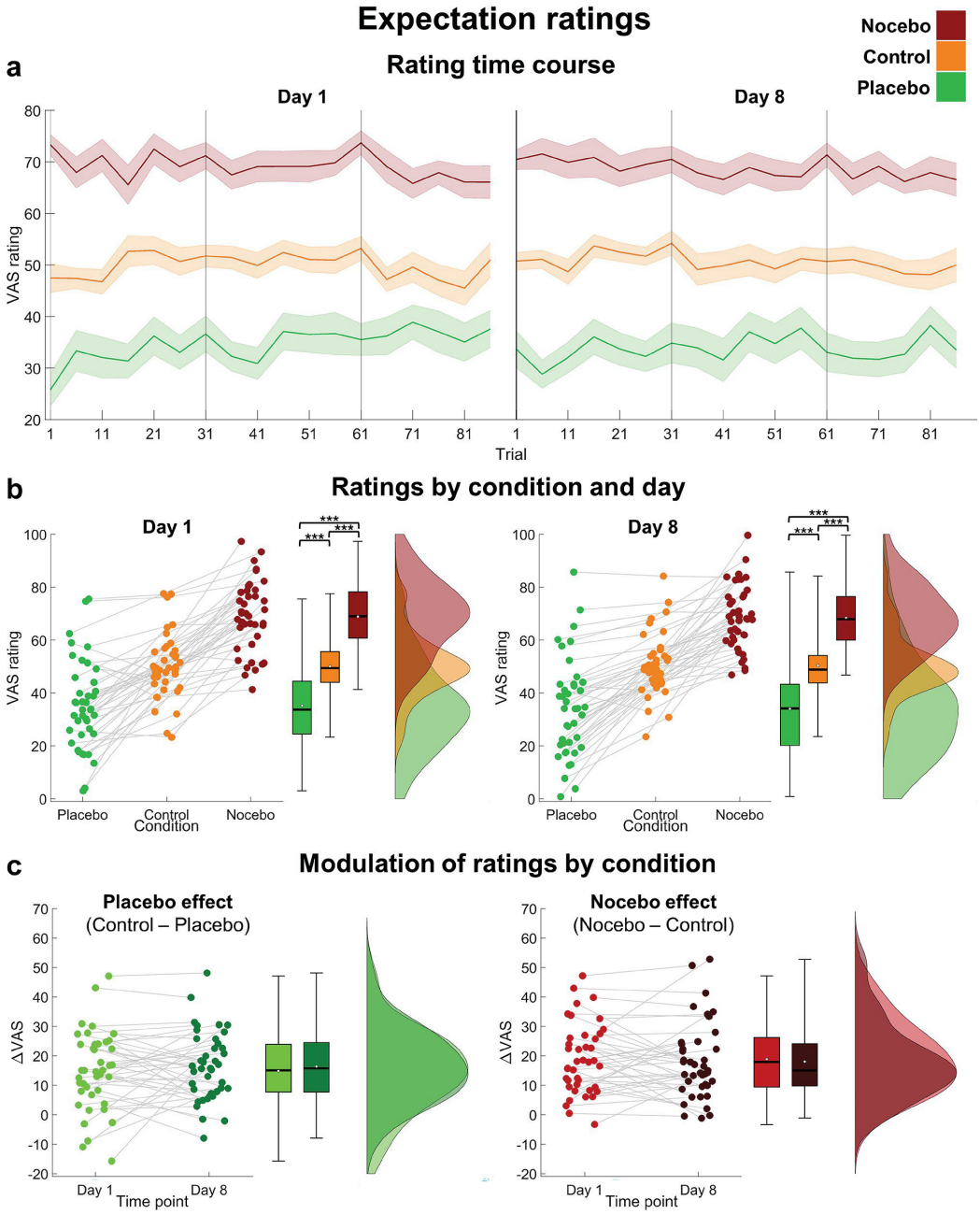

Fig. 2. Expectation ratings. (**a**) Time course of expectation ratings per condition, averaged over five trials. Error bars denote the corrected standard error of the mean (SEM) using the Cousineau-Morey method[32,33]. (**b**) Raincloud plots[34] of expectation ratings per condition on day 1 (left) and day 8 (right). Each dot represents the mean rating of an individual subject per condition and grey lines connect the ratings of the same subject over conditions. The black line inside the boxplots shows the median, the white dot depicts the mean. (**c**) Placebo effect (difference between the control and placebo condition; left) and nocebo effect (difference between the nocebo and control condition; right) in expectation ratings per day. ***$p < .001$.

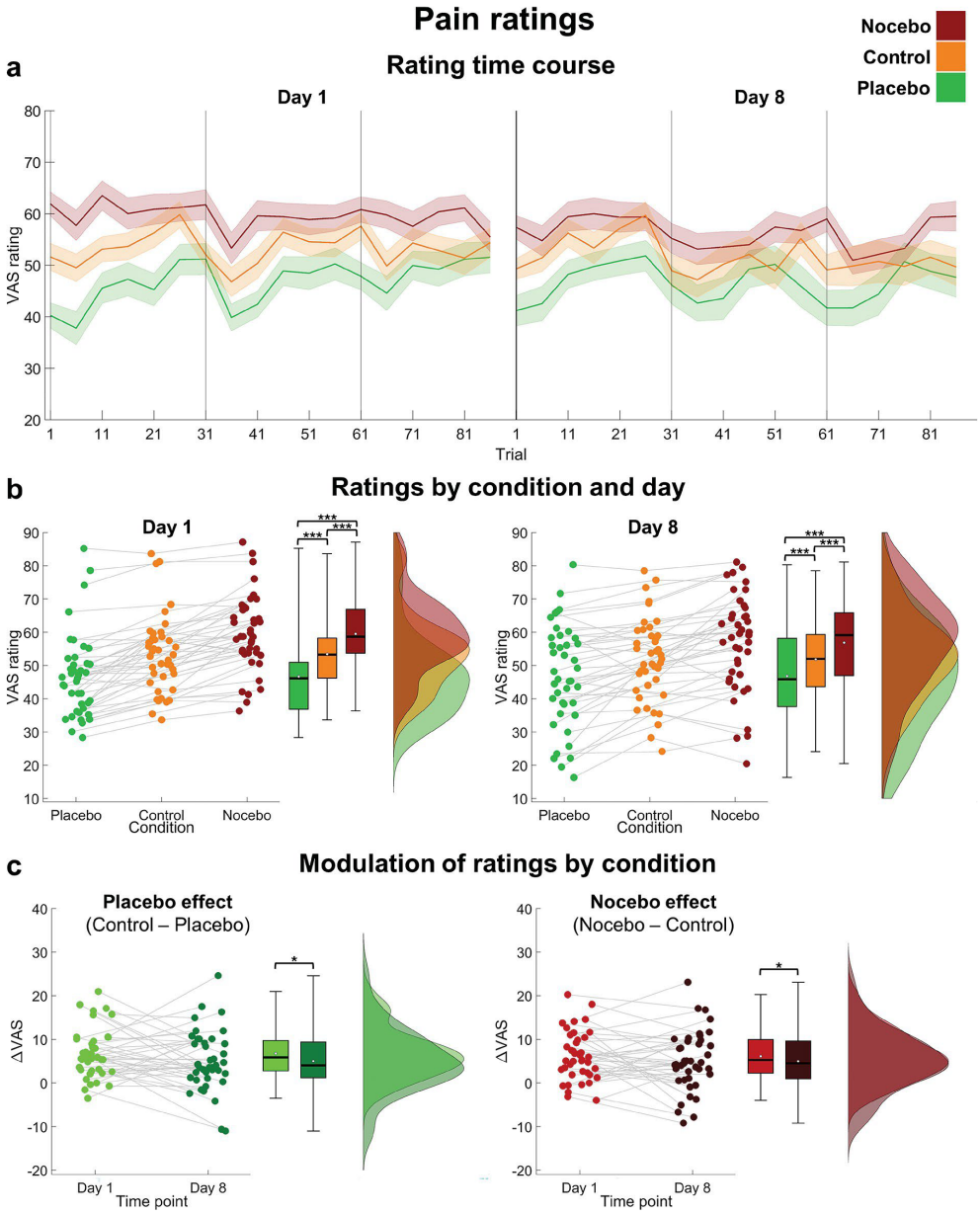

Fig. 3. Pain ratings. (**a**) Time course of pain ratings per condition, averaged over five trials. Error bars denote the corrected standard error of the mean (SEM) using the Cousineau-Morey method[32,33]. (**b**) Raincloud plots[34] of pain ratings per condition on day 1 (left) and day 8 (right). Each dot represents the mean rating of an individual subject per condition and grey lines connect the ratings of the same subject over conditions. The black line inside the boxplots shows the median, the white dot depicts the mean. (**c**) Placebo effect (difference between the control and placebo condition; left) and nocebo effect (difference between the nocebo and control condition; right) in pain ratings per day. *$p < .05$. ***$p < .001$.

	Expectation ratings				Pain ratings			
	df	F	p	η^2_p	df	F	p	η^2_p
Condition	1.14[a]	112.68	< 0.001	0.74	1.13[a]	63.15	< 0.001	0.61
	45.45[a]				50.98[a]			
Time point	1.00[a]	0.13	0.724	< 0.01	1.00[a]	0.58	0.451	0.01
	40.00[a]				40.00[a]			
Condition * Time point	1.22[a]	0.21	0.700	< 0.01	2.00[a]	2.89	0.061	0.07
	48.63[a]				80.00[a]			

Table 1. Results of a repeated measures ANOVA for expectation and pain ratings with condition (placebo, nocebo, control) and time point (day 1, day 8) as factors. [a]The degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity as Mauchly's test of sphericity indicated that the assumption of sphericity was violated ($p < .05$).

	Expectation ratings				Pain ratings			
	df	F	p	η^2_p	df	F	p	η^2_p
Modulation type	1	3.95	0.054	0.09	1	0.13	0.722	< 0.01
	40				40			
Time point (TP)	1	0.06	0.816	< 0.01	1	4.70	0.036	0.11
	40				40			
Modulation type * TP	1	1.62	0.211	0.04	1	0.10	0.760	< 0.01
	40				40			

Table 2. Results of a repeated measures ANOVA for expectation and pain ratings with modulation type (placebo effect, nocebo effect) and time point (day 1, day 8) as factors.

	Expectation ratings				Pain ratings			
	df	F	p	η^2_p	df	F	p	η^2_p
Modulation type	1	3.46	0.070	0.08	1	0.51	0.482	0.01
Residuals	40				40			
Time point (TP)	1	8.60	0.006	0.18	1	5.01	0.031	0.11
Residuals	40				40			
Modulation type * TP	1	2.52	0.120	0.06	1	0.97	0.331	0.02
Residuals	40				40			

Table 3. Results of a repeated measures ANOVA for expectation and pain ratings with modulation type (placebo effect, nocebo effect) and time point (last 5 trials day 1, first 5 trials day 8) as factors.

effect: $t(40) = 7.94$, $p < .001$, $d = 1.24$; nocebo effect: $t(40) = 8.55$, $p < .001$, $d = 1.34$) and pain ratings (placebo effect: $t(40) = 3.17$, $p = .001$, $d = 0.50$; nocebo effect: $t(40) = 3.18$, $p = .001$, $d = 0.50$).

Interestingly, on the descriptive level, placebo and nocebo effects appeared to be even larger in the first trials of day 8 compared to the last trials of day 1 (see Figs. 2a and 3a). This observation was corroborated by an exploratory statistical comparison of placebo and nocebo effects in the last five trials of each condition on day 1 to the first five trials of each condition on day 8 (see Table 3). Post-hoc tests revealed an increase in expectation rating modulation from the last five trials of day 1 ($M = 14.63$, $SD = 13.95$) to the first five trials of day 8 ($M = 19.36$, $SD = 10.64$; $p_{holm} = 0.006$) for both placebo and nocebo effect. Similarly, pain rating modulations were stronger at the start of day 8 ($M = 6.33$, $SD = 6.90$) compared to the end of day 1 ($M = 4.12$, $SD = 4.73$; $p_{holm} = 0.031$).

As the inter-test period was intended to span 7 days but exhibited slight variations between participants (see Methods), we tested whether the actual interval affected the stability of placebo and nocebo effects. There was no significant relationship of the inter-test period with placebo (expectation ratings: $r = .23$, $p = .153$; pain ratings: $r = -.03$, $p = .851$) or nocebo effects on day 8 (expectation ratings: $r = .11$, $p = .478$; pain ratings: $r = -.29$, $p = .061$). Furthermore, there was no significant relationship of the inter-test period with the change in placebo (expectation ratings: $r = .04$, $p = .804$; pain ratings: $r = -.09$, $p = .591$) and nocebo effects (expectation ratings: $r = .01$, $p = .970$; pain ratings: $r = -.14$, $p = .930$) from day 1 to day 8 (calculated as the absolute difference between effects on day 1 and day 8).

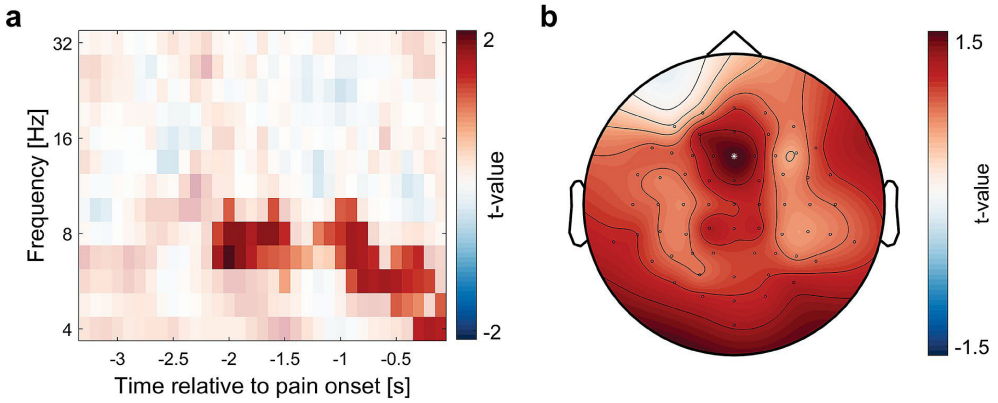

Fig. 4. Differences in EEG power between placebo and nocebo on day 8. (**a**) Time-frequency plot of *t*-values for placebo vs. nocebo in the anticipation phase on day 8 averaged over all cluster electrodes. (**b**) The corresponding topography (peak electrode Fz highlighted with a white star).

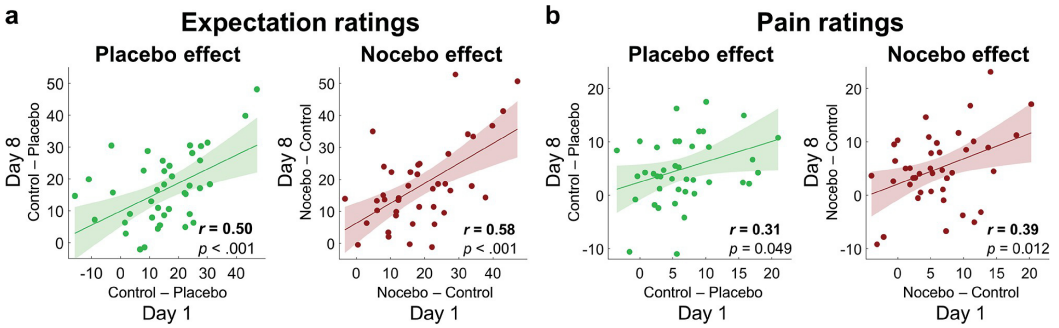

Fig. 5. Prediction of effects on day 8 based on the effects observed on day 1. Correlation plots for expectation (**a**) and pain ratings (**b**), showing the relationship between the individual placebo and nocebo effects on day 1 with the corresponding effects on day 8. Each dot represents one subject.

EEG activity

To assess whether differences in ratings on day 8 were accompanied by changes in brain activity, we compared EEG power between the nocebo and placebo condition. There were significant cluster-corrected differences in theta-to-alpha power during the anticipation phase (-2.1 until − 0.1 s, 4 to 9.5 Hz; $p = .025$, see Fig. 4), suggesting that the expectation effects were not only stable on the behavioral level, but also evident in enhanced low frequency power for nocebo compared to placebo on the neural level. During the pain phase, oscillatory power did not significantly differ between the placebo and the nocebo condition. We further compared EEG power to detect placebo- (placebo vs. control) or nocebo-specific (nocebo vs. control) differences in oscillatory power. There were no significant cluster-corrected differences for these comparisons (all $p > .05$).

Relation of behavioral effects across days

To determine the relation of behavioral placebo and nocebo effects over the measurement days, we correlated placebo and nocebo effects on day 1 with the corresponding effects on day 8. For both expectation and pain ratings, the strength of individual placebo and nocebo effects on day 8 were correlated with the corresponding effects on day 1 (see Fig. 5, all $p < .05$), indicating that effects were not only stable on the group level, but that the individual strength of effects on day 8 was largely determined on how strong effects were on day 1.

Neural correlates of the persistence of placebo and nocebo effects

Next, we assessed whether the neuronal processing on day 1 could predict the persistence of behavioral placebo and nocebo effects. For this aim, we used the individual strength of placebo and nocebo effects on pain ratings on day 8 and tested for a relation with the fMRI activity on day 1 (corrected for the strength of the behavioral

effect on day 1, see Methods). We tested for predictive fMRI activity both in the time frame directly prior to the application of the pain stimulus (anticipation phase) and during pain processing itself (pain phase; see Fig. 1 for details regarding the trial structure).

Individual differences in the placebo effect on day 8 revealed activity differences in the comparison of the placebo condition to the control condition (for detailed results see Table 4). Participants showing higher placebo effects on day 8 showed activity reductions in the amygdala during the anticipation of pain and higher activation in the left and right anterior insula and the right DLPFC during pain perception on day 1 (see Fig. 6a). Analyzing individual differences in the nocebo effect revealed higher activation in the thalamus in subjects showing larger nocebo effects on day 8 in the comparison of the nocebo condition to the control condition during pain perception on day 1 (see Fig. 6b). Additional analyses in other ROIs (see Methods) yielded no significant effects.

Discussion

Examining the persistence of previously induced placebo and nocebo effects over the time-period of one week enabled the assessment of their temporal stability. Both placebo and nocebo effect were revealed to be relatively persistent after one week, even though there was a significant decrease in the strength of both placebo and nocebo effects in pain ratings. However, both effects showed a rebound of effect strength from the last trials of day 1 to the first trials of day 8 and underwent no extinction over the entire time course of the experiment. The comparison of EEG oscillatory activity for nocebo vs. placebo revealed that expectations were represented differentially in anticipatory theta-to-alpha activity on day 8. The persistence of effects was largely dependent on the individual strength of placebo and nocebo effects on day 1, as participants that showed greater effects on day 1 also showed greater effects on day 8. We further investigated the neuronal correlates of the persistence of effects and found that a stronger persistence of placebo effects was predicted by larger placebo-induced modulation of fMRI activity in the amygdala during the anticipation of pain and in the right DLPFC and the bilateral anterior insula during pain processing on day 1. Conversely, the persistence of nocebo effects was correlated with larger nocebo-induced changes in fMRI activity in the thalamus during pain perception.

Our finding of relatively stable placebo and nocebo effects, both on the group and individual level, contribute to the existing body of knowledge on the highly persistent nature of placebo and nocebo effects in clinical settings[10] and expands the understanding of the longer-term stability of placebo effects[5,19]. Importantly, we used a larger number of test trials per session than most previous studies[19–21,23], which led to participants constantly receiving sensory information that did not fit their beliefs on the objective level. Nevertheless, placebo and nocebo effects did not undergo extinction. Thus, our results further challenge classical learning models in the context of placebo and nocebo effects, which would predict that participants learn from their experiences and adjust their expectations over time, leading to a reduction of placebo and nocebo effects[8,9], but highlight the need for more complex interpretations. One possible mechanism is self-reinforcing feedback loops, whereby participants tend to learn more from experiences that align with their expectations, leading to a stabilization of these expectations so that they can withstand potentially invalidating information[8,9]. At least within one session, this may explain the stability of effects.

It is important to note that the placebo and nocebo effects stayed stable between sessions and even increased between the last trials of day 1 and the first trials of day 8. The effects were further found to persist after the change in treatment context from day 1 (MRI lab) to day 8 (EEG lab), in line with previous findings[5]. This suggests that the induced beliefs regarding the efficacy of our treatment were not overwritten by the experience on day 1 but showed a rebound in strength. Other potential explanations for the lack of decrease in expectations include that our combination of verbal instructions and strong conditioning induced highly persistent initial beliefs[23,35], or that there was a shift in expectations from being driven by beliefs to more unconscious associations over time[10]. However, the effects on expectation ratings were remarkably stable, which indicates that participants were aware of their beliefs, suggesting rather a conscious representation of expectations. As it has already been demonstrated that both conditioning[36,37] and verbal instructions alone[5,38] can lead to reliable placebo and nocebo effects, further studies could compare the long-term stability of more conscious (i.e. verbal instructions) versus unconscious methods of expectation induction (i.e. conditioning) to elucidate different factors that might affect the stability of expectation effects.

We observed no difference between placebo and nocebo effects both in terms of strength and stability. This is somewhat surprising given previous evidence suggesting that nocebo effects can be introduced more easily and are more robust against extinction[18,21]. Nevertheless, it has to be considered that the majority of these findings were derived from studies that investigated the course of expectations in one session only[18,20,21]. To our knowledge, we are the first to examine the longer-term stability of negative pain-related expectations. The persistence of both placebo and nocebo effects may be related to the high number of conditioning stimuli that we used (10 per condition), as the number of conditioning stimuli is related to the strength of induced effects[23]. The differential stability of placebo and nocebo effects within shorter time frames has been attributed to higher arousal in nocebo compared to placebo blocks, which impedes learning from experiences and thereby stabilizes nocebo effects[18]. Here, conditions within blocks were pseudo-randomized, which could lead to a similarly high level of arousal for all conditions and therefore similarly low rates of extinction in both placebo and nocebo effects.

Interestingly, we found predictive neural activity for the stability of both placebo and nocebo effects. For placebo effects, this activity was located in areas associated with the DPMS during pain anticipation in the amygdala, and during pain processing in the DLPFC and the anterior insula. The DPMS is proposed to play a pivotal role in placebo and nocebo effects[2,3]. The amygdala exhibits direct connections to classical placebo areas like the vmPFC and the periaquedactual grey (PAG)[2] and decreased activity in the amygdala has been associated with increased placebo effects[4]. It further has been reported to show predictive activity for participants perceiving stimuli as more painful despite receiving an explicitly neutral cream[39]. Additionally, a rightward asymmetry in

Region	Hemi.	MNI Coordinates					p_{FWE}
		X	Y	Z	t		
Placebo Effect as Covariate:							
Placebo < Control during the Anticipation Phase							
Amygdala	R	28	0	-26	4.19		0.021†
Placebo > Control during the Pain Phase							
Anterior Insula	R	40	18	-2	4.85		0.021†
	L	-42	12	-6	4.60		0.040†
DLFPC	R	44	12	40	4.21		0.048†
Nocebo Effect as Covariate:							
Nocebo > Control during the Pain Phase							

Table 4. Peak coordinates and statistics of regions for analyses of the predictive power of brain areas for the persistence of expectation effects, using individual differences in the placebo and nocebo effects on day 8 (controlled for the behavioral effect of day 1, see Methods). Coordinates are in MNI space. DLPFC = Dorsolateral Prefrontal Cortex. Hemi = Hemisphere. †small-volume corrected.

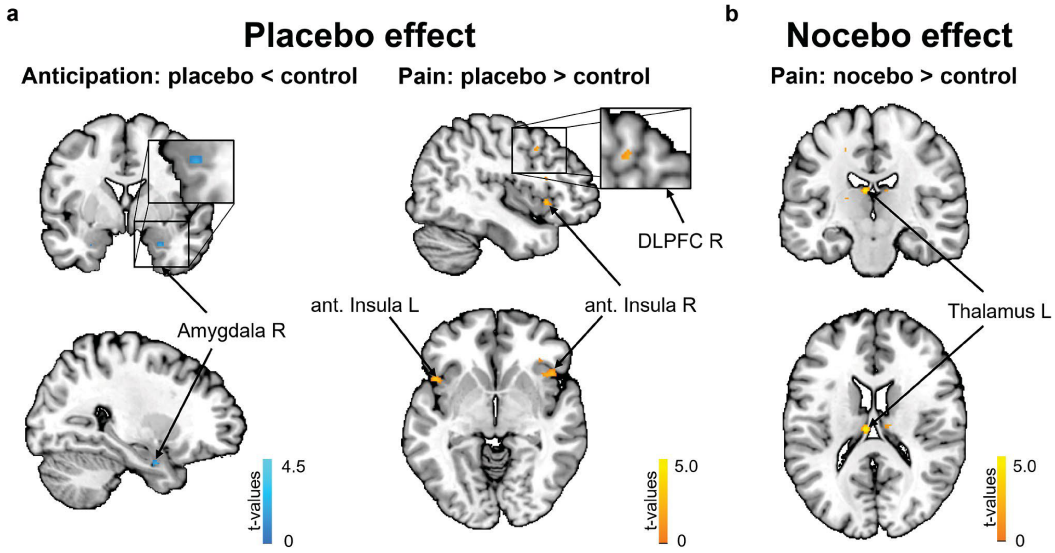

Fig. 6. Neural predictors of placebo and nocebo effect persistence. (**a**) Analyses of the persistence of expectation effects using individual placebo effects of day 8; Blue: lower activation for the placebo condition compared to the control condition in the amygdala during the anticipation phase; Yellow: higher activation in the insula and DLPFC during pain perception in the comparison to control. **(b)** Analyses of the persistence of expectation effects using individual nocebo effects of day 8; Yellow: higher activation in the thalamus during pain perception in the comparison of nocebo to control.

the volume of subcortical limbic structures including the amygdala has been reported for placebo responders compared to non-responders[40]. These findings underline the importance of this structure for the placebo response and the individual differences in response quality. This could be mediated by the important role the amygdala has in threat assessment[41]. A reduced threat perception might lead to higher and more stable placebo effects. As the amygdala is a key structure in associative learning[2,42,43], reduced activity could also be interpreted as a reduction in learning from experiences during the test phase, leading to more pervasive placebo effects.

The DLPFC has been linked to placebo effects in both anticipation of pain and during pain perception[26–28,44,45]. The DLPFC has a pivotal role in the top-down modulation of placebo effects[2]. This could possibly relate to the suppression of learning from prediction errors described by Schenk et al.[8]. Moreover, activity in the DLPFC has been reported to have predictive power for individual placebo responses[44]. In conjunction with our findings, this illustrates the importance of the DLPFC in not only predicting individual placebo responses, but also the individual persistence of these responses. The anterior insula also has often been reported to show increased activation in placebo conditions[2] and further has been reported to have predictive power for individual placebo responses[44]. It also has been marked it as an important node in the salience network[46], and it may also play an important part in evaluating the expected threat level[28,47,48]. Considering the involvement of the amygdala and the anterior insula in affective processing and associative learning, the persistence of placebo effects might depend on the affective evaluation of the stimuli and learning processes triggered by it, with activity in the amygdala mediating this evaluation during the anticipation of pain and the anterior insula mediating the evaluation during pain perception. This effect of the affective evaluation might be extended by a strong top-down modulation by the DLPFC[8].

The persistence of nocebo effects was predicted by increased activity in the thalamus. The thalamus has been reported to encode nociceptive information[49] and nocebo responders showed increased activation in the thalamus compared to non-responders[50]. At first glance, our finding could also be interpreted as an artifact of the thalamus simply encoding higher differences in pain perception between the nocebo and control condition on day 1, leading to higher effects on day 8, however, it has to be kept in mind that we already accounted for the effects on day 1. Thus, a higher nocebo effect on day 1 cannot explain the observed relationship. A more promising explanation is based on the thalamus being linked to conditioning processes regarding pain[51]. Thus, stronger activity in the thalamus could be interpreted as an indicator of a stronger conditioning effect that exerts influence on stimulus processing during the test phase or, in line with the concept of self-reinforcing feedback loops, a stronger learning process from expectation-consistent information[8,9]. The thalamus therefore appears to play an additional role next to stimulus processing in the longer-term maintenance of nocebo effects.

Even though we found no significant behavioral differences between the persistence of nocebo and placebo effects, we see different neural patterns predicting the persistence of these effects. This fits the results of our previous study[28] with differential neural patterns for positive and negative expectations during pain perception.

Additionally, this might indicate that positive and negative valences of expectations rely on differential mechanisms that support their up-keeping. Interestingly, while several areas are connected to placebo and nocebo effects during anticipation of pain and during pain processing[28], out of these only few regions seem to have predictive power for the long-term persistence of these effects.

On day 8, we observed an increased theta-to-alpha EEG power for negative compared to positive expectations during the anticipation of pain. This is in line with previous findings that indicated a high relevance of the pre-stimulus low frequency oscillatory activity that allow for the subsequent modulation of the pain perception[29–31,47,52]. Lower frequency activity, whether altered by spontaneous fluctuations or pain-related expectations, has been shown to affect the pain perception, although the direction of this modulation was inconsistent over studies[29–31,52]. Nevertheless, this points towards an important role of alpha activity for the top-down signaling of expectations that ultimately lead to the modulation of bottom-up sensory information as observed in placebo and nocebo effects[29,53]. As we observed these changes in neural activity one week after the original induction of expectations, this further supports the notion that expectations are not transient but instead result in true, lasting changes in perception. We did not detect any differences in neural processing during the pain phase, which is consistent with prior research and might be due to a transition from the expectation signaling predominant in the anticipation phase to other processes more focused on the signaling of sensory information and prediction errors during pain perception[29,31]. Moreover, there were no significant differences between oscillatory power for both placebo and nocebo compared to control. One possible explanation is that changes in EEG activity were more subtle, in line with the less pronounced behavioral differences between these conditions compared to the contrast between placebo and nocebo.

Taken together, our findings show that the initial experience with a treatment can have long-lasting effects in the same and other contexts. This implies the need to reduce or at least reframe negative experiences in order for them not to impede future treatments. Conversely, this also means that positive experiences can have long-term consequences and should be harnessed. This raises important clinical questions. Notably, the persistence of nocebo effects could serve as one crucial gateway for the development of chronic pain. The emergence of chronic pain is hypothesized to follow a complex interaction of factors, gathered in an integrative psychobiological pain model proposed by Büchel[54]. This model synthesizes the most common approaches for understanding the emergence of chronic pain and underscores the pivotal role of expectations. Following this model, persisting alterations of pain perception by negative expectations (which could also be termed as a stable nocebo effect) could act as a significant driving force, accelerating a vicious cycle leading to chronic pain. However, our findings regarding the persistence of placebo effects suggest that the opposite could also occur, manifesting as a virtuous cycle. This underscores the importance of carefully handling the expectations of patients. The question further arises if the persistence of expectation effects on perception could be an important factor in other disorders. Persistent expectations could e.g. play a role in psychosis, in which the influence of expectations on perception has already been discussed[55].

This study is not without limitations. Our cover story which was based on a sham BCI allowed for a trial-by-trial manipulation of expectations within participants and was thereby a strength of our procedure, but might also be a limiting factor for the generalization of our results to other placebo/nocebo paradigms. Moreover, the stability of expectation effects was only demonstrated for a relatively short time period. Future research should investigate the persistence of placebo and nocebo effects over longer time courses and should ideally incorporate multiple measurements to dissect the temporal trajectory of these effects. Then, the change from the MRI lab on day 1 to the EEG lab on day 8 demonstrated that expectation effects persisted relatively independent of the context in which these effects were induced. However, a direct comparison of fMRI activity between both days was therefore not possible and might be a target for future studies. Finally, it is further important to note the correlative nature of our predictive fMRI analyses. To investigate the causal relationship between fMRI activity on day 1 and behavioral outcomes on day 8, a larger sample size would be necessary.

Overall, our results show that both placebo and nocebo effects can remain stable over one week despite a change in treatment context, and that the stability of the individual effects were determined by distinct neural correlates for positive and negative expectations. Especially for positive expectations areas related to learning, affective processing, and top-down control appear to predict the strength of responses one week after the initial induction of expectations.

Methods

Participants

The final sample of the present study consisted of 41 healthy participants. Initially, 50 participants took part in the first session of the measurement and were included in the previous study[28]. Out of these, 42 participated in a follow-up appointment one week later. As preregistered (German Clinical Trials Register; ID: DRKS00026174), one participant had to be excluded for the lack of expectation effects on day 1, indicated by higher ratings for the placebo compared to the nocebo condition averaged over the entire day, leading to a final sample size of $N = 41$ (26 female; age: $M = 25.6$, $SD = 3.6$ years, range: 18–34 years). Volunteers were recruited via an online job platform. All participants were right-handed, had normal or corrected-to-normal vision, reported no neurological or psychiatric diseases, pain conditions, current medication, substance abuse, or pregnancy, and were non-smokers. They gave written informed consent and were compensated with 15 Euros per hour of participation. Analyses for fMRI data were performed additionally excluding 2 participants due to technical issues leading to incomplete measurements, leading to a final sample size of $N = 39$ for fMRI analyses. The study was approved by the local ethics committee (PV7170). We confirm that all research was performed in accordance with relevant guidelines and regulations.

Procedure

Day 1

The detailed procedure on day 1 has been described elsewhere[28]. Scripts that specify the instructions used can be found in the supplements (see Supplementary Methods S1). In brief, positive, negative, or neutral pain-related expectations were induced using verbal instructions and a conditioning procedure. Participants were informed that they would receive real-time feedback in regard to their current pain sensitivity by means of a BCI (brain-computer-interface). Three images of a brain with the right primary somatosensory cortex highlighted in one of three different colors served as visual cues: A green stimulus represented a state of low pain sensitivity (placebo condition), a red stimulus represented a state of high pain sensitivity (nocebo condition), and a yellow stimulus represented an intermediate state in which no prediction was made (control condition). After a pain calibration (for details see below), participants were conditioned using 10 trials of the placebo and 10 trials of the nocebo condition. This phase was disguised as a calibration of the BCI algorithm to the individual participants. Without the participants knowledge, green cues were always followed by a less painful stimuli (VAS30), red cues were always followed by more painful stimuli (VAS70). After these steps, the test phase was carried out (see below). The pain calibration, conditioning phase and test phase were conducted inside an MRI scanner. For both days, the experiment was programmed using the Psychtoolbox3 (http://psychtoolbox.org/) for Matlab (Version R2021b; The MathWorks).

Day 8

Day 8 took place approximately one week later (actual distance between the two sessions: $M = 7.5$ days, $SD = 1.7$, range: 5–13 days, with a small deviation of the intended span of 7 days due to logistical limitations like the availability of participants or EEG laboratory capacity) and was conducted in an EEG lab. There were only two phases: a pain calibration phase and a test phase. Expectations were not further reinforced on this day, i.e., there was no verbal instruction phase and no conditioning procedure. Participants were only informed that the same algorithm to predict their current state of pain sensitivity as one week prior would be used. Rating responses were given by the participants using a standard keyboard. Instructions and ratings were presented on a monitor with a resolution of 1920 × 1080 at a viewing distance of approximately 100 cm. After the test phase, participants were asked to fill out questionnaires, were debriefed and paid.

Experimental phases

Pain calibration phase A PATHWAY CHEPS (Contact Heat-Evoked Potential Simulator) thermode (https://www.medoc-web.com/pathway-model-cheps), was utilized for pain stimulation. This device has a rapid heating rate of 70 °C/s and a cooling rate of 40 °C/s and is capable of delivering heat stimuli in the range of 30 °C to 55 °C in less than 300 ms. In all phases of the experiment, the baseline temperature was set to 32 °C, and the rise and fall rates were set to 70 °C/s. We used an altered version of the pain calibration by Horing et al.[56]. During the pain calibration, the thermode head was attached to a location directly proximal to the volar mid-forearm. To desensitize the skin, subjects were pre-exposed to 4 brief heat stimuli starting at 42 °C, with each consecutive stimulus increasing by 0.5 °C, up to 43.5 °C. Subsequently, we used a probabilistic tracking procedure for pain threshold determination[57], consisting of eight stimuli of 4 s rated by binary decision (painful or not painful). The temperature of each stimulus was decided by the rating of the stimulus presented before, with a higher temperature chosen when the stimulus before was rated as not painful and a lower temperature when the stimulus before was rated as painful. The final temperature was chosen as the pain threshold. On day 1, a linear regression was employed to determine individual temperatures corresponding to values of VAS30, VAS60 and VAS70 on a visual analog scale (VAS) from 0 ("no pain") to 100 ("unbearable pain") for each participant. On day 8, the same procedure was used to determine the temperature corresponding to VAS60 only. The mean calibrated temperature corresponding to VAS60 on day 1 was 45.48 °C ($SD = 1.28$ °C, $Min = 42.4$ °C, $Max = 48.8$ °C) and 45.94 °C on day 8 ($SD = 1.59$ °C, $Min = 43.2$ °C, $Max = 50.7$ °C).

Test phase On both day 1 and day 8, a test phase of identical procedure was carried out. Participants were informed that they would now receive feedback on their current pain sensitivity from the BCI system on each trial, which could be either highly pain-sensitive (red; nocebo condition), less pain-sensitive (green; placebo condition), or no prediction would be made as our algorithm could not detect a clear-cut state (yellow; control condition). Trials were structured as follows: After being presented with the cue (green, red, or yellow) for 2 s, participants were asked to rate how painful they expected the next stimulus to be on a VAS ranging from 0 to 100 (expectation rating) while the cue was still presented on screen (4 s). Then, there was a pre-stimulus phase of 3.3 s in which a fixation cross was presented (anticipation phase). Next, independently of the cue color, participants always received a painful stimulus of a temperature corresponding to VAS60 for 4 s (pain phase). There were 30 cues of each condition followed by pain stimulation divided into three blocks, summing up to a total of 90 stimuli. The ITI was fully randomized between 2 and 7 s. The order of cues was pseudo-randomized with no more than two direct repetitions of the same condition, and there was a different trial order for day 1 and day 8. During the first block of the test phase, the thermode head was attached to a location directly proximal to the volar mid-forearm. The thermode position was changed to a position directly distal to the volar mid-forearm for the second block and then back to the original position in the third block. Before each block, we applied one pain stimulus of VAS60 without a cue to desensitize the new skin area.

Data acquisition

EEG data

On day 8, continuous EEG data was recorded inside an electrically shielded room using a 64-channel actiCAP and the BrainVision Recorder (BrainProducts, Gilching, Germany). The cap contained 64 active Ag/AgCl

electrodes with 62 electrodes arranged according to the extended 10/20 System and the two remaining electrodes used to record a bipolar horizontal electrooculogram (HEOG). FCz served as reference and Pz served as ground electrode. The cap was connected to two BrainAmp amplifiers with 32 channels each (BrainProducts, Gilching, Germany), powered by rechargeable battery units. Electrode skin impedance was kept below 20 kΩ. EEG data was recorded with a sampling rate of 500 Hz and an amplitude resolution of 0.1 μV. Data was filtered online with a low cut-off filter with a time constant of 10 s and a high cut-off at 1,000 Hz.

fMRI data

For MRI measurements on day 1, a 3T Siemens PRISMA Scanner equipped with a 64-channel head coil was utilized. For the experiment, two sequences were acquired: an EPI BOLD sequence and a field map sequence. Participants were equipped with a 64-channel standard BrainCap MR for 3 Tesla (2020 Version). The EPI BOLD sequence parameters included: TE: 29.0 ms, TR: 1679.00 ms, FOV: 22.4 * 22.4 cm, flip angle: 70°, slice thickness: 2 mm, scan time: 20 min and 17 s, and a total of 715 volumes acquired. One day prior, a T1 image with the following parameters was acquired: T1 FLASH 3D: TE 2.98 ms, TR: 2.3 s, matrix flip angle: 9°, FOV 25.6 * 25.6 cm, TA: 7:22 min.

Preprocessing

EEG data

The Fieldtrip toolbox for Matlab[58] was used for the preprocessing of EEG data. The data were segmented into trials from 1,000 ms prior to cue onset to the end of pain stimulation 15,800 ms following cue onset. The resulting segmented data were filtered (low-pass filter at 150 Hz, high-pass filter at 0.5 Hz) We adapted a recently introduced preprocessing approach (see Hipp et al.[59]). To obtain maximal sensitivity in detecting and removing artifacts, the data was split into low- and high-frequency data (34 Hz low-pass filter and 16 Hz high-pass filter, respectively) and processed in parallel. All single trials were visually inspected and removed for both high- and low-frequency data when containing excessive artifacts. Subsequently, both subsets underwent independent component analysis (ICA) using a logistic infomax algorithm. Components reflecting e.g., blinks, eye- and head movement, or muscle activity were visually identified based on the time course, spectrum, and topography and discarded. Both subsets were re-referenced to the average of all EEG channels and the original reference electrode (FCz) was regained. Finally, all data were subjected to another comprehensive visual scan, and the time axis was adjusted to align with the onset of pain stimulation at t = 0 s. The visual artifact screening process led to the exclusion of 97 of the 3690 recorded trials (2.63%) in total.

Time-frequency decomposition Time-frequency decomposition was conducted for 21 logarithmically spaced frequencies ranging from 4 to 128 Hz (0.25-octave increments) in 0.1 s steps using the multi-taper method based on discrete prolate spheroid sequences (DPSS), adapted from Hipp et al.[59]. For the frequency transformation of frequencies above 25 Hz, high frequency data were used, while for frequencies below 25 Hz, low frequency data were used. Temporal and spectral smoothing were adjusted to match 250 ms and 3/4 octave, respectively, by fixing the time window to 250 ms and adjusting the number of Slepian tapers for frequencies above 16 Hz and using a single taper but adjusting the time window for frequencies up to 16 Hz. The single-trial time-frequency resolved data were averaged per condition for each participant.

fMRI data

Preprocessing of fMRI data was done using the Statistical Parameter Mapping software (SPM 12, Wellcome Department of Imaging Neuroscience, London, UK, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The first two volumes of each block were dropped to get full MRI saturation effects and data then underwent realignment and unwarping, registration to standard space (Montreal Neurological Institute), and spatial smoothing with a 6 mm Gaussian kernel.

Data analysis

Behavioral data

We compared differences in pain and expectation ratings for the different cue conditions at two different time points by computing two-way repeated measures ANOVAs with pain and expectation ratings as outcomes, respectively, and cue type (placebo vs. nocebo vs. control) and session (day 1 vs. day 8) as predictors. Moreover, we compared the relative variability index[60] between conditions using two rmANOVAs with cue type (placebo vs. nocebo vs. control) and session (day 1 vs. day 8) as predictors and the relative variability index of expectation and pain ratings as outcomes, respectively, to determine if the variability in individual ratings differed between conditions and days. For the remaining analyses, we computed the placebo effect (control - placebo) and nocebo effect (nocebo - control) in a way to ensure that positive values always mean higher modulation of ratings in the intended direction. Rating modulations induced by placebo and nocebo effects at the two different time points were examined by computing rmANOVAs for expectation and pain ratings separately, using (1) modulation type (placebo effect vs. nocebo effect) and time point (day 1 vs. day 8), and (2) modulation type (placebo effect vs. nocebo effect) and time point (last 5 trials of each condition on day 1 vs. first 5 trials of each condition on day 8) as predictors. Significant effects in all ANOVAs were followed up by conducting Bonferroni-Holm corrected post-hoc *t*-tests. To assess predictors of the rating modulation on day 8, we correlated the rating modulation during day 1 with the modulation during day 8 (placebo effect with placebo effect, nocebo effect with nocebo effect), separately for expectation and pain ratings. To explore whether the duration of the inter-test period affected the stability of placebo and nocebo effects, we correlated the individual inter-test period with placebo and nocebo effects on day 8 and with the absolute change in placebo and nocebo effects from day 1 to day 8 (e.g. placebo effect on day 8 minus placebo effect on day 1), for both pain and expectation ratings.

EEG data

We compared differences in power between (1) the placebo and nocebo condition, (2) the placebo and control condition, and (3) the nocebo and control condition in the anticipation phase and pain phase separately at each time-frequency-electrode point for high- and low-frequency data. We statistically tested power differences using nonparametric cluster-based permutation tests as implemented in the Fieldtrip toolbox (cluster threshold: $p = .05$, minimum neighbors: 2, number of randomizations: 2000). Statistics were calculated in the time range of -3.3 until −0.1 s for the anticipation phase and 0 until 3.9 s for the pain phase.

fMRI data

Statistical inference Identical to our previous publication[28], for each subject, a Finite Impulse Response model (FIR model) was set up on a time course of 18.4 s starting at the onset of the cue, divided into 11 bins, with a bin roughly covering the duration of one TR (1.679 s compared to 1.675 s). The FIR model was implemented separately for each condition. Data was also corrected for cardioballistic and respiratory artifacts by including them as regressors built with the RETROICOR algorithm of the PhysIO toolbox[61,62].

Contrasts were formed on the first level comparing placebo to control and nocebo to control separately in the anticipation and pain phase. Analyses during the anticipation phase were conducted by using FIR regressors that spanned the period from − 4.275 s to − 0.925 s before the onset of pain (corresponding to bins 4 and 5). For the pain phase, the analyses utilized FIR regressors covering the interval from 0.75 s to 5.8 s after pain onset (corresponding to bins 7, 8, and 9). The contrasts were then used in one-sample *t*-tests on the second level including two covariates. For placebo-related fMRI activity (placebo vs. control), one covariate was formed from the behavioral placebo effects from day 8 to capture the stability of the effects between the two measurements and added on the second position of the design matrix. To assure that only the effects of day 8 had an influence on the brain activity, the placebo effect of day 1 was inserted as a covariate on the first position of the design matrix, assuring an orthogonalization of the vectors and thus only the variance that should go beyond the effect of day 1 remains. Conversely, for nocebo-related fMRI activity (nocebo vs. control), the behavioral nocebo effect on day 1 was included in the first position of the design matrix as a covariate, while the nocebo effect on day 8 was included in the second position. This was done separately for the anticipation and the pain phase. Analyses were corrected for multiple comparisons using FWE correction ($p < .05$).

ROI analyses

Adapted from Wittkamp & Wolf et al.[28], ROI analyses were conducted in the following areas defined by the anatomy based on the Harvard-Oxford atlas: insular cortex, thalamus, ACC, hippocampus and amygdala. Additionally, a ROI analysis was conducted on the DLPFC based on the clusters identified in the meta-analysis conducted by Zunhammer et al.[27] by applying a 15 mm-radius sphere around the two reported peak coordinates (xyz_{MNI}: 42, 11, 33 and xyz_{MNI}: -30, 13, 54) bilaterally. Further, a ROI analysis on the angular gyrus was conducted based on the results of Wittkamp & Wolf et al.[28] with a 15 mm-radius sphere around the peak coordinate during anticipation of pain (xyz_{MNI}: 56, -54, 40) and during pain perception (xyz_{MNI}: 44, -50, 38).

Data availability

Derived data that support the findings of this study are available at https://osf.io/r5ejs/. For additional information and requests, please contact Michael Rose directly.

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M.W.: Software, Validation, Formal Analysis, Investigation, Data Curation, Writing — Original Draft, Writing — Review and Editing, Visualization; C.A.W.: Software, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing — Review and Editing, Visualization; M.R.: Conceptualization, Methodology, Software, Validation, Resources, Writing — Review and Editing, Supervision, Project administration, Funding acquisition. M.W. and C.A.W. contributed equally to this work.

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Competing interests
The authors declare no competing interests.

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5 Zusammenfassung auf Deutsch

Neben dem nozizeptiven Input bestimmt eine Vielzahl an Faktoren die Wahrnehmung von Schmerz. Ein entscheidender Aspekt sind Erwartungen. Diese können beispielsweise bei der Placebo-Hypoalgesie und Nocebo-Hyperalgesie die Schmerzwahrnehmung modulieren. Erste Studienergebnisse deuten darauf hin, dass diese Effekte auch über längere Zeiträume hinweg bestehen bleiben können. Placebo- und Nocebo-Effekte werden auf neuronaler Ebene zumindest teilweise durch ein Netzwerk für absteigende Schmerzmodulierung (DPMS) mediiert. Obwohl die neuronalen Prozesse während der Antizipationsphase maßgeblich für die Bildung von Erwartungen und die Modulierung von Schmerzreizen sind, wurde diese Phase bislang nur in wenigen Studien untersucht.

Diese Dissertation umfasst drei Studien, deren Ziele es sind, (1) Gemeinsamkeiten und Unterschiede zwischen den neuronalen Repräsentationen positiver und negativer Erwartungen in der Antizipations- und Schmerzphase zu untersuchen, (2) die zeitliche Stabilität von Erwartungseffekten auf die Schmerzwahrnehmung zu evaluieren und neuronale Prädiktoren für die Stabilität dieser Effekte zu identifizieren, sowie (3) zu testen, ob eine bewusste Modulation von Erwartungen die Schmerzempfindung beeinflusst.

In allen Studien wurden die Erwartungen von Versuchspersonen hinsichtlich eines bevorstehenden Schmerzreizes in jedem Versuchsdurchlauf manipuliert. In der ersten Studie wurden EEG- und fMRT-Daten erhoben, während die Versuchspersonen vermeintliches Feedback zu ihrer derzeitigen Schmerzempfindlichkeit erhielten. Eine Woche später wurde dieselbe Stichprobe für die zweite Studie erneut eingeladen, um die Stabilität der Effekte zu überprüfen. In der dritten Studie sollten die Teilnehmenden ihre Erwartungen entsprechend eines Hinweisreizes anpassen, wobei wie in der zweiten Studie EEG-Daten aufgezeichnet wurden.

Die Ergebnisse der ersten Studie zeigten, dass positive und negative Erwartungen während der Antizipationsphase neuronal ähnlich repräsentiert waren, während sich die neuronale Aktivität während der eigentlichen Schmerzverarbeitung unterschied. Dieses Muster fand sich sowohl in der anterioren Insula als auch in Arealen des DPMS, einschließlich des ACC, DLPFC und vmPFC, wieder. Mithilfe einer kombinierten EEG-fMRT-Analyse konnte gezeigt werden, dass Aktivität im präfrontalen Kortex und in der anterioren Insula während der Antizipationsphase der Aktivität im ACC zeitlich vorausging. In der zweiten Studie wurde gezeigt, dass sowohl positive als auch negative Erwartungen über den Zeitraum von einer Woche relativ stabile Effekte auf die Schmerzwahrnehmung hatten. Diese Stabilität ließ sich durch die bereits eine Woche zuvor gemessene neuronale Aktivität vorhersagen. Dabei erwies sich die Aktivität im DLPFC, in der anterioren Insula und in der Amygdala als relevant für die Stabilität positiver Erwartungen, während die Aktivität im Thalamus mit der Stabilität negativer Erwartungen zusammenhing. Zudem spiegelten sich Erwartungen in oszillatorischer Aktivität im Theta- bis Alpha-Band wider. Die dritte Studie zeigte, dass bewusst modulierte Erwartungen die Wahrnehmung von Schmerzreizen beeinflussen konnten und zu Veränderungen in hochfrequenter oszillatorischer Aktivität während der Schmerzphase führten.

Die hier präsentierten Ergebnisse verdeutlichen die Relevanz von einzigartigen neuronalen Prozessen während der Antizipationsphase für die nachfolgende Schmerzmodulation. Es wurde gezeigt, dass die Areale des DPMS, insbesondere der präfrontale Kortex, sowie die anteriore Insula eine wichtige funktionelle Rolle bei der Generierung und langfristigen Stabilisierung von Erwartungen spielen. Die Ergebnisse ziehen zudem klinische Implikationen nach sich, insbesondere hinsichtlich der hohen Stabilität von Placebo- und Nocebo-Effekten sowie der Wirksamkeit bewusster Erwartungsmodulationen.

6 Zusammenfassung auf Englisch

Besides the nociceptive input, the percept of pain is shaped by many factors. One prominent factor is expectations, as observed in placebo hypoalgesia and nocebo hyperalgesia. There are indications that this influence can even last over longer periods of time. On a neurobiological level, the descending pain modulatory system (DPMS) may partially mediate placebo and nocebo effects. Although they appear crucial for the initiation of expectations for the subsequent modulation of pain, only few studies investigated the neural processes during the anticipation phase.

This dissertation consists of three studies aimed at (1) assessing similarities and differences between the neuronal representations of positive and negative expectations during the anticipation and pain phase, (2) evaluating the temporal stability of expectation effects on pain perception and identifying neural predictors for the persistence of effects, and (3) testing whether a conscious modulation of expectations without deceptive information affects pain perception.

In all studies, expectations regarding an upcoming pain stimulus were manipulated on a trial-by-trial basis. In the first study, EEG and fMRI data were recorded while participants supposedly received real-time feedback on their pain sensitivity on every trial. In the second study, the same sample of participants was re-invited to the lab one week later to test for the stability of effects. Participants in the third study were instructed to consciously modulate their expectations in line with a visual cue, while, as in the second study, EEG data were recorded.

The first study revealed that positive and negative expectations were similarly represented in neural activity during the anticipation phase, and activity only differentiated during the actual pain processing. Strikingly, this pattern was mirrored in the anterior insula and areas of the DPMS including the ACC, DLPFC, and vmPFC. The combined EEG-fMRI analysis showed an earlier onset of activity in the prefrontal cortex and anterior insula compared to activity in the ACC during the anticipation phase. In the second study, it was shown that both positive and negative expectations had lasting effects on pain perception over one week. The stability of effects could be predicted by neural activity recorded during the initial day of expectation induction, with activity in the DLPFC, anterior insula, and amygdala being related to the stability of positive expectations, while activity in the thalamus predicted the stability of negative expectations. Moreover, expectations were reflected in theta-to-alpha oscillatory activity. The third study showed that pain ratings could be modulated when participants consciously adjusted their expectations, which also led to changes in high-frequency oscillatory activity during the pain phase.

The results presented here highlight the relevance of unique anticipatory processes for the subsequent modulation of pain perception. It was shown that areas of the DPMS, especially the prefrontal cortex, and the anterior insula play an important role in the generation of expectations and their longer-term stabilization. The results bear clinical implications, particularly in regard to the high stability of both placebo and nocebo effects and the efficacy of conscious expectation modulations.

7 Erklärung des Eigenanteils an den Publikationen

Wittkamp, C. A.*, Wolf, M.-I.*, & Rose, M. (2024). The neural dynamics of positive and negative expectations of pain. *eLife*, *13*, RP97793. https://doi.org/10.7554/eLife.97793.3

Maren-Isabel Wolf designed, programmed and conducted the experiment, analyzed the data and wrote the manuscript, all in cooperation with Christoph Arne Wittkamp.

Wolf, M.-I.*, Wittkamp, C. A.*, & Rose, M. (2024). Differential neural activity predicts the long-term stability of the effects of positive and negative expectations on pain. *Scientific Reports*, *14*(1), 27874. https://doi.org/10.1038/s41598-024-77693-z

Maren-Isabel Wolf designed, programmed and conducted the experiment, analyzed the data and wrote the manuscript, all in cooperation with Christoph Arne Wittkamp.

Wolf, M.-I.*, Wittkamp, C. A.*, & Rose, M. (in preparation). *The influence of consciously generated expectations on pain perception*.

Maren-Isabel Wolf designed, programmed and conducted the experiment, analyzed the data and wrote the manuscript, all in cooperation with Christoph Arne Wittkamp.

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*Co-first authorship

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Vielen Dank!

9 Lebenslauf

entfällt aus datenschutzrechtlichen Gründen

10 Tools

ChatGPT version 4, OpenAI (https://uhhgpt.uni-hamburg.de), was used for the following purposes:

- Improving sentences and passages for their readability and flow
- Grammar and spelling checks

11 Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe, insbesondere ohne entgeltliche Hilfe von Vermittlungs- und Beratungsdiensten, verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe. Das gilt insbesondere auch für alle Informationen aus Internetquellen.
Soweit beim Verfassen der Dissertation KI-basierte Tools („Chatbots“) verwendet wurden, versichere ich ausdrücklich, den daraus generierten Anteil deutlich kenntlich gemacht zu haben. Die „Stellungnahme des Präsidiums der Deutschen Forschungsgemeinschaft (DFG) zum Einfluss generativer Modelle für die Text- und Bilderstellung auf die Wissenschaften und das Förderhandeln der DFG“ aus September 2023 wurde dabei beachtet.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich damit einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Datum Unterschrift