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The neural and behavioral mechanisms of the modulation of pain by positive and negative expectations

Dissertation

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vorgelegt von:

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1. Synopsis

1.1 Introduction

The percept forming in the mind is rarely a linear depiction of what receptor cells are emitting as a signal towards the brain. Perceiving the world is not a straightforward inference from sensory input but a complex process that is formed by a compound of multiple factors. This includes social influences such as the socioeconomic status (Dorner et al., 2011), psychological influences such as the attention in visual perception (Boynton, 2005) and hearing (Auerbach & Gritton, 2022), and biological factors such as the intraepidermal nerve fiber density for pain perception (Mouraux et al., 2012). All these factors interact with the input of the sensory neurons to form the perception. Out of this compound one of the most important factors with a profound impact on the perception is the expectation about the stimulus, meaning expectations about its intensity or pleasantness and unpleasantness or the expectation, that a treatment will change the perception of the stimulus (Atlas & Wager, 2012).

1.1.1 Background

While the impact of expectations on perception is well known (Amanzio et al., 2013; Atlas & Wager, 2014; Petersen et al., 2014), the processes that form the expectations in the brain are still under investigation. Evidence has been provided, that expectations form our perception of the world in general such as in the domain of taste (Luo et al., 2024), in the domain of visual perception (e.g., Piedimonte et al., 2024) or in the domain of the emotional processing of faces (Baker et al., 2022). However, the domain of perception in which the impact of expectations is studied most intensively is pain perception, and in our studies, we therefore focused on the impact of expectations on pain as well. In the pain domain it has been shown that inducing the positive expectation that a stimulus will hurt less, either because of a treatment or because of the expectation of a reduced intensity, has led to hypoalgesia (Atlas & Wager, 2012; Montgomery & Kirsch, 1997) and inducing the negative expectation that a stimulus will hurt more has led to hyperalgesia (Colloca & Benedetti, 2007). This alteration of pain perception is termed the placebo effect in the case of positive expectations and the nocebo effect in the case of negative expectations. How these expectations are generated and the mechanisms underlying them are still under discussion. In the most

common model expectations are seen as the result of the generative process of predictive coding. In this model, perception is the result of a process of matching incoming sensory data with top-down predictions (or expectations) called the prior (Büchel et al., 2014; Edwards et al., 2012). This prior is then in turn altered by what is called the prediction error, the mismatch between the sensory data and the former top-down prediction. This model allows us to understand expectations as something that is changing from experience to experience and is underlying the process of learning.

Derived from this model of pain modulation by expectations, the question of the induction of the pain modulating expectations was raised. Evidence has been presented for conditioning and verbal suggestion playing an important role in the emergence of placebo and nocebo effects (Bąbel et al., 2017). This is also true for observational learning (Bräscher et al., 2018; Colloca & Benedetti, 2009). Importantly, all these pathways rely on the deception of the participants. Differing to these approaches an open-label-placebo framework used for example by Kaptchuk et al. (2010) does not rely on deception but patients are openly told that they will receive a placebo instead of a treatment. Interestingly, without the deception, participants still show robust placebo responses (Kaptchuk et al., 2010; von Wernsdorff et al., 2021) and self-regulation of pain experiences has also been shown to alter pain experiences (Woo et al., 2015). These results raise the question if deception is necessary for placebo and nocebo effects or if participants can voluntarily influence their expectations regarding upcoming stimuli and use these to alter their experiences.

Once the expectation towards a painful stimulus is induced, the valence of this expectation is important. The question has to be raised if negative expectations and positive expectations are similar in their mechanisms. Evidence has been presented that they might differ in respect to their proneness for conditioning (Colloca et al., 2008, 2010). Therefore, it might be reasonable to assume that positive and negative expectations might differ in other aspects of their mechanisms as well. However, the investigation of the different mechanisms of positive and negative expectations is complex, as to successfully investigate the similarity of positive and negative expectations they must be induced by a comparable paradigm. This is a difficult task in placebo and nocebo research because of the importance of the underlying rationale for the suggestions (Locher et al., 2017). Therefore, a way to induce expectations has to be found that allows for both positive and negative expectations to be reasonable. Besides how they are learned, the question of the stability of expectations is of great

interest. Following the presumption of the generative model, an expectation should be altered by prediction errors and following simple learning paradigms should undergo extinction if not reinforced (Jepma et al., 2018). However, evidence has been presented that expectations might be relatively stable and behave as self-fulfilling prophecies over the course of the experiments (Jepma et al., 2018). Further the possibility has been discussed that expectations can get solidified to a belief, a long lasting conviction, though it remains unclear at which point and how an expectation gets solid enough to be a belief, that is difficult to change, and therefore induces long-term alterations of perception (e.g., in chronic pain: Boersma & Linton, 2006). Again, this appears to be different for positive and negative expectations, with evidence for a higher persistence of negative expectations that is discussed to stem from higher arousal in negative expectations (Colagiuri & Quinn, 2018; Colloca et al., 2010). Investigating both valences is therefore important to fully understand the stability of expectations. It also remains unclear to which degree individual traits impact the process of pain modulation by expectations. One trait associated pain modulation seems to be the certainty in ascending sensory signals, with evidence that it the certainty is impacting placebo effects but also clinical pain reporting (Kuperman et al., 2020).

1.1.2 How expectations shape pain perception

Considering the influence of expectations on the perception of pain, the question arises about the neural mechanisms that alter the experience. Pain perception is altered by expectations on multiple levels of the networks shaping the perception with evidence for modulation already at the level of the spinal cord (Eippert, Finsterbusch, et al., 2009; Geuter & Büchel, 2013; Tinnermann et al., 2017). It has further been shown that placebo effects (or positive expectation effects) are reliant on the endogenous opioid system, which can be derived from the hampering effects of naloxone, an opioid antagonist, on placebo effects (Benedetti, 1996; Grevert et al., 1983; J. D. Levine et al., 1978; J. D. Levine et al., 1979). Moreover, there is additional evidence suggesting the involvement of the cannabinoid system (Benedetti et al., 2011). Unalike the role of opioids, the role of dopamine in placebo effects is currently disputed with evidence for an involvement of dopamine (de la Fuente-Fernández et al., 2001), but also against it (Kunkel et al., 2024). Less research has been done for

nocebo effects, though the role of cholecystokinin is under discussion with evidence of proglumide (a cholecystokinin antagonist) blocking nocebo effects (Benedetti et al., 1997).

The evidence for endogenous opioids taking an important role in placebo effects fits the work of Basbaum & Fields (1978, 1984), describing a descending and ascending pain modulatory system with the periaqueductal gray (PAG) projecting via the rostral ventromedial medulla (RVM) to the dorsal horn of the spinal cord. Importantly, following the GABA-disinhibition hypothesis, among cannabinoids μ-opioids have a key role in this system, being the main driver for GABAergic interneurons in the PAG (Lau & Vaughan, 2014) and the RVM (Fatt et al., 2024). The GABAergic interneurons are important gateways in an inhibitory system for pain perception. One conclusion of this could be that placebo effects might therefore rely at least partially on the GABAergic interneurons activated by opioids, even though there seem to be other factors than opioids contributing to the effect (Gracely et al., 1983). Remarkably, research indicates that naloxone's impact on placebo effects is observed at a neurological level above that of the midbrain. Eippert and Bingel (2009) provide evidence that naloxone obstructs the pathway linking the rostral anterior cingulate cortex (rACC) to the periaqueductal gray (PAG). This finding implies that pain modulation processes associated with opioids must also be operative at levels higher than the midbrain and brainstem.

Fitting this evidence, the PAG-RVM-dorsal horn axis described by Basbaum and Fields is not the only axis responsible for pain perception, but there is a complex matrix of cortical and subcortical brain areas responsible for the processing of pain, a network of brain areas often coined as the Descending Pain Modulatory System (DPMS; Büchel, 2022; Geuter, Koban, et al., 2017; Tu et al., 2022), or the Descending Pain Control System (Eippert, Bingel, et al., 2009). This network consists of cortical areas as the dorsolateral prefrontal cortex (DLPFC), ventromedial prefrontal cortex (vmPFC), rACC and anterior insula, and further spans subcortical areas such as the amygdala, thalamus, and hypothalamus. Importantly, these areas project to the PAG, which in turn projects to the RVM and the axis described above. This network is thought to be responsible for the top-down-modulation of pain perception and the complex structure of the network already gives reason to assume that many factors are responsible for how pain is perceived. To fully understand this network and how we perceive pain in the brain, it has again to be considered that pain is an actively

constructed experience determined by expectations and modified by learning (Wiech, 2016). It further has been shown that several mechanisms of pain modulation by expectations change brain activity in these areas (Woo et al., 2015) , and that this is specifically the case for pain perception that is independent of stimulus intensity differences(Woo et al., 2017). It can therefore be concluded, that in the DPMS some areas are responsible for the modulation of pain perception by expectations (Atlas & Wager, 2014; Geuter, Boll, et al., 2017; Tu et al., 2022; Wager & Atlas, 2015; Zunhammer et al., 2021). However if positive and negative expectation effects rely on the same networks is still under discussion with evidence for distinct representations (Bingel et al., 2011; Freeman et al., 2015; Fu et al., 2021; Shi et al., 2021; Shih et al., 2019) competing with evidence for shared representations (Amanzio et al., 2013; Amanzio & Palermo, 2019; Colloca & Benedetti, 2007; Palermo et al., 2015; Rossettini et al., 2023; Schmid et al., 2015). This again raises the question of the similarity of positive and negative expectations and specifically if they are processed similarly in the brain. Interestingly, the activation of areas associated with pain perception can be subdivided even more in a signature associated with stimulus intensity, the Neurologic Pain Signature (NPS; Wager et al., 2013), and the Stimulus Intensity Independent Pain Signature (SIIPS; Woo et al., 2017), a signature capturing activity orthogonal to the NPS, reflecting pain processing that does not depend on stimulus intensity differences reflected in physical stimuli differences. The two signatures underline that pain processing in the brain is complex. Remarkably, placebo effects have been associated with changes in activity in the SIIPS, but less so with changes in the NPS (Botvinik-Nezer et al., 2023).

Importantly, the complexity of the network responsible for the active construction of pain perception is not only evident in the large network of areas involved, but also by its temporal division. It can be assumed that information about features of a stimulus is already present in the brain before the stimulus occurs (Wiech et al., 2014). This information is part of preparatory processes in the brain that already influence what we will perceive. The presence of these processes is supported by evidence of preparatory activity measured with fMRI, e.g., in the prefrontal cortex (Wager et al., 2004) and DLPFC (Watson et al., 2009). Further, EEG oscillatory activation in the period before the stimulus in the alpha and beta band (Nickel et al., 2022; Strube et al., 2021) or the theta-band (Taesler & Rose, 2016) was associated with altered subsequent pain perception. Interestingly, this is not limited to higher areas

of the brain but evidence has been presented for anticipatory activity in the spinal cord, influencing behavior already on that lower level before a stimulus occurs (Stenner et al., 2025). In conclusion, a complex matrix of areas spanning from the DLPFC and vmPFC down to the spinal cord is influencing pain perception during the anticipation of pain and during pain perception. The timing of these processes and the mechanisms, however, remain unclear, especially the differences of the valences of expectations regarding these processes. The processing of pain perception altered by expectations is a therefore a complex phenomenon that takes place in large networks in the brain and that is reliant on multiple systems in the brain and multiple psychological mechanisms.

1.1.3 Research Questions

In the work presented here we wanted to investigate the mechanisms underlying the modulation of pain by expectations. We aimed to understand the differences between a positive and negative valence of expectations regarding their behavioral mechanisms and their neural correlates and underlying processes. To better understand these processes, the temporal division between the processes and systems must be considered. Therefore, in Study 1 we investigated the processes combining EEG for the good temporal resolution and fMRI for the good spatial resolution in the anticipation of pain and during pain perception. To investigate the expectation modulation, we used a paradigm that manipulated expectations in both the negative and positive direction. In Study 2 we tested how stable the induced expectations were depending on their valence and which neural correlates the stability of expectations has. In Study 3 we focused on the question if both positive and negative expectations must be manipulated by deception, or if an overt approach can elicit comparable effects.

1.2 Methods

1.2.1 The paradigm

Study 1 and Study 2 relied on a newly developed paradigm of pain using a sham-Brain-Computer-Interface (BCI) based on EEG measurements. The BCI was

used to give sham feedback about the pain sensitivity of participants with the feedback inducing positive and negative expectations. Study 1 (N = 55) was based on data measured on the same day as the induction of expectations. Study 2 (N = 42) combined this data (day 1) with data that was obtained from the same participants of Study 1 approximately one week after the induction of expectations (day 8) without reinforcing expectations, this means that no conditioning or verbal suggestions were delivered on the day of the second measurement. On day 1, participants were told that their brain activity would be measured via the EEG cap and that they would be given feedback based on their brain activity about their pain sensitivity via cue images in the form of a colored brain area either in green for less pain sensitivity, red for higher pain sensitivity, or in yellow. The yellow-colored brain area was used as a cue for a control condition, in which participants were told that the algorithm of the BCI had no clear result either because their brain state was changing too quickly or because the measurement was not successful. This condition was designed to induce a neutral expectation towards the painful sensation.

On the day of the first measurement, after participants were verbally instructed, they underwent pain calibration and a conditioning procedure pairing green colored brain areas with less painful stimuli (VAS 30) and red colored brain areas with highly painful stimuli (VAS 70). The conditioning procedure was masked as a calibration of the algorithm of the BCI by telling participants that an individual threshold had to be created and that for this purpose, the algorithm would wait for clearcut results for the classification of brain activity in either high or low pain sensitive states, hence that they would not see yellow colored brain areas. Following the conditioning procedure, a test phase was conducted pairing the three cue images (red, yellow and green) with, unbeknownst to the participants, always the same intensity of a painful stimulus (VAS 60). Participants were shown the cue image and subsequently had to rate their expectations towards the next painful stimulus on a VAS Scale (0-100) ranging from no pain to unbearable pain. The expectation rating was followed by a presentation of a fixation cross lasting 3.3 seconds. This period was used to capture neural activity that corresponds to the preparation for the painful stimulus. Following this period, a painful stimulus of 4 seconds was presented that subsequently had to be rated by participants on an identical VAS scale as the expectation rating. The same test phase was used on Study 2, but without repeating conditioning and verbal instructions upfront, which made it possible to test the stability of expectations induced in Study 1.

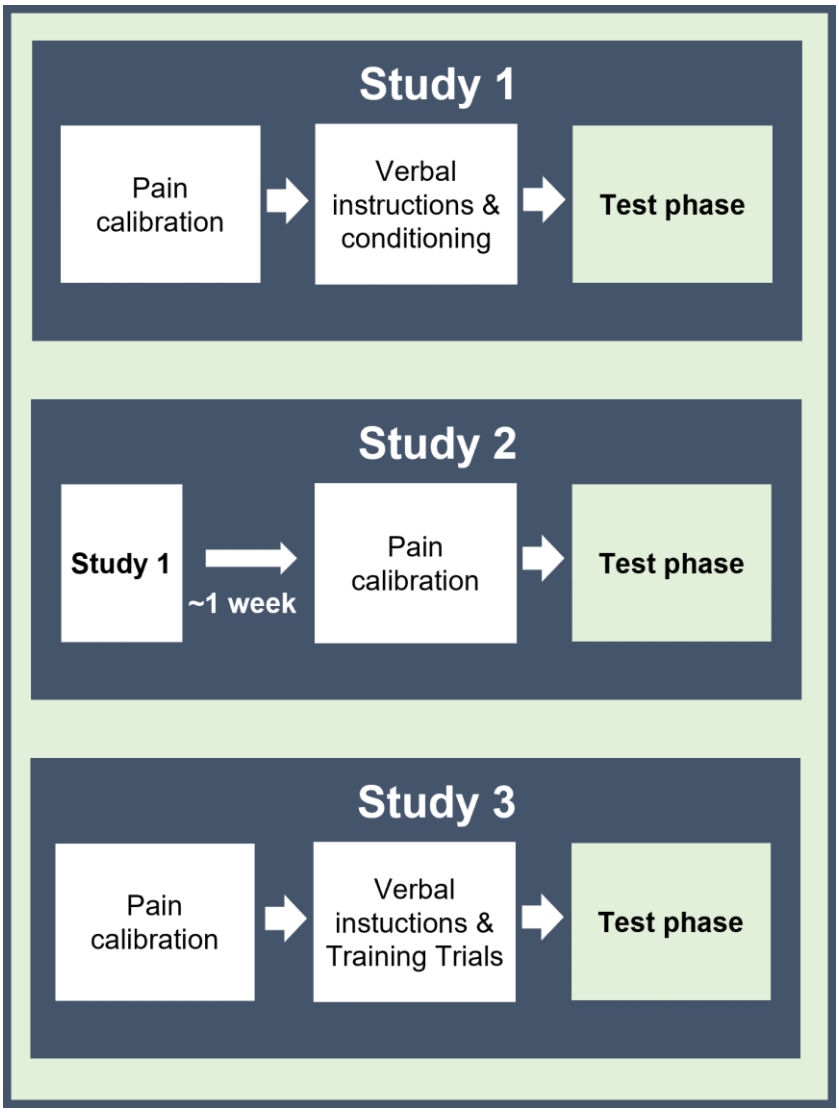

Figure 1. Sequence of events in Study 1, Study 2, and Study 3. Test phases and pain calibration were identical in all three studies. Study 2 was conducted approximately one week after Study 1, without reinforcement of expectations. In Study 3, no deception about the nature of the study was conducted, but participants were openly told to change their expectation based on a cue.

In Study 3 (N = 42), the structure of the test phase was nearly identical. However, the paradigm was altered in the way that participants were not deceived about a sham-BCI but were told that they themselves are able to influence their pain perception by creating expectations towards the painful stimuli. Participants were told that pain can be modulated by expectations but also by voluntary action. Identical cue images to Study 1 and 2 were subsequently used as cues for participants to voluntarily create a positive, neutral, or negative expectation. These cues were presented before

a painful stimulus (60 VAS) of always the same intensity without participants being aware of this. Identically to Study 1 and 2 participants were then asked to rate their expectations and the pain perception. Before the test phase, participants underwent a training consisting of six trials identical to the test phase. The training was used to familiarize participants with their task.

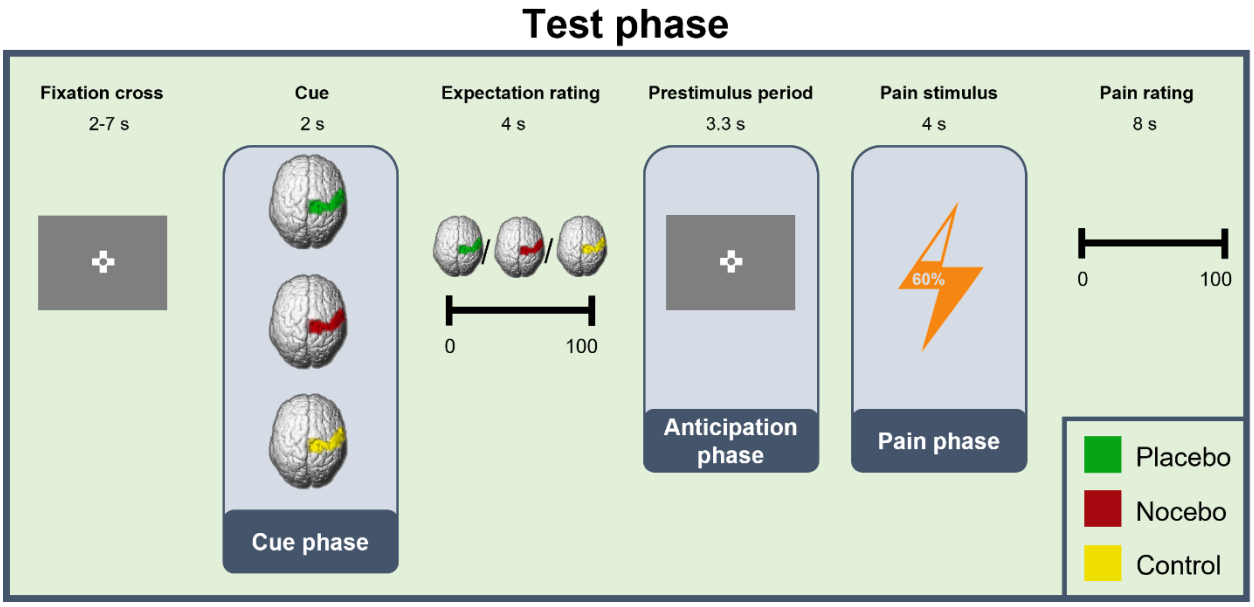

Figure 2. Depiction of a single test trial. A fixation cross shown during the inter-trial-interval was followed by a presentation of a cue image for 2 seconds. Participants then had to rate their expectation towards the upcoming painful stimulus in a time window of 4 seconds. Another fixation cross was shown for 3.3 seconds in the prestimulus period. Subsequently, a painful stimulus calibrated to the intensity of 60 VAS was presented plateauing for 4 seconds. Participants then had to rate the intensity of the stimulus. This figure was reused and adapted from (Wolf et al., 2024) licensed under CC BY 4.0

1.2.2 Technical implementation

Study 1

Study 1 was conducted as a combined EEG-fMRI measurement. FMRI data was recorded using a 3T Siemens PRISMA Scanner and a 64-channel head coil. EEG data was recorded simultaneously using a custom 64-channel BrainCap-MR for 3 Tesla containing 64 passive sintered Ag/AgCl electrodes arranged according to the

10/20 System, as well as one ECG electrode, and recorded using the BrainVision Recorder software (Version 1.10, BrainProducts, Gilching, Germany). The FCz was used as the reference electrode and the Pz was used as the ground electrode.

Study 2 and 3:

Study 2 and 3 were conducted in an electrically shielded room using a 64-channel actiCap, containing 64 active Ag/AgCl electrodes with 62 electrodes. Electrodes were arranged to the extended 10/20 System using FCz as the reference and the Pz as the ground electrode and two additional electrodes as horizontal electrooculogram (HEOG) electrodes.

Heat Stimulation

In all three studies, pain stimuli were applied via a PATHWAY CHEPS (Contact Heat-Evoked Potential Simulator) thermode (https://w ww.medoc-web.com/pathway-model-cheps). The PATHWAY CHEPS has a rapid heating rate of 70 °C/s and a cooling rate of 40 °C/s and can deliver heat stimuli in the range of 30 °C to 55 °C in less than 300 ms. For the studies, baseline temperature was set to 32° C and rise and fall rate were set to 70 °C/s. Participants were calibrated using an adapted version of a calibration established by Horing et al. (2019).

1.3 Results summary

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

In Study 1 we aimed to investigate the neural representations of the modulation of pain by expectations and their temporal organization. For this study, data derived from skin conductance response (SCR), EEG, behavior, and fMRI was collected. For preprocessing of all data modalities please see the method section in the paper (Wittkamp et al., 2024). Using condition as a predictor for behavioral data (placebo vs. nocebo vs. control), differences in mean pain ratings by condition and differences in mean expectation ratings by condition were compared separately in two repeated-measures ANOVAs (n = 50). Significant main effects for condition in both expectation and pain ratings marked a successful induction of expectation effects for both positive

and negative expectations. Post-hoc tests revealed that pain and expectation ratings were higher in the nocebo condition compared to the control condition and lower in the placebo condition compared to the control condition. Expectation effects were induced in 47 out of 50 subjects successfully. Further, the expectation effects, consisting of the difference of control to placebo for the placebo effect and the difference of nocebo to control for the nocebo effect, were highly correlated across subjects for both expectation (r=0.64, p<.001) and pain ratings (r=0.30, p=.033). Therefore, participants with high placebo effects were also showing high nocebo effects.

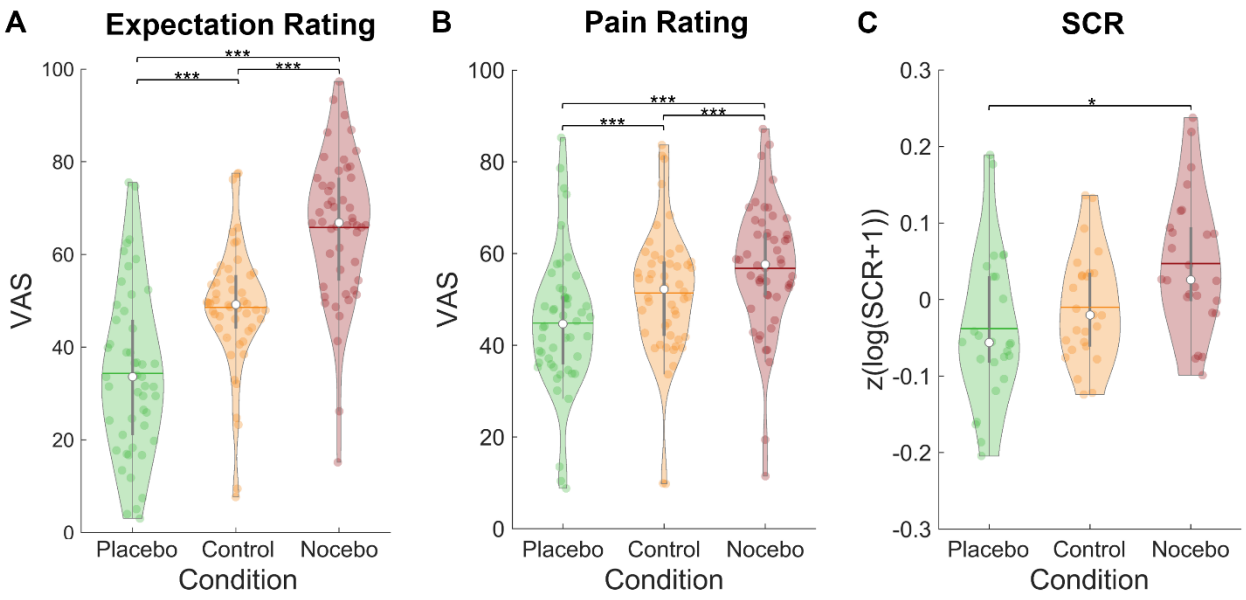

Figure 3. Mean expectation (A) and pain ratings (B) on a visual analogue scale separately for each condition (n = 50). (C) Mean skin conductance responses in the three conditions (n = 26). White dots = mean, horizontal lines = median, thick gray vertical lines = upper and lower quartile, colored dots = pain ratings of individual participants per condition. *p<0.05. **p<0.01. ***p<0.001., this figure and the figure description were reused and remained unmodified from Wittkamp et al., (2024), licensed under CC BY 4.0.

Rating differences between the conditions were supported by significant differences in phasic SCR during the pain phase. The response window for SCR during pain was defined by visual inspection of the curve to discover the peak and set between 2 and 7.5 seconds after pain onset. A repeated measures ANOVA was conducted using the factor cue type (placebo vs. nocebo vs. control) as predictor and SCR as

outcome (n = 26). Post-hoc tests revealed larger SCR responses in the nocebo condition compared to the placebo condition, but no significant differences between the control condition and the placebo or nocebo condition, supporting the notion of differences in pain perception levels between the placebo and nocebo condition.

FMRI analyses were conducted on the first level using a finite impulse response model (FIR model) for each of the three conditions separately on a time course of 18.4 s starting at the cue onset and divided into 11 bins. Each bin covered the duration of 1.675 seconds. Differently to the canonical HRF model (hemoglobin response function model), the FIR model does not make assumptions about the onset of a stimulus, therefore it allowed us to examine non-time locked effects in the anticipation phase. To test if the induced pain modulations led to changes of brain activity comparable to other studies, the SIIPS score was calculated for each of the bins of the FIR model separately for each condition. The values were consequently compared in a rmANOVA (n = 45) which revealed significant differences between the placebo and nocebo condition during pain perception, marking significant differences in the perception of pain during the pain phase. No significant differences were found during the anticipation of pain, suggesting distinct processes during anticipation of pain and pain perception.

Second Level analysis (n = 45) was conducted using a flexible factorial design, comparing common effects (placebo and nocebo vs. control) and distinct effects (placebo vs. nocebo) of the expectation conditions using *t*-contrasts. The common effects were additionally masked using the *F*-contrast between the placebo and nocebo conditions (threshold at $p<.05$ uncorrected) to exclude effects rooting only in the differences of these conditions, only. Analyses were conducted in the phase of anticipation of pain (–4.275 s until –0.925 s relative to pain onset in bins 4 and 5) and in the phase of pain perception (0.75 s until 5.8 after pain onset in bins 7,8, and 9). Additional ROI analyses were conducted in the ACC, insula, thalamus, amygdala, DLPFC and hippocampus.

The analyses revealed a dynamic pattern of activity from pain anticipation to pain perception. During anticipation of pain, common effects of placebo and nocebo effects were present in areas of the DPMS such as, e.g., the bilateral DLPFC, right vmPFC, left anterior insula and bilateral ACC, areas closely associated with the DPMS. During pain perception, areas such as the bilateral DLPFC, right vmPFC, left anterior

insula and thalamus differentiated their activity patterns for placebo and nocebo effects. In these areas, placebo effects now led to higher activity in comparison to nocebo effects. This proposes a common representation of positive and negative expectations during the anticipation phase and a differentiation of processes during pain perception that could be the driver of the changed pain perception.

An analysis of combined EEG data and fMRI data (n = 41) was administered on the significant peak voxels derived from the analysis of common effects of positive and negative expectations. Around the significant peaks, ROIs were formed by 10 mm spheres. ROIs contained the left anterior insula (xyzMNI: –28, 22,–6), left thalamus (−6,–12, 4), left (xyzMNI: –2, 40,–4) and right ACC (xyzMNI: 4, 42, 12), right vmPFC (xyzMNI: 14, 56,–14), left (xyzMNI: –32, 18, 36) and the right DLPFC (xyzMNI: 40, 24, 36). For this analysis, single-trial mean beta values for each ROI were extracted, estimating the hemodynamic responses measured in fMRI.

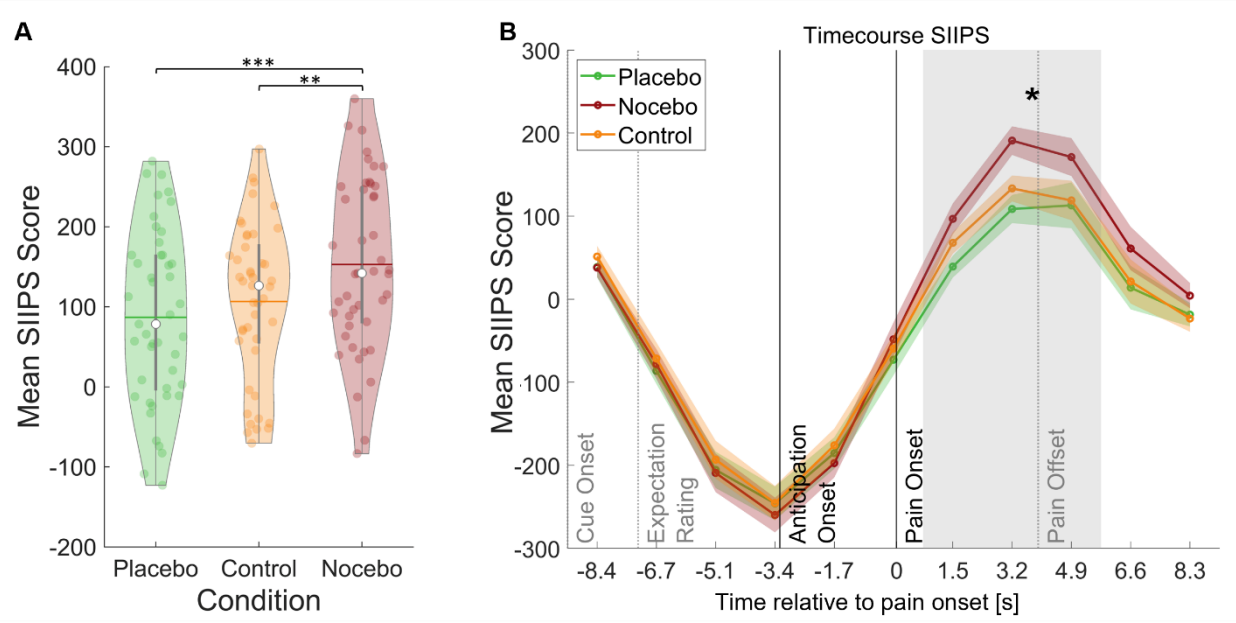

Figure 4. (A) Mean SIIPS score per condition for all time-points during pain perception. White dots = mean, horizontal lines = median, thick gray vertical lines = upper and lower quartile, colored dots = pain ratings of individual participants per condition. *p<0.05. **p<0.01. ***p<0.001. (B) Mean SIIPS 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. This figure and the figure description were reused and remained unmodified from Wittkamp et al., (2024), licensed under CC BY 4.0.

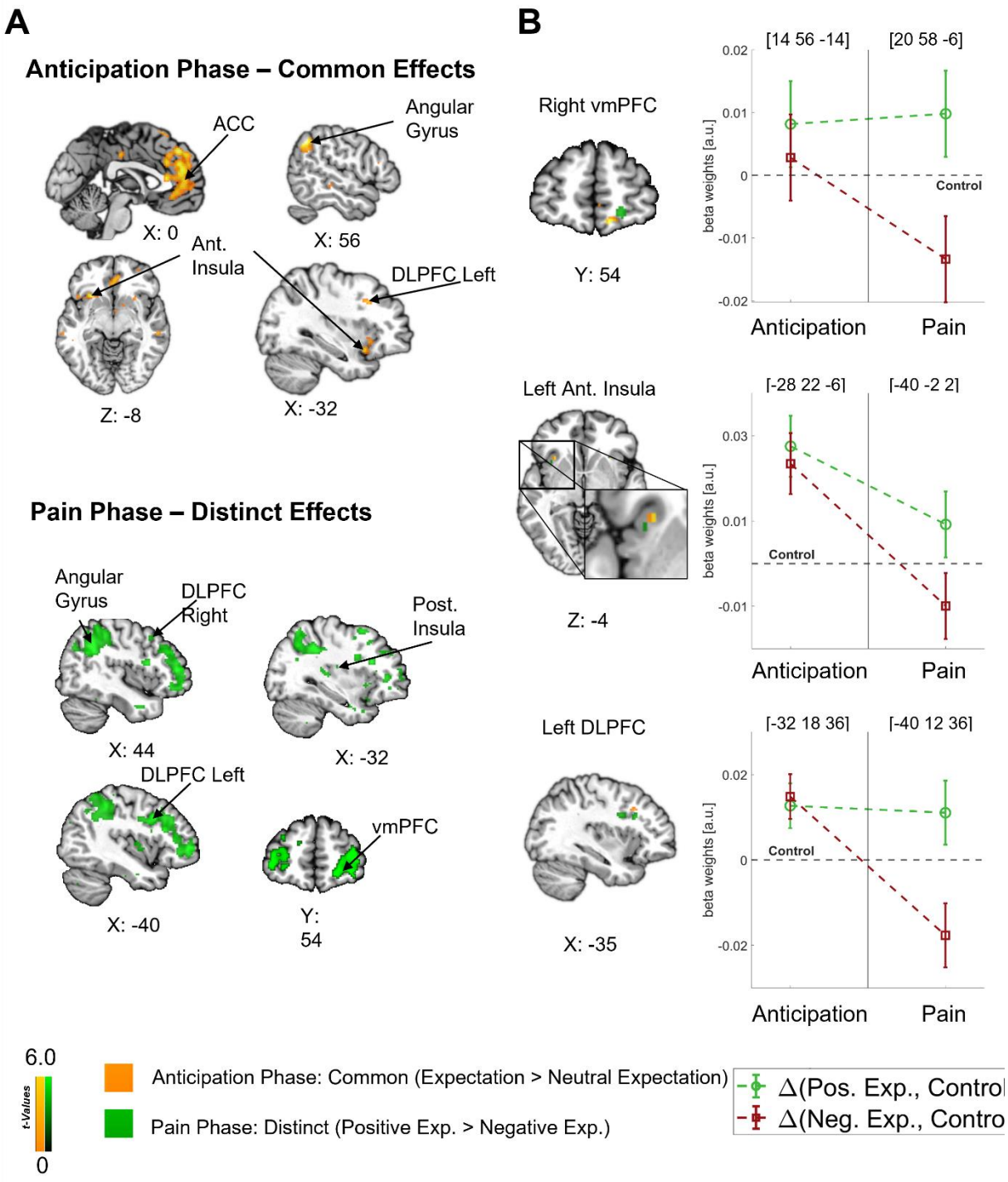

Figure 5. (A) Top: Common effects during pain anticipation (expectation > neutral expectation) at *p*<.001 (uncorrected for display purposes) show widespread higher activity for both positive and negative expectations compared to the control condition. Bottom: Distinct effects (positive > negative) during 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. This figure and the figure description were reused and remained unmodified from Wittkamp et al., (2024), licensed under CC BY 4.0

Beta estimates were calculated on preprocessed MR data using the toolbox GLMsingle (Prince et al., 2022), by fitting a boxcar function with the length of 1.679 s to the onset of the anticipation phase. These betas were then paired with time-frequency EEG data to calculate Spearman's rank correlation coefficients, resulting in one time-frequency-resolved correlation pattern for each participant and for each ROI. EEG time-frequency data was calculated for each electrode over the time-course of the whole trial. Time-frequency decomposition was adapted from Hipp et al. (2011), therefore it 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). Time-frequency data from cue onset until pain offset and beta estimates were correlated on the group-level. Fishers-z transformed correlations were tested against zero (cluster threshold: p=0.05, minimum neighbors: 2, number of randomizations: 2000) using nonparametric cluster-based permutation tests derived from the toolbox Fieldtrip (Oostenveld et al., 2011). Interestingly, this analysis revealed significant correlations between fMRI activity and EEG oscillatory power in several clusters that were graded temporally. The first cluster that correlated negatively with left DLPFC activity ranged from theta to low gamma band activity and was found already in the phase of expectation ratings. A second cluster spanning from theta to low gamma band correlated negatively with activity in the left anterior insula during the very early anticipation phase. The third cluster spanned the gamma frequency range and correlated positively with activity in the right ACC, set at the end of the anticipation phase and the start of the pain phase. Considering these significant clusters, a temporal organization of the processes in the DLPFC, anterior insula and ACC can be derived, that can be further differentiated by the oscillatory patterns that mark these processes.

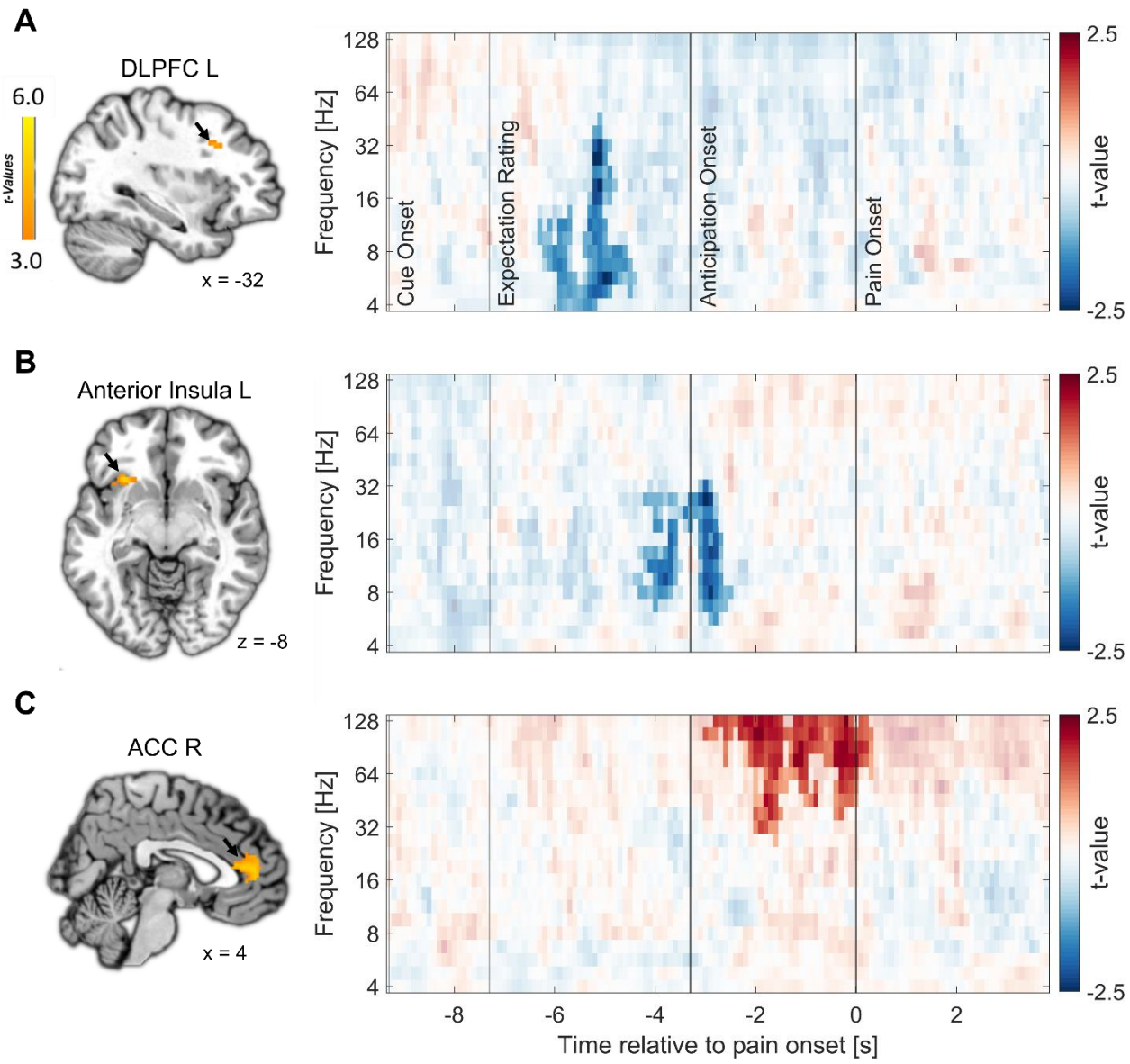

Figure 6. Correlation of single-trial hemodynamic responses with time-frequency resolved EEG activity in the left dorsolateral prefrontal cortex (DLPFC) (A), left anterior insula (B), and right anterior cingulate cortex (ACC) (C) during the anticipation phase, ordered by the timing of observed correlations 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<.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). This figure and the figure description were reused and remained unmodified from Wittkamp et al., (2024), licensed under CC BY 4.0

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

Study 2 was designed to investigate the stability of expectation effects and its neural correlates. Similar to Study 1, repeated-measures ANOVAs were conducted for pain ratings and expectations ratings, respectively. Differently to Study 1, additionally to cue type (placebo vs. nocebo vs. control), the session was implemented as a predictor, comparing the behavioral data collected in Study 1 to data that was collected from identical subjects (n=42) around one week after Study 1 (day 1 vs. day 8). Expectation ratings as well as pain ratings were significantly affected by conditions, with nocebo ratings being significantly higher than control ratings and placebo ratings and placebo ratings being significantly lower than control ratings. For both expectation and pain ratings, no significant effect of time point and no interaction effect was found, indicating the lack of significant differences between day 1 and day 8. To test, if the effects of day 1 and day 8 are similar, we computed the placebo effect (control – placebo) and the nocebo effect (nocebo – control) and used it as a predictor in a rmANOVA for both expectation ratings and pain ratings. For expectation ratings no differences in rating modulation were found between day 1 and day 8. However, pain ratings were significantly different between day 1 and 8, marked by significant post-hoc test for both placebo effects and nocebo effects, indicating a decline in strength of

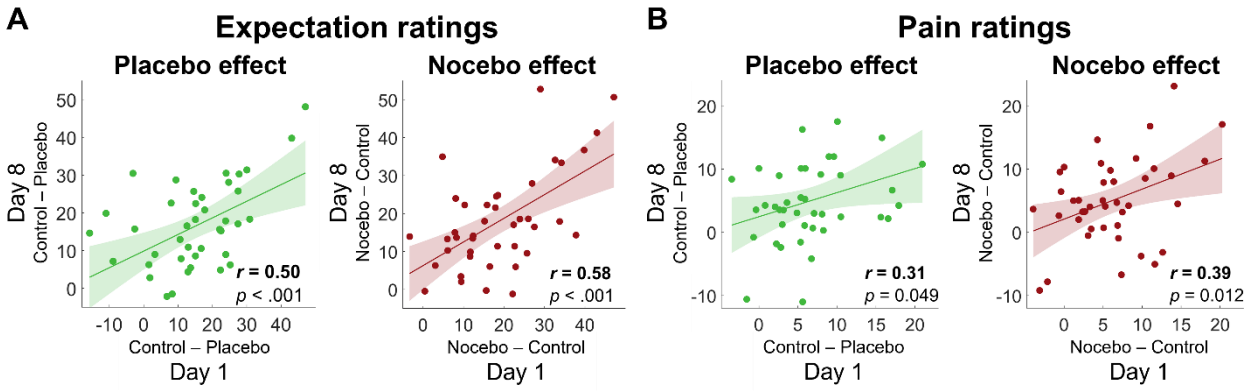

Figure 7. Prediction of effects on day 8 based on the effects observed on day 1. Correlation plots for expectation (A) and pain ratings (B), showing the relationship between the individual placebo and nocebo effects on day 1 with the corresponding effects on day 8. Each dot represents one subject. This figure and figure description were reused and slightly adapted from (Wolf et al., 2024) licensed under CC BY 4.0

the effects. Nonetheless, the effects were still highly evident on day 8, shown by a medium effect size (d = 0.73) for differences in pain ratings in both placebo and nocebo effects at day 8. Further, the significant effects were still present even in the last block of the experiment. The rmANOVA using the placebo and nocebo effects as predictors further revealed no significant effect for the comparison of placebo and nocebo effects and no significant interaction of day and effect type, indicating no difference in strength of placebo and nocebo effects on day 1 or day 8. Additionally, there was a significant correlation between the individual strength of placebo and nocebo effects on the corresponding days in both expectation and pain ratings, marking the individual strength of effects on day 1 as a determinant of the effect strength on day 8.

To investigate the neural correlates of the stability of the placebo and nocebo effect, we used fMRI data collected at day 1. Analogous to Study 1, first level analyses were conducted using a FIR model for each of the three conditions on a time course of 18.4 s starting at the cue onset. The time course was divided into 11 bins, each covering the duration of 1.675 seconds. Differently to Study 1, contrasts comparing placebo to control and nocebo to control in the anticipation phase from − 4.275 s to − 0.925 s before the onset of pain (corresponding to bins 4 and 5) and during pain perception from 0.75 s to 5.8 s after pain onset (corresponding to bins 7, 8, and 9) were calculated on the first level. On the second level these contrasts were then paired with two covariates in one-sample *t*-tests (n=42), using the individual strength of placebo and nocebo effects to investigate the neural correlates of their persistence. The covariate of interest for placebo-related activity was formed from the behavioral placebo effects of day 8, the second covariate was formed from the behavioral placebo effects of day 1 and included at the first position in the design matrix to ensure that the covariates were orthogonalized. This was done to assure that only the variance from day 8 going beyond the effects of day 1 remained. For the nocebo-related activity the respective nocebo effects were used as covariates. Identical ROIs to Study 1 were used for analyses with an additional ROI on the angular gyrus based on the results of Study 1. Analyses revealed that participants with higher placebo effects at day 8 showed activity reductions in the amygdala during pain anticipation and heightened activation in the anterior insula and right DLPFC during pain perception on day 1 (see Figure 10). Also, during pain perception, participants having high nocebo effects at day 8 showed higher activation in the thalamus.

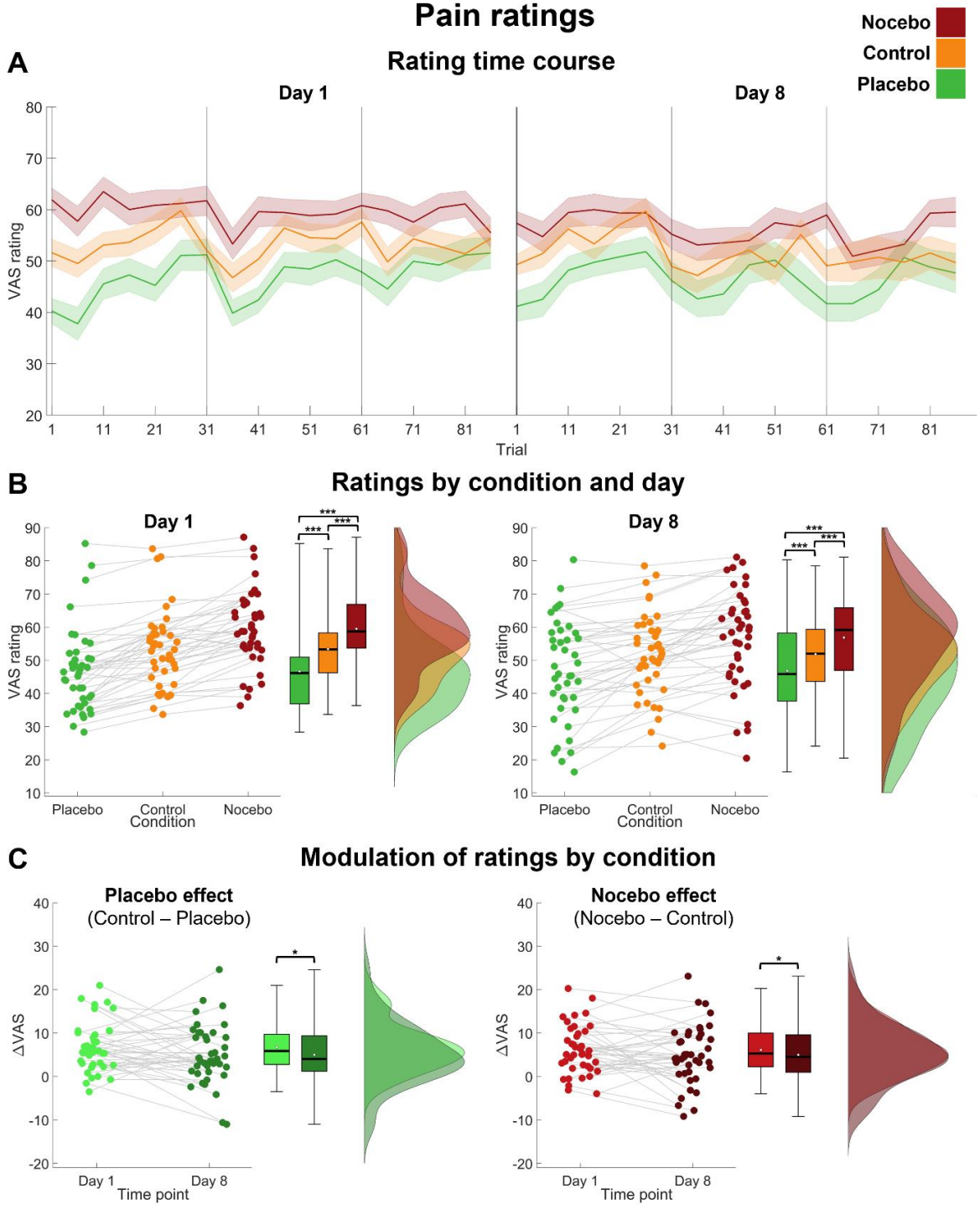

Figure 8. Pain ratings. (A) Time course of pain ratings per condition, averaged over five trials. Error bars denote the corrected standard error of the mean (SEM) using the Cousineau-Morey method. (B) Raincloud plots of pain ratings per condition on day 1 (left) and day 8 (right). Each dot represents 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. This figure and figure description were reused and slightly adapted from (Wolf et al., 2024) licensed under CC BY 4.0

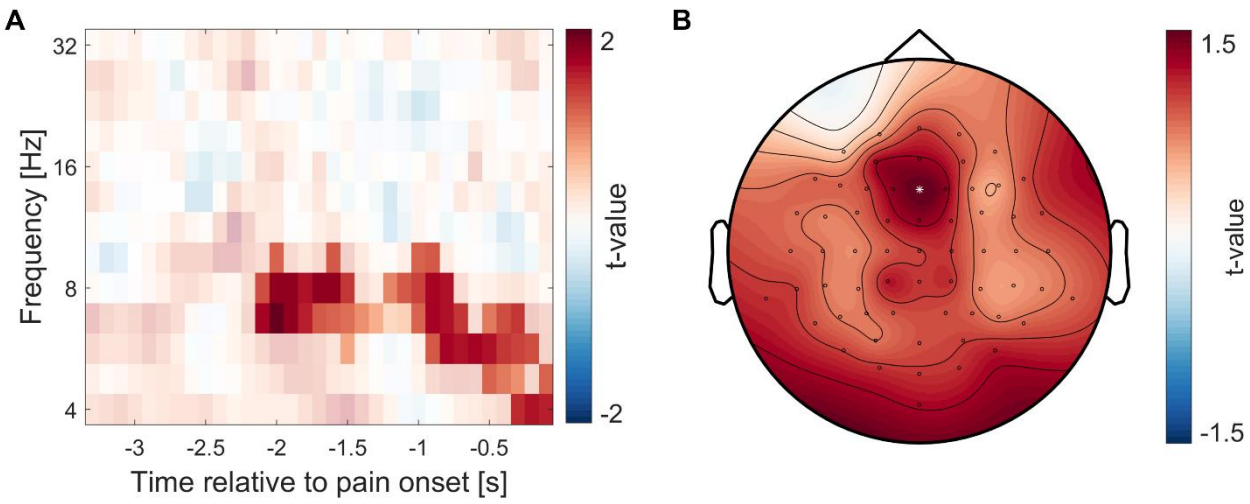

Figure 9. Differences in EEG power between placebo and nocebo on day 8. (A) Time-frequency plot of t-values for placebo vs. nocebo in the anticipation phase on day 8 averaged over all cluster electrodes. (B) The corresponding topography (peak electrode Fz highlighted with a white star). This figure and figure description were reused and slightly adapted from (Wolf et al., 2024) licensed under CC BY 4.0

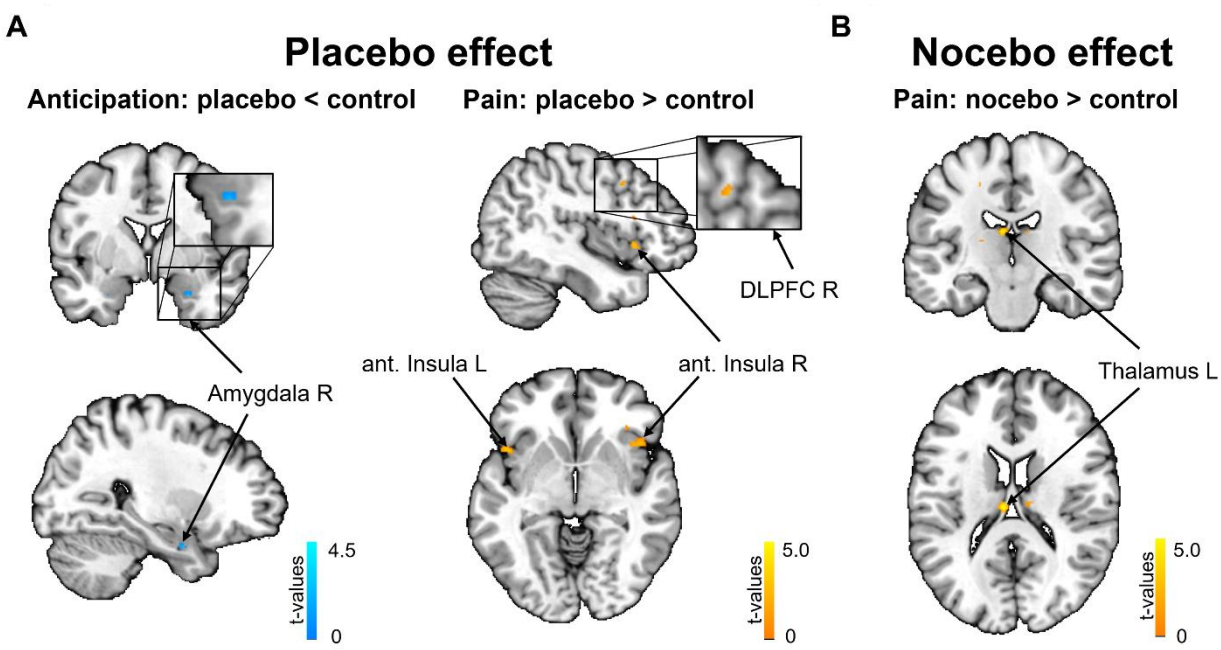

Figure 10. Neural predictors of placebo and nocebo effect persistence. (a) Analyses of the persistence of expectation effects using individual placebo effects of day 8; Blue: lower activation for the placebo condition compared to the control condition in the amygdala during the anticipation phase; Yellow: 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. This figure and figure description were reused and slightly adapted from (Wolf et al., 2024) licensed under CC BY 4.0

1.3.3 Study 3: Increased pain modulation by voluntarily generated expectations

In Study 3, we aimed to test for the effects of voluntarily generated expectation modulations. Further, we aimed to compare these effects with the placebo and nocebo effects of Study 1. However, expectation ratings for Study 3 are hard to interpret as they differed to the expectation ratings of Study 1 and Study 2. Instead of only capturing the current expectation ratings also served as a tool to steer expectations. Therefore, no usable conclusions can be derived from their analysis. Thus, for the analysis of Study 3 by using condition as a predictor in a repeated measures-ANOVA (n=42) we tested for effects of expectation modulation type on pain ratings only. The main effect of condition (positive vs. neutral vs. negative expectations) reached significance,

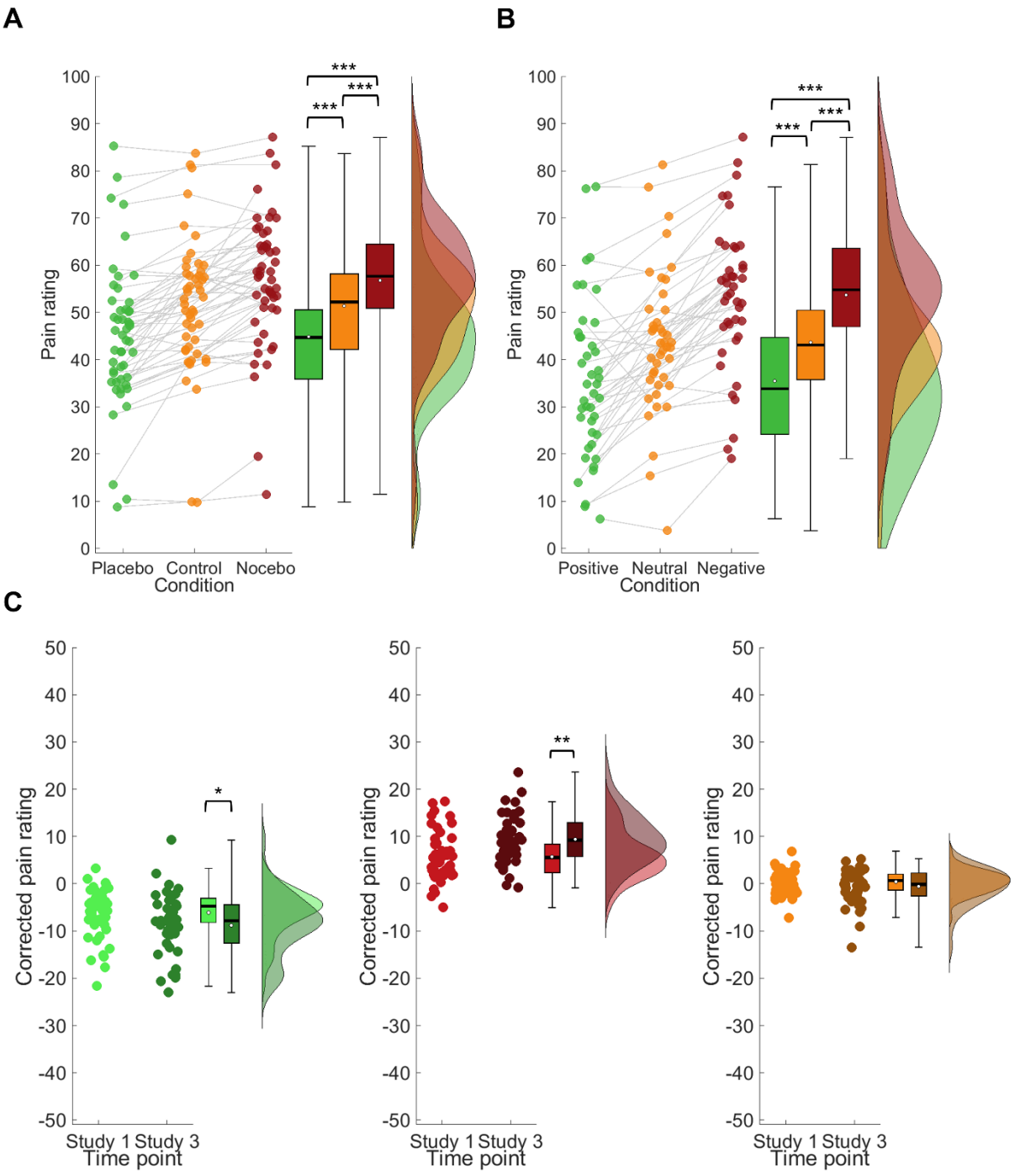

Figure 11. Raincloud plots of pain ratings of (A) Study 1 with classical placebo and nocebo effects in comparison to (B) Study 3 with voluntarily generated expectations in all three conditions. Dots represent individual average rating of subjects per condition. Grey lines connect the ratings of the subjects over the different conditions. Black thick line = Median. White dot = Mean. (C) Comparison of pain ratings corrected by calculating the difference of the pain ratings of each subject to the overall mean pain rating of each subject. *p<0.05. **p<0.01. ***p<0.001.

corrected for violation of sphericity using Greenhouse-Geisser correction (F(1.438, 58.972) = 79.776, p < .001, η^2 = 0.661). Additional post-hoc-tests revealed significant differences between all of the three conditions, for the comparison of placebo (M = 35.47 , SD = 16.77) and nocebo (M = 53.64, SD = 15.43; t(41) = -10.228 , p_{holm} < 0.001), the comparison of placebo and control (M = 43.64, SD = 14.91; t(41) = -5.569, p_{holm} < .001) and the comparison of nocebo and control (t(41) = 10.45, , p_{holm} < .001). To then test for differences between Study 1 and Study 3 we calculated corrected pain ratings by calculating the difference of the pain ratings of each subject to the overall mean pain rating of each subject respectively. This was done to ensure that group pain level differences are not the driving factor in the analysis. In mixed ANOVA, study was added as a grouping variable and condition was added as the predictor. A significant main effect of condition was found (F (1.43,128.63) = 146.59, p < .001, η^2 = 0.62) paired with a significant interaction of study and condition (F (1.49,128.63) = 6.18, p = .004, η^2 = 0.06). However, no significant main effect of study was found (F (1,90) = 1.13, p = .29). Conditional post-hoc tests for the interaction revealed a significant difference between the pain modulation associated with nocebo in Study 1 (M = 5.79, SD = 5.01) and negative pain modulation in Study 3 (M = 9.45, SD = 5.38; t(90) = -3.39, p_{holm} = .001) with the nocebo condition showing smaller values than the negative modulation condition of Study 3, and a significant difference for pain modulation associated with placebo (M = -6.15, SD = 5.09) in Study 1 and the positive pain modulation in Study 3 (M = -8.75, SD = 6.68; t(90) = 2.12, p_{holm} = .037) again with a higher effect in Study 3. The control conditions were not significantly different. To test for differences in the positive and negative expectation effect in Study 3, we computed the effects as the difference between pain ratings for positive expectation and in the control condition (control – positive expectation) and of negative expectations and the control condition (negative expectation – control) and compared these effects in a paired t-test. No significant difference in strength of these effects was found (t(41) = 1.04, p = 0.3).

To underline the rating differences of pain between the conditions, we analysed time-frequency EEG data. The data was preprocessed using the Fieldtrip toolbox for Matlab (Oostenveld et al., 2011). The data was segmented into trails staring 1,000 ms before cue onset and ending 15,800 ms after cue onset. After filtering the data with a low-pass filter at 150 Hz and a high-pass filter at 0.5 Hz, identically to Study 1 and Study 2 the data was split into low and high frequency data using a low-pass filter at

34 Hz for the low frequency data and a high-pass filter at 16 Hz for the high frequency data. The data was then processed in parallel to maximize sensitivity for the detection and the removement of artifacts (Hipp et al., 2011). Single trial data was subsequently visually inspected and removed if heavy artifacts were detected. Next, the data was inspected using independent component analysis (ICA) to remove blink, eye-movement, and head-movement artifacts as well as muscle artifacts. After preprocessing both data subsets were re-referenced to the average of all EEG channels to regain the reference electrode used during the recording session (FCz).

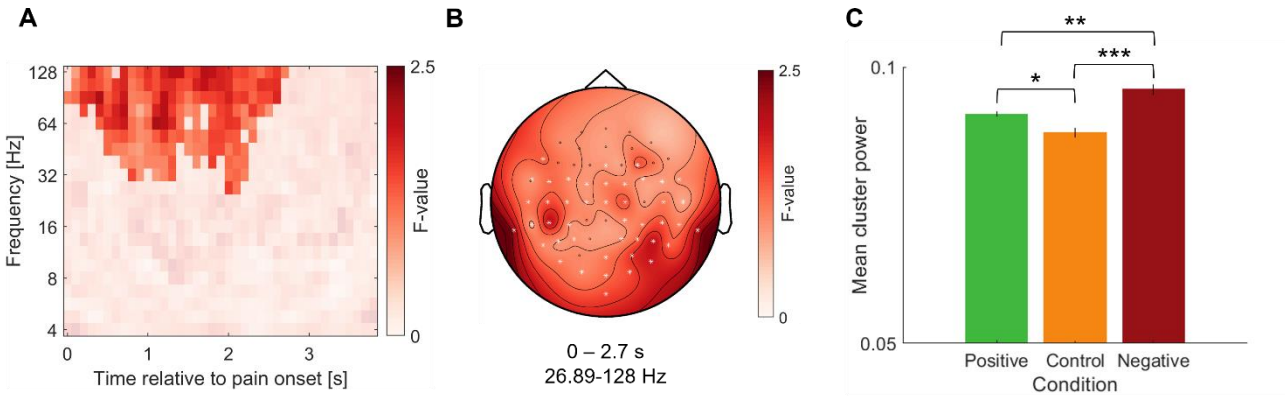

Figure 12. (A) Time-frequency plot of *F*-Values comparing all three conditions during pain perception, averaged over all electrodes within the cluster and masked for non-significant time-frequency points. (B) Topography of gamma effects over all frequencies and time-points of the cluster shown in (A). Cluster channels are highlighted with a white star. (C) Extracted mean power in the cluster per condition used for the rmANOVA. Error bars represent the corrected standard error of the mean using the Cousineau-Morey method (Cousineau, 2005; Morey, 2008). *p<0.05. **p<0.01. ***p<0.001.

For the analysis of EEG data, time frequency data was analysed using a univariate rmANOVA during the phase of pain perception (0 s to 3.9 s after pain onset) with nonparametric cluster-based permutation tests, revealing significant differences in the gamma band from 26.89 -128 Hz between the three expectation conditions (p = .017). Differences in power between the conditions were further tested in a rmANOVA, that compared the extracted mean power of each cluster per participant and per condition. This analysis revealed a significant main effect of condition (F(1.37, 56.35)

= 16.84, $p < .001$). The negative expectation condition had higher power within the analysed cluster (M = 0.096, SD = .082) compared to the positive (M= 0.092, SD = 0.078, $p_{holm} = .003$) and neutral expectation condition (M = 0.088, SD = 0.075, $p_{holm} < .001$). Additionally, the neutral expectation condition was significantly different to the positive expectation condition ($p_{holm} = .016$). The differences in neural processing during pain perception support the notion of different perceptions in the three conditions, making a mere reporting bias improbable.

1.4 Discussion

The aim of this dissertation was to gain insight into the neural and behavioral mechanisms underlying the modulation of pain by either positive or negative expectations. To investigate these mechanisms, we developed a paradigm that successfully induced expectations in all the three present studies. Additionally, we investigated the neural correlates of pain modulation by positive and negative expectations using EEG and fMRI. We were able to show that the expectations induced by our paradigm had significant effects on the perception. Not only was the induction of expectations in Study 1 successful, which can be seen in significant differences in expectation ratings, but the induced expectations also altered the pain perception in a significant manner, with significant differences in pain ratings between all three expectation conditions (positive vs. neutral vs. negative). In Study 2 we could further show that these expectations were highly stable, indicated by a reliably induced pain modulation about one week after the induction of expectations and effects being still highly evident at the second measurement. In Study 3, we provided data for the effectiveness of voluntarily generated expectations for the modulation of pain, providing evidence that deception might not be needed for the emergence of pain altering expectations. For the neural processing of expectations, we demonstrated in Study 1 that a complex network of areas contributes to pain modulation, exhibiting differential activation between positive and negative expectations during pain perception, while also showing more common effects of these expectation valences in the anticipation of pain. Harnessing the good temporal resolution of the EEG and the good spatial resolution of the fMRI, we were further able to show that the processes during the anticipation of pain can be temporally differentiated. In Study 2 we could show that neural activity correlated with the stability of pain modulation in the right

DLPFC, amygdala and thalamus. Furthermore, during the anticipation of pain significant differences in the alpha-to-theta band were found, indicating distinct processes in the preparation of pain perception. In Study 3, we found significant differences in the gamma-band during pain perception between all three conditions, marking differences in pain processing induced by voluntarily generated expectations. Thus, the findings presented here extent the current understanding of the modulation of pain by expectations.

1.4.1 Behavioral effects of positive and negative expectations

To better understand the mechanisms of expectations, we investigated the differences between the valences, their stability, and the sources of their induction. In Study 1 we were able to show that our paradigm based on a sham-BCI was able to induce successful expectation effects in 47 out of 50 subjects. The successful induction of expectation effects was supported by significant differences in the SIIPS score between the placebo and nocebo condition as well as between the nocebo and control condition, and by significant differences in SCR responses in the placebo and nocebo condition. These objective measures support the notion that the paradigm was suitable to induce positive and negative expectations on a trial-by-trial basis outside of possibly biased self-reports in the form of pain and expectation ratings, making it possible to investigate the differences and similarities of positive and negative expectations.

In Study 2 we examined the stability of positive and negative expectations respectively. Participants underwent the paradigm without reestablishing their expectations approximately one week after their participation in Study 1. Despite participants again receiving 90 stimuli of the same intensity and thus providing many learning possibilities, we were able to show that both valences of expectations led to pain modulation in a highly stable manor. These findings support a body of literature describing stable expectation effects (Ashar et al., 2017; Colloca & Benedetti, 2006; Whalley et al., 2008). However, our findings are opposed to studies showing evidence for more robust nocebo effects, that are also more easily induced (Colagiuri & Quinn, 2018; Colloca et al., 2008) as we found no differences between placebo and nocebo effects in their stability. The similarity of positive and negative expectations regarding their stability when induced by the same paradigm suggests similar mechanisms for the modulation of pain by positive and negative expectations. Additionally, aside from

the similarities between positive and negative expectations, our results allow us to make inferences about the underlying mechanisms involved in the learning of these expectations. The stability of expectation modulation despite multiple learning possibilities for participants in the form of 90 trials in each session challenges classical learning models and supports the alternative explanations such as self-reinforcing feedback loops, with participants learning more from experiences that support their expectation, supporting therefore the stabilization of these expectations (Jepma et al., 2018; Schenk et al., 2017). This aspect of expectations might lead to long-term alterations of perceptions. If the stable expectation effects found here are solidified enough to create long lasting beliefs remains unclear though. In future studies it would be interesting to test participants again one year after the induction of expectation effects. This would be further interesting for the research of individual traits contributing to the stabilization of expectation effects.

Another interesting aspect of pain modulating expectations is how they can be induced. In Study 3 we tested voluntarily generated expectations and compared them to expectations induced by verbal suggestion and conditioning in Study 1. Voluntarily generated expectations led to significant positive and negative pain modulation effects, shown by higher ratings in the negative expectation condition and lower ratings in the positive expectation condition. These effects were supported by significant differences in time-frequency data between all three conditions during pain perception, indicating significant differences in neural processing of the painful stimulus. Moreover, the comparison of the expectation effects of Study 3 to the expectation effects of Study 1 revealed even higher positive and negative expectation effects in Study 3, providing evidence for larger effects of voluntarily generated expectations. The paradigm of voluntarily generated expectations parallels that of open-label-placebos (OLPs), a term describing the overt use of placebo effects. Interestingly, in line with our results Locher et al. (2017) found no differences in the effectiveness of placebo effects and OLPs, if OLPs are provided with a profound rationale. This further opens the discussion if deception is a necessity for placebo or nocebo effects. However, the are some differences between our paradigm and OLP. The paradigm of Study 3 is relying more on the concepts of self-agency and control. The sense of self-agency is discussed to impact pain perception (Borhani et al., 2017; Büchel, 2023) and is driven in this study by the participants experiencing the possibility to change their pain perception. The second factor for the pain modulation in Study 3 could be the feeling of control, which

has been discussed to be an important factor in pain perception (Habermann et al., 2025). The additional harnessing of this effects might be an explanation why the effects found in Study 3 are even stronger than in the placebo and nocebo conditions of Study 1, in which participants never gain the feeling of self-agency or control. These results underline that we do not have fully understood how expectations that influence pain perception are formed. We may have a too simplistic view on these expectations as something that has to be derived from deception. Interestingly, the effects were again found for both valences, marking no difference of the direction of pain perception. This supports that when induced by the same paradigm the effects are very comparable.

1.4.2 Neural mechanisms of positive and negative expectations

The analysis of neural data again strongly supports the role of the DPMS for pain modulation by expectations. In key areas of the DPMS such as the DLPFC, rACC, anterior insula and vmPFC neural representations of expectation modulation were found in Study 1. The importance of many of these areas was discussed in the literature before (Wager & Atlas, 2015; Zunhammer et al., 2021). Our results now strongly support, that the anticipation of pain and pain perception are two distinct phases, suggesting that preparatory processes take place in the anticipation of pain and not just mere pre-activation. This can be concluded from the differentiation of activity of positive and negative valences. While we saw mainly common effects in areas strongly associated with the DPMS such as the rACC, the DLPFC, and the vmPFC, for placebo and nocebo during pain anticipation in Study 1, we saw mainly differential effects during pain processing in partially the same areas as the DLPFC and vmPFC, indicating distinct mechanisms in the two phases. This temporal differentiation of effects is further supported by the results of Study 2 that show different areas correlating with the stability of effects during anticipation of pain and pain perception, again providing evidence for distinct underlying mechanisms. Lastly, this is supported by the analysis of the SIIPS. While there are significant differences between positive and negative expectations during pain perception, no such significant differences were found during pain anticipation.

These results further allow to investigate the similarity of positive and negative expectations. The differential effects found during pain perception in Study 1 might

reflect the differences in pain modulation, which are themselves reflected in different pain intensity perception marked by significant pain ratings in the positive and negative expectation conditions. On the other hand, the common effects seem to underline similar processes in the anticipation of pain of both valences. However, it is still possible that even though the activation was found in the same areas, different subcircuits in these areas might be the source for the response. Importantly, in Study 2 the differences in the mechanism of positive and negative expectations were supported by significant differences of the placebo and nocebo conditions reflected in a significant time-frequency cluster in the anticipation of pain spanning 4-9.5 Hz and spanning the time of -2.1 seconds until -0.1 seconds. Similar to the study of Taesler and Rose (2016), in which painful and not-painful stimuli at the pain-threshold could be differentiated by activation in the theta-band in the anticipation of pain, this differentiation of positive and negative expectations could be seen in alpha-to-theta band activation. It must be noted that this is a timeframe immediately before the painful stimulus that might not be well depicted in fMRI data, as this is a timeframe that falls into a bin in the FIR-analysis of Study 1 that overlaps the anticipation phase and pain phase (-0.925 s before pain onset to 0.75 s after pain onset). Therefore, it is possible that these differences are a direct preparation for the pain stimulus reflecting the altered pain perception about to happen. In Study 2 the differences in neural processing of positive and negative expectations were further supported by differences in the correlates of stability in fMRI activity in the positive and negative expectation condition. While during pain perception activity in the right DLPFC and bilateral anterior insula correlated with the stability of positive expectation effects, in the negative expectation effect it was activity in the left thalamus. The DLPFC has been connected to placebo effects in many studies (Atlas & Wager, 2014), both during pain anticipation (Watson et al., 2009) and pain perception (Zunhammer et al., 2021). Its importance for placebo effects further has been supported by studies showing that when its neuronal processing is altered, the placebo effect can be either enhanced or diminished (Egorova et al., 2015; Krummenacher et al., 2010). With our data we were now able to show that it is connected to the stability of the placebo effect also. Like the DLPFC, the anterior insula was shown to play a role both during anticipation of pain and pain perception in Study 1, and for the stability of placebo effects in Study 2. The anterior insula is an area connected to placebo effects in many studies (Atlas & Wager, 2014; Kober et al., 2008; Wager et al., 2004) and further has been discussed to be an

important node in the network in the assessment of threat (Taesler & Rose, 2016; Wiech et al., 2010), marking a possible multimodal role of the insula in placebo effects (Horing & Büchel, 2022), with the anterior insula being a hub that possibly interconnects different networks. Importantly, noxious stimuli are inherently aversive, threat inducing and associated with fear conditioning (Hayes & Northoff, 2012; Meulders, 2020). These results and the multimodal role of the anterior insula support the role of affective processes in expectation effects.

Using neural results, we can also make assumptions about the question how positive and negative expectations can be induced. Participants showing less amygdala activation were showing more stable placebo effects on day 8 with the way we set up our predictors controlling for the mere strength of placebo effects. Therefore, higher activation in the amygdala might have led to less stable placebo effects. Similar to other brain areas, opioid activity in the amygdala correlates with placebo effect strength (Helmstetter et al., 1998). Like the insula the amygdala is associated with threat assessment (Johansen et al., 2010) and additionally associated with cued fear (Lonsdorf, Haaker, Fadai, et al., 2014) or contextual fear (Lonsdorf, Haaker, & Kalisch, 2014). Less activation therefore might reflect a reduction in these emotional mechanisms, which in turn might benefit the generation of stable positive expectations towards the unpleasant pain stimuli. Additionally, the amygdala is described as playing a key role in fear conditioned analgesia (Schafer et al., 2018), suggesting a possible role of conditioning in the genesis of stable positive expectations. Interestingly, also the thalamus, the area correlating with negative pain expectation stability, has been discussed to be linked to conditioning processes (Jensen et al., 2015). The results found here might indicate the role of a successful conditioning in the stability of effects of negative expectations. Moreover, this might be a hint towards differential mechanisms underlying the conditioning of positive and negative expectations that were described in the literature before (Colloca et al., 2010), that are possibly based on the differences in associated emotions, e.g. in the amount of threat or fear associated with the painful stimulus. In conclusion, while the behavioral data indicates no real differences between the pain modulation effects of positive and negative expectations regarding their stability and sources of induction, the neural data presented here does indicate differences in the underlying mechanisms.

We identified a temporal organization of the processes in the anticipation of pain and during pain perception in Study 1 derived from the correlation of time-

frequency data and fMRI results as well as from the differentiation of effects in the fMRI from the anticipation of pain towards pain perception. If these neural representations reflect preparatory processes for pain perception, the representations should reflect steps of the Bayesian predictive coding model. However, it remains unclear how this temporal organization is connected to steps of these processes. It can only be assumed that the areas associated with the anticipation of pain are connected to different steps of the formation of the prior that consequently shifts perception during the painful stimulus. Moreover, it remains unclear how and where in the brain the prediction error is calculated and how it influences the next formation of a prior. Interestingly, the prefrontal cortex has been connected to the suppression of learning from prediction errors in the ventral striatum (Schenk et al., 2017). As we found the DLPFC correlating with the stability of the placebo effect, the role of the DLPFC in the suppression of the learning of prediction errors might move into focus in future studies. This is further the case as the DLPFC also seems to play a pivotal role at a very early stage of the anticipation phase, as seen in our combined analysis in Study 1. However, the process associated with this early stage remains speculative.

While we observed results in the fMRI for more common effects in the anticipation of pain and more distinct effects during pain perception in Study 1, the EEG revealed a differential effect of positive and negative expectations in Study 2 during pain anticipation and no significant effect during pain perception. In Study 3 however, we found no significant effect in the EEG data during the anticipation of pain, but significantly different activation during pain perception for all conditions. The inconsistency of effects in the comparison of EEG and fMRI raises the question of whether the two methods measure the same. To understand this inhomogeneity, the distinct temporal profiles of EEG and fMRI must be considered when analyzing the data. While the recording rate of EEG is very fast and only limited by processing power and storage room for the recorded data, fMRI recordings depend on the slow BOLD signal. While EEG can therefore record processes in the brain in nearly real-time, the fMRI data is dependent on models that estimate a possible BOLD response such as for example the HRF or on an estimation by the researcher when a possible response would occur. Additionally, it must be noted that EEG activity does not correlate directly with heightened hemodynamic activity measured by fMRI as it has to be differentiated regarding the frequency band (Kilner et al., 2005), with high frequency correlating positively with BOLD activity measured by the fMRI and low frequencies correlating

negatively with BOLD activity. Therefore, high BOLD responses do not necessarily transfer to large time-frequency effects, especially in the environment of the fMRI scanner, as the data quality of EEG measured inside the fMRI environment is not as good as it would be when measured in an EEG laboratory. This is even true after correcting for the most common artifacts that are found in combined measurements (Scrivener, 2021; Warbrick, 2022). The lack of EEG results during pain perception or pain anticipation outside of the combined analysis in Study 1 could therefore partially be due to the bad data quality. However, no significant results were found during pain perception in Study 2 as well, which raises the question of the suitability of EEG measurements for thermode heat pain. Many areas participating in pain modulation are not on the cortical surface and therefore difficult to measure with EEG (Jackson & Bolger, 2014; Whittingstall et al., 2003), as they are more prone to artifacts and have a weaker signal in general.

Summed up, our data suggests both common and differential mechanisms for positive and negative expectations in their neural processing. The differential effects may be based on the differential affective aspects of positive and negative expectations, especially in the assessment of threat and salience.

1.4.3 Individual differences in responses to expectations

In Study 1 and Study 2 correlations of placebo and nocebo effects in Study 1 and between the placebo effects on day 1 and day 8 and nocebo effects on day 1 and day 8 were found. These correlations indicate that subjects are not prone to one valence of the expectation spectrum but that subjects are either more driven by expectations or less. This might be a hint towards latent traits that mark a responsiveness to placebo and nocebo effects which was suggested by some researchers in earlier studies (e.g. Kuperman et al., 2020) and could be a gateway to understand the emergence of chronic pain. In a unifying model, Büchel (2023) described a possible mechanism for expectations to influence the emergence of chronic pain, especially in the form of a viscous cycle (Edwards et al., 2012). Given the results presented here for highly stable pain modulation effects the connection between expectations and the emergence of chronic pain appears to be probable. Therefore, the behavioral results found in the three studies might have interesting implications for the treatment chronic pain. Importantly, our data in Study 2 suggests,

that if positive expectations are stable, the opposite of viscous cycles, virtuous cycles are also possible (Clark, 2024; Jepma et al., 2018; Wolf et al., 2024). Therefore, harnessing positive expectations could be a good way to prevent the emergence of chronic pain. However, in a clinical environment, individuals often have contact to situations (e.g., dentist, emergency care) that are deeply connected to painful experiences, inducing structural expectations towards the pain experiences, that are difficult to change (Seriès & Seitz, 2013). When considering the importance of areas such as the anterior insula and amygdala for stable expectation effects, the role of emotional processes in the generation of stable expectations is suggested. Emotions promoted by these structural expectations could therefore lead to even more stable nocebo effects than the manipulation in our second study achieved and must be considered when planning interventions.

The importance of expectations of course raises the question of possible markers for the proneness of expectation effects. Indeed there have been many attempts to find possible traits for the responsiveness to placebo effects (Kang et al., 2023; Kern et al., 2020; Peciña et al., 2013), with the best prediction being an aggregate of factors from Ego-Resiliency, Altruism, Straightforwardness, and Angry Hostility, explaining up to 25% of variance of the placebo response (Peciña et al., 2013). However, this result has still to be supported by a study of a larger cohort and over multiple placebo paradigms. Another new approach to predict placebo responsiveness is measuring the certainty in ascending sensory signals, a construct measured by the newly developed Focused Analgesia Selection Test (Kuperman et al., 2020) that is supposed to capture the individual differences in the precision of the perception of sensory signals. This paradigm is supported by the finding that participants showing high scores in the Focused Analgesia Selection Test also show high variability in the rating of clinical pain and higher placebo responses. However, it still must be shown how stable these relationships are in larger cohorts, as well. Nonetheless, predicting how responsive patients or participants are to placebo or nocebo effects could be very useful in the future treatment of multiple diseases. Considering that voluntarily generated expectations seem to be even more impactful on perception, harnessing this approach could also prove useful. Remarkably, even less is known about the relationship between voluntarily generated pain modulation and the placebo and nocebo effects. Especially about the traits that might predict the proneness to these effects. A reasonable question is if placebo and nocebo are

interconnected in their predictive traits in that sense, that participants showing high placebo and nocebo effects do show good capabilities in manipulating their pain experience. More research in this field is needed.

1.4.4 Limitations and future directions

For the clinical practice the question of the strength of placebo and nocebo effects or positive and negative expectation effects is of utmost importance and therefore this question is discussed broadly in the literature (Colloca et al., 2008; Freeman et al., 2015; Fu et al., 2021). Importantly, the strength of placebo and nocebo effects highly depends on the paradigm, especially the control condition. Inducing no expectation towards a stimulus is an unfeasible task, as participants themselves will always form an expectation. However, to compare the strength of the placebo and nocebo effect respectively, the difference to the control condition has to be computed. If the control condition is not free of expectations towards a direction of stimulus intensity, results will be biased. Therefore, the control condition must be formed with utmost carefulness. Though, even then the medium to induce placebo or nocebo effects is of relevance. If placebo or nocebo effects are induced by a medium that is inherently threatening, such as for example a TENS device that uses electric currents (e.g., Colagiuri & Quinn, 2018), it is reasonable to assume that nocebo effects might be boosted and placebo effects might be blunted by the induction method itself. Inversely, the approach to induce expectation effects using a cream (e.g., Schenk et al., 2014) might trigger experiences of healing, thus boosting the placebo effect in comparison to the nocebo effect. In the optimal case, a relatively neutral device to induce expectations would be used. In conclusion, the comparison of the strength of placebo and nocebo effects is not a simple endeavor. Moreover, this raises the question of the comparability of paradigms in the expectation modulation literature. In clinical trials, the question of the correct comparison for a treatment is of utmost importance (Turner, 2012). Derived from the possible differences in strength of positive or negative expectations, the comparability of positive and negative expectation paradigms is not given per se. In the paradigm developed for the presented studies we tried to induce no directed expectation for the control condition and used a BCI as a relatively neutral way to not induce positive or negative affect. Nonetheless, not inducing expectations at all is merely impossible and the insight into the strength of

positive and negative expectation effects might therefore be limited. A standardized paradigm that tackles the problems described here and that would be used over several studies and laboratories might help in the investigation of positive and negative expectation effects and their neural correlates, however this would come with a toll on generalizability (Zunhammer et al., 2021). Another important aspect in the research of pain modulation is, that pain perception is often treated as a one-dimensional process with pain either being high or low, which has to be seen critically, as there was evidence provided against this dichotomous classification (Crawford Clark et al., 2002; Crisman et al., 2024). However, in the expectation literature and often beyond basic research pain intensity measures remain dominant (Jaaniste et al., 2019) and in the studies presented here the multidimensional aspects of pain perception were not taken into account, limiting possible insights. In future research considering the multidimensionality of pain perception might reveal hidden aspects of expectation effects, especially on emotional aspects of these processes. This can improve the understanding of the contributors to positive and negative expectation effects.

The data presented here regarding the induction of expectation effects and the stability of these expectation effects raise the important question of how these findings can be salvaged for the treatment of, for example, chronic pain. One possible mechanism that might be of interest in future studies that investigate the treatment of chronic pain is the feeling of self-agency and control (Habermann et al., 2025), which in our data apparently boosted expectation effects in Study 3. Another aspect of interest might be induction of expectation without deception. Nonetheless, while our data suggests these paths for research, more studies are needed to investigate these mechanisms.

1.4.5 Conclusion

The present dissertation identified common mechanisms of the modulation of pain by positive and negative expectations on the behavioral level. The strength of effects of positive and negative expectations were comparable and the effects remained comparably stable over the time-course of one week. Further, both positive and negative expectations could be induced voluntarily by participants of Study 3 and led to comparable effects on pain perception.

However, on the neural level similarities as well as differences between positive and negative expectation effects were found. Both expectation-effects were closely connected to areas of the DPMS for both valences, and positive and negative expectation effects shared preparatory effects in the anticipation of pain found in the fMRI analysis of Study 1. The effects differentiated during pain processing, marking distinct processes in the anticipation of pain and pain processing, and depicting differences of the neural correlates of positive and negative expectations. Additionally, a significant difference between positive and negative expectations during the anticipation of pain was found in the time-frequency analysis of Study 2, suggesting also distinct processes during pain anticipation. Further, the stability of expectation effects appears to be promoted by activation or deactivation of distinct areas for positive and negative expectations, with the thalamus being an important driver for stable negative expectations during pain processing and the anterior insula and the DLPFC being important drivers for the stability of positive expectations during pain processing, while less amygdala activation appeared to drive stable positive expectation effects during pain anticipation. These differences between the valences in their neural correlates give reason to assume different mechanisms driving these effects. More research is needed to better understand the common and distinct mechanisms of positive and negative expectations.

2. Abbreviations

ACC	Anterior cingulate cortex
Ant. insula	Anterior insula
BOLD-response	Blood oxygen level dependent response
DLPFC	Dorsolateral prefrontal cortex
DPMS	Descending pain modulatory system
ECG	Electrocardiogram
EEG	Electroencephalography

FCz – electrode	Frontal central zero electrode
FIR	Finite impulse response
Fz - electrode	Frontal zero electrode
fMRI	Functional magnet resonance imaging
GABA	Gamma-aminobutyric acid
HRF	Hemoglobin response function
ICA	Independent component analysis
MRI	Magnet resonance imaging
NPS	Neurologic pain signature
OLP	Open label placebo
PAG	Periaqueductal grey
Pz - electrode	Parietal zero electrode
RVM	Rostral ventral medulla
ROI	Region of interest
rACC	Rostral anterior cingulate cortex
SCR	Skin conductance response
SD	Standard deviation
SEM	Standard error of the mean
SIIPS	Stimulus intensity independent pain signature
SPM	Statistical parametric mapping
TENS	Transcutaneous electrical nerve stimulation
vmPFC	Ventromedial prefrontal cortex

3. Tools and Bibliography

Tools

ChatGPT version 4, OpenAi: chatgpt.com

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- Sentences and grammar for the readability
- Sorting the abbreviations chapter alphabetically
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4. Publications

4.1 The neural dynamics of positive and negative expectations of pain

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The neural dynamics of positive and negative expectations of pain.

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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 para- digm 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 nega- tive expectations, such as their varying correlations with the amount of prior experience and differ- ences 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 simulta- neous 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 (posi- tive, negative, or neutral expectations) followed by an anticipatory period in which different expecta- tions 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 expec- tations (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 expec- tations 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 nega- tive 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 nega- tive 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, partic- ipants 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 expecta- tion), 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

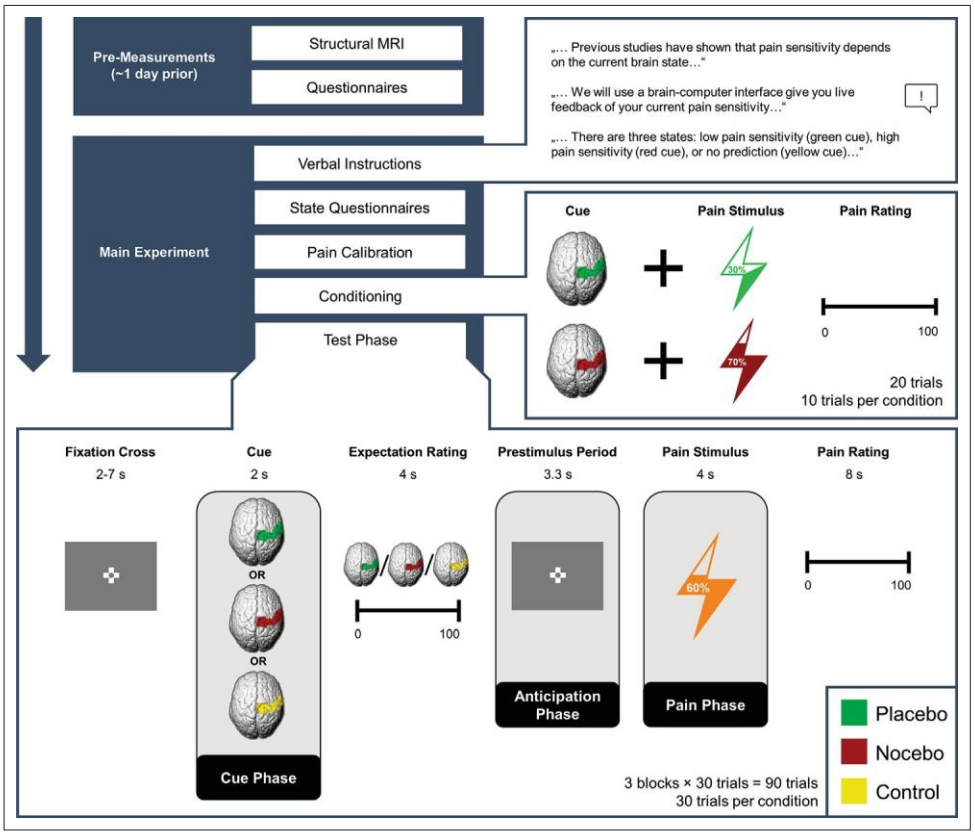

Figure 1. Experimental Procedure. Structure of the experiment including pre-measurements and the main experiment. Expectations were generated using a sham brain-computer interface (BCI), i.e., participants were told that they would receive real-time feedback regarding their pain sensitivity (verbal instructions) 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 expecta- tions. 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, partici- pants 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 antic- ipation 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

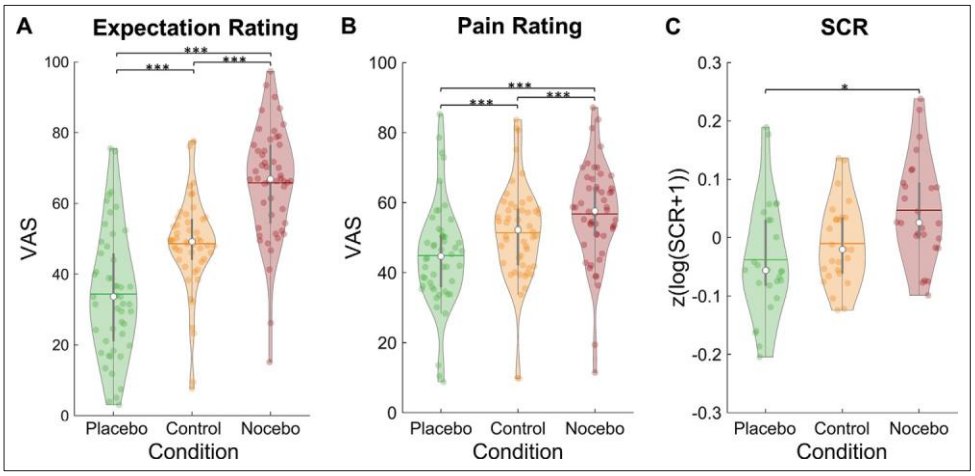

Figure 2. Expectation ratings, pain ratings, and skin conductance response by condition. Mean expectation (**A**) and pain ratings (**B**) on a visual analogue scale separately for each condition (n = 50). (**C**) Mean skin conductance responses in the three conditions (n = 26). White dots = mean, horizontal 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, $\hat{\eta}_p^2$=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, $\hat{\eta}_p^2$=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, $\hat{\eta}_p^2$=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

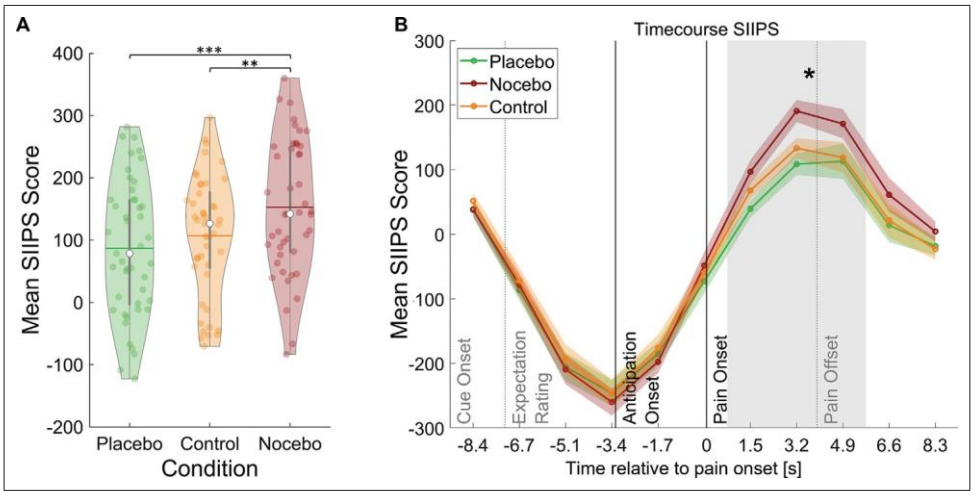

Figure 3. Stimulus intensity independent pain signature (SIIPS) scores by condition. (**A**) Mean SIIPS score per condition for all time-points during pain perception. White dots = mean, horizontal lines = median, thick gray vertical lines = upper and lower quartile, coloured dots = pain ratings of individual 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 differ- ences 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 condi- tion ($t_{(44)} = 4.79$, $p<0.001$) 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

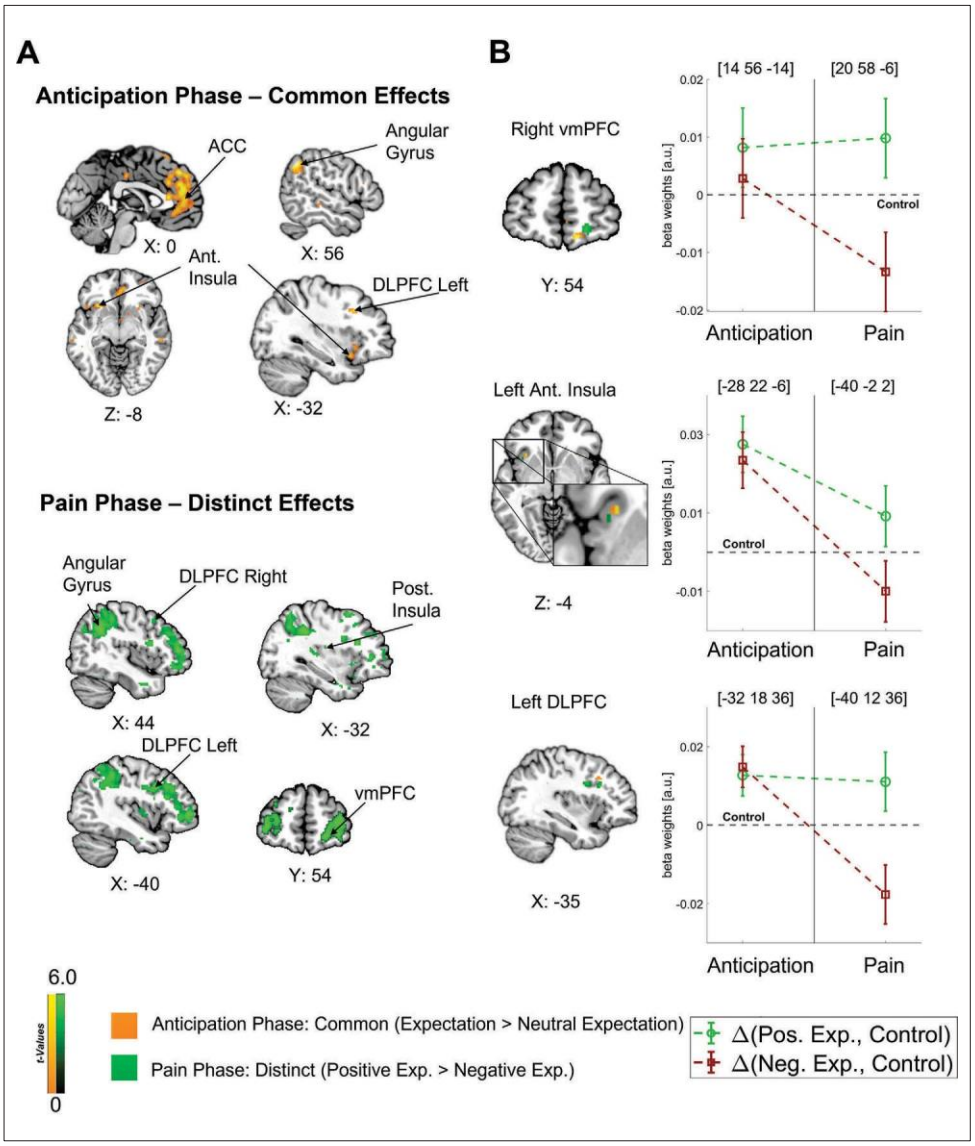

Figure 4. Differentiation of effects during the anticipation and pain phase. (**A**) Top: Common effects during pain anticipation (expectation > neutral expectation) at p<0.001 (uncorrected for display purposes) show widespread higher activity for both positive and negative expectations compared to the 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.

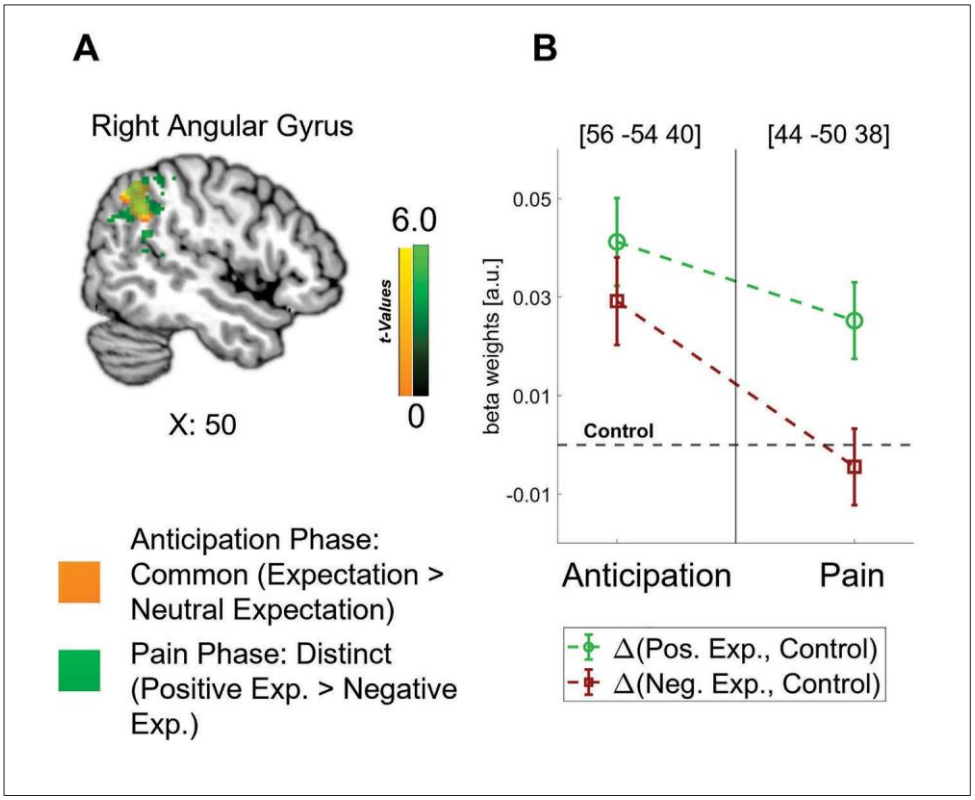

Figure 5. Representation of expectations in the angular gyrus. (**A**) Overlap between common effects of expectations during the anticipation phase (expectation > neutral expectation; yellow) and distinct effects of positive and negative expectations during the pain phase (positive > negative; green) 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 > nega- tive: 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 expecta-
tions 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

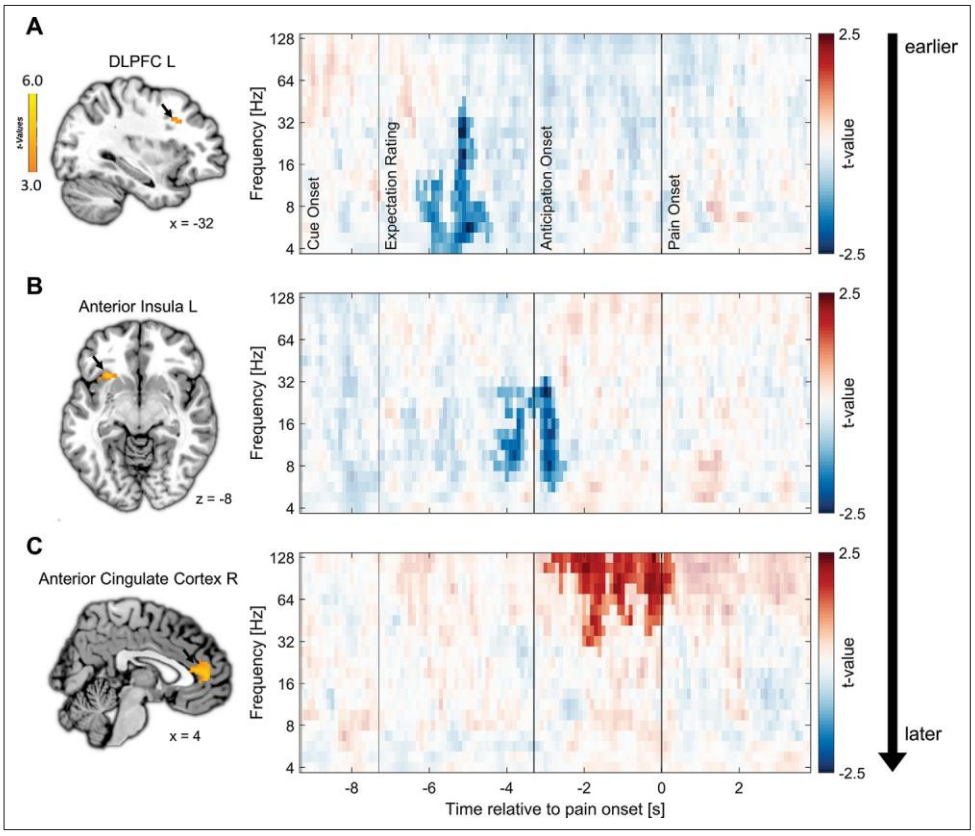

Figure 6. Relation of fMRI activity with EEG oscillatory power. Correlation of single-trial hemodynamic responses with time-frequency resolved EEG activity in the left dorsolateral prefrontal cortex (DLPFC) (**A**), left anterior insula (**B**), and right anterior cingulate cortex (ACC) (**C**) during 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 nega- tive 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 subse- quent 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 interven- tions (***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 demon- strated 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 inte- gration 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 expec- tations 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***). Accord- ingly, 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, compa- rable 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 condi- tions 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 condi- tions 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 vari- ability 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 exam- ination 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 gener- ation 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 mainte- nance 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***). Expec- tation 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 condi- tions, 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 partic- ipants, 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 restric- tions 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 expec- tation 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 imped- ance 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 synchro- nized 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 compo- nents. 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-trans- formed 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, Well- come 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. Further- more, 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 correc- tion 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 (***Oosten- veld 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 compar- ison 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 identi- fied 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 GLMs- ingle 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 admin- istration, Writing – review and editing; Michael Rose, Conceptualization, Resources, Software, Super- vision, 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 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* = 4I). 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

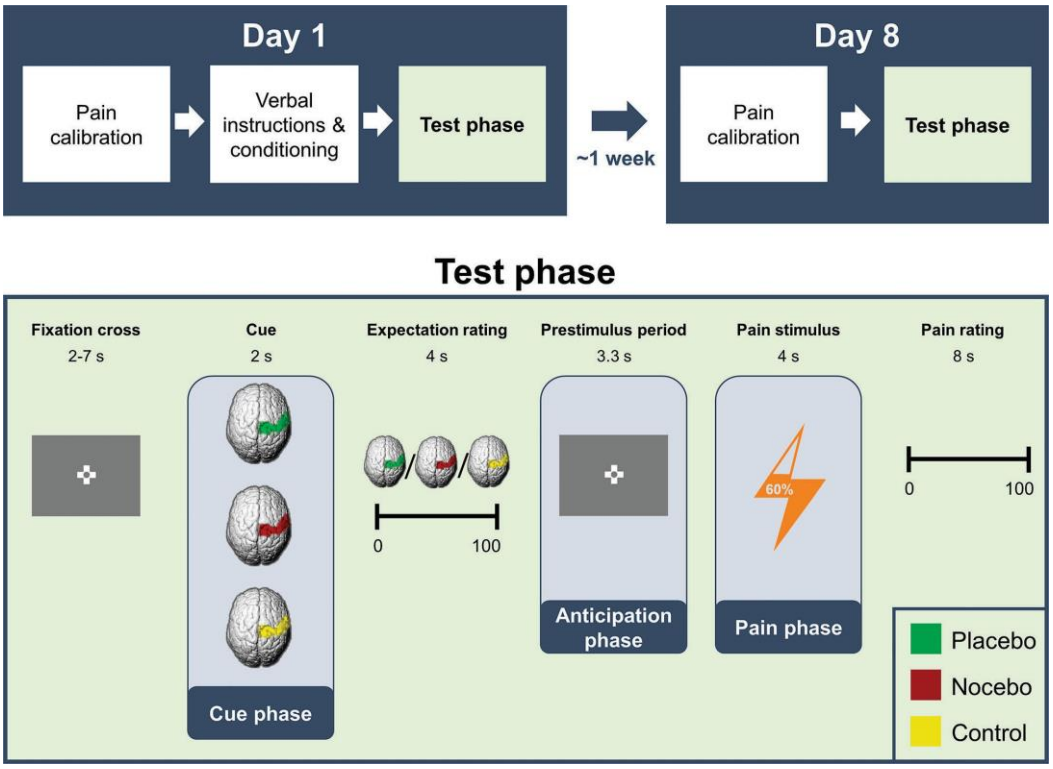

Fig. 1. Experimental structure. The experiment consisted of two sessions approximately one week apart (day 1 and day 8). On day 1 only, positive and negative pain-related expectations regarding visual cues were induced using verbal instructions and a conditioning procedure. On both day 1 and day 8, a 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

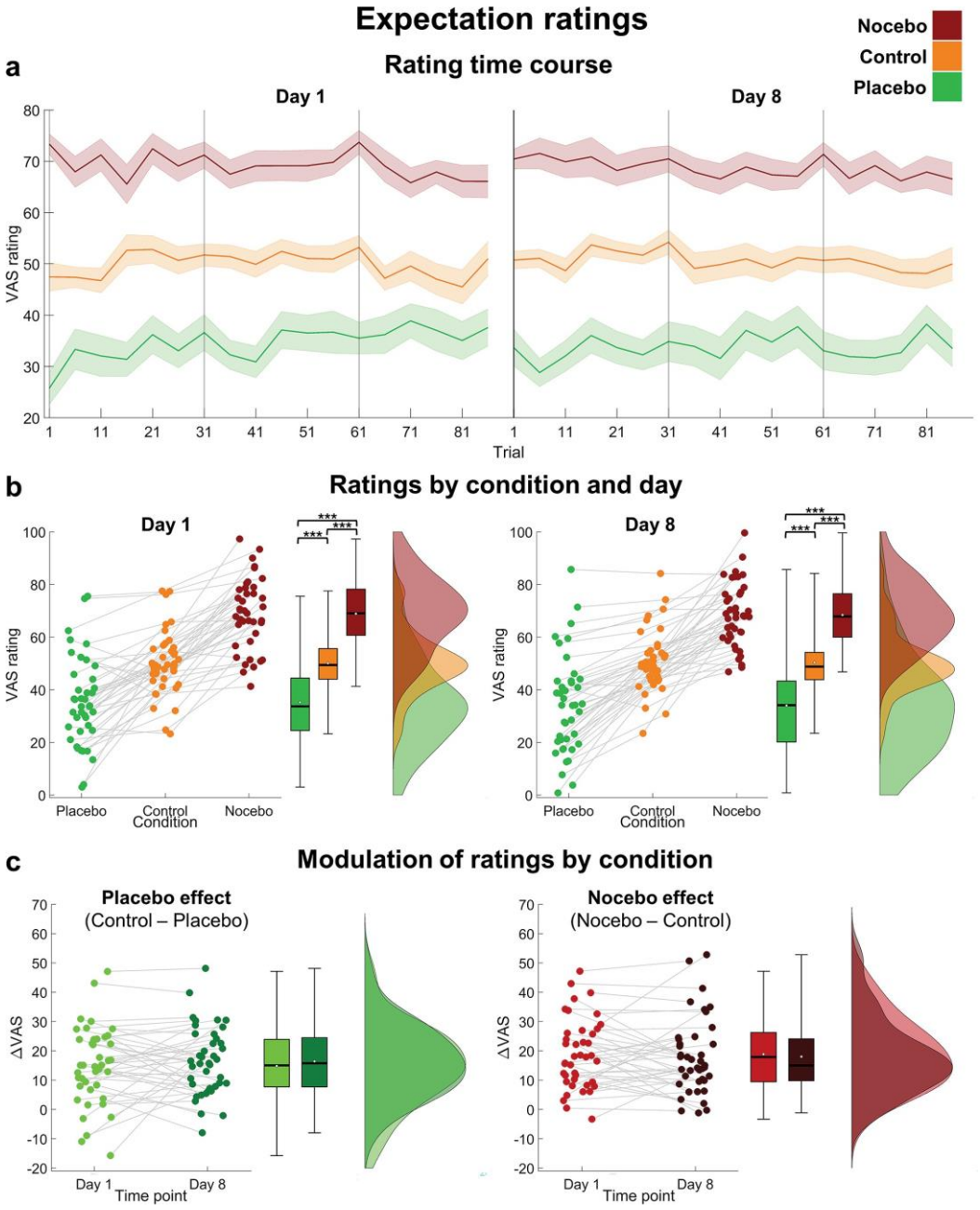

Fig. 2. Expectation ratings. (**a**) Time course of expectation ratings per condition, averaged over five trials. Error bars denote the corrected standard error of the mean (SEM) using the Cousineau-Morey method[32,33]. (**b**) Raincloud plots[34] of expectation ratings per condition on day 1 (left) 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. ***$p < .001$.

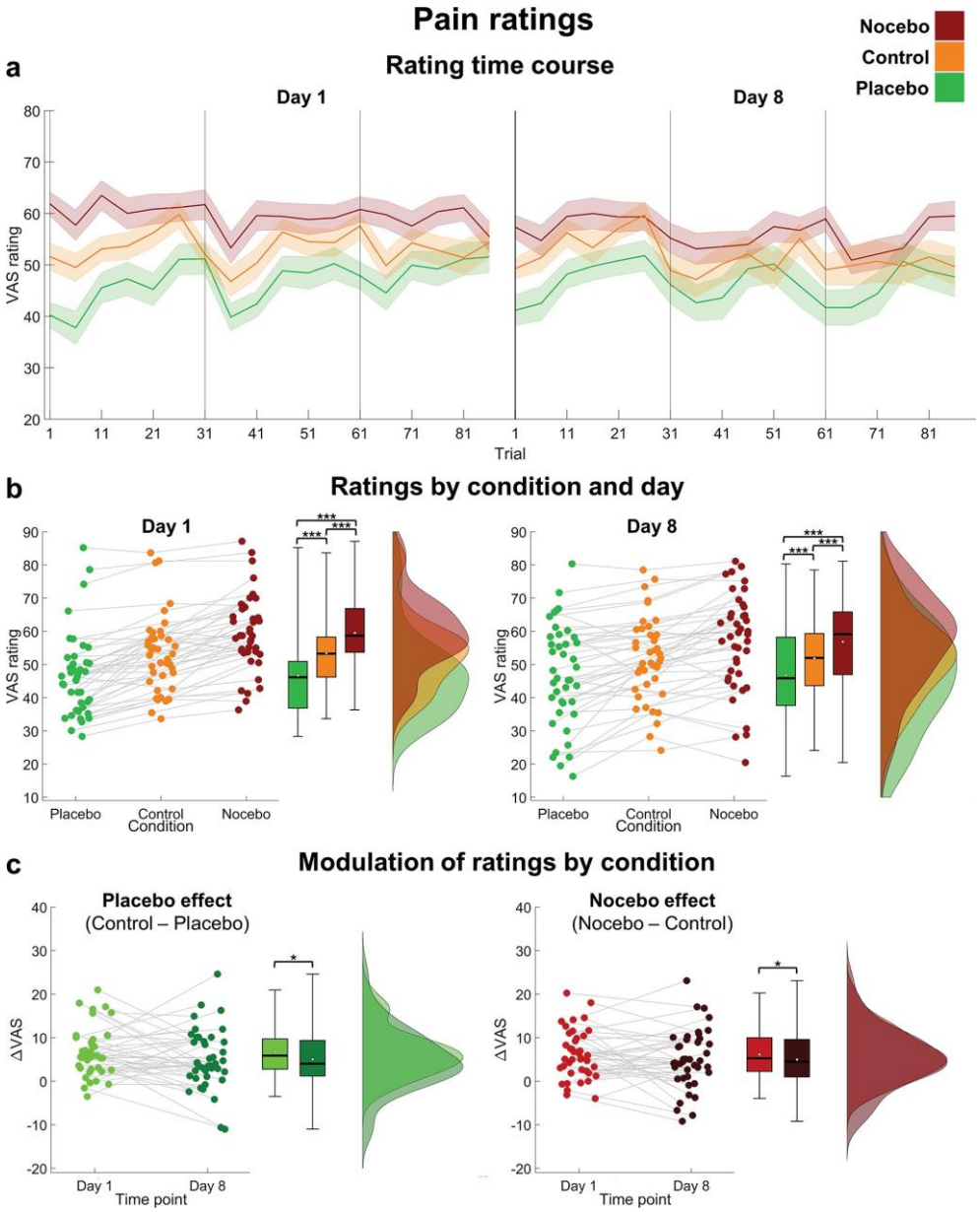

Fig. 3. Pain ratings. (**a**) Time course of pain ratings per condition, averaged over five trials. Error bars denote the corrected standard error of the mean (SEM) using the Cousineau-Morey method[32,33]. (**b**) Raincloud plots[34] of pain ratings per condition on day 1 (left) and day 8 (right). Each 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	$\eta 2_p$	df	F	p	$\eta 2_p$
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	$\eta 2_p$	df	F	p	$\eta 2_p$
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	$\eta 2_p$	df	F	p	$\eta 2_p$
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).

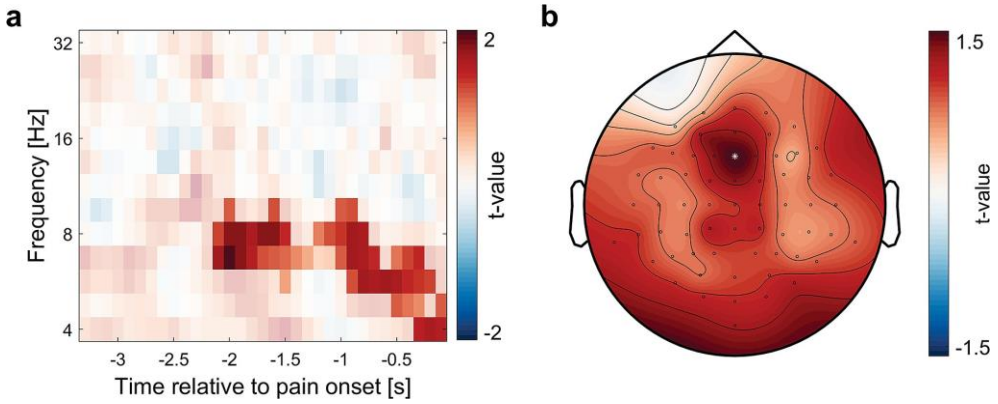

Fig. 4. Differences in EEG power between placebo and nocebo on day 8. (**a**) Time-frequency plot of *t*- values for placebo vs. nocebo in the anticipation phase on day 8 averaged over all cluster electrodes. (**b**) The corresponding topography (peak electrode Fz highlighted with a white star).

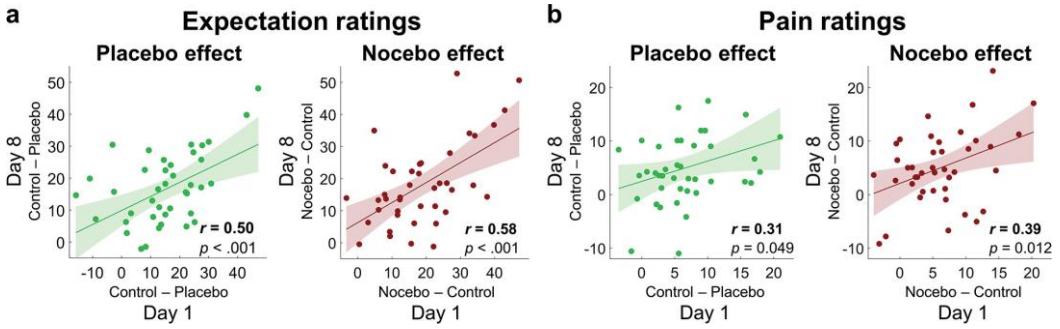

Fig. 5. Prediction of effects on day 8 based on the effects observed on day 1. Correlation plots for expectation (**a**) and pain ratings (**b**), showing the relationship between the individual placebo and nocebo effects on day 1 with the corresponding effects on day 8. Each dot represents one subject.

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 ehavioral

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			t	p_{FWE}
		X	Y	Z		
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						
Thalamus	R	-6	-18	18	4.80	0.016†

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.

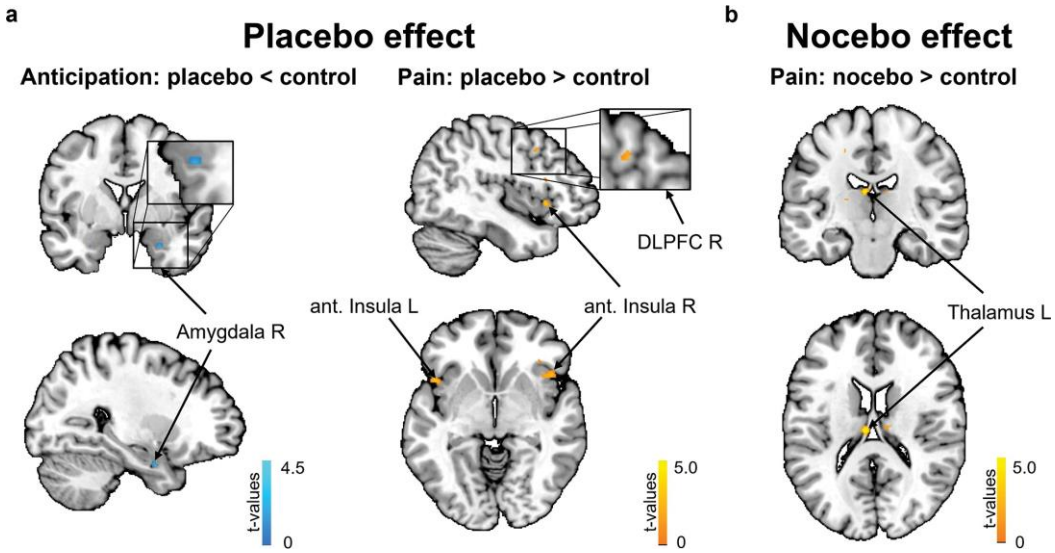

Fig. 6. Neural predictors of placebo and nocebo effect persistence. (**a**) Analyses of the persistence of expectation effects using individual placebo effects of day 8; Blue: lower activation for the placebo condition compared to the control condition in the amygdala during the anticipation phase; Yellow: 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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Author contributions

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.

Additional information

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4.3 Increased pain modulation by voluntarily generated expectations

- *In publication process -*

7. Zusammenfassung/ Summary

Die Wahrnehmung der Umwelt ist stark von Erwartungen geprägt. Dies gilt auch für die Wahrnehmung von Schmerzreizen. Die positive Erwartung, dass ein Schmerzreiz weniger schmerzhaft werden wird, vermindert den empfundenen Schmerz. Sie führt also zu Hypoalgesie. Im Gegensatz dazu erhöht die negative Erwartung, dass ein Schmerzreiz sehr schmerzhaft sein wird zu Hyperalgesie, also einer verstärkten Schmerzwahrnehmung. Diese Effekte sind auch als Placebo-Effekt im Falle der positiven Erwartung und als Nocebo-Effekt im Falle der negativen Erwartung bekannt. Während diese Effekte gut untersucht sind, ist weniger darüber bekannt, welche Mechanismen diesen Effekten zu Grunde liegen. Insbesondere bleibt unklar, ob positive Erwartungen und negative Erwartungen den gleichen Mechanismen folgen. Das gilt z.B. dafür, ob sie die gleichen Effektstärke zeigen, für die Stabilität der Erwartungen und für die Möglichkeiten, diese Erwartungen zu induzieren. Unklar ist zudem, ob Täuschung für das erfolgreiche Induzieren von Erwartungen notwendig ist und ob sich die Erwartungseffekte in ihren neuronalen Korrelaten unterscheiden.

In drei Studien wurden deshalb die Mechanismen der Schmerzmodulation durch positive und negative Erwartungen beleuchtet. In Studie 1 wurde mit einer Kombination aus EEG und fMRT die neuronalen Korrelate der Schmerzmodulation durch positive und negative Erwartungen untersucht, die durch ein vorgetäuschtes Brain-Computer-Interface induziert wurden. Dabei zeigte sich, dass die Erwartungen die Schmerzwahrnehmung der Versuchspersonen beeinflussten und dass positive und negative Erwartungen in der Phase der Vorbereitung auf den Schmerz vor allem gemeinsame Effekte im Gehirn aufweisen. Jedoch differenzierten sich diese Effekte während der Schmerzwahrnehmung in den meisten Arealen. Mit Hilfe des EEGs konnte zudem eine zeitliche Organisation der Effekte im Gehirn ausgearbeitet werden. In Studie 2 wurde die Stabilität der Erwartungseffekte sowie die neuronale Grundlage dieser Stabilität untersucht. Dabei ergab sich, dass die Induktion von sowohl positiven und negativen Erwartungen zu stabilen Veränderungen der Wahrnehmung ca. eine Woche nach der Induktion geführt haben. Es zeigte sich auch, dass die Areale im Gehirn, die diese Stabilität unterstützen, sich zwischen positiven und negativen Erwartungen unterscheiden. Stabile positive Erwartungen wurden durch mehr Aktivität in der anterioren Insula, mehr Aktivität im DLPFC und durch verringerte Aktivität in der Amygdala unterstützt. Stabile negative Erwartungen gingen ausschließlich mit mehr Aktivität im Thalamus einher. In Studie 3 aufgezeigt werden, dass Täuschung für eine erfolgreiche Induktion von positiven oder negativen Erwartungen nicht notwendig ist. Zusammenfassend wurden in den Verhaltensdaten der drei Studien keine bedeutsamen Unterschiede zwischen positiven und negativen Erwartungseffekten auf die Schmerzwahrnehmung gefunden. In den neuronalen Daten lassen sich jedoch neben Gemeinsamkeiten in der Verarbeitung auch Unterschiede erkennen, die möglicherweise Unterschiede in den zugrunde liegenden Mechanismen andeuten.

The perception of the environment is heavily influenced by expectations. This is also the case for the perception of pain. The positive expectation that an upcoming stimulus will hurt less leads to hypoalgesia. In the opposite however, the negative expectation that a stimulus will hurt more leads to hyperalgesia. This phenomena are also known as the placebo effect in the case of positive expectations and the nocebo effect in the case of negative expectations. While these effects are well known and researched, less is known about the underlying mechanisms of the positive and negative expectation effects and if they underlie the same set of rules. This pertains, for example to whether the effect strength of positive and negative expectations is comparable. Further, the stability of this expectation effects still needs to be investigated as well as the means to induce these expectation effects, as it remains unclear if deception is necessary for the induction of expectations.

In three studies, the mechanisms of pain modulation through positive and negative expectations were investigated. Study 1 employed a combination of EEG and fMRI to examine the neural correlates of pain modulation by positive and negative expectations that were induced using a sham brain computer interface. It was found that during the anticipation of a painful stimulus, positive and negative expectations primarily led to shared effects in the brain. However, during pain perception, in multiple brain areas a differentiation between the effects emerged with higher activation for the positive expectation condition compared to the negative expectation condition. Furthermore, through EEG, it was possible to reveal a temporal organization of the effects in the brain during pain anticipation. Study 2 explored the stability of expectancy effects and the neural basis for this stability. It was observed that the induction of both positive and negative expectations resulted in stable changes in perception approximately one week after the expectation induction. Moreover, the brain regions supporting this stability were found to differ between positive and negative expectations. Stable positive expectations were associated with increased activity in the anterior insula, increased activity in the DLPFC, and reduced activity in the amygdala. In contrast, stable negative expectations were associated with increased activity in the thalamus. Study 3 demonstrated that deception is not necessary for the successful induction of positive or negative expectations. In summary, the behavioral data of the three studies revealed no significant differences between the effects of positive and negative expectations on pain perception. However, among the neural data, both commonalities and differences in processing were observed, which may indicate variations in the underlying mechanisms of positive and negative expectations.

8. Declaration of contribution on publications

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

Christoph Arne Wittkamp: Conceptualization, Data curation, Software, Formal analysis, Validation, Investigation, Visualization, Methodology, Writing – original draft, Project administration, Writing – review and editing

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Christoph Arne Wittkamp: Software, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing — Review and Editing, Visualization

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10. Curriculum Vitae

Entfallen aus datenschutzrechtlichen Gründen

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: ……