# Cognitive and Emotional Influences on Placebo Analgesia and Nocebo Hyperalgesia

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**Table of Content**

**Abstract**

The perception of pain can be modulated by a variety of factors such as biological/pharmacological treatments as well as potent cognitive and emotional manipulations. Placebo and nocebo effects are among the most prominent examples for such manipulations. Placebo and nocebo manipulations cause reliable psychological and physiological changes, although the administered agent or treatment is inert. The present dissertation aimed at investigating the role of cognitive and emotional influences in the generation of placebo and nocebo effects on pain perception. In addition, the feasibility of solely psychological placebo manipulations to alter the perception of pain was tested.

Two commonly discussed preconditions for the generation of placebo and nocebo effects are prior *experiences* (i.e., past encounter of drug effects) and *expectations* (i.e., positive or negative attitudes towards an intervention). So far, research on placebo and nocebo effects relied on the administration of sham interventions, which resembled medical treatments like inert pills, creams or injections. However, such experimental procedures deal with confounds due to earlier experiences and expectations resulting from the individual's history with medical interventions. Accordingly, the implementation of a placebo manipulation that is completely new to an individual, seems necessary to disentangle the contribution of experience and expectation for the induction of placebo and nocebo effects.

To this end, in Experiment 1 the level of experience and expectation regarding a placebo-nocebo treatment was stepwise manipulated across three different experimental groups. To avoid any resemblances to earlier experiences and individual expectations, a mere psychological placebo-nocebo treatment was chosen that was new to all participants. They were instructed that visual black and white stripe patterns had been found to reliably alter the perception of pain. One group of participants received only the placebo-nocebo instruction (expectation), a second group experienced a placebo-nocebo treatment within a conditioning phase (experience) but no instruction, and a third group received the combination of both that is a placebo-nocebo instruction and a placebo-nocebo conditioning (experience + expectation).

It was shown that only the experience + expectation group revealed significantly higher pain ratings and physiological responses during nocebo, compared to placebo trials of the

succeeding test phase. These findings demonstrate that the induction of a mere psychological placebo-nocebo effect on pain is in principle possible. Most important, results indicate that such effects most likely rely on both, a positive treatment experience, due to the encounter of an effective intervention (placebo conditioning), and a positive expectation about the intervention (placebo instruction).Besides experience and expectation, the current mood state has been shown to modulate pain and to impact the induction of placebo and nocebo effects. In this vein it has been demonstrated that placebo effects come along with positive affect, while nocebo effects often occur together with elevated feelings of anxiety. To clarify the interaction of emotions and placebo-nocebo manipulations on pain perception, in Experiment 2 the paradigm of Experiment 1 was modified. Instead of black and white stripe patterns, positive and negative emotional pictures were presented, which either cued pain increase (nocebo) or pain decrease (placebo). Two experimental groups were compared, which differed with regard to the instructed contingency of positive pictures serving as placebo and negative pictures serving as nocebo cues or vice versa (congruent vs. incongruent). Results indicate that the differentiation of placebo and nocebo trials (behaviorally and physiologically) was more pronounced for the congruent compared to the incongruent group. However, in the incongruent group, affective pain ratings were also significantly higher for nocebo (positive pictures) than placebo (negative pictures) trials, similar to the congruent group. These findings demonstrate that a placebo-nocebo manipulation is capable to dampen and even reverse the originally pain augmenting effect of negative emotions.

The results of Experiment 2 were further corroborated in Experiment 3, when the design was adapted to the fMRI scanner, and again a congruent and an incongruent experimental group were compared. Behavioral, physiological and neurophysiological markers of pain processing revealed a differentiation between nocebo and placebo conditions that was present irrespective of the experimental group. In addition, the fMRI analysis revealed an increased engagement of prefrontal areas for the incongruent group only, supposedly reflecting the reinterpretation or appraisal process when positive pictures were cueing negative outcomes.

Taken together, the results of the present studies showed (a) that it is possible to induce a placebo-nocebo effect on pain solely by a psychological manipulation, (b) that both,

prior experiences and positive expectation, are necessary preconditions for this placebo-nocebo effect, (c) that the impact of negative emotion on pain can be dampened and even reversed by placebo-nocebo manipulations, and (d) that most likely a cognitive top-down process is crucial for the induction of (psychological) placebo-nocebo effects.

These results significantly enhance our understanding of psychological mechanisms involved in the induction of placebo-nocebo effects. Further, a fruitful foundation for future studies is provided, which will need to determine the contributions of primarily nocebo or placebo responses mediating the effects as demonstrated in the present studies. In a long-term perspective, the present findings may also help to exploit placebo effects and prevent from nocebo effect in clinical contexts by further elucidating crucial psychological factors that contribute to the placebo and nocebo response.

## Zusammenfassung

Die Wahrnehmung von Schmerz kann durch eine Vielzahl von Faktoren beeinflusst werden, darunter biologische und pharmakologische Interventionen sowie potente kognitive und emotionale Manipulationen. Placebo- und Nocebo-effekte gehören mit zu den eindrucksvollsten Beispielen für die Wirksamkeit derartiger Manipulationen. Placebo- und Nocebo-Behandlungen können zu manifesten psychologischen und physiologischen Veränderungen führen, obwohl die verabreichten Substanzen frei von Wirkstoffen bzw. den angewandten Scheinbehandlungen keine Wirkung zugeschrieben wird. In der vorliegenden Dissertation wurden kognitive und emotionale Einflussfaktoren auf die Induktion von Placebo- und Nocebo-Effekten bei der Wahrnehmung von Schmerz untersucht. Darüber hinaus sollte die Möglichkeit zur Verwendung rein psychologischer Placebo-Nocebo Manipulationen für die Modulation von Schmerz getestet werden.

Zwei zentrale Voraussetzungen für die Erzeugung von Placebo und Nocebo-Effekten sind vorherige Erfahrung (z.B. auf Grund früherer Erfahrungen mit einem Medikament) und Erwartung (z.B. eine positive oder negative persönliche Einstellung gegenüber einer Therapie). Bisher basierte die Forschung zu Placebo- und Nocebo-Effekten vornehmlich auf Ergebnissen von Untersuchungen die Schein-Behandlungen oder Leerpräparate einsetzten wie z.B. Tabletten, Cremes oder Injektionen, die herkömmlichen medizinischen Interventionen sehr ähnlich sind. Jedoch ergibt sich bei einem derartigen experimentellen Vorgehen stets das Problem einer Konfundierung der Ergebnisse durch den Einfluss früherer Erfahrungen oder der individuellen Erwartungshaltung an die Behandlung, die aus einer Vorgeschichte medizinischer Therapieerlebnissen herrührt. Daraus leitet sich die Notwendigkeit von anderweitigen, dem Probanden völlig unbekannten Placebo-Interventionen ab, um die jeweilige Beteiligung von Erwartungs- und Erfahrungsprozessen für die Induktion von Placebo- und Nocebo-Effekten bestimmen zu können.

Zu diesem Zweck wurden in Experiment 1 Erwartung und Erfahrung in drei Experimentalgruppen stufenweise und unabhängig voneinander manipuliert. Um einer Ähnlichkeit zu früheren Behandlungs-Erfahrungen und dadurch abgeleiteten Erwartungen vorzubeugen, wurde ein rein psychologisches Placebo-Nocebo Verfahren herangezogen, das mit Sicherheit allen Teilnehmern unbekannt war. Sie wurden darüber informiert, dass die Betrachtung von schwarz-weißen Streifenmustern eine wissenschaftlich bestätigte Wirkung

auf die Schmerzwahrnehmung hätte. Eine Gruppe der Teilnehmer erhielt lediglich eine Placebo-Nocebo Instruktion (Erwartung), eine zweite Gruppe erlebte tatsächlich die Kopplung von zwei verschiedenen Streifenmustern mit unterschiedlich starken Schmerzreizen während einer Konditionierungs-Phase (Erfahrung) bekam aber keine Instruktion und eine dritte Gruppe erhielt sowohl die Placebo-Nocebo Instruktion als auch die Placebo-Nocebo Konditionierung (Erfahrung + Erwartung). Es konnte gezeigt werden, dass während der anschließenden Testphase lediglich die kombinierte Erfahrung + Erwartung Gruppe signifikant unterschiedliche Schmerzratings und physiologische Reaktionen auf die Schmerzreize während der Placebo- im Vergleich zu den Nocebo-Durchgängen aufwies. Diese Ergebnisse belegen, dass die Induktion eines rein psychologischen Placebo-Nocebo Effektes auf die Schmerzwahrnehmung prinzipiell möglich ist. Besonders hervorzuheben ist dabei die Notwendigkeit beider Prozesse, nämlich einer tatsächlichen Erfahrung der Wirksamkeit der Therapie (Placebo-Nocebo Konditionierung) und einer positiven Erwartung hinsichtlich der Intervention (Placebo-Nocebo Instruktion).

Neben Erfahrung und Erwartung, hat die momentane Stimmung entscheidenden Einfluss auf die die Induktion von Placebo- und Nocebo-Effekten einerseits, sowie generell auf die Wahrnehmung von Schmerz andererseits. In diesem Zusammenhang konnte gezeigt werden, dass Placebo-Effekte mit einer Verbesserung der Stimmung einhergehen, Nocebo-Effekte hingegen häufig von gesteigerter Angst begleitet sind. Um die Interaktion von Emotionen und Placebo-Nocebo Manipulationen zu eruieren, wurde das in Experiment 1 etablierte Paradigma angewendet und modifiziert. Anstelle von Streifenmustern, wurden positive und negative emotionale Bilder präsentiert, die entweder eine Schmerz-Verstärkung (Nocebo) oder eine Schmerz-Linderung (Placebo) anzeigten. Zwei Experimentalgruppen wurden miteinander verglichen, die sich hinsichtlich der Kontingenz von positiven Bildern als Placebo- und negativen Bildern als Nocebo-Indikator, bzw. umgekehrt, positiven Bildern als Nocebo- und negativen Bildern als Placebo-Indikator, unterschieden (kongruent vs. inkongruent). Es zeigte, dass die Unterscheidung (Schmerzratings und physiologische Reaktionen auf den Schmerzreiz) zwischen Placebo- und Nocebo-Durchgängen in der kongruenten Gruppe stärker ausgeprägt war als in der inkongruenten Gruppe. Allerdings waren die affektiven Schmerzratings der inkongruenten Gruppe ebenfalls in Nocebo-Durchgängen (positive Bilder) signifikant höher als in Placebo-Durchgängen (negative Bilder), ähnlich zur kongruenten Gruppe. Die Daten zeigen damit, dass eine Placebo-Nocebo

Manipulation in der Lage ist, die genuin Schmerz verstärkende Wirkung negativer Emotionen abzuschwächen und sogar umzukehren.

Die Befunde aus Experiment 2 konnten zusätzlich in Experiment 3 gestützt werden, welches das zuvor getestete Design ins fMRT überführte und gleichermaßen eine kongruente und eine inkongruente Experimentalgruppe miteinander verglich. Verhaltensmaße sowie physiologische und neurophysiologische Korrelate der Schmerzwahrnehmung ergaben eine eindeutige Differenzierung zwischen Placebo- und Nocebo-Durchgängen, unabhängig von der Experimentalgruppe. Darüber hinaus zeigte sich in der inkongruenten Bedingung eine verstärkte präfrontale Aktivierung für den Vergleich von Nocebo- und Placebo-Durchgängen, was potenziell auf einen zusätzlichen Re- Interpretations- oder Appraisal-Prozess zurückzuführen ist, der sich einstellt, wenn ein positives Bild eine negative Konsequenz vorhersagt.

Zusammengefasst zeigen die vorliegenden Studien, dass es (a) möglich ist einen Placebo-Nocebo Effekt mit einer rein psychologischen Manipulation hervorzurufen, dass (b) im Fall rein psychologischer Placebo-Nocebo Manipulationen sowohl Erfahrung als auch positive Erwartung notwendig sind, dass (c) der Einfluss negativer Emotionen auf Schmerz mittels einer Placebo-Nocebo Manipulation reduziert und sogar umgekehrt werden kann und (d) höchstwahrscheinlich ein kognitiver (Neu-) Bewertungsprozess für die Induktion (psychologischer) Placebo-Nocebo Effekte essentiell ist.

Die Ergebnisse tragen zum Verständnis der beteiligten psychologischen Prozesse bei der Induktion von Placebo-Nocebo Effekten erheblich bei. Darüber hinaus stellen die verwendeten Paradigmen eine vielseitige Ausgangsposition für zukünftige Studien dar, die klären müssen, ob für die gefunden Ergebnisse vornehmlich Placebo- oder Nocebo-Effekte verantwortlich sind. Perspektivisch könnten die vorliegenden Befunden helfen, die psychologischen Grundlagen der Placebo-Nocebo Antwort näher zu beleuchten und damit sogar im klinischen Kontext zum Ausschöpfen von Placebo- sowie zur Vorbeugung von Nocebo-Effekten beizutragen.

# 1. Introduction

*"The magnitude of pleasure reaches its limit in the removal of all pain. When such pleasure is present, so long as it is uninterrupted, there is no pain either of body or of mind or of both together."*

(Epicurus)

## 1.1 The Perception and Modulation of Pain

In healthy humans, pain is a highly adaptive bodily alarm signal that informs the organism of potential tissue damage and prevents from serious harm (Wall, McMahon, & Koltzenburg, 2006). Apart from its protective character, pain is a complex, multidimensional experience involving a variety of different reactions: motor, behavioral, humoral and emotional (Birbaumer & Schmidt, 2010). Its multidimensionality is also reflected in the definition of pain by the international association for the study of pain (IASP) that states pain to be : "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'' (Merskey et al., 1979).

### 1.1.1 The Processing of Pain: Basic Mechanisms and Neural Correlates

The above descriptions render the *perception of pain* - that is the conscious interpretation of nociceptive information - as a dynamic, and to a large degree psychologically mediated and moderated experience. From a physiological perspective, nociception is specific somatosensory information that is encoded in the periphery and transferred to the brain. The free nerve endings of the nociceptive axons, C- and Aδ- fibers, constitute the beginning of the nociceptive signal cascade and are located throughout the organism. Nociceptors can be differentiated with regard to the quality/modality of the stimulation they respond to (or respond to preferably). A large group of nociceptors, so called polymodal nociceptors, react in response to different qualities, thus depicting a nice example for the dynamicity of pain already on the physiological level. Those stimulation qualities that can be separated from each other are mechanical, chemical and heat (Magerl & Treede, 2011; Ringkamp & Meyer, 2008). Regarding the signal transduction of a heat pain stimulus that is applied to the skin, five different stages need to be accomplished before entering the central nervous system: heat is absorbed by the skin, reaches the nociceptor terminals, is transduced into a change of

membrane potential and - given the threshold is exceeded - the signal is propagated to the central nervous system (Ringkamp & Meyer, 2008).

As an example, the processing of nociceptive heat is encoded by the activation of the polymodal (heat- and chemo sensitive) transient receptor potential (TRP), more precisely, the vanilloid receptor TRPV1. It can be activated by heat (> 43°C) or by chemical substances like capsaicin resulting in the influx of $Na^+$ and $Ca^{2+}$ ions. The ion influx generates a sensor- or receptor potential that is subsequently encoded into a series of action potentials. The resulting frequency of action potentials is proportional to the strength of the initial influx of positive ions providing a means of magnitude encoding of the peripheral stimulation (see Magerl & Treede, 2011). Nociceptors of the skin are located close to the inner surface of the epidermis and, as already mentioned, transduce information mainly by Aδ delta and C fibers, which differ with regard to their conductive velocity due to myelination and caliber. On hairy skin, for instance on the volar site of the forearm, heat pain perception is mediated by type-II A-fibers, which are responsible for the early sensation of a sharp first pain. C-fiber mechano-heat-sensitive nociceptors constitute the delayed and prolonged sensation of the so called second pain. The differences in timing of the two sensations are due to the described variations of the fibers conduction velocity (see Ringkamp & Meyer, 2008). After entering the dorsal horn ganglion of the spinal cord, action potentials are transduced mainly by glutamatergic neurotransmission, and the nociceptive input is propagated (in part) via the spinothalamic tract onto the lateral and medial nuclei of the thalamus (D'Mello & Dickenson, 2008). Two supraspinal, neuronal systems can be differentiated with regard to their primary role within the processing of nociceptive information: the lateral system, mainly encoding for sensory discriminative components of pain, and the medial system, encoding the affective, motivational component of the resulting pain percept (Price, 2000; Treede & Apkarian, 2008).

The actual perception of pain is represented in vast neuronal networks that encompass a number of cortical and subcortical structures which in concert code for the different aspects of pain (Apkarian, Bushnell, Treede, & Zubieta, 2005; Melzack, 2001; Peyron, Laurent, & García-Larrea, 2000). Functional imaging studies investigating the processing of pain frequently, albeit not consistently, report the activation of structures like the somatosensory cortex, the motor cortex, the anterior cingulate cortex (ACC) /anterior midcingulate cortex (aMCC), the insula, the prefrontal cortex (PFC), the thalamus and the cerebellum (Apkarian,

et al., 2005; Price, 2000). The somatosensory cortex receives input from the lateral nuclei of the thalamus, whereas the ACC receives input mainly from the medial portions of the thalamus and further provides the PFC with nociceptive input. The cerebellum receives direct input from the spinothalamic tract and is one of the subcortical structures which were commonly found to respond to pain, besides the caudate-putamen, the amygdala and the periaqueductal grey (PAG). Accordingly, sensory, discriminatory aspects of pain are encoded in the somatosensory, lateral thalamic and cerebellar portions of the brain, instead affective and cognitive components of pain are represented dominantly in the anterior (mid)cingulate cortex, and insular and prefrontal areas (Apkarian, et al., 2005).

Anatomical differences might be further qualified by the functional aspect of different pain components. The perception of the so-called first pain is likely represented by an early activation of the primary sensory cortex, informing about the precise location of bodily threat. The second pain sensation is mainly represented by the ACC and provides information relevant for even longer periods of time such as recovery from an injury (Treede & Apkarian, 2008). The classic concept of the pain matrix (e.g., Tracey & Mantyh, 2007), which was inspired by the neuromatrix of pain (Melzack, 1989, 2001), proposes a specific neuroanatomical representation of pain. However, just recently this concept entered a controversial debate and the specificity of its assumptions were questioned (Iannetti & Mouraux, 2010; Legrain, Iannetti, Plaghk, & Mouraux, 2011).

Based on findings that revealed the neuronal representation of pain to share substantial overlap with other sensory modalities, rather than being pain-specific, the concept of a *saliency* matrix in contrast to the classical *pain* matrix was proposed. This modified theoretical concept reflects the involved allocation of attention and processing resources towards behaviorally relevant information, which is not necessarily pain-specific (Legrain, et al., 2011). In this vein a series of studies provided evidence for the responsiveness of pain-associated brain areas in conditions other than the actual perception of pain. It was shown that areas like the ACC or the insula, which were found to be activated during the processing of pain, also responded when observing the pain of others (Lamm, Decety, & Singer, 2011). A study that nicely captured this aspect of empathy for pain was conducted by Singer et al. (2004). They scanned participants in a functional magnet resonance scanner while their partners were standing next to them and received a series of painful stimulations. The brain

activations were similar to first person responses to pain, although solely the partner was shocked. In a similar study participants were presented pictures of potentially harmful and harm-free scenarios (e.g., a finger being cut by a scissors vs. a finger being photographed close to a scissor). The painful pictures elicited brain activity patterns which again were very similar to the neural responses found for the actual perception of pain (Jackson, Meltzoff, & Decety, 2005). It has also been found that the processing of facial expressions of pain elicits brain responses similar to those of actually perceiving pain (Simon, Craig, Miltner, & Rainville, 2006). In addition, in a recent study on the mutual effects of pain and emotion, the presentation of pain faces was found to modulate the processing of concomitantly administered pain stimuli (Reicherts, Gerdes, Pauli, & Wieser, 2013), while the perception of pain also altered the processing of emotion. In another study it could be shown that even the feeling of social rejection, which was induced by excluding a participant during a simple computer animated ball game, was capable of eliciting activity in pain-associated areas (Eisenberger, Lieberman, & Williams, 2003).

Besides the common neural representations of various somatosensory and emotional states, a recent series of fMRI studies, which encompassed a large data set (more than 100 participants) and provided different experimental pain paradigms, revealed activity in the ventrolateral thalamus, the somatosensory cortex, the dorsal posterior insula to be specific for (heat) pain and distinguishable from other salient events such as social rejection. These findings reveal - at least to a certain degree - a brain signature that specifically corresponds to the physical representation of pain (Wager et al., 2013). Taken together, these results demonstrate the perception and processing of pain and pain related information to be of high individual and inter-individual relevance. Further, the neural substrates of pain were shown to share substantial commonalities with other highly salient sensory or emotional experiences; however, at least to a certain degree, pain is represented by a specific neural signature.

### 1.1.2 Mechanisms of Pain Regulation

As discussed in the previous section, the perception of pain is represented by the engagement of different brain areas. Likewise the regulation of pain (i.e., the exacerbation or the reduction of pain) can arise from the interactions of several cortical and subcortical brain structures that constitute the so called nociceptive control system (for an overview, see Fields,

Basbaum, & Heinricher, 2006). The nociceptive control system encompasses cortical areas such as the PFC, the ACC and the insular cortex, as well as subcortical structures like the amygdala and the hypothalamus. All these structures can evolve pain facilitating or inhibitory effects via projections to midbrain areas such as the PAG. The PAG acts on the nociceptive signal transmission at the level of the dorsal horn, via projections to the rostral ventromedial medulla (Fields, et al., 2006; Tracey & Mantyh, 2007) and thereby promotes the release of pain modulating endogenous opioids. Opioids can evolve a pain inhibitory effect in the spinal cord as well as on the cortical level. All components of the nociceptive control system, as reviewed so far, are rich in opioid receptors, and the administration of opioids results in inhibition of nociceptive responses (Fields, 2004). The release of endogenous opioids might result from cognitive and/or affective processes, leading to a decrease of the perception of pain (Bushnell, Ceko, & Low, 2013; Wiech, Ploner, & Tracey, 2008). In this vein, dose dependent changes of brain activity in structures that encode sensory and affective components of pain were demonstrated in study conducting positron emission tomography (PET), and administering different concentration of remifentanil, a μ opioid receptor agonist (Wagner et al., 2001). Further, endogenous opioids were found to be candidate mediators of placebo analgesia, since the systematic blockade of opioids due to the administration of the receptor antagonist naloxone resulted in a complete loss of the analgesic placebo effect (Levine, Gordon, & Fields, 1978). These findings were further corroborated by brain imaging studies revealing the transmission and binding of opioids during placebo analgesia (Zubieta et al., 2005).

Taken together, these findings demonstrate that the perception of pain can be shaped by the activation of nociceptive control mechanisms, which can be initiated by psychological processes and critically involve the transmission of endogenous opioids.

### 1.1.3 Measures of Pain

As described above, pain is a multidimensional phenomenon and as such provokes responses on multiple system levels of an organism. Accordingly, the measurement of pain targets at various parameters that show alterations in response to pain. In the context of experimental pain stimulation, commonly assessed measures comprise pain ratings, autonomic physiological responses, (functional) neurophysiological measures, nociceptive reflexes and sometimes even facial responses.

Pain ratings can be obtained for instance using a numerical ratings scale (NRS) which commonly consists of 10 or more steps and additionally can provide verbal descriptors of the pain intensity or quality. In addition, visual analogue scales (VAS) can be used which demand a participant to mark a line between two verbal anchors (e.g., no pain vs. unbearable pain) corresponding to the strength of the pain experience. The pain rating equals the distance of the marking and the anchor. VAS can be easily used and are assumed to be less prone to recency effects within a series of pain ratings compared to categorical NRS (Gracely, 2006; Price, Bush, Long, & Harkins, 1994; Price, McGrath, Rafii, & Buckingham, 1983).

At the autonomic level, alterations of skin conductance (SC), which indicate the activation of the sympathetic nervous system, were shown to reliably differentiate between different intensities of pain stimulations (Breimhorst et al., 2011). In addition, heart rate (HR) was demonstrated to increase as a function of heat pain stimulation and further was correlated with verbal pain reports (Loggia, Juneau, & Bushnell, 2011). Both measures, HR and SC, were also found to respond to the modulation of pain caused by the presentation of emotionally relevant, visual stimuli (Rhudy, Williams, McCabe, Russell, & Maynard, 2008). Another commonly used measure of pain perception is the peripheral nociceptive withdrawal reflex (R-III) which can be elicited by electrical stimulation of the sural nerve and is discussed as an indirect measure of spinal nociceptive processing (Sandrini et al., 2005). In addition, the perception of intense pain is accompanied by a distinct facial expression that encompasses a set of prototypical facial muscle movements, which can be evaluated by observation (Kunz, Mylius, Schepelmann, & Lautenbacher, 2004) or even by the measurement of facial muscle activity using surface electromyography (Reicherts, et al., 2013).

Further, the processing of pain can be captured by applying measures of the central nervous system, for instance electroencephalography (EEG) or functional magnetic resonance imaging (fMRI). Due to its high temporal resolution, the measurement of EEG is especially suitable to capture very early pain signals. The administration of short painful stimuli, for instance an electrical current or an laser stimulus, reliably elicits a somatosensory or laser evoked-potential (SEP/ LEP) which consists of typical components, such as a negative deflection around 150 ms after stimulus onset and a later positive peak around 260 ms (Bromm & Lorenz, 1998; Garcia-Larrea, Frot, & Valeriani, 2003). In contrast to EEG, fMRI is of much lower time resolution, but instead provides high spatial resolution and allows capturing

activation of small brain areas and even the spinal cord (for an overview see: Somborski & Bingel, 2010; Tracey, 2008; Treede & Apkarian, 2008). Brain structures, which are frequently targeted during functional imaging studies, are part of the pain or salience matrix as reviewed in section 1.1.1.

The measures as introduced above were found to respond to the administration of pain and can provide crucial insight for the experimental research of pain perception or its modulation. The assessment of pain responses should rely on multiple measures and optimally incorporates measures of cognitive-verbal responses, physiological-humoral responses, and behavioral responses (see Apkarian, et al., 2005; Price, et al., 1994; Treister, Kliger, Zuckerman, Aryeh, & Eisenberg, 2012). This is even more crucial since the modulation of pain (Bushnell, et al., 2013; Rhudy, et al., 2008) as well as the modulation of emotion (Lang & Bradley, 2010; Lang, Greenwald, Bradley, & Hamm, 1993) is documented on all of these levels.

## 1.2 Psychological Modulation of Pain

As already introduced, the perception of pain is to a large degree psychologically mediated, as such the perception of pain is prone to modulations by psychological mechanisms. In two fMRI studies hypnotic suggestions were applied which resulted in alterations of the affective or the sensory dimension of pain, selectively (Hofbauer, Rainville, Duncan, & Bushnell, 2001; Rainville, Duncan, Price, Carrier, & Bushnell, 1997). Participants were instructed to regulate either the sensory or the affective component of an administered thermal pain stimulus. Depending on the instruction, either sensory areas such as the somatosensory cortex or affective pain processing areas such as the ACC showed increased activity. These findings provide additional evidence for the two dimensions of pain, and more importantly, further reveal the huge impact of psychological manipulations on the perception of pain. Besides the modulation by hypnosis, many other psychological mechanisms and strategies impact the processing of pain. The probably most frequently investigated psychological modulators are current mood (Wiech & Tracey, 2009), expectations about the magnitude of an upcoming pain stimulus (Keltner et al., 2006; Koyama, McHaffie, Laurienti, & Coghill, 2005; Ploghaus et al., 2001; Porro et al., 2002; Sawamoto et al., 2000), and the allocation of attention towards or away from nociceptive input (Van Damme, Legrain, Vogt, & Crombez, 2010; Villemure & Bushnell, 2002).

### 1.2.1 Attention

As mentioned above, the neural pain matrix likely serves as a saliency detector ensuring the processing of crucial sensory information (Legrain, et al., 2011). Thus, the processing of pain and the allocation of attention are closely linked processes. It could be shown that variations of the focus of attention modulates the perception of pain, such that distraction leads to decreased perception of pain while concentrating on pain results in elevated perception of pain (e.g., Quevedo & Coghill, 2007).

The impact of attention on pain can be investigated by applying simple (Frankenstein, Richter, McIntyre, & Remy, 2001) or intensive multisensory (Mühlberger, Wieser, Kenntner-Mabiala, Pauli, & Wiederhold, 2007) attention distraction manipulations. Moreover, more complex experimental designs can be used that provide control for the actual engagement of attention (see Price, Hirsh, & Robinson, 2008). For instance, in cross modality attention paradigms two stimuli of different sensory qualities are presented at the same time (a pain stimulus and a second stimulus of another modality) and participants are asked to detect changes in one of the modalities at a time (Price, Hirsh, et al., 2008). Similarly, during primary task paradigms the focus of attention is manipulated by presenting participants a task that engages working memory capacities, while a task irrelevant pain stimulus is administered (e.g., Buhle & Wager, 2010; Petrovic, Petersson, Ghatan, Stone-Elander, & Ingvar, 2000). In both paradigms, the modulation of pain can be captured by effects on pain measures (ratings, physiological, and neural responses) while the interfering influence of pain is depicted in the primary task performance (Price, Hirsh, et al., 2008). In line with a limited capacity model of attention, a distractive task would absorb attentive resources which in the following cannot be engaged in the processing of pain that is consequently perceived as less intense (Eccleston & Crombez, 1999). However, the interaction of attention and pain is highly dynamic and relies on both, bottom up variations (e.g., pain stimulus quality or magnitude) and top down modulators (e.g., motivational relevance of a goal which competes against a pain sensation for the available processing resources, see Van Damme, et al., 2010).

Functional imaging studies reveal effects for distraction from pain in the somatosensory cortex, the ACC, the thalamus and the insula (Bantick et al., 2002; Wiech, et al., 2008), which is most likely due to the engagement of the descending nociceptive control system (Wiech, et al., 2008). These findings were further corroborated by a recent functional

imaging study investigating the impact of a working memory task on cortical, subcortical - and what is most intriguing - spinal correlates of pain. It was demonstrated that reduced pain perception during working memory engagement was also reflected in reduced activity in the dorsal horn of the corresponding spinal segment. This modulation was in part reversed by the administration of the opioid-receptor antagonist naloxone, thus providing compelling evidence for the descending modulation of pain being mediated by opioids (Buhle, Stevens, Friedman, & Wager, 2012).

Most likely, attentional processes moderating pain incorporate similar mechanisms as found in other sensory modalities (Gilbert & Sigman, 2007; Wiech, et al., 2008). However, pain might be special, regarding its high salience. When a painful stimulus is novel and intense, it will most probably break through any attentive filter (Legrain, Perchet, & Garcia-Larrea, 2009). Conversely in other modalities such as vision, even huge alterations of a visual scene might be overlooked when they are not expected or an individual is distracted (e.g., change blindness, see Simons & Rensink, 2005).

The investigation of pain modulation by any psychological manipulation may often involve a modulation of attention as well. For instance the presentation of complex visual emotional stimuli implies a general capture of attention, solely by its observation, and thereby modulates the perception of pain compared to a low level control condition (Reicherts, et al., 2013). Accordingly, it is difficult to separate the modulation of pain by attention and emotion from each other. In an attempt to disentangle the influence of emotion and attention on pain, participants were presented odors of different valence and additionally were administered painful stimuli (Villemure, Slotnick, & Bushnell, 2003). Participants were asked to focus either on the valence of the odor or on the sensation of the pain stimulus. The sensation of pain was independently modulated by the focus of attention and the valence of the presented odor, which suggests separate mechanism that are involved during the modulation of pain by attention and emotion (Villemure, et al., 2003). In another experiment that compared the attentional and emotional modulation of pain, participants were presented affective pictures and were told to focus either on the picture content or on a concomitantly administered pain stimulus, while EEG was recorded (Kenntner-Mabiala, Andreatta, Wieser, Mühlberger, & Pauli, 2008). It was found that attentional and affective manipulations resulted in a different modulation of behavioral and neurophysiological (somatosensory evoked potentials)

measures of pain. The attention manipulation specifically impacted sensory pain ratings, while emotional picture content altered ratings for both dimensions of pain. Moreover, picture content was found to alter the early (N150) component of the SEP, resulting in higher amplitudes for negative pictures, while attention manipulation resulted in elevated amplitudes of P260 when focusing on the pain stimulation (Kenntner-Mabiala, et al., 2008).

Both mechanisms, the modulation of pain by attention and emotion, supposedly converge on the descending pain control system (Wiech, et al., 2008). In a very recent review article the impact of attentional manipulations on pain was summarized as to mainly alter the sensory discriminative component of pain, reflected on the neural level by changes in the somatosensory and insular cortex. In contrast, the emotional modulation of pain seems to result mainly in changes of the affective component of pain, reflected by variations of activity in the PFC and the ACC, coding for the motivational component of pain (Bushnell, et al., 2013; Loggia, Mogil, & Bushnell, 2008).

### 1.2.2 Emotion

Studies that focused on the manipulation of pain by emotions found that pain is reduced by the induction of positive mood, while pain is increased during the induction of negative affective states (Bushnell, et al., 2013; Wiech & Tracey, 2009). A variety of different methods and stimulus types have been used to experimentally induce affective mood states in participants, such as the presentation of happy or sad pieces of music (Roy, Lebuis, Hugueville, Peretz, & Rainville, 2012; Zhao & Chen, 2009), pleasant and unpleasant odors (Marchand & Arsenault, 2002; Villemure, et al., 2003), affectively toned excerpts of stories (Zelman, Howland, Nichols, & Cleeland, 1991), movie clips (Weisenberg, Raz, & Hener, 1998), affective facial (pain) expressions (Reicherts, et al., 2013; Senkowski, Kautz, Hauck, Zimmermann, & Engel, 2011), and affective pictures which represent probably the most intensively investigated stimulus category (Rhudy, Williams, McCabe, Nguyen, & Rambo, 2005; Rhudy, et al., 2008; Roy, Piché, Chen, Peretz, & Rainville, 2009).

The motivational priming hypothesis by Lang (1995) lends an explanatory framework for these effects. The theory states that emotion processing is linked to behavioral reactions via two distinct orthogonal motivational systems. The appetitive motivational system is accounting especially for approach and hedonic behaviors, while the defensive motivational system is responsible for withdrawal responses. The implications of the theory were

intensively investigated within the modulation of the startle reflex, an automatic protective reaction in response to sudden stimuli, most often elicited in experimental contexts by the presentation of short burst of white noise (Lang, Bradley, & Cuthbert, 1990). Accordingly, the presentation of positive affective stimuli results in the activation of the appetitive system and thereby facilitates approach behaviors and at the same time inhibits defensive reactions as can be measured by a decrease of the startle response (Lang & Bradley, 2010). Vice versa the presentation of negative affective foreground stimuli primes the defensive motivational system and facilitates the startle response (e.g., Bradley, Codispoti, Cuthbert, & Lang, 2001). The motivational priming hypothesis has successfully been tested also for the modulation of pain by emotion, revealing similar results as for the modulation of the startle response. For instance, Meagher and colleagues (2001) presented participants emotional pictures drawn from the International affective picture system (IAPS, Lang, Bradley, & Cuthbert, 1999) and found pain threshold measures to be modulated by the emotional content, such that male participants revealed higher pain threshold after watching erotic compared to neutral pictures. Theses preliminary findings were further corroborated by following experiments, demonstrating that behavioral pain responses (pain ratings) and physiological pain responses (skin conductance responses, heart rate and the nociceptive withdrawal RIII-reflex) were increased by negative and decreased by positive picture content (Rhudy, et al., 2005; Rhudy, et al., 2008). Two similar experiments showed that pain ratings and early SEP (N150) were increased during negative and decreased during positive picture presentation (Kenntner-Mabiala, et al., 2008; Kenntner-Mabiala & Pauli, 2005). Further it could be demonstrated that highly arousing emotional pictures resulted in a decrease of a later component of the SEP (P260) irrespective of picture valence, suggesting the altered processing of pain supposedly driven by elevated capture of attention. In line with that, emotional modulation of pain was found to be further moderated by the level of arousal of the presented stimuli: picture valence determined the direction of pain modulation (either increase or decrease), while the level of arousal determined its magnitude (Rhudy & Meagher, 2001; Rhudy, et al., 2008). Highly arousing positive emotional stimuli revealed more pronounced decreases in pain than low arousing stimuli. Regarding negative stimuli this relationship held true as well, such that highly compared to moderately arousing affective stimuli resulted in higher pain increase (Rhudy, et al., 2008). It is hypothesized that a continuous increase of negative emotional arousal even may become analgesic (e.g., during life threatening danger), similar to stress induced

analgesia, found predominantly in the animal model (Rhudy & Meagher, 2000; Rhudy, et al., 2008).

The neuronal correlates of emotional influences on pain suggest the engagement of the descending pain control system (Wiech & Tracey, 2009) that increases or decreases neuronal activation in response to pain respectively. In one of the very few functional imaging studies that investigated the impact of emotional pictures on central and peripheral pain responses Roy et. al. (2009) found that the increased perception of pain during the presentation of negative compared neutral and positive affective pictures resulted in enhanced activity of sensory pain-associated areas like the paracentral lobule, the thalamus and the anterior insula; especially the parahippocampal gyrus and the amygdala were found to respond to negative affect as well.

### 1.2.3 Expectation

Negative emotions, such as anxiety, which increase the perception of pain, may also arise from manipulations of expectations regarding the strength of an administered pain stimulus. In this vein Ploghaus al. (2001) conducted an fMRI study and instructed participants that they would be administered low and high painful heat stimuli cued by two different visual signals, which resulted in low or high levels of (anticipatory) anxiety. However, in certain trials, a low pain stimulus was falsely cued to be highly painful, resulting in increased pain perception compared to correctly cued pain stimuli, although the exact same level of heat was administered. On the neuronal level, exacerbated pain perception came along with elevated responses in hippocampus and entorhinal cortex -which share connections to the amygdala - and were found in earlier studies to be activated during anxiety induction or threat conditioning paradigms (Phelps et al., 2001; Ploghaus, et al., 2001). These results demonstrate that expectations impact the current mood state and can critically shape the processing of pain. In another study which addressed the impact of expectation on pain, participants were informed about the contingency of a visual cue and a heat pain stimulus. It could be shown that positive expectation - induced by cueing low pain stimulation and administering high pain stimuli - resulted in a decreased perception of pain and reduced activity of pain-associated areas like the thalamus, the insula and the ACC when compared to conditions that were correctly cued as highly painful (Koyama, et al., 2005). In a related manner, Kong et al. (2013) found that pain stimuli were perceived as less painful, if these were falsely signaled to be only

moderately painful, relative to an earlier learning phase. The authors found that neural connectivity of a frontoparietal control network and the rACC were predicting expectation effects on the perception of pain. These findings suggest a top down evoked pain modulating process and suppose individual differences in functional connectivity to mediated the responsiveness to pain modulation by expectation (Kong, et al., 2013). The manipulation of expectancy is one of the basic processes which is addressed during placebo interventions when an individual is expecting an inert treatment to have a tremendous impact on his symptoms. In the next paragraphs, placebo effects - and the opposite nocebo effect - will be described with special focus on placebo analgesia that is the reduction of pain by a placebo treatment.

## 1.3 The Placebo and Nocebo Effect: Definition, Methodology and Mechanisms

In his textbook on placebo effects, Fabrizio Benedetti describes the ancient roots of the placebo effect and its exploitation as early as the advent of medical interventions in human history. The very first treatments relied on bizarre assumptions about the effectiveness of interventions and were quasi ignorant to the physiological and anatomical preconditions. Successful medicaments like "*bezoar* […which was…] believed to be the crystallized tear from the eye of a dear bitten by a snake", (Benedetti, 2009, p. 2) were prescribed in a variety of medical situations. Probably their effect relied to some degree on artifacts like spontaneous remission or the lacking of any *real* malady or disease. However, effects were certainly also mediated by the expectations and beliefs of the patients who trusted in the therapy of a convincing and charismatic doctor or shaman. Today, we would most probably understand these processes as indicators of a real psycho-biologically mediated symptom change, namely placebo effects (Benedetti, 2009). A very broad but in the same manner comprehensive definition of the placebo effect describes it as: "a genuine psychological or physiological effect, in a human or another animal, which is attributable to receiving a substance or undergoing a procedure, but is not due to the inherent powers of that substance or procedure" (Stewart-Williams & Podd, 2004, p. 326). Analogously, the nocebo effect is defined as a placebo effect as well -since it also depends on an completely inert agent - but in contrast to a placebo, the nocebo effect relies on the induction of a negative expectation that results in an increase or worsening of negative symptoms (Benedetti, 2008, p. 43).

In medical and pharmacological research that aims at evaluating the effectiveness of a treatment, placebo groups serve as a control rather than a treatment itself. The randomized double-blind placebo controlled trials (RCT) represent the gold standard (Benedetti, 2009; Tracey, 2010) in this field of research. During an RCT, participants are randomly allocated to a verum treatment or a placebo group, while participants and investigator are blinded to the group allocation (Enck, Bingel, Schedlowski, & Rief, 2013). Anyhow, the adequate interpretation of the results may be complicated due to the underestimation of a potentially involved placebo effect resulting from the way participants were instructed: uncertain expectation about receiving verum or placebo can confound the treatment outcome. The evaluation of the actual amount of a placebo effect relies on the comparison of a waiting control or natural history group that receives no treatment at all compared to a placebo condition. The placebo group is identical with regard to all aspects of the verum treatment except that the agent administered is inert (Benedetti, 2009). Even more precise and elaborate are the so called balanced placebo designs (Ross, Krugman, Lyerly, & Clyde, 1962) which incorporate all possible permutations of 1) intervention: drug vs. placebo administration and 2) instruction of the participant: receiving a verum vs. receiving a placebo. These designs are much more seldom realized since they involve the ethical issue of purposely deceiving patients and in addition demand a large total sample size. Nevertheless, essential insights can be drawn from experiments like these. Recently it was shown that the way of introducing a clinical intervention on pain drastically moderates its outcome, although the actual treatment remains the same (Rief & Glombiewski, 2012). The authors compared the impact of different experimental instructions that varied according to the probability of receiving a placebo during the study (0% vs. 50% vs. 100 %). In addition, two different placebo substances were compared: a conventional passive placebo vs. an active placebo[1] that was a nasally administered spray containing a small dosage of capsaicin. The results show that an instruction of having received a placebo by chance (50%) evokes smaller responses compared to the belief of having received a drug for sure. These findings seem especially crucial since the experimental instruction corresponds to the procedure of a standard RCT - as reviewed

[1] Active placebos, in contrast to classical placebos, evoked a physical sensation in the participant, what is especially crucial to overcome so called onset effects that are common after the administration of real drugs. Onset effects can hamper the blinding of experimental group allocation directly after the start of a study (Enck, Bingel, Schedlowski, & Rief, 2013; Rief & Glombiewski, 2012).

above - which is widely accepted. It might be assumed that the induction of an uncertain 50:50 expectation probably does not capture the complete placebo effect a treatment (potentially) contains and may lead to the overestimation of the pure drug effect.

Placebo responses need to be separated from a number of methodological interferences like regression to the mean resulting from multiple symptom assessment, report biases in doctors, experimenters or highly motivated participants, as well as from the natural course of a disease, the typically occurring fluctuations of symptoms and spontaneous remission (Benedetti, 2008; Tracey, 2010; Wager & Fields, in press). Experimental trials are capable to overcome many of the aforementioned confounds by applying rigid methodology and providing measures that are less prone to subjective response patterns. Moreover, in the laboratory, treatment history and time course of a disease is often less crucial. Further the application of within-subjects designs provide adequate statistical comparisons - and especially when investigating pain - symptom induction(i.e., pain stimulation) is highly reliable and can be individually adjusted to each participant (Benedetti, 2009). In this vein, placebo effects that were demonstrated on the basis of subjective measures were criticized to merely rely on a response bias in favor of the a priori hypothesis. However, a plethora of studies provide evidence for the manifestation of placebo effects on various system levels such as the behavioral (e.g., movement, pain ratings) or (neuro-) physiological level (brain responses, SCR, hormones) for an overview see (Price, Finniss, & Benedetti, 2008).

Regarding the frequency of placebo responses, the estimate by Beecher (1955) that around 30 % of all of his patients responded to placebo, is more of an anecdote than accumulated scientific evidence (Benedetti, 2009). Actually, the probability of responding to placebos and the magnitude of the resulting placebo effect relies on a number of highly variable influences such as the method or experimental protocol to induce a placebo, trait variables within the patient/participant and contextual factors that may support or hamper the successful induction (Benedetti, 2009; Pecina et al., 2013; Price et al., 1999). Although placebo and nocebo effects can be observed in various circumstances encompassing diverse physiological and psychological response systems, probably the best investigated modality is pain, and many of the putative mechanisms underlying the generation of placebo and/or nocebo effects have been investigated by administering pain (Benedetti, 2009; Wager & Fields, in press).

The following section will focus on the modulation of pain in placebo and nocebo designs, and will elaborate the involved processes and modulating context factors.

### 1.3.1 Placebo Effects and Pain: The Impact of Experience and Expectancy

Probably the most crucial factors which placebo and nocebo effects rely on are (a) prior experiences, for example the encounter of positive drug effects or negative side effects, and (b) expectations that can be shaped by a history of earlier experiences or induced by verbal instructions or observation of role models (Colloca, Sigaudo, & Benedetti, 2008; Price, Finniss, et al., 2008; Vögtle, Barke, & Kröner-Herwig, 2013). From an experimental point of view, experiences can be shaped by classical (placebo) conditioning procedures. The sensation of pain (unconditioned response, UR) is repeatedly reduced by a drug (drug effect on the brain = unconditioned stimulus, UCS) which is delivered as a pill (conditioned stimulus, CS). After a series of repetitions, the pill itself becomes a cue for pain relief (CS+) which is capable of evoking a reduction of pain (conditioned response, CR) (Wager & Fields, in press).

Experiences and expectations interact with each other and can be mutually supported or inhibited for instance when one process approves or negates the other. Acting in concert, experience and expectation result in stronger placebo responses than being manipulated separately (Amanzio & Benedetti, 1999; Bingel et al., 2011; Voudouris, Peck, & Coleman, 1990). In a comprehensive model, (see ***Fig. 1***) Benedetti et al. (2003), described the relation of experience and expectation for generating the placebo response. Positive expectations affect particularly conscious processes such as pain sensation and induce a placebo response. The effect of experience instead is two-fold: for instance after a pharmacological conditioning procedure, a CS+ (e.g., a placebo pill) can result in the modulation of unconscious processes and elicit a CR (e.g., the secretion of hormones), but further is also capable of creating expectations that may impact conscious processes.

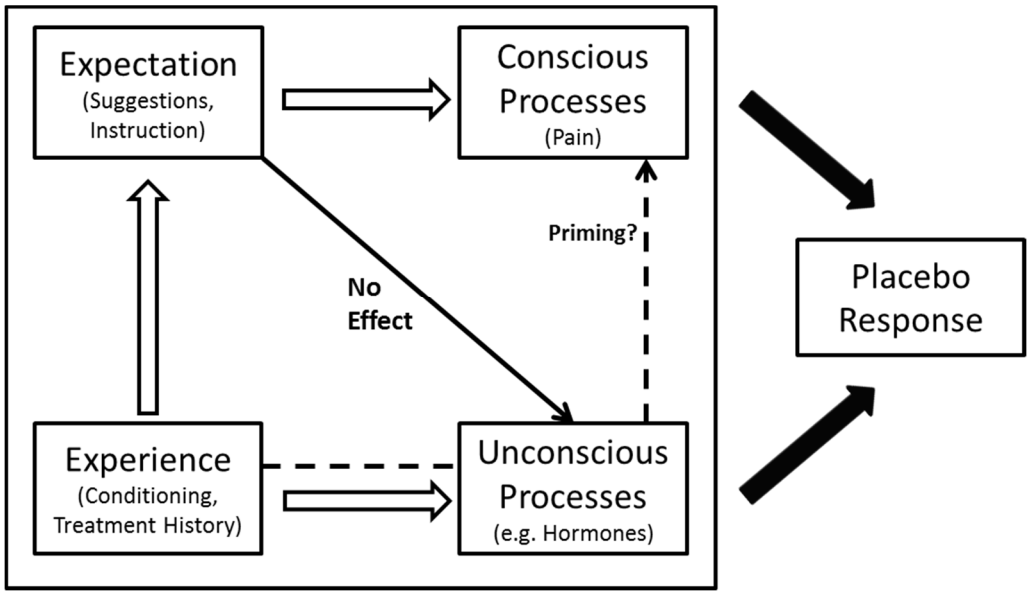


***Fig. 1*.** A model to describe the establishment of placebo responses; slightly modified according to Benedetti et al. (2003).

However, this concept probably needs to be refined to a certain degree since just recently, a conditioning procedure alone was demonstrated to be sufficient for inducing placebo analgesia (Jensen et al., 2012). What is more, in a second experiment, even the subliminal presentation of the CS+ was sufficient to evoke a placebo response, and by that bypassed a conscious expectation (see the dashed line in ***Fig. 1***), likely engaging a priming process (Jensen, et al., 2012).

In an elegant attempt to disentangle the influence of expectation and experience for the constitution of placebo effects two studies were performed to determine the impact of each respective process (Voudouris, Peck, & Coleman, 1989; Voudouris, et al., 1990). The authors compared the impact of a placebo suggestion (analgesic cream) in combination with or without an additional placebo conditioning procedure (placebo cream is paired with lower pain stimulation, control cream is paired with higher pain stimulation) for the resulting placebo response. It was found that a conditioning procedure was enough to induce placebo analgesia, but that it was most pronounced when combined with an additional placebo instruction. A placebo instruction alone, which was designed to induce a positive expectation in the participants, did not produce a significant placebo response. Consequently the authors conclude that a conditioning procedure and the resultant manipulation of experience would be the more relevant aspect for inducing placebo analgesia (Voudouris, et al., 1990). In

another attempt to investigate these essential factors contributing to the placebo effect, Montgomery and Kirsch (1997) applied a similar paradigm and first surreptitiously lowered the level of pain stimulation in a placebo conditioning phase to generate the impression of a pain easing effect of a sham analgesic cream. Afterwards, one group of participants was debriefed about the conditioning procedure, while the second group was kept blinded regarding the experimental manipulation. The results of the subsequent test phase revealed significant placebo responses solely in the deceptive group, suggesting that expectation was indispensable for generating the placebo response, whereas the contribution of learning alone was found to be much lower. An additional regression analysis, comparing the contribution of expectancy and learning to the actual placebo response, revealed that nearly all the variance could be explained by expectancy, whereas the contribution of experience was rather low (Montgomery & Kirsch, 1997). In the meantime a number of studies aimed at further elucidating the characteristics of the involved learning mechanisms. Most often, combined placebo instructions plus placebo conditioning paradigms were realized. In this vein, it could be shown that the length of the learning history - i.e., the number of trials during the placebo conditioning phase - moderates the magnitude of the placebo response and its stability over time (Colloca & Benedetti, 2006; Colloca, Petrovic, Wager, Ingvar, & Benedetti, 2010). Furthermore, it could be demonstrated that a nocebo effect (i.e., the elevated perception of pain compared to a control condition) can be elicited faster than a placebo effect and persists even after a short learning interval (Colloca, et al., 2010). What is more, it could be shown that a placebo learning experience not necessarily needs to be accomplished on a first person level, even the mere observation of a social model was sufficient to induce a placebo response in the observer (Colloca & Benedetti, 2009).

### 1.3.2 Nocebo Hyperalgesia

In contrast to the amount of research focusing on the placebo effect and pain, the converse nocebo effect has been investigated to a much lesser degree. This is likely due to ethical issues which come up when purposely aiming at worsening a symptom or even inducing a negative psychological or physiological situation (Benedetti, 2009). The impact of a negative suggestion on a pain treatment was demonstrated by Dworkin et al. (1983) who could reverse the analgesic effect of nitrous oxide - an analgesic - by a negative instruction which the participants received prior to drug administration. In a related manner, the analgesic effect of a potent painkiller (the opioid agonist remifentanil) was completely blocked

by telling the participants that the analgesic drug infusion was stopped, although the drug administration was actually continued (Bingel, et al., 2011). Some of the involved processes for the induction of nocebo effects seem similar to those determined for the placebo effect, which are experience and expectation. However, expectancy seems to play a key role especially for nocebo effects. In a study by Colloca et al. (2008) participants were either told that an electrical sham stimulation would have an analgesic or hyperalgesic effect, which was further supported in half of the experimental groups during a placebo or nocebo conditioning phase. While placebo effects relied on the actual experience - generated during the conditioning phase - nocebo effects were accomplished solely by a negative instruction (Colloca, Sigaudo, et al., 2008). In a following study it could be shown that the strength of the learning experience - which was manipulated by varying the length of the conditioning phase - was crucial for the induction of a placebo effect, though less relevant for the magnitude of the nocebo effect (Colloca, et al., 2010). Just recently, it could be shown that the observation of someone showing elevated pain signals after the application of a sham hyperalgesic procedure promoted a nocebo driven hyperalgesic effect in the observer (Vögtle, et al., 2013). These findings thereby further underscore the relevance of social learning processes for the actual treatment outcome.

The biological basis of the nocebo effect was investigated in a set of studies by Benedetti and colleagues. Based on the findings that revealed the administration of proglumid, a cholecystokinin (CCK) antagonist, to increase opiate-analgesia and placebo-analgesia (Benedetti, 1996), its potential role for the modulation of nocebo effects was investigated. The application of proglumid was found to abolish the pain increasing effect of a nocebo manipulation that encompassed the application of a sham hyperalgesic medication (Benedetti, Amanzio, Casadio, Oliaro, & Maggi, 1997). The transmission of CCK is further a common finding during the experience of anxiety (Lovick, 2008). Since the induction of a nocebo effect involves the anticipation of a negative outcome that is potentially anxiogenic, elevated transmission of CCK is likely. In this vein, Benedetti et al. (2006) compared the effects of an anxiolytic benzodiazepine and a CCK antagonist on nocebo hyperalgesia. They could show that administration of both drugs blocked the nocebo effect, but that solely during benzodiazepine administration physiological responses of anxiety were reduced as well. These findings demonstrate the specificity of the CCK-ergic transmission during nocebo conditions. Wiech and Tracey (2009) hypothesized the transmission of CCK in the PAG to be a nocebo-

specific - placebo-opposing - mechanism that probably should be explored in more detail within futures studies.

Taken together, the studies on placebo analgesia and nocebo hyperalgesia as reviewed above compellingly demonstrate the relevance of experience and expectancy for the induction and moderation of placebo and nocebo effects. However, all these studies have been performed using agents or treatments such like pills, creams or syringes that may be linked to prior experiences with medical interventions or medications. It seems inevitable to think of a placebo/nocebo manipulation that does not interfere with any earlier experience of a participant when investigating the contribution of experience and expectancy to the establishment of placebo and nocebo effects.

### 1.3.3 Neuronal Correlates of Placebo Analgesia and Nocebo Hyperalgesia

The advent of functional brain imaging techniques allowed investigating the neural underpinnings of the involved mechanism during the induction of placebo effects on pain. Probably one of the most influential fMRI studies in this field was conducted by Wager et al. (2004). They manipulated the participants' expectation and experience about a sham analgesic cream during a placebo conditioning phase, analogously to the study by Montgomery and Kirsch (1997). They surreptitiously lowered the level of pain stimulation on patches of placebo-treated skin, whereas they applied always the same amount of pain on a control site. Afterwards participants entered a test phase, and received the same pain level on all stimulation sites. They found higher pain ratings for pain stimulation of control sites compared to placebo-treated patches of skin, which was also reflected on the neural level by higher activation of areas that were found to be activated during pain processing such as the anterior insula, the contralateral thalamus and the rACC. Moreover, during an anticipation phase (before the actual pain stimulation started) they found elevated activity in the DLPFC and midbrain structures, which the authors interpret as a prefrontal initiation of subcortical opioid transmission. The actual involvement of endogenous opioids and brain structures that revealed high density of opioid receptors such as the PAG, the DLPFC, the insula and the rACC during placebo analgesia could be shown in two complementary studies that measured PET and the binding potential of $^{11}$C-radiolabeled carfentanil, a μ-opioid receptor agonist (Wager, Scott, & Zubieta, 2007; Zubieta, et al., 2005). The activation of the rACC and midbrain structures during placebo analgesia could be replicated in a number of following studies

(Bingel, Lorenz, Schoell, Weiller, & Buchel, 2006; Geuter, Eippert, Hindi Attar, & Buchel, 2013). The results are interpreted with regard to the activation of the descending nociceptive control system during placebo conditions. This was further corroborated by a functional connectivity analysis revealing concurrent activity of the rACC and the PAG, and also by demonstrating the modulation of pain signals in the dorsal horn of the spinal cord (Eippert et al., 2009; Eippert, Finsterbusch, Bingel, & Buchel, 2009).

Evidence from functional brain imaging data for nocebo hyperalgesia instead, is much rarer. A study that investigated the impact of negative outcome expectations -that is at least a crucial shared feature with regard to nocebo paradigms- found elevated responses of hippocampus and entorhinal cortex to be involved during the anxiety-related increase of pain (Ploghaus, et al., 2001). One of the very few imaging studies that actually administered a nocebo manipulation was conducted by Kong et al. (2008). Participants were led to believe that a sham acupuncture treatment would result in augmented pain perception, which was further supported by a nocebo conditioning phase. Results of the subsequent test phase showed elevated signals in pain-associated areas like the ACC and the insula, and in addition, the left hippocampus. Moreover, enhanced functional connectivity between the hippocampus and the bilateral insula, the ACC, the pre- and postcentral gyrus suggests a key role of the hippocampus for the induction of nocebo hyperalgesia. A recent fMRI study by Bingel and colleagues (2011) that investigated the interaction of negative expectations with the effectiveness of a pain killer also found that the loss of the drug effect due to a verbal (nocebo) instruction was paralleled by elevated hippocampal activity. A recently published functional imaging study revealed, that even at the level of spinal cord nociceptive signaling is enhanced during nocebo compared to control conditions (Geuter & Buchel, 2013).

### 1.3.4 Additional Influences on the Placebo Effect

Placebo responses were found to be modulated by the characteristics of the treatment itself as well as trait variables of the individual receiving a placebo. With regard to the first aspect, Kaptchuk and colleagues (2010) compared the effectiveness of a placebo pill with sham acupuncture and found stronger long term effects for the sham acupuncture. In addition, the placebo acupuncture was more compelling and effective in convincing the patients of having received the verum treatment, compared to the patients that solely were taking placebo pills. The authors interpret these findings in light of the relevance of medical

rituals and physical interventions which support a positive treatment outcome. In a related manner, it was found that the price of a medicament is a putative predictor for its effectiveness, such that an expensive (placebo) pain killer was found to be more effective than a cheap one (Waber, Shiv, Carmon, & Ariely, 2008). Just recently this finding was further corroborated in a brain imaging study comparing two placebos differing in market price. The pricy placebo was found to elicit stronger behavioral effects, which were also mirrored on the neural level by higher activation of the rostral ACC (Geuter, et al., 2013).

With regard to the treatment surrounding, Benedetti (2009) postulated that the study of placebo and nocebo effects actually represents the investigation of the psychosocial context of medical interventions. For instance, the treatment surroundings or the interaction of patient and caregiver crucially impact on the outcome of an intervention (Benedetti, 2009). Besides, personality traits were found to modulate the placebo response. In this vein it was shown that optimistic participants expressed stronger analgesic placebo responses (Morton, Watson, El-Deredy, & Jones, 2009), the same was true for highly suggestible participants (De Pascalis, Chiaradia, & Carotenuto, 2002). In addition, participants that were found to be especially sensitive to external rewards were found to be better placebo responders (Scott et al., 2007). In a very recent experiment that combined personality measures, placebo responses and the associated neural opioid transmission, it could be shown that participants scoring high in resilience and agreeableness (an attitude encompassing for instance straightforwardness and compliance) revealed stronger placebo analgesia and higher endogenous opioid transmission (Pecina, et al., 2013). All these attempts to identify characteristics of placebo responders seem fruitful future directions, pointing at individually tailored treatments and maximizing placebo effects. However, since many of the conducted studies aiming at inter individual differences and placebo responsiveness so far, deal with small sample sizes and reveal inconsistent results, findings need to be interpreted with caution (Colloca, Klinger, Flor, & Bingel, 2013).

### 1.4 Emotions interact with Placebo and Nocebo Effects on Pain

The studies as reviewed so far demonstrate that cognitive and emotional manipulations as well as placebo and nocebo procedures heavily alter the perception of pain. Supposedly, the modulation of pain by placebo, nocebo and emotions rely on shared mechanism and involve similar processes. A recent review article discusses the central role of

emotions for the induction of placebo effects and hypothesizes that a placebo response is (in part) due to the reduction of negative and induction of positive emotions in the recipient (Flaten, Aslaksen, Lyby, & Bjørkedal, 2011). Indeed it could be shown that a placebo manipulation resulted in a decrease of negative affect (Scott, et al., 2007). On the contrary, it was found that the induction of anxiety due to the announcement of an aversive electrical shock during the experiment reduced the effectiveness of a placebo manipulation, indicating the influence of concurrent mood on treatment efficacy (Lyby, Forsberg, Åsli, & Flaten, 2012). Likewise, nocebo effects were found to come along with - or even are the result of - anxiety that is induced when anticipating a negative outcome or symptom worsening (Benedetti, et al., 1997). Moreover, the involved neurotransmitter CCK was found to specifically mediate nocebo responses on the one hand and was also shown to be involved during acute stress and feelings of anxiety on the other. The strong connections of nocebo inductions and negative emotions become further apparent by their shared neural substrates: Increased activation of a hippocampal, parahippocampal network was found during the exacerbation of pain during anxiety inductions (Ploghaus, 2001) or negative picture processing (Roy, et al., 2009), and also for the induction of nocebo hyperalgesia (Kong, et al., 2008) or the blockade of analgesia by instruction (Bingel, et al., 2011). In addition, the modulation of pain by emotion is discussed to rely on the activation of the descending pain modulatory system (Bushnell, et al., 2013; Wiech & Tracey, 2009), which was also found to mediate the analgesic effect of placebo treatments (Eippert, Bingel, et al., 2009)

Therefore the question arises, how the modulation of pain by emotion, would interact with a placebo manipulation. Such research would allow evaluating the relevance of concurrent affective information for placebo-nocebo inductions. At the same time it would be possible to determine, whether the modulation of pain by emotion is a stimulus-driven, bottom-up mechanism or whether it can be additionally shaped by a top-down driven process arising from placebo-nocebo manipulations.

### 1.5 Aim of this Dissertation

In the following, three experiments will be presented, which were designed to further elucidate essential, psychological preconditions and processes that are involved in the establishment and modulation of placebo-analgesia and nocebo-hyperalgesia as well as the modulation of pain by emotion and cognition.

As reviewed in section **1.3.1** the probably most commonly discussed mechanism mediating placebo and nocebo effects are prior *experiences* - i.e., learning processes that involves the encounter of positive or negative treatment effects -, on the one hand, and *expectancy* - i.e., assumptions or attitudes about a treatment resulting from verbal instructions or prior experiences - on the other hand. The separation of experience and expectation processes is not possible when applying sham treatments which participants in any resembling way might already have experienced earlier. Therefore, the examination of the independent contributions of both processes inevitable asks for a treatment that most probably nobody would have encountered before. Therefore in **Experiment 1,** a non-pharmacological placebo-nocebo intervention was implemented to manipulate stepwise and independently the level of experience and expectation across three different groups of participants, and further to evaluates the feasibility of a mere psychological placebo-nocebo treatment. Based on earlier studies which administered medication-like placebo interventions, it was hypothesized that placebo-nocebo effects would be largest when a placebo-nocebo conditioning procedure would be combined with an accordant placebo-nocebo instruction. In addition, experience alone (only placebo-nocebo conditioning) or expectation alone (only placebo instruction) supposedly might result in a significant (but smaller) physiological and behavioral differentiation between placebo and nocebo conditions as well, however, whether a mere psychological placebo treatment would be sufficient to modulate the processing of pain in general, was to be examined.

In addition to learning and expectation processes, **Experiment 2** and **Experiment 3** focused on the role of emotions for the induction of placebo-nocebo effects**.** The modulation of pain by positive and negative emotional pictures was adapted into a psychological placebo-nocebo paradigm to evaluate the influence of affective valence on the induction and magnitude of a placebo-nocebo response. In addition, it was investigated whether emotional pain modulation is prone to an additional placebo-nocebo manipulation. Therefore in **Experiment 2**, two groups of participants were compared that received orthogonal placebo-nocebo instructions in accordance with a placebo-nocebo conditioning phase. One group of participants was told that positive emotional pictures will decrease the perception of pain (placebo) and negative emotional pictures will increase the perception of pain (nocebo). A second group was told the exact opposite (positive pictures = nocebo; negative picture = placebo). In a control group, which received no specific placebo-nocebo instruction, the

principal capacity of the selected affective pictures to actually modulate pain was determined. It was hypothesized that - given the assumptions of additive congruency effects - positive pictures cueing placebo and negative pictures cueing nocebo (congruent group) would result in elevated differentiation of placebo and nocebo conditions for physiological (facial electromyography, SCR) and behavioral pain measures compared to the incongruent group (negative pictures cueing placebo and positive pictures cueing nocebo). This hypothesis is based on the findings as reviewed in 1.4, showing a close connection of positive affect and placebo, as well as negative affect and nocebo responses.

To elucidate the neural underpinnings of these effects, in **Experiment 3** the paradigm established in Experiment 2 was transferred to an fMRI study. It was hypothesized, based on findings from functional imaging studies investigating nocebo hyperalgesia and placebo analgesia (see 1.3.3), to find elevated activation in placebo-mediating areas such as the DLPFC and the rACC when comparing placebo with nocebo trials. Further, the placebo-nocebo manipulation was assumed to result in the modulation of brain structures that were commonly found to be involved in the processing of pain. Therefore, it was expected that the comparison of nocebo and placebo trials would result in elevated activity of the ACC, the insular, and the somatosensory cortex. In accordance with the literature on neural correlates of nocebo-hyperalgesia and negative emotions impacting pain, the involvement of the hippocampus and/or adjacent areas moderating the nocebo response was assumed. Analogue to the argumentation described for Experiment 2, results were expected to be more pronounced for the congruent compared to the incongruent placebo-nocebo manipulation. Further, group differences were explored to evaluate possibly diverse mechanism across the two experimental conditions.

Overall, the three studies presented in this dissertation were intended to advance our understanding of cognitive-emotional mechanisms involved in the generation of placebo-nocebo responses and to further scrutinize the impact of emotion on pain. Furthermore, the feasibility of mere psychologically mediated placebo and nocebo effects to actually modulate the perception of pain should be investigated.

## 2. Experiment 1: The Contribution of Expectancy and Experience in a Psychologically Induced Placebo Analgesia Paradigm

Prior experiences and positive expectations are discussed as crucial mediators of placebo effects, see Stewart-Williams & Podd (2004) for a thorough overview. Both, experiences and expectations are shaped by a variety of processes, of which several have already been investigated in the context of placebo analgesia such as social observational learning (Colloca & Benedetti, 2009), classical conditioning (Bingel, et al., 2006; Colloca & Benedetti, 2006; Jensen, et al., 2012; Lui et al., 2010), or verbal instruction (Amanzio & Benedetti, 1999; Benedetti, et al., 2003; Voudouris, et al., 1990). Accordingly, patients or individuals receiving medical care, frequently encounter clinical interventions in association with stimuli or contexts like syringes, medications, white coats or the hospital itself which may serve as cues (CS+) associated with an actual drug or treatment (UR). These cues may be capable of eliciting conditioned reactions themselves (CR) like symptom decrease - or in the case of nocebo - symptom increase. Importantly, these experiences also shape future expectations which may modulate placebo or nocebo effects also. Consequently, investigating experience and expectancy effects on placebo mechanisms separately is rather difficult. When using any placebo agent that is somehow familiar to the participant and/or is linked to a learning history of medical interventions it might be impossible to separate effects of expectation and experience. To disentangle experience and expectation, it seems indispensable to think of placebo manipulations that are free of any previous experience, learning history, expectation, or pharmacological plausibility to the participant. So far, placebo experiments cannot distinguish experience and expectation effects because they almost always were conducted using medicine-like treatments such as inert creams (Bingel, et al., 2006; Wager, et al., 2004), pills (Kaptchuk et al., 2006), prickling nasal sprays (Rief & Glombiewski, 2012) or involved procedures that resemble medical, pain easing interventions such as the (sham) application of low current electrical stimulation (Colloca, et al., 2010).

The present study aimed at manipulating expectation and experience independently from each other and therefore realized a merely psychological placebo paradigm presenting stimuli which are not associated with any medical treatment effect or intervention. Experience and expectation were experimentally manipulated to determine whether each manipulation itself or only both in concert are capable of eliciting a placebo-nocebo effect on the perception

of pain. To this end, we compared three groups of participants. One group was only instructed about analgesic or pro-algesic effects of two specific types of black and white stripe patterns (expectation). The second group only experienced heat stimuli being more or less painful in association with two different black and white stripe patterns (experience). The third group finally, was instructed about the analgesic/pro-algesic effect of the visual stimuli and additionally experienced the heat stimuli being more or less painful during a conditioning procedure accordant with the placebo-nocebo instruction (expectation + experience). To measure the impact of the different placebo-nocebo manipulations on pain, pain ratings (sensory and affective) and physiological measures that were found to respond to the perception of pain such as facial electromyography (EMG) (Reicherts, et al., 2013) and SCR (Breimhorst, et al., 2011; Loggia, et al., 2011) were obtained. We hypothesized that each of the three placebo-nocebo manipulations would be capable of altering the perception of pain, thus resulting in higher pain ratings and elevated physiological responses following the administration of pain during nocebo compared to placebo trials. In accordance with the literature on medicinally or pharmacologically plausible placebo treatments (e.g., sham electrical stimulation or analgesic cream, see Colloca & Benedetti, 2006; Voudouris, et al., 1990) we further expected the strongest effects for the group receiving the combined placebo manipulation (expectation + experience) compared to the two other groups.

## 2.1 Method

### 2.1.1 Participants

Sixty-five participants (32 women; mean age 23.62 years, *SD* = 3.18) were recruited at the University of Würzburg and participated in the study. Participants received course credit or € 12 as compensation. None of them had taken any analgesic medication 24h prior to the test session (self-report). Participants were randomly allocated to one of the three experimental groups, which varied according to the written instructions and experimental manipulations: experience, expectation, or, experience + expectation (for further details see ***Fig. 3*** and paragraph 2.1.2). Participants filled out questionnaires on state and trait anxiety (Spielberger trait and state anxiety inventory, STAI-T/S) (Laux, Glanzmann, Schaffner, & Spielberger, 1981; Spielberger, 1970), positive and negative mood, (Positive and Negative Affect Schedule, PANAS, Krohne, Egloff, Kohlmann, & Tausch, 1996; Watson, Clark, & Tellegen, 1988), pain catastrophizing (PCS) (Meyer, Sprott, & Mannion, 2008; Sullivan, Bishop, & Pivik,

1995), life orientation, that is dispositional optimism and pessimism (LOT by Glaesmer, Hoyer, Klotsche, & Herzberg, 2008), and sensitivity for reward and punishment (SPSRQ) (Torrubia, Ávila, Moltó, & Caseras, 2001) in the German translation (Hewig & Hagemann, 2002: Der SPSR Fragebogen von Torrubia, Ávila, Moltó & Caseras, unpublished German translation, University of Trier, personal communication). Further, socio-demographic information and personal attitudes towards pain were assessed. The groups did not statistically differ with regard to state or trait anxiety or state mood, attitudes towards pain or any other of the psychometric measures. Only the analysis of the mean age revealed a significant difference between the three groups, $F(2,62) = 5.87$, $p = .005$, with the experience group ($M$ = 24.65 years; $SD$= 3.45) and the expectation + experience group ($M$= 24.39 years; $SD$= 2.45) being somewhat older than the expectation group ($M$=21.86; $SD$ = 3.18), for further details see **Tab. 1**. All subjects had normal or corrected-to-normal vision, and no current or prior history of chronic pain, neurological, or psychiatric disorders (self-report). The experimental procedure was approved by the institutional review board of the Medical Faculty of the University of Würzburg.

**Tab. 1**. Sample Description of Experiment 1

| Measure | Exp. (n=20) | | Expect.(n=22) | | Expect.+Exp.(n=23) | | | |
|---|---|---|---|---|---|---|---|---|
| | M | SD | M | SD | M | SD | $F(1,64)$ | $p$ |
| Pain threshold (°C) | 46.58 | 2.74 | 46.55 | 2.20 | 46.55 | 2.18 | 0.00 | .99 |
| Age | 24.65 | 3.45 | 21.86 | 2.96 | 24.39 | 2.44 | 5.87 | .01 |
| LOT_R | 7.00 | 3.67 | 7.91 | 4.45 | 8.09 | 4.07 | 0.43 | .66 |
| PCS_Sum | 19.65 | 11.44 | 19.27 | 8.99 | 15.95 | 6.67 | 1.06 | .35 |
| STAI_Trait_Sum | 35.20 | 7.86 | 39.86 | 9.21 | 36.43 | 9.73 | 1.53 | .22 |
| STAI_State_Sum | 31.05 | 8.33 | 32.64 | 5.75 | 32.04 | 5.19 | 0.31 | .73 |
| PSQ_Total | 59.60 | 18.15 | 59.45 | 19.88 | 59.35 | 20.10 | 0.00 | .99 |
| PANAS_Positive | 32.42 | 6.37 | 29.00 | 5.44 | 31.39 | 5.46 | 1.96 | .15 |
| Panas_Negative | 11.84 | 4.39 | 10.86 | 0.71 | 11.61 | 1.78 | 0.79 | .46 |
| SPSRQ_Punishment | 37.30 | 4.37 | 38.50 | 4.66 | 37.43 | 4.28 | 0.47 | .62 |
| SPSRQ_Reward | 40.65 | 4.00 | 37.95 | 4.91 | 40.13 | 4.89 | 2.04 | .14 |

***Note:*** LOT= Life Orientation Test; PCS= Pain Catastrophizing Scale; STAIT/S= State/Trait Anxiety Inventory; PSQ = Pain Sensitivity Questionnaire; PANAS= Positive Affect/Negative Affect Schedule; SPSRQ= Sensitivity for Punishment, Sensitivity for Reward Questionnaire; Exp. = Experience Group; Expect. = Expectation Group; Expect. + Exp. = Expectation + Experience Group.

### 2.1.2 Experimental Groups and Placebo-Nocebo Manipulation

The three experimental groups varied according to (a) the instruction given prior to the experiment, and to (b) the procedure of the first phase of the experiment (placebo

conditioning). Two groups, the expectation and the expectation + experience group, were informed that previous research proved that pictures displaying vertical or horizontal black and white stripe patterns (see ***Fig.* 2**)are capable of increasing or decreasing the perception of pain, respectively. This combined placebo-nocebo instruction was given to induce a distinct, orthogonal expectation in the participants about the effects of these visual stimuli on their pain perception. The third group - experience - instead was told to take part in an experiment that was designed to basically investigate the perception of heat pain. For further details and the exact wording of the instruction see **Suppl. 1**. After reading the instruction, the expectation + experience group and the experience group underwent a placebo-nocebo conditioning phase, watching the placebo or the nocebo cues while receiving low heat pain stimuli (pain threshold temperature) or high heat pain stimuli (pain threshold + 1°C), respectively. This procedure was intended to make the participants actually experience the effectiveness of the placebo/nocebo cues, and to allow them to learn the contingency between the visual placebo/nocebo cues and high/low pain stimuli (experience). The expectation group watched a central fixation cross and received in random order the same number of high on low painful heat stimuli (pain threshold vs. threshold + 1°C) analogous to the two other groups. As a consequence, all groups received the same amount of heat stimulation during the experiment. For further details about the manipulation see ***Fig.* 3** and paragraph 2.1.8 describing the procedure.

### 2.1.3 Visual Placebo/Nocebo Cues

Placebo and nocebo cues consisted of vertically and horizontally oriented black and white stripe patterns (see ***Fig.* 4**). Stimuli were counterbalanced across participant regarding their function as an indicator for placebo or nocebo, respectively. Since Lui et. al. (2010) reported differences in the effectiveness of inducing a placebo effect depending on cue color, e.g., using a red or green pill, we avoided to use colored stimuli. The visual placebo and nocebo cues had a resolution of 756 x 756 pixels and were presented centrally on a 17” computer screen, around 80 cm away from the participants.

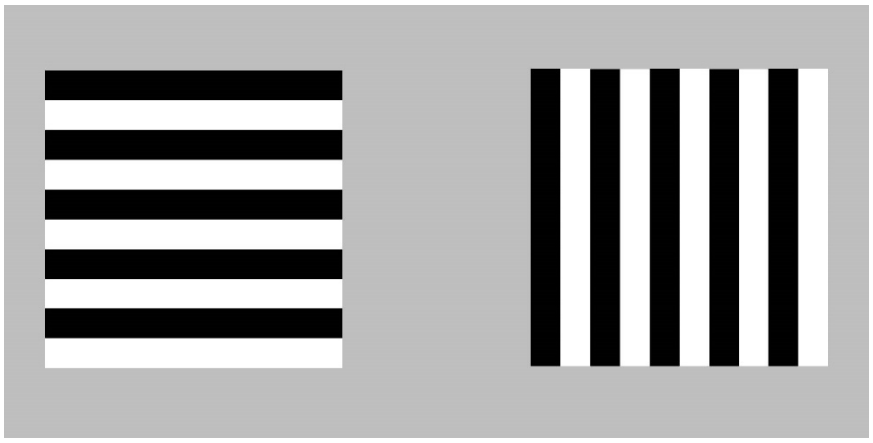

***Fig. 2***. Placebo and nocebo cues.

### 2.1.4 Pain Threshold Assessment

The pain threshold procedure was explained to the participants via written instruction on the computer screen. Thermal heat stimuli were delivered using a Somedic MSA thermal stimulator (Somedic Sales AB, Hörby, Sweden) and a Peltier thermode with an active surface of 25 x 50 mm. The thermode was attached to the participants' non-dominant forearm, while they held a stop device in their other hand. According to the method of limits procedure (Gescheider, 1985) the participants were asked to press the stop signal as soon as they felt the heat to start being painful. This written instruction was presented as a reminder throughout the whole threshold assessment. A series of 10 gradually ascending heat stimuli starting at 32°C, heating up (and cooling down) with 1°C/s were presented to the participants and the resulting mean threshold temperature was used as reference temperature for the following experiment. The average pain threshold temperature was $M$ = 46.56 °C, $SD$ = 2.34°C (groups did not differ from each other, $F < 1$), see **Tab. 1**.

### 2.1.5 Thermal Pain Stimulation and Pain Ratings

During the conditioning phase, two different levels of pain stimuli were delivered. For placebo trials the individual thermal pain threshold was administered whereas during nocebo trials the threshold temperature + 1°C was administered. During the subsequent test phase pain threshold + 1°C was administered for placebo as well as for nocebo trials. This procedure is similar to the design by Colloca and Benedetti (2006), which also consisted of two conditions (placebo vs. control) except that electrical stimulation instead of heat pain was administered. During all trials the heat stimulation started from a baseline temperature defined as 10°C

lower than the individual nocebo temperature and rose with a ramp of 5°C/s until the target temperature was achieved. The temperature remained on the plateau for 3 s and then cooled down to the baseline temperature with a ramp of 1°C/s. Participants rated the pain stimuli regarding pain intensity and pain unpleasantness by adjusting a red mark on a 100 step, digitized visual analogue scale (VAS) ranging from 0 = no pain at all to 100 = unbearable pain using a PC keyboard.

### 2.1.6 Skin Conductance Measurement

Two 22/10 mm Ag/AgCl surface electrodes, filled with electrode cream (concentration of 0.5% NaCl) were attached to the thoroughly cleaned thenar and hypothenar eminence of the participants' non-dominant hand. Skin conductance was recorded with a sampling rate of 1000 Hz, constantly applying 0.5V, using a V-Amp amplifier (Brain Products Inc., Munich, Germany) and recording software (Brain Vision Recorder). SC signals were segmented in time windows of 20 s after visual stimulus onset and a 1000 ms pre-stimulus baseline was subtracted. Skin conductance responses were quantified as highest positive deflection in two respective time windows. For responses to the visual placebo/nocebo cues a peak interval from 1 s to 6 s and for pain responses an interval from 6 s to 19 s was used. Two participants showed no SCR during the whole test phase and therefore were excluded from the data analysis.

### 2.1.7 EMG Measurement

EMG was recorded from M. corrugator supercilii, M. orbicularis oculi and M. zygomaticus major on the left side of the face (Dimberg & Petterson, 2000) using bipolar montages of 13/7 mm Ag/AgCl surface-electrodes according to guidelines established by Fridlund and Cacioppo (1986). Facial muscles were chosen based on earlier findings which suggest facial pain responses in these or closely adjacent areas (Kunz, et al., 2004; Prkachin, 1992). The EMG raw signal was measured with a V-Amp amplifier (Brain Products Inc., Munich, Germany) at a sampling rate of 1000 Hz. Raw signals were rectified and filtered off-line with a 30 Hz high-pass, a 500 Hz low-pass, a 50 Hz notch filter and integrated with a moving average using 200 ms time windows. EMG responses to the placebo/nocebo cues were scored as mean activity from 0 to 3 s after visual cue onset as a change in activity from a -1000 ms baseline before trial onset. Pain associated EMG responses were scored as the mean activity during an interval of 9 to 12 s after placebo/nocebo cue onset (= 6 s to 9 s after heat stimulus onset) as

change in activity from a -1000 ms baseline before trial onset. Data quality was evaluated by visual inspection and resulted in the exclusion of 5 participants (4 from the expectation, 1 from the experience group) due to excessive artifacts.

### 2.1.8 Procedure

On arrival, participants were randomly assigned to one of the three experimental groups, read and signed informed consent that included the first part of the experimental manipulation (information about the stripe pattern induced analgesia vs. mere heat pain processing experiment, see **Suppl. 1** and **Suppl. 2**), answered socio-demographic questions and filled out the questionnaires on state anxiety (STAI-S) and current mood state (PANAS). Then, the individual pain threshold was assessed. Afterwards EMG and SCR electrodes were attached and participants were instructed about the distinction of sensory and affective pain components (Price, et al., 1983) and the usage of visual analogue scales for both pain dimensions. Afterwards participants completed three training trials consisting of a centrally presented fixation cross (20 s) as well as the application and rating of a highly painful heat stimulus (pain threshold +1°C). Subsequently participants proceeded to the conditioning phase consisting of 30 trials (15 placebo, 15 nocebo trials) which was followed by the test phase consisting of 20 trials (10 placebo, 10 nocebo). During each trial a placebo or nocebo cue was presented in the center of the screen for 20 s. After 3 s the thermal stimulation was started, reached target temperature after about 2 s, and remained on the target level for 3 s. After the temperature had cooled down to the baseline level, participants were asked to rate the pain intensity and unpleasantness on a VAS. Each trial was separated by an inter-trial interval (ITI) of 4-5 s (interval was randomized) presenting a central fixation cross. During the conditioning phase the expectation + experience and the experience groups were presented nocebo cues and received highly painful heat stimuli (pain threshold + 1°C) or were presented placebo cues and received moderately painful heat stimuli (pain threshold temperature). The expectation group watched solely fixation crosses and received the same number of pain stimulations like the other groups. In the subsequent test phase all participants watched the placebo and nocebo cues again (see ***Fig. 2***) while they received only the highly painful heat stimulation (threshold + 1°C). After the test phase participants rated the nocebo and placebo cues regarding valence, arousal and threat (9 point Likert scale) and evaluated how intensive, and unpleasant they remembered the pain sensation after the respective cue (placebo vs. nocebo) using a 100 point VAS (this time, scale starting from the middle position, VAS =50).

Finally, participants filled out the remaining questionnaires (PCS, SPREQ, LOT, PSQ) and were informed about the actual purpose of the study.

### 2.1.9 Statistical Analysis

The ratings for valence, arousal and threat of the placebo and nocebo cues at the end of the experiment as well as the ratings of recalled pain were analyzed with separate 2-factorial repeated-measures ANOVAs. The within-subjects factors were placebo-nocebo (2 levels, placebo vs. nocebo cue) and the between-subjects factor group (3 levels: expectation + experience [Expect+Exp] vs. experience [Exp] vs. expectation [Expect]). Pain ratings, SCR and EMG responses of the **conditioning phase** were analyzed using separate 3-factorial repeated measures ANOVAs. The within-subjects factor were pain stimulation level (2 levels: placebo vs. nocebo), time (3 levels, each consisting of the mean of 5 consecutive trials: Trials 1-5 vs. Trials 6-10 vs. Trials 11-15) and the between-subjects factor was group (2 levels: Expect+Exp vs. Exp). Since the Expectation group was watching only fixation crosses during the conditioning phase, a separate analysis was conducted with the within-subjects factor pain stimulation level (2 levels: high vs. low) and a factor of time (3 levels: Trials 1-5 vs. Trials 6-10 vs. Trials 11-15). Regarding the **test phase** pain ratings, SCR and EMG responses were analyzed with a separate 3-factorial repeated measures ANOVAs with the within-subjects factor placebo-nocebo (2 levels: placebo vs. nocebo), the within-subjects factor time (2 levels, each consisting of the mean of 5 consecutive trials: Trials 1-5 vs. Trials 6-10) and the between-subjects factor group (3 levels: Expect+Exp vs. Exp vs. Expect). When necessary, Greenhouse-Geisser corrections of degrees of freedom were applied. Post-hoc comparisons were realized using planned contrasts or pair-wise *t*-tests. A priori significance level was set at $p < .05$. Associations of psychometric measures and placebo-nocebo outcomes were analyzed using linear correlation analysis of questionnaire scores and nocebo vs. placebo differences of the test phase conducted for sensory and affective pain ratings as well as skin conductance measures.

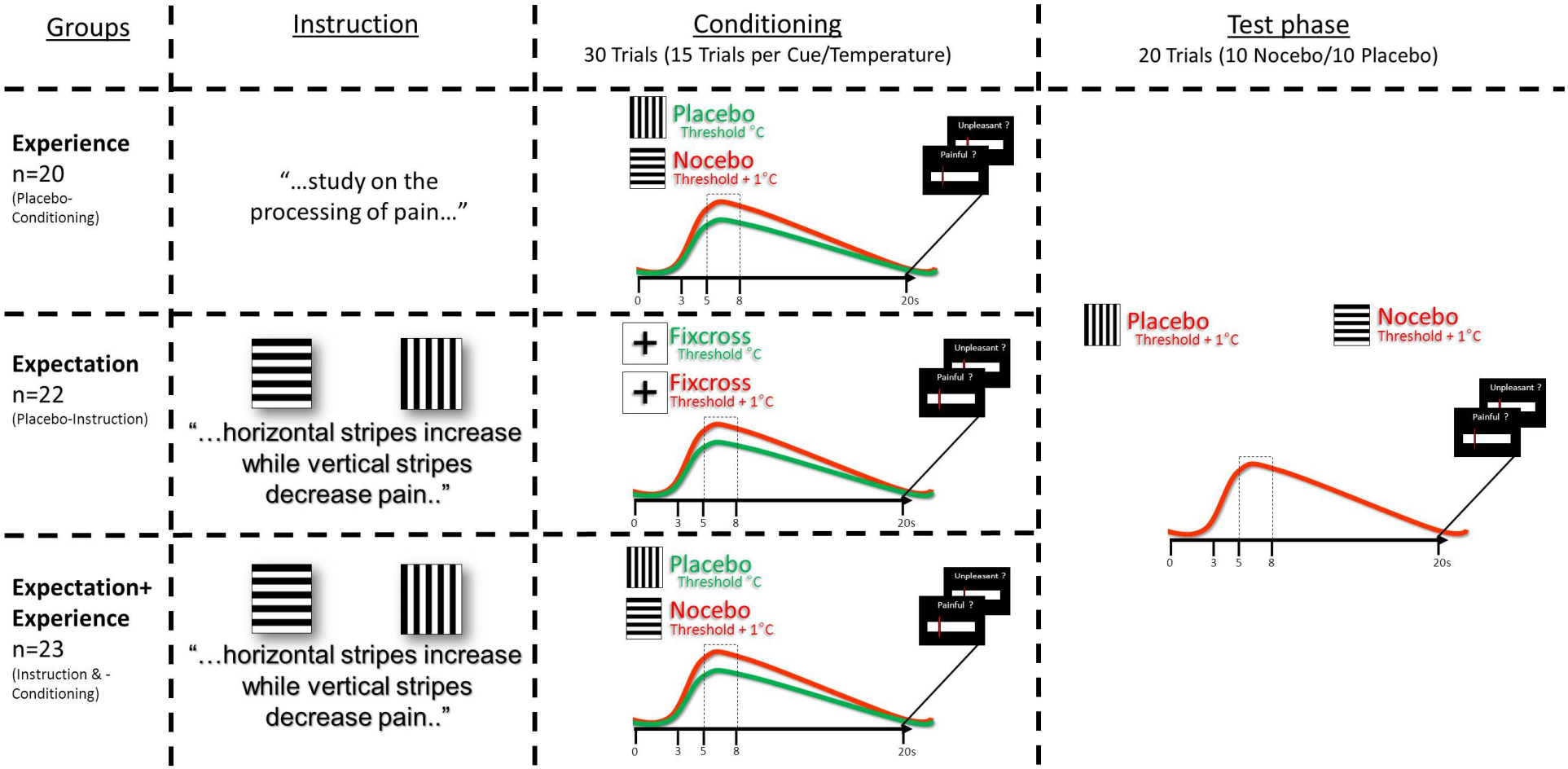


***Fig. 3.*** Experimental procedure of Experiment 1. The three experimental groups varied in terms of the instruction ("…heat pain experiment…" vs. placebo-nocebo instruction) and the type of cues, presented during the conditioning procedure (fixation cross vs. stripe pattern). In the experience and expectation + experience group, placebo cues were paired with low pain stimuli (green) and nocebo cues with high pain stimuli (red). During the test phase, all participants watched the same cues (placebo vs. nocebo) and were administered the high pain stimuli (red), only.

## 2.2 Results

### 2.2.1 Conditioning Phase

#### 2.2.1.1 SCR in Response to Placebo and Nocebo CUE (1-6s after Cue Onset) for the Expectation and Expectation + Experience Group

The analysis of skin conductance responses for the nocebo/placebo cue revealed a significant effect of time, $F(2,82) = 4.75$, $p = .01$, $\eta_p^2 = .10$, due to higher responses in the beginning of the conditioning phase (Trials 1-5) compared to the end (Trials 11-15), $F(1,41) = 7.93$, $p < .01$, $\eta_p^2 = .16$. No differences were found for placebo vs. nocebo cues, experimental group or their respective interactions (all *ps* > .20).

#### 2.2.1.2 SCR in Response to PAIN (6-19s after Cue Onset) for Expectation and Expectation + Experience

The analysis of skin conductance responses to the heat pain stimuli revealed a significant effect for the level of pain stimulation, $F(1,62) = 16.37$, $p < .001$, $\eta_p^2 = .21$, as a result of higher responses following nocebo compared to placebo pain stimulation. Also a significant effect of time was found, $F(2,124) = 21.74$, $p < .001$, $\eta_p^2 = .26$, contrasts revealed higher responses during Trials 1-5 compared to Trials 6-10, $F(1,41) = 20.37$, $p < .001$, $\eta_p^2 = .33$, and Trials 11-15, $F(1,41) = 17.80$, $p < .001$, $\eta_p^2 = .303$, respectively (see **Suppl.3 ).**

#### 2.2.1.3 EMG in Response to PAIN (9-12s after Cue Onset) for Expectation and Expectation + Experience

**M. corrugator supercilii**

The analysis of EMG responses to the heat pain stimuli revealed a non-significant trend for the interaction of time and pain stimulation level, $F(2,80) = 2.71$, $p = .10$, $\eta_p^2 = .06$. This was most likely due to higher corrugator activity for the nocebo compared to placebo stimulations during Trials 6-10, $t(41) = 1.87$, $p = .07$, and Trials 11-15, $t(41) = 1.73$, $p = .09$. In the beginning of the conditioning phase, this comparison was far from significance, Trials 1-5, $t(41) = 0.31$, $p = .76$.

**M. orbicularis oculi**

Responses of M. orbicularis oculi were higher for nocebo pain stimuli, $F(1,40) = 5.55$, $p = .02$, $\eta_p^2 = .12$. In addition, the two groups differed with regard to their responses over time

as shown by a significant interaction of group and time, $F(2,80) = 3.40$, $p = .05$, $\eta_p^2 = .08$, due to higher M. orbicularis oculi responses in Expect+Exp during Trials 11-15 compared to Exp, $t(40) = 3.44$, $p = .001$. In general, Expect+Exp showed higher orbicularis responses than Exp, $F(1,40) = 6.27$, $p = .02$, $\eta_p^2 = .14$.

**M. zygomaticus major**

The analysis of M. zygomaticus major responses revealed a nearly significant interaction of time and group, $F(2,80) = 3.00$, $p = .06$, $\eta_p^2 = .07$ that is most likely due to marginal higher zygomaticus responses during Trials 11-15 in Expect+Exp compared to Exp, irrespective of the pain stimulation level, $t(40) = 1.83$, $p = .08$.

#### 2.2.1.4 Sensory Pain Ratings for Expectation and Expectation + Experience

Pain intensity ratings were higher for the nocebo pain stimulation, $F(1,41) = 139.76$, $p < .001$, $\eta_p^2 = .773$ and increased over time, $F(2,82) = 4.53$, $p = .014$, $\eta_p^2 = .10$. These effects were further qualified by a significant interaction of stimulation level and time, $F(2,82) = 12.91$, $p < .001$, $\eta_p^2 = .24$. Separate ANOVAs for the two pain stimulation levels showed a significant effect of time only for the nocebo stimulation, $F(2,84) = 11.00$, $p < .001$, $\eta_p^2 = .21$, with higher rating for later trials. In addition, a significant 3-way interaction of Pain Stimulation Level x Time x Group was due to differences of the two groups regarding pain ratings for high nocebo compared to low placebo pain stimulations across time, $F(2,82) = 4.31$, $p = .017$, $\eta_p^2 = .10$. Post hoc comparisons revealed that in Expect+Exp ratings increased more strongly over time for the placebo stimulation (Trials 1-5 compared to Trials 6-10 and; Trials 1-5 compared Trials 11-15, $t(22) = 2.45$, $p = .02$, $t(22) = 2.30$, $p = .03$, respectively). In Exp ratings were higher for nocebo Trials 1-5 compared to the nocebo Trials 6-10, $t(19) = 2.14$, $p = .05$, but then remained on a similar level until the end of conditioning phase, nocebo Trials 6-10 compared to nocebo Trials 11-15, $t(19) = 0.22$, $p = .83$ (see **Suppl. 4**).

#### 2.2.1.5 Affective Pain Ratings for Expectation and Expectation + Experience

Pain unpleasantness ratings revealed a similar picture as the pain intensity ratings. High nocebo pain stimulation was rated as more unpleasant than low placebo pain stimuli, $F(1,41) = 126.10$, $p < .001$, $\eta_p^2 = .76$, and this effect was further qualified by a significant interaction of pain stimulation and time, $F(2,82) = 20.67$, $p < .001$, $\eta_p^2 = .34$. Separate ANOVAs for the two pain stimulation levels showed a significant effect of time only for the high nocebo stimulation, $F(2,84) = 9.12$, $p < .001$, $\eta_p^2 = .18$, with higher rating for later trials. In addition, a

marginal significant 3-way interaction of Pain Stimulation Level x Time x Group, $F(2,82) = 2.94$, $p = .059$, $\eta_p^2 = .07$, showed differences for the two groups regarding pain ratings for higher compared to low pain stimulation across time. Post hoc comparisons revealed that ratings of Expect increased more strongly from Trials 1-5 compared to Trials 6-10, $t(19) = 2.88$, $p = .01$, than this was the case in Expect+Exp (Trials 1-5 compared to Trials 6-10, $t(22) = 2.06$, $p = .05$), (see **Suppl. 5**).

#### 2.2.1.6 SCR in Response to PAIN (6-19s after Cue Onset) for Expectation

The analysis of SCR in response to the heat pain stimuli revealed only a significant effect of the factor time, $F(2,42) = 8.90$, $p = .002$, $\eta_p^2 = .30$. Planned contrasts revealed higher SCR during Trials 1-5 compared to Trials 6-10, $F(1,21) = 9.43$, $p = .006$, $\eta_p^2 = .31$, and Trials 11-15, $F(1,21) = 11.59$, $p = .003$, $\eta_p^2 = .36$, respectively (see **Suppl. 3**)

#### 2.2.1.7 EMG in Response to PAIN (9-12s after Cue Onset) for Expectation

**M. corrugator supercilii**

The analysis of M. corrugator supercilii EMG responses for high pain stimuli revealed no significant effects, regarding level of pain stimulation, time or group, (all $p$s < .21).

**M. orbicularis oculi**

The analysis of M. orbicularis oculi responses revealed a marginal significant interaction of time and pain stimulation level, $F(1,18) = 2.68$, $p = .08$, $\eta_p^2 = .13$, most likely due to marginal significantly higher activity in response to the high stimulation during Trials 6-10 when compared to the low pain stimuli, $t(18) = 1.81$, $p = .09$.

**M. zygomaticus major**

The analysis of M. zygomaticus major pain responses revealed a similar pattern, though the interaction of pain stimulation level and time failed to reach even marginal significance, $F(1,18) = 2.21$, $p = .13$, $\eta_p^2 = .11$.

#### 2.2.1.8 Sensory Pain Ratings for Expectation

Pain intensity ratings were higher for high pain stimuli, $F(1,41) = 112.58$, $p < .001$, $\eta_p^2 = .84$. This effects were further qualified by a significant interaction of pain stimulation level and the time course of the conditioning, $F(2,42) = 5.03$, $p = .01$, $\eta_p^2 = .19$. Separate ANOVAs for the two pain stimulation levels showed a significant effect of time only for the high pain

stimulation, $F(2,42) = 5.47$, $p = .023$, $\eta_p^2 = .21$, with higher rating for later trials: Trials 1-5 compared to Trials 6-10, $F(1,21) = 5.28$, $p = .03$, $\eta_p^2 = .20$, and Trials 6-10, compared to Trials 11-15, $F(1,21) = 3.19$, $p = .09$, $\eta_p^2 = .13$ (see **Suppl. 4**).

#### 2.2.1.9 Affective Pain Ratings for Expectation

Pain intensity ratings were higher for the high stimulation, $F(1,21) = 31.88$, $p < .001$, $\eta_p^2 = .60$ . This effects were further qualified by a significant interaction of pain stimulation level and the time course of the conditioning, $F(2,42) = 8.37$, $p = .002$, $\eta_p^2 = .29$. Separate ANOVAs for the two pain stimulation levels showed a significant effect of time only for the high stimulation, $F(2,42) = 3.85$, $p = .05$, $\eta_p^2 = .16$, with higher rating for later trials: Trials 1-5 compared to Trials 6-10, $F(1,21) = 4.84$, $p = .04$, $\eta_p^2 = .19$, Trials 1-5 compared to Trials 11-15, $F(1,21) = 4.03$, $p = .06$, $\eta_p^2 = .16$ (see **Suppl. 5**).

### 2.2.2 Test Phase

#### 2.2.2.1 Test Phase: SCR in Response to Placebo and Nocebo CUES (1-6s after Cue Onset)

The analysis of skin conductance responses to the placebo/nocebo cues revealed no significant effects (all *p*s > .30).

#### 2.2.2.2 Test Phase: SCR in Response to PAIN (6-19s after Cue Onset)

The analysis of SCR in response to the heat pain stimuli revealed a significant 3 way interaction of Placebo-Nocebo x Time x Group, $F(2,60) = 3.16$, $p = .05$, $\eta_p^2 = .10$. This was due to higher SCR during nocebo Trials 1-5 compared to placebo Trials 1-5, in the Expect+Exp group only, $t(21) = 2.90$, $p = .009$. Whereas all other comparison for placebo vs. nocebo trials, separate for group and time did not reach significance (all *p*s > .12) (see ***Fig.* 4**).

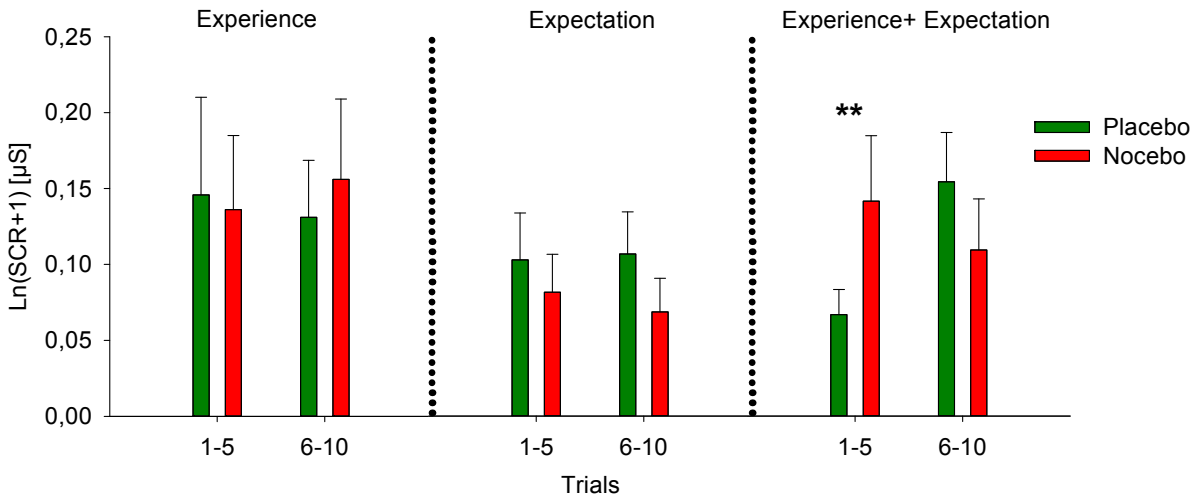


***Fig. 4.*** SCR (Means and *SEM*) during the test phase in response to the pain stimulation are depicted separately for each experimental group, split by Trials 1-5 and 6-10 of the test phase;** = $p < .01$.

#### 2.2.2.3 Test Phase: EMG Pain Responses

The analysis of **M. corrugator supercilii** EMG responses to the heat pain stimuli revealed no significant effects of placebo-nocebo, time or group, (all *ps* >. 12). **M. orbicularis oculi** responses were higher during the second half of the test phase, $F(2,58) = 4.44$, $p = .04$, $\eta_p^2 = .07$. This effect varied across the different groups as shown by a significant interaction of time and group, $F(2,58) = 3.43$, $p = .04$, $\eta_p^2 = .11$. Post hoc tests revealed significant differences between Trials 1-5 and Trials 6-10 for Exp, $t(18) = 2.25$, $p = .04$, and Expect+Exp, $t(18) = 2.54$, $p = .02$. However, this difference was far from significance for Expect, $t(18) = -0.88$, $p = .39$. The analysis of **M. zygomaticus major** pain responses revealed a marginally significant effect of time, $F(2,58) = 3.95$, $p = .052$, $\eta_p^2 = .05$. This is most likely due to higher amplitudes during the second half of the test phase, irrespective of the experimental group or placebo-nocebo condition.

#### 2.2.2.4 Test Phase: Sensory Pain Ratings

Pain intensity ratings were higher for nocebo compared to placebo trials, $F(1,62) = 9.37$, $p = .003$, $\eta_p^2 = .131$, irrespective of experimental group, $F(2,62) = 1.49$, $p = .24$, $\eta_p^2 = .05$, or time interval of the test phase, $F(1,62) = 0.52$, $p = .47$, $\eta_p^2 = .01$. There was no general difference in pain ratings across the experimental groups, $F(2,62) = 1.51$, $p = .23$, $\eta_p^2 = .05$ (see ***Fig.* 5**).

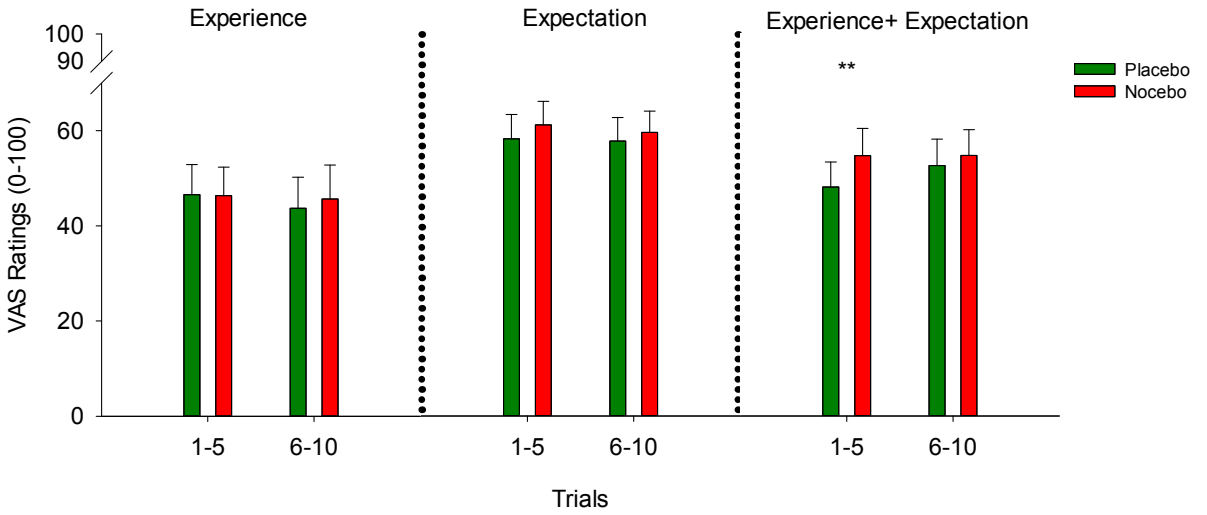


**Fig. 5.** Means and SEM of the sensory pain ratings are depicted for each experimental group, split by Trials 1-5 and 6-10 of the test phase; * = $p < .05$; ** = $p < .01$.

### 2.2.2.5 Test Phase: Affective Pain Ratings

Pain unpleasantness ratings revealed a significant effect of placebo-nocebo, $F(1,62) = 12.37$, $p = .001$, $\eta_p^2 = .17$, due to higher ratings for nocebo compared to placebo trials. This effect was further qualified by significant interaction of placebo-nocebo and group, $F(2,62) = 4.28$, $p = .02$, $\eta_p^2 = .12$. Separate ANOVAs for each group revealed a significant effect of placebo-nocebo trials for the combined Expect+Exp group, $F(1,22) = 16.47$, $p = .001$, $\eta_p^2 = .43$, instead this effect was not significant for Exp, $F(1,19) = 0.02$, $p = .88$, $\eta_p^2 = .001$ or Expect, $F(1,21) = 2.81$, $p = .11$, $\eta_p^2 = .12$ (see ***Fig.* 6**).

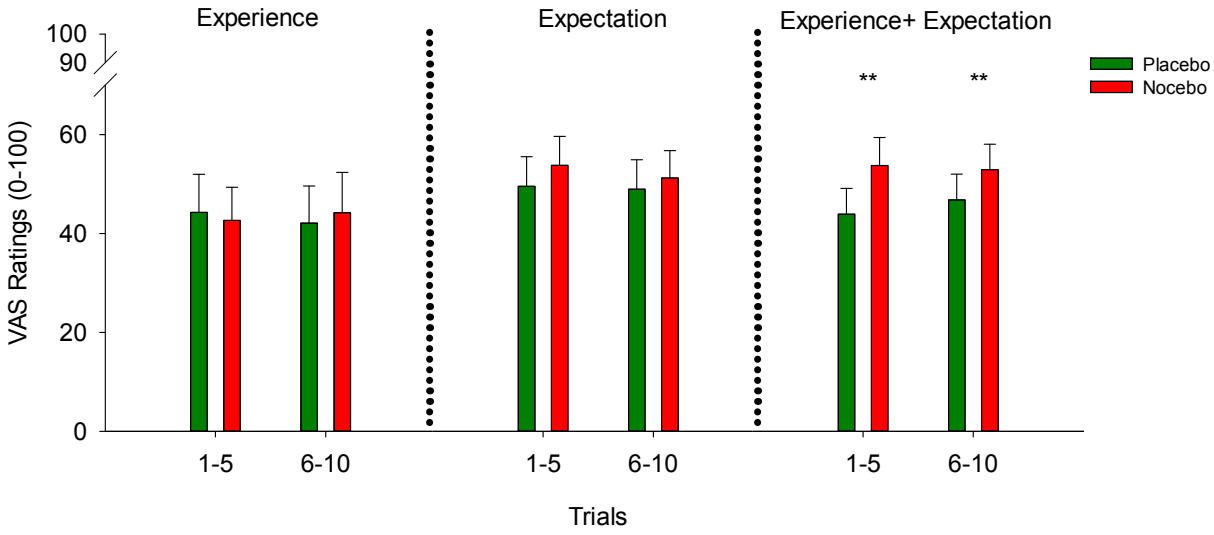


***Fig. 6.*** Means (+ *SEM*) of the affective pain ratings are depicted for each experimental group, split by Trials 1-5 and 6-10 of the test phase; ** = $p < .01$.

### 2.2.3 Affective Cue Ratings and Ratings of Recalled Pain

The analysis of the valence ratings revealed no difference for the visual placebo and nocebo cues, $F(1,62) = 0.19$, $p = .66$, $\eta_p^2 = .003$, irrespective of the experimental group. Similarly, threat ratings were not different for placebo and nocebo cues, $F(1,62) = 0.23$, $p = .64$, $\eta_p^2 = .004$, in both groups. The analysis of the arousal ratings revealed a significant interaction of the placebo/nocebo cue and experimental group, $F(2,62) = 3.93$, $p = .03$, $\eta_p^2 = .11$, due to higher arousal ratings for placebo compared to nocebo cues in Expect+Exp ($t(22) = 2.40$, $p = .03$) only (see ***Fig.* 7**).

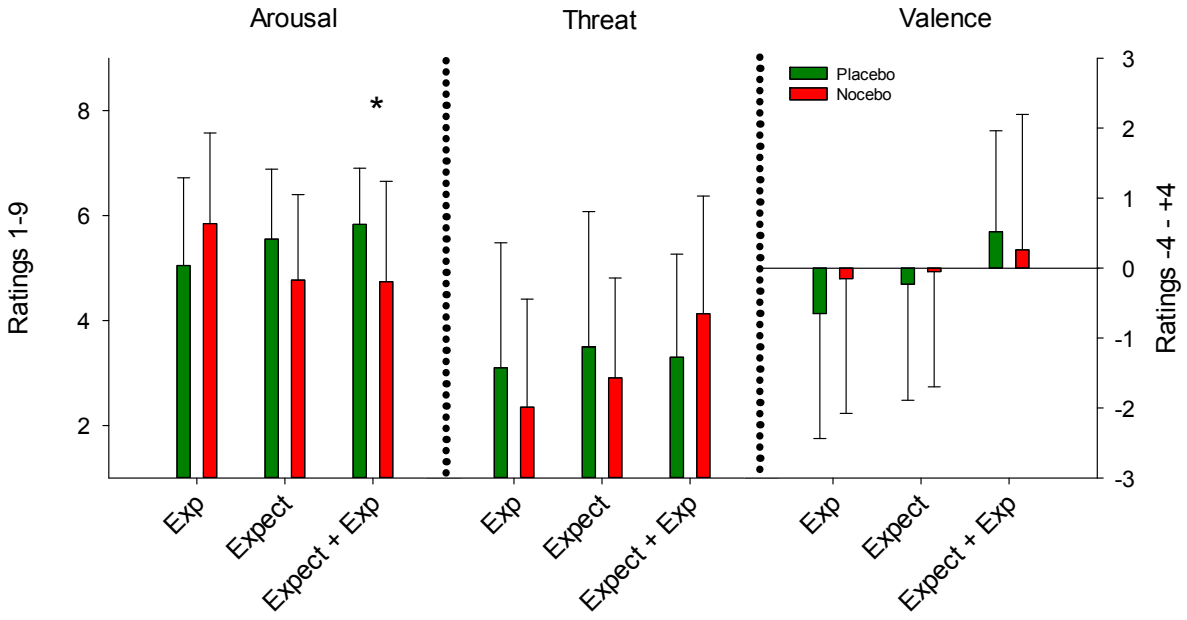


***Fig. 7.*** Means (+ *SEM*) of ratings for valence, arousal and threat are depicted separately per experimental group; *$p$. < .05

**Ratings of recalled sensory pain** were higher for nocebo compared to placebo trials, $F(1,62) = 22.35$, $p < .001$, $\eta_p^2 = .27$. A marginal significant effect of group, $F(2,62) = 2.63$, $p = .08$, $\eta_p^2 = .08$, is a result of higher recalled ratings in Expect compared to Exp, $t(62) = 2.18$, $p = .03$. Further the marginal interaction of placebo-nocebo and group, $F(2,62) = 2.54$, $p = .09$, $\eta_p^2 = .08$, is most probably due to higher differentiation between placebo and nocebo trials in Expect+Exp compared to Exp (independent $t$-test for nocebo–placebo difference scores: $t(43) = 2.68$, $p = 01$) while the other comparison did not reach significance. **Ratings of recalled affective pain** were higher for nocebo compared to placebo trials, $F(1,62) = 22.38$, $p < .001$, $\eta_p^2 = .27$, for all groups. The interaction, $F(2,62) = 2.25$, $p = .11$, $\eta_p^2 = .07$ as well as the factor group, $F(2,62) = 1.66$, $p = .20$, $\eta_p^2 = .05$, did not reach significance (see ***Fig.* 8**).

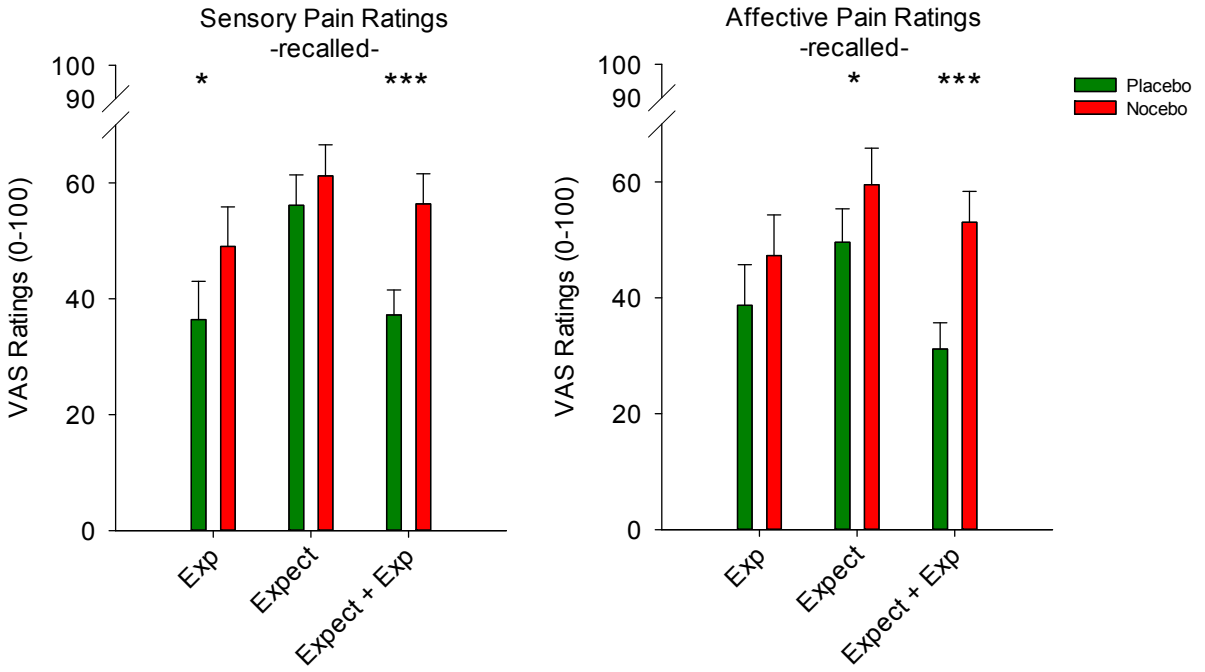


***Fig. 8.*** Means (+ *SEM*) of the sensory and affective ratings for recalled pain conducted at the end of experiment; in contrast to the pain ratings during the experiment, scales started on a default position VAS = 50, instead of VAS = 0; **p*. < .05; ***p*. < .01; ****p*. < .001.

### 2.2.4 Psychometric Measures and Placebo-Nocebo Effects

No significant associations of questionnaire scores and any of the placebo-nocebo measures (i.e., pain ratings, SCR or facial EMG responses) were found.

## 2.3 Discussion

The present study aimed at investigating the contribution of experience, expectation and its combination to modulate the perception of pain within a placebo-nocebo paradigm, which relied on a mere psychological manipulation.

The results reveal a reliable differentiation between placebo and nocebo trials during the test phase only for the combined experience + expectation group as indicated by the significant differences of the pain ratings. In addition, higher SCR for early nocebo compared to placebo trials during the test phase were also found for the combined experience + expectation group only. This result underscores the findings from the pain ratings and clearly suggests that these results cannot be ascribed to a mere report bias (Benedetti, 2009). Instead, they indicate a modulation of pain perception due to the placebo-nocebo manipulation on a subjective and physiological level. The results of the facial EMG only revealed a differentiation between high and low pain stimulation during the conditioning

phase, whereas there was no difference during the test phase. SCR were also more pronounced for high compared to low pain stimulation during the conditioning phase. Subjective ratings of the placebo and nocebo cues at the end of the experiment showed only for the combined expectation + experience group a difference regarding emotional arousal, which was rated higher for the placebo compared to nocebo cues. Taken together, these findings show that it is possible to induce a placebo-nocebo effect on pain by mere psychological means. However, when administering a psychological placebo-nocebo intervention it seems inevitable to rely on both central processes that mediate placebo and nocebo effects, namely distinct expectations about the efficacy of a treatment, and the preceding experience of the treatment´s potency.

### 2.3.1 Conditioned Placebo Analgesia

In the present study, the impact of experience, expectation and its combination on the induction of a placebo nocebo effect modulating the perception of pain was tested. A conditioning procedure alone did not suffice to induce a stable differentiation between placebo and nocebo trials during the test phase. However, there is also evidence for purely conditioned placebo effects which does not involve any further placebo suggestions (e.g., informing a participant to receive a potent pain killer), although it is rather scarce. Voudouris and colleagues (1990) employed a placebo paradigm, in which they administered a placebo cream and compared four different groups, thereby manipulating expectation (analgesic cream vs. control cream) and experience (placebo conditioning vs. mere pain stimulation). They found a significant analgesic effect for the group that underwent a placebo conditioning procedure, whereas the mere instruction was not sufficient to induce a placebo effect. Amanzio and Benedetti (1999) could show that a pharmacological conditioning procedure using morphine resulted in a subsequent analgesic placebo response when participants were administered saline, but thought they were treated with an antibiotic solution free of any analgesic effect. Although the conditioning alone (without any specific analgesic expectation) revealed significant effects, these were much smaller than in another experimental condition in which participants thought the saline injection to be a dose of morphine (expectation + conditioning) (Amanzio & Benedetti, 1999). Benedetti and colleagues state that conditioning is especially crucial for the induction of a placebo effect when the focused mechanism or physiological system is independent of aware cognitions (e.g., in the case of the conditioned secretion of hormones) (Benedetti, et al., 2003).

Thus, in contrast to conditioned placebo paradigms that provide a physical agent (e.g., creams) as a conditioned stimulus (Geuter, et al., 2013; Voudouris, et al., 1990; Wager, et al., 2004), a stripe pattern might be less easily associated with a pain easing effect. In this vein, studies on classical fear conditioning revealed that a negative or even fear related CS+ result in stronger CR than a neutral or fear unrelated CS+ (Ohman & Mineka, 2001). Accordingly, one may expect a cream (CS+) to be more easily linked to a pain easing mechanism (UR) and consequently resulting in a stronger CR than stripes serving as CS+. Moreover, the strength of the UCS might be crucial for the induction of the conditioned placebo response as well. In favor of this hypothesis, studies on fear conditioning (Glenn, Lieberman, & Hajcak, 2012) and conditioned immune-suppression in animals (Ader & Cohen, 1975) found higher levels of CR as a result of stronger US suggesting that a placebo conditioning should probably provide high contrast between the US (pain stimulation level) that follows the CS+ (placebo) and the CS- (control) during the conditioning phase. The pairing of visual, pain unrelated cues (CS+/CS-) with just slightly different levels of heat (US) might result in only slightly alterations of pain processing (UR) causing only small placebo-nocebo effects (CR). Therefore a psychological placebo paradigm assumedly depends on additional cognitive support such as a placebo instruction.

However, very recently Jensen et al. (2012) could induce a placebo analgesic effect using a behavioral conditioning procedure without any verbal placebo suggestion. They presented participants facial stimuli that were combined with high or low painful heat stimuli during a placebo conditioning phase. During a subsequent test phase, pain ratings were significantly higher after watching nocebo compared to placebo and even newly introduced control faces, although the actual level of pain stimulation was identical. Additionally, the perception of placebo and nocebo cues did not need to be conscious, as shown in a second experiment that presented placebo and nocebo faces subliminally during the test phase (Jensen, et al., 2012). However, the results relied on pain ratings only and the question remains whether the described effects also generalize to the physiological level. A probably crucial methodological difference of the study by Jensen et al. in comparison to the large majority of experiments conducted in the field of placebo conditioning was the way of instructing the participants. Participants were told that they would watch faces and receive thermal stimuli during the following experiment which was designed to investigate the impact of implicit and explicit learning on the perception of pain (Jensen, et al., 2012). Furthermore,

participants were observed from an experimenter sitting close behind them and repeating aloud all verbal pain reports the participants gave during the experiment. This might induce expectations and demand effects in the participants (Orne, 2009). In addition, during the test phase participants were presented so called “booster trials”, faces coupled with their respective level of pain stimulation during the conditioning phase (high pain stimuli + nocebo faces; low pain stimuli + placebo faces) to avoid early extinction. Altogether, the length of the placebo conditioning phase, the booster session and the explicit instruction during the experiment by Jensen et al (2012) clearly exceed the potency of the placebo conditioning procedure of the present study. These methodological differences might explain the rather small effect in experience group when comparing placebo and nocebo trials during the test phase. However, pain ratings during the conditioning phase as well as the ratings of recalled pain revealed that the participants of the experience group were actually aware of the differences between placebo and nocebo trials but failed to transfer these differences into the test phase. These results suggest that the present manipulation resulted in no or rather weak conditioned responses (placebo analgesia/nocebo hyperalgesia) although participants learned about the contingency between the cues and the respective pain stimulation level.

### 2.3.2 Instructed Placebo Analgesia

In the present study, no placebo effect was found for the mere instruction (expectation) group. This is in line with earlier findings which showed no placebo effects when comparing verbal suggestions with placebo conditioning procedures (Voudouris, et al., 1990). However, there is also evidence that verbal instructions may be sufficient to produce placebo effects or have at least a strong impact on subsequent treatments or interventions. Amanzio and Benedetti (1999) compared the influence of verbal instruction and pharmacological placebo conditioning with opioidergic and non-opioidergic agents, and the blockade by its respective antagonist in a study that contained 12 experimental conditions. They showed that the belief of having received morphine (actually saline) increased pain tolerance and that this effect can be reversed by the application of naloxone, a morphine antagonist. This suggests an analgesic placebo effect mediated by endogenous opioids that relies only on expectancy. Further, it was shown that the impact of potent painkillers could be dampened and even reversed by giving a contradictory instruction (Bingel, et al., 2011; Dworkin, et al., 1983). Similarly, a single announcement of the likely occurrence of negative symptoms was enough to induce nocebo hyperalgesia (Colloca, et al., 2010). In a different set of studies with patients

suffering from irritable bowel syndrome, greater pain relief during experimental rectal balloon distension was found when patients were informed to receive an opioidergic medication with 100% probability vs. receiving the same medicament and being told that it might be a placebo (50% chance of receiving the drug) (Vase, Robinson, Verne, & Price, 2005). Even more impressing, Kaptchupk and colleagues (2010) could show that the administration of a medication that was clearly announced as a placebo in combination with detailed information about the characteristics of a placebo and the involved mechanism of action, resulted in significant symptom reduction compared to a control group. Taken together, an individual's expectation was demonstrated to tremendously impact on a treatment outcome, what might be especially crucial in the clinical context, (for a detailed overview see: Enck, et al., 2013). However, many of the studies as reviewed above provided either a placebo instruction that was pharmacologically plausible (Colloca & Benedetti, 2006; Geuter, et al., 2013) or elaborately aimed at the participants' compliance (Kaptchuk, et al., 2010). In contrast, the present study solely provided a short, rather implausible written instruction, which alone did not suffice to induce a placebo response. Accordingly, further investigation on the most compelling composition of a patient information/placebo seem a promising future direction.

### 2.3.3 Combined Placebo Instruction and Placebo Conditioning

The combined experience + expectation group was the only to show a stable placebo-nocebo differentiation on the behavioral as well as on the physiological level. These results fit well with earlier findings that demonstrate the strongest placebo effect when participants where first told that they would receive a potent analgesic treatment and afterwards underwent a placebo conditioning phase (Colloca & Benedetti, 2006; Voudouris, et al., 1990). The present results extend these earlier findings with regard to the type of placebo procedure that was "administered" to the participant. In the present case the placebo consisted of a mere psychological non-pharmacologically driven procedure that was explained to the participants and provided only moderate levels of persuasiveness as supposedly demonstrated by the zero findings in the expectation group. Likewise, the conditioning procedure alone (experience group) was also not sufficient to modify the perception of pain during the test phase. This is probably the result of the missing attribution of the perceived pain relief to be the direct consequence of observing the visual placebo cue. In favor of this view, a recent review article (Atlas & Wager, 2012) stated placebo and nocebo paradigms to be conceptually separate from experimental designs that solely evoke stimulus expectations

in the participant. For instance, when cues predict high or low pain stimulation (e.g., Ploghaus, et al., 2001) but do not indicate the action of a sham treatment that might be responsible for the pain easing effect (e.g., a placebo painkiller). In a similar vein, Montgomery and Kirsch (1997) conducted a seminal study to investigate the impact of different placebo conditioning manipulations. One group of participants was aware of the variation of pain stimulation level during the placebo conditioning phase, whereas the other was unaware of the manipulation and should believe that the altered sensations were the result of a sham analgesic cream. They could show that only participants in the deceptive group showed significant placebo responses. Accordingly, the results of the present experiment suggest that when dealing with rather weak and only moderately suggestive placebo instructions ("…stripes that will ease your pain…"), it is necessary to confirm their effectiveness and potency by an actual experience.

### 2.3.4 Limitations and Outlook

In contrast to the SCR results that support the behavioral findings of the placebo-nocebo manipulation, the measurement of pain-associated EMG responses during the test phase failed to provide a clear differentiation between placebo and nocebo trials. In an earlier study we could show that facial EMG is a suitable measure of facial pain signals (Reicherts, et al., 2013). In line with this result, higher M. orbicularis oculi activation was found in response to the high compared to low pain stimulation during the conditioning phase of the present study. In contrast to other experiments that focused on eliciting clearly visible facial reactions in response to pain (Kunz, Chen, Lautenbacher, Vachon-Presseau, & Rainville, 2011), we applied rather low levels of heat pain regarding the magnitude and the length of the stimulation. Therefore, the absent differentiation between placebo and nocebo conditions during the test phase - when the actual level of pain stimulation was identical - might be a result of the insufficient power of the psychological placebo manipulation to alter facial pain responses likewise. To increase the probability of detecting potential differences, the application of longer and more painful stimuli might be necessary; however, such design variations introduce new methodological issues regarding the number of trials or the length of the experiment, which need to be considered carefully.

Besides the zero findings for the facial EMG, the analysis of psychological variables that were discussed to affect placebo responsiveness and the actual placebo effect (e.g., optimism

or mood state) revealed no associations with the present physiological and behavioral effects. A possible explanation might be the rather small sample sizes of each experimental cell and the fact that stable placebo effects were restricted to the combined experience + expectation group. Accordingly, the interpretation with regard to a moderating influence of psychological traits and placebo responsiveness in the two other groups is rather difficult.

Regarding the psychometric measures as well as all other conducted analyses, the exclusion of non-responders would have probably resulted in more pronounced effects. However, such an approach results in an increased total sample size and seems less favorably when trying to compare the impact and feasibility of different manipulations with each other. In the present study the placebo-nocebo manipulation was designed to maximize the differentiation between the two conditions and therefore provided a combined placebo-nocebo instruction, informing the participants that one visual cue would reduce while the other would increase the perception of pain. This design is similar to the paradigm introduced by Colloca et al. (Colloca & Benedetti, 2006; Colloca, Sigaudo, et al., 2008) who successfully induced placebo and nocebo effects and in addition provided a neutral control conditioning. Accordingly, future experiments might comprise an additional control condition to more precisely disentangle the present findings as a result of mainly placebo analgesia or nocebo hyperalgesia.

### 2.3.5 Conclusion

The present study could show that in principle it is possible to induce alterations of pain perception by a merely psychological placebo-nocebo manipulation that does not rely on any earlier associative learning experiences such as past medical treatments or interventions. The results suggest that the combination of an induced expectation and its subsequent affirmation are sufficient to change physiological responses and behavioral measures of pain, although the treatment itself is only moderately convincing. This opens a new perspective on the study of mechanisms involved in the formation of placebo effects, and the presented paradigm seems well suited for this purpose. Furthermore, the investigation of methodological aspects such as the length and characteristics of the placebo conditioning phase (Colloca & Benedetti, 2006; Colloca & Miller, 2011; Colloca, et al., 2010) or the level of persuasiveness of the placebo instruction (Enck, et al., 2013; Kaptchuk, et al., 2010) seem crucial - open - research questions.

## 3. Experiment 2: The Interaction of Affective Stimuli and Placebo Analgesia

Placebo manipulations, like the administration of analgesic sham treatments, were shown to successfully reduce the perception of pain (Benedetti, 2009; Price, Finniss, et al., 2008). Likewise, nocebo procedures, using agents or treatments supposed to worsen bodily symptoms were found to increase the perception of pain (Benedetti, et al., 2003; Geuter & Buchel, 2013; Kong, et al., 2008). As shown in Experiment 1, it is also possible to induce a combined placebo-nocebo effect on pain by psychological manipulations comprising the induction of a positive expectation and the actual experience of the manipulation to be effective. Similarly, the induction of mood by presenting emotional stimuli, like music (Roy, et al., 2012; Roy, Peretz, & Rainville, 2008), films (Weisenberg, et al., 1998), emotional stories (Zelman, et al., 1991), pleasant and unpleasant odors (Villemure, et al., 2003), was also found to reliably modulate the perception of pain. Probably the most intensively investigated emotional stimuli in this context are affective pictures (Kenntner-Mabiala, et al., 2008; Kenntner-Mabiala & Pauli, 2005; Kenntner-Mabiala, Weyers, & Pauli, 2007; Meagher, et al., 2001; Rainville, Bao, & Chrétien, 2005; Rainville, Roy, Piche, Chen, & Peretz, 2009; Rhudy & Meagher, 2001; Rhudy, et al., 2008). Previous research demonstrated that positive emotional pictures reliably decrease and negative emotional picture increase the perception of pain (Villemure & Bushnell, 2002; Wiech & Tracey, 2009) see also 1.2.2.

In a recent review article, Flaten and colleagues comprehensively describe the broad interactions of emotion and placebo manipulations on pain (Flaten, et al., 2011). The authors provide evidence that placebo analgesia and the processing/experience of emotion share substantial commonalities in terms of neuronal structures and involved neurotransmitter systems. For example, dopamine was found to be released during both, placebo analgesia (Scott, et al., 2007) and reward processing (Schultz, 2007). Likewise, the transmission of endogenous opioids is considered a central mechanism underlying placebo analgesia (Zubieta, et al., 2005) and was shown to be also enhanced during the processing of positive emotions (Koepp et al., 2009). Furthermore, it was reported previously that placebo and nocebo treatments alter an individual's emotional state. In this vein, nocebo treatments, were reported to elevate feelings of anxiety (Colloca & Benedetti, 2007) while placebo treatments were shown to decrease physiological and subjective stress (Aslaksen & Flaten, 2008b). With regard to the physiological and psychological consequences of emotion processing, negative

and positive emotional stimuli are hypothesized to activate a defensive and appetitive motivational system, respectively (Kenntner-Mabiala & Pauli, 2005; Lang, 1995; Rhudy, et al., 2008). Finally, the modulation of pain by placebo/nocebo effects or the modulation of pain by emotion, both seem to incorporate the nociceptive control system (Eippert, Bingel, et al., 2009; Tracey & Mantyh, 2007).

Based on the profound interactions and similarities of emotion processing and placebo analgesia, a seminal neuroimaging study addressed whether placebo effects could be induced for the processing of negative emotion, similarly to the induction of placebo analgesia (Petrovic et al., 2005). It could be shown that the administration of a sham drug, introduced as an anxiolytic benzodiazepine, decreased negative affect accompanied by neuronal activity in circuits reported previously to be involved in the mediation of placebo analgesia, such as the DLPFC and the rACC (Petrovic, et al., 2005). These results demonstrate the close link between pain, emotion and placebo effects and suggest a common higher order mechanism to be involved in the mediation of placebo effects per se, instead of a unique process at work specifically involved during placebo analgesia.

Based on these findings, the question arises to what degree placebo manipulations and affective processing interact with each other while modulating pain: Do positive and negative emotional stimuli alter a placebo analgesic effect and, conversely, is the modulation of pain by affective stimuli modified by a placebo manipulation? To investigate the interaction of placebo analgesia/nocebo hyperalgesia and affective pain modulation, the present study compared two groups of participants which were instructed that positive pictures were found to decrease and negative pictures to increase the perception of pain (congruent group) or vice versa (incongruent group). Both groups first underwent a placebo conditioning phase: the congruent group received high (pain threshold +1°C) or low (pain threshold temperature) heat pain stimuli during the presentation of negative vs. positive pictures; for the incongruent group, the contingencies were reversed. Afterwards, both groups underwent a test phase and watched again positive and negative affective pictures, but this time, they received solely the high pain stimuli. To determine subjective and physiological responses to the emotional stimuli, ratings of valence and arousal for the affective pictures were collected prior to the experiment, and facial EMG and SCR were recorded during the whole experiment. To capture the modulation of pain by the emotional placebo manipulation (placemo), physiological

signals (SCR, facial EMG) in response to the pain stimulation were recorded and sensory and affective pain ratings were conducted. It was hypothesized that the differentiation of pain responses (pain ratings and physiological responses) between placebo and nocebo trials during the test phase would be more pronounced for the congruent group compared to the incongruent group.

## 3.1 Method

### 3.1.1 Participants

Fifty-three participants (26 women) were recruited from the University of Würzburg. Five participants had to be excluded from the final sample (four in the congruent group, one in the incongruent group, due to exceeding threshold temperatures n = 3, lacking compliance or missing understanding of the rating procedure n = 2). The remaining sample of forty-eight participants were on average 23.33 years old (*SD* = 3.99) and consisted of twenty-five women and twenty-three men. Participants received course credit or € 12 as compensation. None of them had taken any analgesic medication for the last 24 h prior to the test session (self-report). Participants were randomly allocated to one of the two experimental groups, which varied according to the different experimental manipulations: congruent vs. incongruent, for further details see ***Fig.* 10**. Participants completed questionnaires on state and trait anxiety (Spielberger trait and state anxiety inventory, STAI-T/S) (Laux, et al., 1981; Spielberger, 1970), positive and negative mood, (Positive and Negative Affect Schedule, PANAS, Krohne, et al., 1996; Watson, et al., 1988) pain catastrophizing (PCS) (Meyer, et al., 2008; Sullivan, et al., 1995), life orientation, (i.e. dispositional optimism and pessimism, Glaesmer, et al., 2008), and sensitivity to reward and punishment (SPSRQ) (Torrubia, et al., 2001) German translation by Hewig & Hagemann, 2002: Der SPSR Fragebogen von Torrubia, Ávila, Moltó & Caseras, unpublished German translation, University of Trier, personal communication). Further, socio-demographic information and personal attitudes towards pain were assessed. At the end of the experiment participants completed a test evaluating their proneness to suggestive instructions (Carleton University Responsiveness to Suggestions Scale, CURSS, (Carleton University Responsiveness to Suggestions Scale, CURSS, Spanos et al., 1983). The two groups did not differ statistically from each other with regard to any of the collected measures, for further details see **Tab. 2**. All subjects had normal or corrected-to-normal vision, and no current or prior history of chronic pain, neurological or psychiatric disorders (self-report). The

experimental procedure was approved by the institutional review board of the Medical Faculty of the University of Würzburg.

**Tab. 2.** Sample Description of Study 2

| Measure | Con (n=22) | | Incon (n=26) | | | |
|---|---|---|---|---|---|---|
| | *M* | *SD* | *M* | *SD* | *F*(1,47) | *p* |
| Pain threshold temperature in °C | 45.27 | 2.48 | 45.25 | 3.07 | 0.00 | .99 |
| Age | 23.91 | 4.26 | 22.85 | 3.76 | 0.84 | .36 |
| LOT_R | 6.36 | 3.46 | 7.65 | 4.96 | 1.05 | .31 |
| PCS_Sum | 16.14 | 7.94 | 15.85 | 6.53 | 0.02 | .89 |
| STAI_Trait_Sum | 34.59 | 8.42 | 39.42 | 10.24 | 3.12 | .08 |
| STAI_State_Sum | 35.14 | 5.67 | 34.73 | 5.36 | 0.06 | .80 |
| PSQ_Total | 4.44 | 1.24 | 4.44 | 1.29 | 0.00 | .99 |
| PANAS_Positive | 29.82 | 6.98 | 30.04 | 4.39 | 0.02 | .89 |
| PANAS_Negative | 11.05 | 1.36 | 11.88 | 2.67 | 1.77 | .19 |
| SPSRQ_Punishment | 7.50 | 4.86 | 9.92 | 4.59 | 3.15 | .08 |
| SPSRQ_Reward | 10.55 | 3.54 | 10.88 | 3.44 | 0.11 | .74 |
| CURSS_Subjective | 6.59 | 3.16 | 7.31 | 3.54 | 0.49 | .49 |

***Note:*** LOT= Life Orientation Test; PCS= Pain Catastrophizing Scale; STAIT/S= State/Trait Anxiety Inventory; PSQ = Pain Sensitivity Questionnaire; PANAS= Positive and Negative Affect Schedule; SPSRQ= Sensitivity to Punishment, Sensitivity to Reward Questionnaire; CURSS= Carleton University Responsiveness to Suggestion Scale; Con= Congruent Group, Incon= Incongruent Group.

### 3.1.2 Experimental Groups and Placebo Manipulation

Two different experimental groups were realized and varied according to (a) the instruction given prior to the placebo conditioning phase and to (b) the contingency of affective stimulus type and pain stimulation level during placebo conditioning. The congruent group was informed according to well-established findings from the literature (e.g., Wiech & Tracey, 2009) that positive pictures would reduce pain perception (placebo cue), while negative pictures would increase pain perception (nocebo cue). The incongruent group was told the exact opposite, such that positive pictures would increase the perception of pain (nocebo cue) and negative pictures would decrease the perception of pain (placebo cue). After reading the instruction, both groups underwent a placebo-nocebo conditioning phase, similar to Experiment 1 (experience + expectation): while watching the positive pictures the congruent group received low pain stimuli (pain threshold temperature) and higher pain stimuli (pain threshold + 1°C), while watching negative affective pictures. For the incongruent group the contingency of affective pictures and pain level was reversed. During the

subsequent test-phase, participants of both groups watched positive and negative pictures and received the higher pain stimuli only. For further details about the manipulation see ***Fig.* 10** and paragraph 2.1.8, describing the procedure.

### 3.1.3 Control Condition: Emotional Modulation of Pain

In a separate group, the efficacy of the selected emotional pictures to actually modulate the perception of pain was tested. During the placebo conditioning phase the participants of the control condition (n = 20) watched the positive and negative pictures, pseudorandomly accompanied by high and low painful heat stimuli. This was intended to provide the same amount of heat stimulation in all experimental groups prior to entering the test phase, but prevent the control group from learning any contingency of picture content and pain level. In the following test-phase the control group watched positive and negative emotional pictures and received solely high pain stimuli, analogue to the congruent and incongruent group. Data from the test phase was used to determine the genuine impact of the selected affective pictures on the perception of pain.

### 3.1.4 Emotional Pictures Serving as Placebo / Nocebo Cues

Placebo and nocebo cues consisted of 10 emotional pictures (5 negative and 5 positive [2]), drawn from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2008). Pictures were selected based on normative valence and arousal ratings to create two pictures sets that were clearly discriminative with regard to emotional valence, but highly similar with regard to emotional arousal. Positive and negative pictures served as placebo and nocebo cues, according to the experimental group. Before starting the actual experiment, participants watched every emotional picture for 20 s and evaluated valence and arousal using the Self-Assessment Mannekin (SAM; Bradley & Lang, 1994). Usage of the SAM scale was illustrated to the participants via written instruction on the screen. The visual placebo and nocebo cues had a resolution of 1024 x 768 pixels and were presented centrally on a 17” computer screen, around 80 cm away from the participants.

[2] IAPS catalog numbers of positive: 1710, 2071, 2303, 4599, 8185 and negative: 1050, 3230, 6570, 9040, 9440, pictures.

### 3.1.5 Pain Threshold Assessment

Thermal heat stimuli were delivered using a Somedic MSA thermal stimulator (Somedic Sales AB, Hörby, Sweden) and a Peltier thermode with an active surface of 25 x 50 mm identical to Experiment 1. Likewise, thermal pain threshold assessment was analogue to Experiment 1, see 2.1.4.

### 3.1.6 Thermal Pain Stimulation and Pain Ratings

During the conditioning phase, two different levels of pain stimulation were delivered analogue to Experiment 1: for placebo trials the individual thermal pain threshold temperature was administered whereas during nocebo trials threshold temperature + 1°C was administered. During the subsequent test phase pain threshold + 1 °C was administered during both placebo and nocebo trials. Participant rated the pain stimuli in terms of pain intensity and pain unpleasantness, using a digitized visual analogue scale (VAS) ranging from 0 = no pain at all to 100 = unbearable pain. For further details see 2.1.5.

### 3.1.7 Skin Conductance Measurement

Like in Experiment 1, skin conductance was measured using two 22/10 mm Ag/AgCl surface electrodes, filled with electrode cream (concentration of 0.5% NaCl) attached to the thenar and hypothenar eminence of the participants' non-dominant hand. The skin conductance data were segmented in time windows of 20 s after emotional picture onset. SCR were quantified as highest positive deflection in two respective time windows: for responses to the emotional pictures cueing placebo or nocebo, a peak interval from 1 s to 6 s after trial onset was used; for pain-associated responses an interval from 6 s to 19 s after trial onset was used. Regarding the first peak search interval, a 1 s pre-visual-stimulus baseline was subtracted from the signal whereas for pain responses a 1 s baseline starting from 5 s after trial onset was applied (pain stimulation started 3 s after trial onset and reached plateau level after 2 s). For details regarding data recording and preprocessing, see 2.1.6.

### 3.1.8 EMG Measurement

Like in Experiment 1, EMG was recorded from M. corrugator supercilii, M. orbicularis oculi and M. zygomaticus major. Facial muscles were chosen based on earlier findings, which suggest facial pain responses in these or closely adjacent areas (Kunz, et al., 2004; Prkachin, 1992). Moreover, activity of M. corrugator supercilii and M. zygomaticus major was reported previously to be sensitive indices of affective picture processing (Bradley, et al., 2001). EMG

responses to the emotional pictures cueing placebo or nocebo were scored as difference score comprising the mean activity from 0 to 3 s after picture onset and the activity from a 1000 ms baseline before visual stimulus onset. Pain-associated EMG responses were scored as the mean activity during an interval of 9 to 12 s after placebo-nocebo cue onset (= 6 to 9 s after heat stimulus onset) as change in activity from a 1 s pre-trial onset to 3 s post trial onset baseline interval (-1 to +3 s), to level out cue responses following the emotional pictures. Data quality was evaluated by visual inspection to exclude excessive artifacts. For further details see 2.1.7.

### 3.1.9 Procedure

After arrival, participants signed informed consent (see **Suppl. 6**) and individual pain threshold was assessed (see 3.1.5). Afterwards participants answered socio-demographic questions and filled out the questionnaire on state anxiety (STAI-S) and current mood state (PANAS). Then, participants completed three pain familiarization trials consisting of a centrally presented fixation cross (20 s) and the application and rating of the high pain stimulus (pain threshold +1°C). Afterwards, EMG and SCR electrodes were attached. In the following, participants received further information regarding the experiment, which included the first part of the experimental manipulation (congruent group: "… research revealed that positive emotional pictures decrease and negative emotional picture increase the sensation of pain, …"; incongruent group: information was the exact opposite), for further details see **Suppl. 7** and **Suppl. 8**. Participants were instructed on the PC screen about the subsequent emotional picture rating procedure comprising the self-assessment manikin, watched all emotional pictures on the screen (single picture presentation for 20 s) and rated them for valence and arousal. Afterwards participants were presented a thumbnails of all visual placebo or nocebo stimuli respectively and were asked to rate how much they expected the presented pictures to alter their perception of pain during the experiment on a 10 point Likert scale, ranging from not at all = 0 to very much =9. Thereafter, participants proceeded to the second part of the experimental manipulation that is the placebo-nocebo conditioning phase, which consisted of 30 trials (15 placebo, 15 nocebo). During this phase, participants were presented nocebo cues and received highly painful heat stimuli (threshold + 1°C) or were presented placebo cues and received moderately painful heat stimuli (pain threshold temperature). In the subsequent test phase participants watched the placebo and nocebo cues again while they received the highly painful heat stimuli (threshold + 1°C) only. The test phase consisted of 20 trials (10 placebo,

10 nocebo), for further details see ***Fig.* 10**. During each trial (conditioning and test phase), the placebo or nocebo cues (positive vs. negative pictures) were presented in the center of the screen for 20 s. After 3 s, the thermal stimulation was started, reached the target temperature after about 2 s and remained on the target level for a plateau of 3 s. Thereafter the temperature cooled down to baseline level. After picture offset (20 s) participants were asked to rate the pain intensity and unpleasantness on the VAS. Each trial was separated by an ITI of 4-5 s (interval was randomized), presenting a central fixation cross. After the test phase, participants evaluated how they remembered the pain sensation after the placebo and the nocebo cues on a 100 point visual analogue scale (this time scale was starting from the middle position, VAS =50). Thereafter, participants filled out the remaining questionnaires (PCS, SPSRQ), completed the test for suggestibility (CURSS) and were informed about the actual purpose of the study.

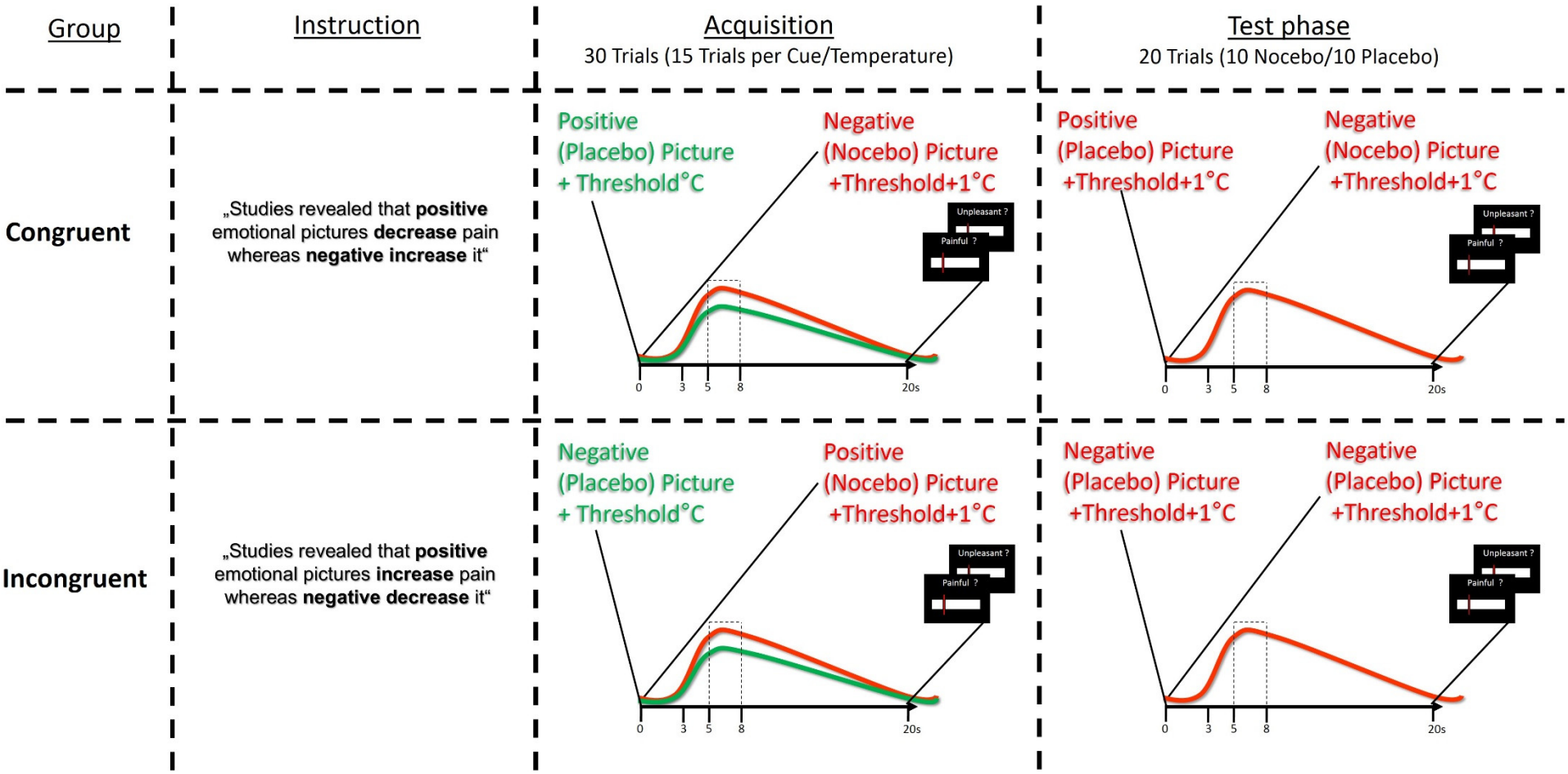


***Fig. 9***. Experimental procedure of Experiment 2 and 3. The congruent and incongruent group varied in terms of the instructed influences of emotion on pain (positive emotion decrease and negative emotion increase pain = congruent; or vice versa = incongruent) and the contingency of positive vs. negative pictures combined with high pain stimuli (red, nocebo) vs. low pain stimuli (green, placebo) during the acquisition phase. In the test phase, participants of both groups watched again positive and negative pictures and were administer the high pain stimuli (red), only.
In contrast to Experiment 2, Experiment 3 took place in the fMRI scanner

### 3.1.10 Statistical Analysis

For the **control group**, the affective picture ratings (valence and arousal) and pain ratings (sensory and affective pain) during the test phase were analyzed by employing separate repeated-measures ANOVAs with the within-subjects factor picture content (2 levels, positive vs. negative). **Affective picture ratings** of the **congruent vs. incongruent group** were analyzed by employing separate 2-factorial repeated-measures ANOVAs for valence and arousal with the within-subjects factor picture content (2 levels, positive vs. negative) and the between-subjects factor experimental group (2 levels: congruent vs. incongruent). Ratings of **expected pain modulation** were analyzed by employing a 2-factorial repeated-measures ANOVAs with the within-subjects factors placebo-nocebo (2 levels: placebo vs. nocebo cues) and the between-subjects factor experimental group (2 levels: congruent vs. incongruent). Data obtained during the **conditioning phase** from the **congruent vs. incongruent group** such as the pain ratings (intensity and unpleasantness), SCR (cue and pain), and EMG (cue and pain) responses were analyzed using separate 3-factorial repeated measures ANOVAs. For cue responses, a within-subjects factor picture content (2 levels: positive vs. negative) was defined, whereas regarding the pain responses a factor stimulation level (2: high vs. low pain stimulation) was applied. Furthermore, for both, cue and pain responses, the within-subjects factor time (3 levels: mean of Trials 1-5 vs. Trials 6-10 vs. Trials 11-15) and the between-subjects factor experimental group (2 levels: congruent vs. incongruent) was defined. Regarding the **test phase,** pain ratings, SCR and EMG responses were analyzed using separate 3-factorial repeated measures ANOVAs containing the within-subjects factors picture content (2 levels: positive vs. negative) for cue responses, and the factor placebo-nocebo (2 levels: placebo vs. nocebo trials) for pain responses. For both the cue and the pain responses, the within-subjects factor time (2 levels: mean of Trials 1-5 vs. Trials 6-10), and the between-subjects factor experimental group (2 levels: congruent vs. incongruent) was applied. **Ratings of recalled pain** were analyzed by employing separate 2-factorial repeated-measures ANOVAs for pain intensity and pain unpleasantness with the within-subjects factor placebo-nocebo (2 levels, placebo vs. nocebo trials) and the between-subjects factor experimental group (2 levels: congruent vs. incongruent). The interaction of the **placebo-nocebo manipulation and affective pain modulation** across the experimental groups and the control group was explored by employing a one-way ANOVA with the between-subjects factor group (3 levels: congruent vs. incongruent vs. control) for differences scores comparing sensory and affective pain ratings

after negative minus positive pictures during the test phase. In a similar vein, to evaluate the impact of the placebo-nocebo manipulation on affective picture modulation of pain, t-test were calculated for sensory and affective pain ratings during the test phase, comparing positive and negative picture trials of the congruent against to the incongruent group. When necessary, Greenhouse-Geisser corrections of degrees of freedom were applied. Post-hoc comparisons were realized using planned contrasts or pair-wise *t*-tests. Significance level was defined as $p < .05$. Associations of psychometric measures and placebo-nocebo outcomes were analyzed using linear correlation analysis of questionnaire scores and nocebo vs. placebo differences of the test phase conducted for sensory and affective pain ratings as well as for skin conductance measures.

## 3.2 Results

### 3.2.1 Control Group: Emotional Modulation of Pain and Affective Picture Evaluation

The analysis of the **arousal** ratings revealed a significant effect for picture content, $F(1,19) = 11.47$, $p = .003$, $\eta_p^2 = .37$, with negative pictures being rated as more arousing than positive pictures. The analysis of the **valence** ratings also revealed a significant effect of picture content, $F(1,19) = 270.65$, $p < .001$, $\eta_p^2 = .93$, due to negative pictures being rated as more negative than positive pictures. The analysis of the **sensory** pain ratings revealed a significant effect for picture content, $F(1,19) = 8.87$, $p = .008$, $\eta_p^2 = .32$, due to higher pain ratings when watching negative compared to positive pictures. Similarly, the analysis of the **affective** pain ratings revealed a significant effect for picture content, $F(1,19) = 11.61$, $p = .003$, $\eta_p^2 = .38$, due to higher pain ratings after negative compared to positive pictures (see ***Fig.* 11**).

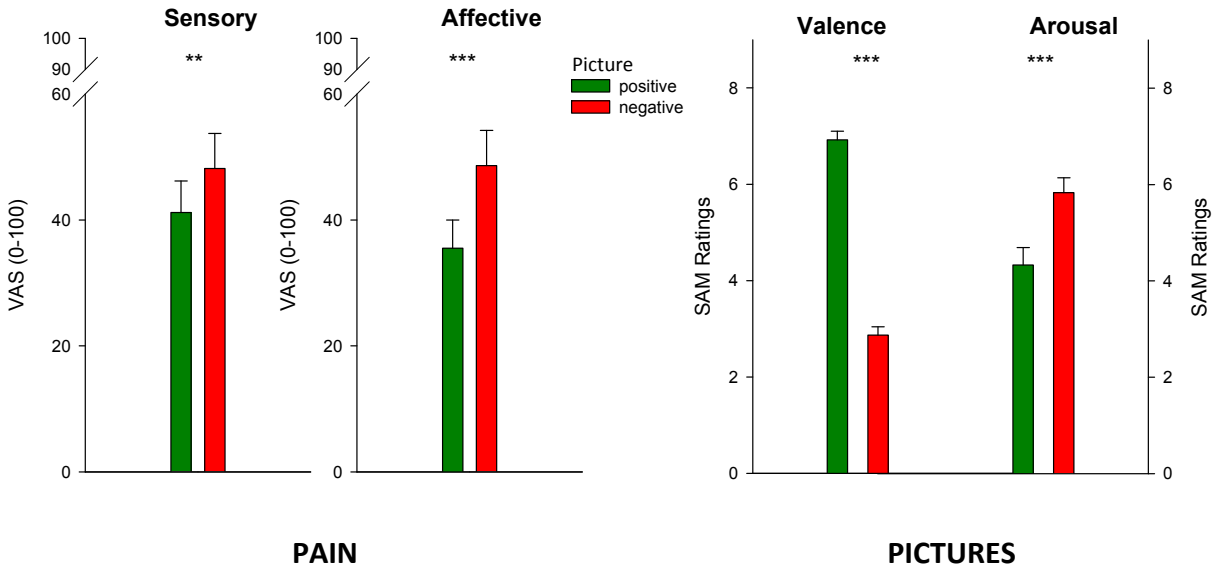


***Fig. 10.*** Control group: Means (+*SEM*) of the sensory and affective pain ratings (left) and the ratings for valence and arousal (right) are depicted separately for positive and negative affective pictures; ** $p < .01$;*** $p < .001$.

### 3.2.2 Congruent vs. Incongruent Group: Ratings of Valence and Arousal for the Emotional Pictures Cueing Placebo or Nocebo Trials and Expected Pain Modulation

The analysis of the **arousal** ratings revealed a significant effect for picture content, $F(1,46) = 31.62$, $p < .001$, $\eta_p^2 = .41$, with negative pictures being rated as more arousing than positive pictures. However, this effect was not further modulated by the factor group. The analysis of **valence** revealed a significant effect for picture content, $F(1,46) = 472.30$, $p < .001$, $\eta_p^2 = .91$, with negative pictures being rated as more negative than positive pictures, irrespective of the experimental group (see ***Fig.* 12**). The analysis of the expected pain modulation revealed a significant effect of placebo-nocebo, $F(1,46) = 62.63$, $p < .001$, $\eta_p^2 = .58$. The interaction of placebo-nocebo and group showed a non-significant trend, $F(1,46) = 2.58$, $p = .12$, $\eta_p^2 = .05$, which was likely due to higher expected pain for nocebo pictures in the congruent compared to the incongruent group, $t(46) = 2.12$, $p = .04$. However, the groups did not differ from each other regarding to the expected modulation of pain by placebo cues, $t(46) = -.85$, $p = .40$.

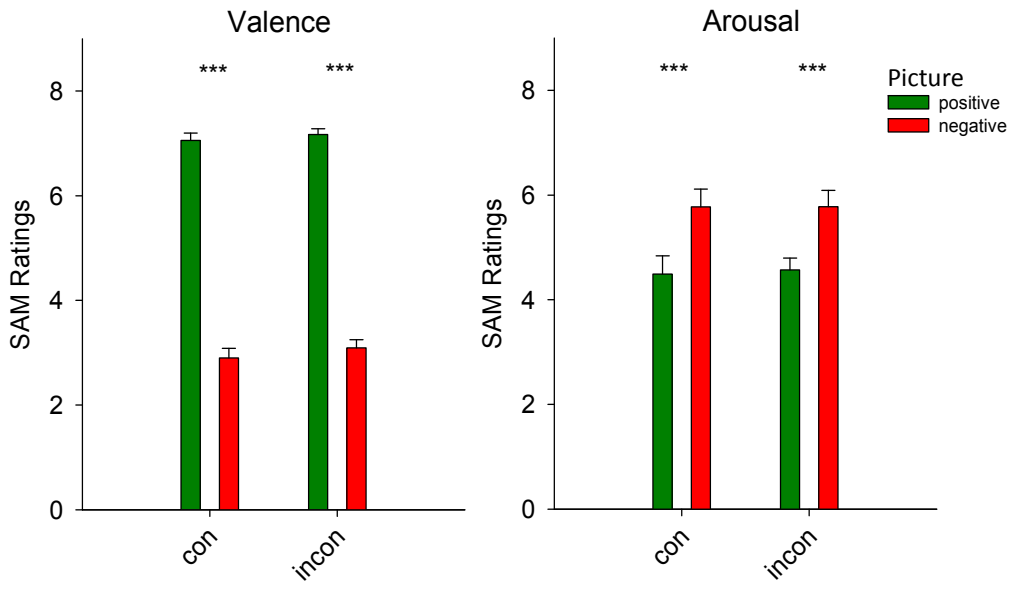


***Fig. 11.*** Mean (+ *SEM)* of the ratings for valence and arousal are depicted separately per experimental group; *** $p < .001$.

### 3.2.3 Conditioning

#### 3.2.3.1 Sensory Pain Ratings for Low (Placebo) and High (Nocebo) Pain Stimulation

The analysis of the sensory pain ratings revealed a significant effect of pain stimulation level, $F(1,46) = 94.12$, $p < .001$, $\eta_p^2 = .67$, due to higher ratings for the higher pain stimuli. This effect was further qualified by a significant interaction of pain level and experimental group, $F(1,46) = 6.53$, $p = .01$, $\eta_p^2 = .12$, which resulted from a stronger differentiation between high and low pain stimulation in the congruent ($\Delta M = 25.47$, $SEM = 3.82$; $t(21) = 6.66$, $p < .001$) than in the incongruent ($\Delta M = 14.85$, $SEM = 2.04$; $t(25) = 7.27$, $p < .001$) group. In addition, a significant interaction of time and stimulation level was found, $F(2,92) = 3.53$, $p = .03$, $\eta_p^2 = .07$. Separate ANOVAS for each stimulation level revealed a significant effect of time only for the low pain stimulation, $F(1,46) = 5.76$, $p = .004$, $\eta_p^2 = .11$, as a result of higher ratings for Trials 1-5 compared to Trials 6-10, $F(1,47) = 5.69$, $p = .02$, $\eta_p^2 = .11$, and for Trials 1-5 compared to Trials 11-15, $F(1,47) = 8.11$, $p = .007$, $\eta_p^2 = .15$.

#### 3.2.3.2 Affective Pain Ratings for Low (Placebo) and High (Nocebo) Pain Stimulation

The analysis of the affective pain ratings revealed a significant effect of stimulation level, $F(1,46) = 113.59$, $p < .001$, $\eta_p^2 = .71$, due to higher ratings for high pain stimuli. Similar to the sensory pain ratings, this effect was further qualified by a nearly significant interaction of pain level and experimental group, $F(1,46) = 3.98$, $p = .052$, $\eta_p^2 = .08$. The interaction is a result

of stronger differentiation between high and low pain stimulation in the congruent (Δ *M* = 30.72, *SEM* =3.64) than in the incongruent group (Δ *M* = 21.03, *SEM* = 3.23). In addition, a significant interaction of time and stimulation level was found, $F(2,92) = 17.32$, $p < .001$, $\eta_p^2 = .27$, due to higher differences between higher and low pain stimulation during later trials. The analysis of high minus low difference scores revealed that during Trials 1-5 pain ratings were less different than in Trials 6-10, $F(1,47) = 27.45$, $p < .001$, $\eta_p^2 = .37$ or Trials 11-15, $F(1,47) = 20.94$, $p < .001$, $\eta_p^2 = .31$. High vs. low differences scores of Trials 6-10 were quite similar to those of Trials 11-15, $F(1,47) = 0.51$, $p = .48$, $\eta_p^2 = .01$.

#### 3.2.3.3 EMG in Response to Positive and Negative Pictures

**M. corrugator supercilii**

The analysis of M. corrugator supercilii EMG responses to the emotional pictures revealed a significant effect of picture content, $F(1,46) = 15.55$, $p < .001$, $\eta_p^2 = .25$, due to higher decrease in corrugator activity in response to positive than to negative pictures. No further effect or any other interaction reached significance (all *ps* > .16).

**M. orbicularis oculi**

The analysis of the M. orbicularis oculi responses revealed a significant effect for the factor group, $F(1,46) = 4.49$, $p = .04$, $\eta_p^2 = .09$, (congruent group: *M* = -0.03 *SD* = 0.35; incongruent group: *M* = 0.24, *SD* = 0.51) which was further qualified by a marginal significant interaction of experimental group and picture valence, $F(1,46) = 3.28$, $p = .08$, $\eta_p^2 = .07$. This marginal significant interaction most likely was due to slightly higher differences between positive and negative pictures within the incongruent group, $t(25) = 1.71$, $p = .10$; the same comparison was far from significance in the congruent group, $t(21) = -0.84$, $p = .41$. Besides, the analysis revealed a marginal significant effect of time, $F(2,92) = 2.86$, $p = .07$, $\eta_p^2 = .06$, which was probably due to higher responses during Trials 1-5 compared to Trials 6-10, $F(1,46) = 4.14$, $p = .05$, $\eta_p^2 = .08$, and compared to Trials 11-15, $F(1,46) = 5.75$, $p = .02$, $\eta_p^2 = .11$.

**M. zygomaticus major**

The analysis of M. zygomaticus major responses revealed a marginal significant interaction of time and picture valence, $F(1,92) = 2.72$, $p = .07$, $\eta_p^2 = .06$, which occurred most likely due to the nearly significant comparison of positive minus negative difference scores of Trials 6-10 compared to Trials 11-15 (planned contrast, $F(1,47) = 3.69$, $p = .06$, $\eta_p^2 = .07$). In

addition, a significant effect for the factor experimental group was revealed, due to higher responses of the incongruent compared the congruent group, $F(1,46) = 10.77$, $p = .002$, $\eta_p^2 = .19$.

#### 3.2.3.4 EMG in Response to Pain for Low (Placebo) and High (Nocebo) Pain Stimulation

**M. corrugator supercilii**

The analysis of M. corrugator supercilii responses only revealed a marginally significant interaction of pain stimulation level and time, $F(1,46) = 2.30$, $p = .11$, $\eta_p^2 = .05$, which most likely resulted from a nearly significant higher activity during high compared to low pain stimulation during Trials 6-10, $t(47) = 2.00$, $p = .052$ (all other comparisons $p > .90$).

**M. orbicularis oculi**

The analysis of M. orbicularis oculi responses revealed a significant effect of pain stimulation level, with higher responses for high pain stimulation, $F(1,46) = 6.29$, $p = .016$, $\eta_p^2 = .12$. Further, a marginal significant effect of time $F(2,92) = 2.84$, $p = .06$, $\eta_p^2 = .06$, was due to higher responses during Trials 1-5 compared to Trials 6-10, $F(1,46) = 6.00$, $p = .02$, $\eta_p^2 = .12$.

**M. zygomaticus major**

The analysis of M. zygomaticus major revealed a significant effect of pain stimulation level due to higher responses during high compared to low pain stimulation, $F(1,46) = 3.65$, $p = .06$, $\eta_p^2 = .07$. Further, only the factor time reached marginal significance, $F(1,46) = 1.98$, $p = .14$, $\eta_p^2 = .04$, most likely due to nearly significant higher responses during Trials 1-5 vs. Trials 6-10, $F(1,46) = 3.63$, $p = .06$, $\eta_p^2 = .07$.

#### 3.2.3.5 SCR in Response to Positive and Negative Pictures

The analysis of SCR to the emotional pictures revealed a significant effect of time, $F(2,92) = 6.05$, $p = .003$, $\eta_p^2 = .12$. SCR were marginally increased during the beginning of the conditioning phase: Trials 1-5 compared to Trials 6-10, $F(1,46) = 3.89$, $p = .054$, $\eta_p^2 = .08$, and Trials 1-5 compared to Trials 11-15, $F(1,46) = 3.97$, $p = .052$, $\eta_p^2 = .08$. This effect was further qualified by a significant interaction of picture content and time, $F(1,92) = 4.94$, $p = .01$, $\eta_p^2 = .10$, due to higher responses during the first five positive compared to negative picture presentations, $t(47) = 2.18$, $p = .04$. In contrast, during later trials of the conditioning phase,

cue responses for positive and negative pictures did not differ from each other, Trials 6-10, $t(47) = -1.50$, $p = .14$, and Trials 11-15, $t(47) = -1.50$, $p = .65$.

#### 3.2.3.6 SCR in Response to Pain for Low (Placebo) and High (Nocebo) Pain Stimulation

The analysis of skin conductance responses to the heat pain stimuli revealed a significant effect for the level of pain stimulation, $F(1,46) = 22.42$, $p < .001$, $\eta_p^2 = .33$, as a result of higher responses following high compared to low pain stimulation. Further, a significant effect of time was found, $F(2,92) = 22.47$, $p < .001$, $\eta_p^2 = .33$, due to higher responses during Trials 1-5 compared to Trials 6-10, $F(1,46) = 35.78$, $p < .001$, $\eta_p^2 = .44$, and Trials 11-15, $F(1,46) = 32.42$, $p < .001$, $\eta_p^2 = .41$, as revealed by planned contrasts.

### 3.2.4 Test Phase

#### 3.2.4.1 Sensory Pain Ratings of Placebo and Nocebo Trials

The analysis of the sensory pain ratings revealed a significant effect of placebo-nocebo, $F(1,46) = 10.03$, $p = .003$, $\eta_p^2 = .18$, due to higher ratings for nocebo compared to placebo trials. This effect was further qualified by a significant interaction of placebo-nocebo and experimental group, $F(1,46) = 4.58$, $p = .04$, $\eta_p^2 = .09$. Separate ANOVAS for each group revealed a significant effect of placebo-nocebo only for the congruent group, $F(1,21) = 7.26$, $p = .01$, $\eta_p^2 = .26$, as a result of higher ratings for nocebo compared to placebo trials. In contrast, the same analysis revealed no significant effect for the incongruent group, $F(1,25) = 1.71$, $p = .20$, $\eta_p^2 = .06$. In addition, a significant effect of time was found, $F(1,46) = 5.29$, $p = .03$, $\eta_p^2 = .10$, due to higher ratings during the first compared to the second half of the test phase. The factor group was far from significance, $F(1,46) = 0.24$, $p = .63$, $\eta_p^2 = .01$, (see ***Fig.* 13**).

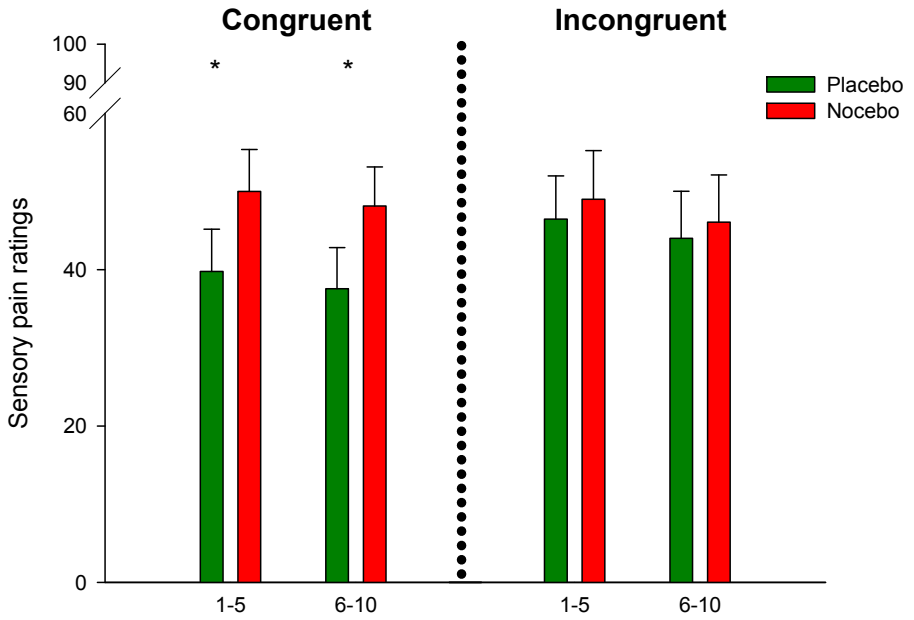


***Fig. 12.*** Means (+*SEM*) of sensory pain ratings during the test phase are depicted separately for the congruent and incongruent group, split by Trials 1-5 and 6-10; * $p < .05$.

#### 3.2.4.2 Affective Pain Ratings of Placebo and Nocebo Trials

The analysis of the affective pain ratings revealed a significant effect of placebo-nocebo, $F(1,46) = 29.84$, $p < .001$, $\eta_p^2 = .39$, due to higher ratings for nocebo compared to placebo trials. This effect was further qualified by a significant interaction of placebo-nocebo and experimental group, $F(1,46) = 4.24$, $p = .045$, $\eta_p^2 = .08$. Separate ANOVAS for each group revealed a significant effect of placebo-nocebo for both, the congruent group, $F(1,21) = 22.53$, $p < .001$, $\eta_p^2 = .52$, and the incongruent group, $F(1,25) = 7.29$, $p = .01$, $\eta_p^2 = .23$, as a result of higher ratings for nocebo compared to placebo trials, respectively. However, the effect of the placebo-nocebo manipulation was more pronounced in the congruent group (Δscore: $M = 17.42$, $SEM = 3.67$) compared to the incongruent group (Δscore: $M = 7.88$, $SEM = 2.92$). The factor group was not significant, $F(1,46) = 0.63$, $p = .43$, $\eta_p^2 = .01$, (see ***Fig.* 14**).

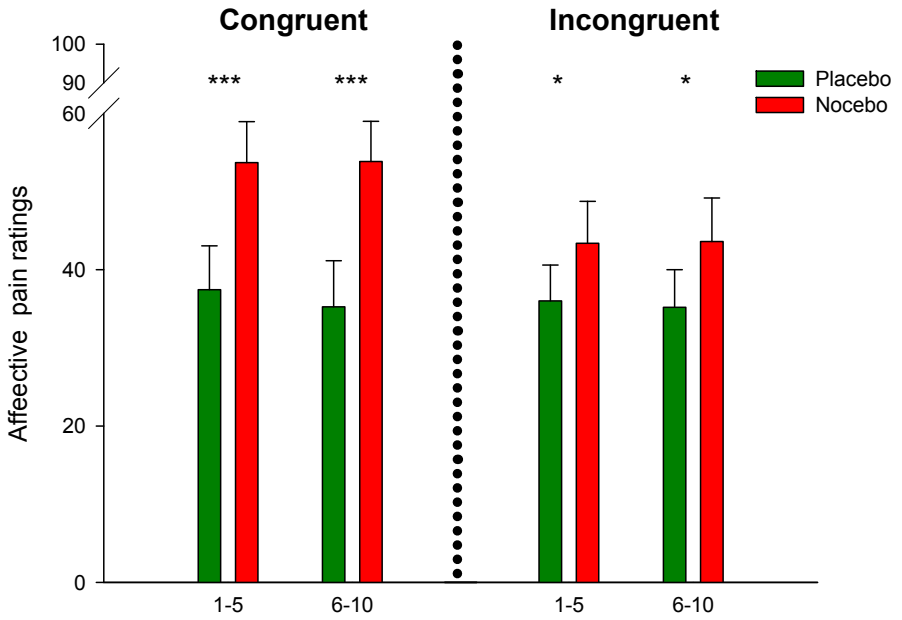


***Fig. 13***. Means (+ *SEM*) of the affective pain ratings during the test phase are depicted separately for the congruent and incongruent group, split by Trials 1-5 and 6-10; * $p < .05$;*** $p < .001$.

### 3.2.4.3 EMG in Response to Positive and Negative Pictures

**M. corrugator supercilii**

The analysis of **M. corrugator supercilii** responses to the emotional pictures revealed a significant effect of picture content, $F(1,46) = 13.51$, $p = .001$, $\eta_p^2 = .23$, due to higher corrugator relaxation in response to positive pictures and stronger activation in response to negative pictures. No further effect or any other interaction reached significance (all *ps* > .17).

**M. orbicularis oculi**

The analysis of **M. orbicularis oculi** responses revealed a marginal significant interaction of picture content and time, $F(1,46) = 3.72$, $p = .06$, $\eta_p^2 = .08$, most likely due to higher orbicularis activation in response to positive compared to negative pictures during the second half of the test phase, $t(47) = 2.70$, $p = .01$. In contrast, the same comparison was not significant during the first half of the test phase, $t(47) = -0.88$, $p = .38$.

**M. zygomaticus major**

The analysis of **M. zygomaticus major** responses revealed a significant effect of picture content, $F(1,46) = 4.45$, $p = .04$, $\eta_p^2 = .09$, with higher activation in response to positive compared to negative pictures, for details see **Tab. 3**.

**Tab. 3.** Facial Muscular Responses (EMG) to Emotional Pictures (0-3 sec) during the Test Phase

| Muscle | Picture type | Trial | Con (n=22) | | Incon (n=26) | |
|---|---|---|---|---|---|---|
| | | | M | SD | M | SD |
| Cor. | pos | 1-5 | -0.07 | 0.64 | -0.08 | 1.27 |
| | | 6-10 | -0.22 | 0.84 | -0.49 | 1.33 |
| | neg | 1-5 | 0.35 | 0.97 | 0.07 | 1.43 |
| | | 6-10 | 0.28 | 0.47 | 0.06 | 1.52 |
| Oo. | pos | 1-5 | 0.14 | 0.67 | -0.22 | 1.56 |
| | | 6-10 | 0.27 | 0.78 | 0.25 | 1.15 |
| | neg | 1-5 | 0.18 | 1.16 | 0.14 | 0.96 |
| | | 6-10 | -0.01 | 0.82 | -0.56 | 1.91 |
| Zyg. | pos | 1-5 | 0.04 | 1.04 | -0.2 | 1.19 |
| | | 6-10 | 0.19 | 1.03 | 0.04 | 1.73 |
| | neg | 1-5 | -0.04 | 0.36 | -0.55 | 1.58 |
| | | 6-10 | -0.54 | 1.52 | -0.42 | 0.78 |

***Note:*** Cor. = M. corrugator supercilii; Oo. =M. orbicularis oculi; Zyg. = M. zygomaticus major; pos = positive pictures; neg = negative pictures.

#### 3.2.4.4 EMG Pain Responses during Placebo and Nocebo Trials

**M. corrugator supercilii**

The analysis of M. corrugator supercilii responses to the heat pain stimuli revealed no significant effects of placebo-nocebo, time or group, (all *ps* >. 17).

**M. orbicularis oculi**

The analysis of M. orbicularis oculi responses revealed no significant effects of placebo-nocebo, time or group, (all *ps* > .09).

**M. zygomaticus major**

Similarly, the analysis of M. zygomaticus major pain responses revealed no significant effects, (all *p*s > .32).

### 3.2.4.5 SCR in Response to Positive and Negative Pictures

The analysis of SCR to the emotional pictures during the test phase solely revealed a significant interaction of time and experimental group, $F(1,46) = 4.65$, $p = .04$, $\eta_p^2 = .09$, likely due to slightly higher responses of the incongruent group compared to the congruent group during Trial 1-5 of the test phase, $t(46) = 1.54$, $p = .13$. In contrast, SCR of both groups were quite similar during Trials 6-10 of the test phase, $t(46) = 0.01$, $p = .94$. Neither the factor picture content nor any other effect or interaction reached significance, (all *p*s > .22).

### 3.2.4.6 SCR in Response to Pain During Placebo and Nocebo Trials

The analysis of SCR to pain revealed a significant 3-way interaction of Placebo-Nocebo x Time x Group, $F(1,46) = 4.75$, $p = .03$, $\eta_p^2 = .09$, which was the result of higher SCR for nocebo compared to placebo trials during Trials 1-5 of the test phase in the congruent group only, $t(46) = 2.31$, $p = .03$ (see ***Fig.* 14**). All other effects were not significant, (all *p*s > .14).

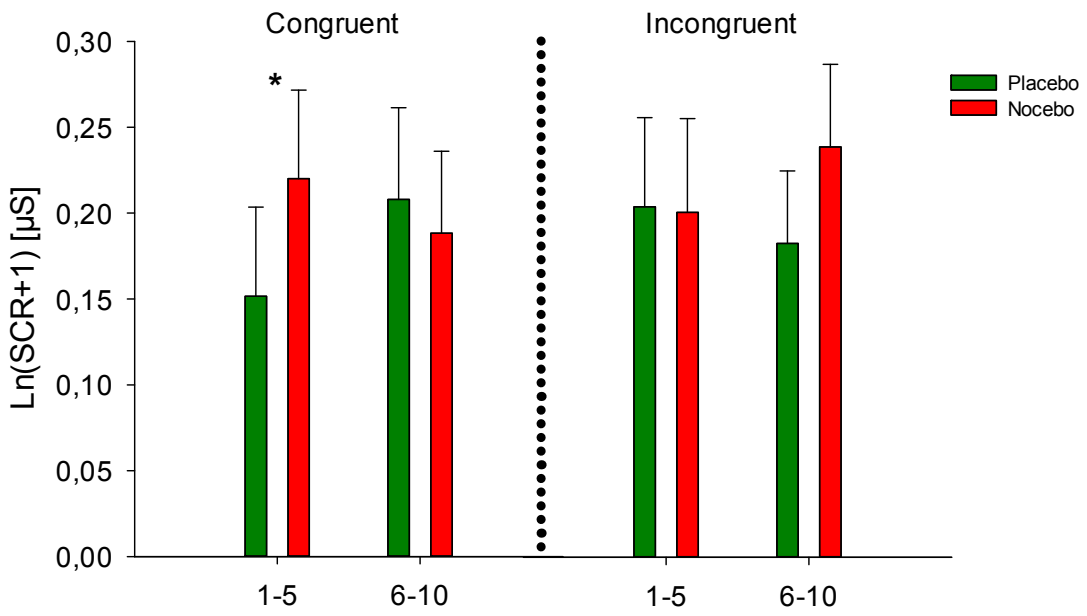


***Fig. 14.*** SCR (Means + *SEM*) during the test phase in response to the pain stimulation are depicted separately for each experimental group, split by Trials 1-5 and 6-10; * $p < .05$.

### 3.2.5 Ratings of Recalled Sensory and Affective Pain

The analysis of the ratings for recalled **sensory** pain revealed a significant effect of placebo-nocebo, due to higher ratings of recalled pain for nocebo compared to placebo trials, $F(1,46) = 32.83$, $p < .001$, $\eta_p^2 = .42$. This effect was further qualified by a marginal significant interaction of placebo-nocebo and group, $F(1,46) = 3.09$, $p = .09$, $\eta_p^2 = .06$, most likely due to

a stronger differentiation between nocebo and placebo trials in the congruent group (Δ *M* = 24.00, *SEM* = 5.05; *t*(21) = 4.75, *p* < .001) compared to the incongruent group (Δ *M* = 12.73, *SEM* = 4.06; *t*(25) = 3.13, *p* = .004). The analysis of the ratings for recalled **affective pain** revealed similar results. The significant effect of placebo-nocebo, *F*(1,46) = 33.99, *p* < .001, $\eta_p^2$ = .43, was due to higher ratings for recalled nocebo compared to placebo trials. This effect was further qualified by a marginal significant interaction of placebo-nocebo and group, *F*(1,46) = 3.03, *p* =. 08, $\eta_p^2$ = .07. Post hoc *t*-tests showed marginally significant higher ratings for recalled nocebo trials in the congruent group compared to the incongruent group, *t*(46) = 1.97, *p* = .06. In contrast, the recalled ratings for placebo trials did not differ between the two groups, *t*(46) = 0.17, *p* = .87.

### 3.2.6 Psychometric Measures and Placebo-Nocebo Effects on Pain

No significant associations of questionnaire scores and nocebo > placebo differences values of the pain ratings or SCR pain responses during the test phase were found.

### 3.2.7 Comparison of Pain Ratings after Negative vs. Positive Pictures during the Test Phase; Congruent Group vs. Incongruent Group

*T*-tests comparing **sensory** pain ratings during the test phase across the two experimental groups revealed no significant difference for positive, *t*(46) = 0.37, *p* = .71, or negative pictures, *t*(46) = 1.36, *p* = .18. The comparison of **affective** pain ratings revealed no difference for positive pictures, *t*(46) = 0.91, *p* = .37. However, the comparison of negative pictures revealed significantly higher pain ratings in the congruent group compared to the incongruent group, *t*(46) = 2.65, *p* = .01, (congruent: *M* = 53.73, *SD* = 24.03 vs. incongruent: *M* = 35.59, *SD* = 23.45).

### 3.2.8 Comparison of Pain Ratings during the Test Phase for the Congruent Group, Incongruent Group and the Control Group

To scrutinize the impact of the placebo-nocebo manipulation on the affective modulation of pain, difference scores of pain ratings after negative minus positive pictures were compared across the two experimental groups and the control group. A one-way ANOVA for **sensory** pain ratings revealed a significant effect of group, *F*(2,67) = 6.15, *p* = .004. *T* tests revealed higher difference scores of the congruent compared to the incongruent group, *t*(65) = 3.37, *p* = .001, but similar to the control group, *t*(65) = 0.87, *p* =.39. In contrast, the difference scores of the incongruent group were significantly smaller

compared to the control group, $t(65) = 2.40$, $p = .02$. Difference scores of the **affective** pain ratings revealed a similar but even more pronounced pattern of results. A one-way ANOVA revealed a significant effect of group, $F(2,67) = 16.59$, $p < .001$. *T* tests showed that the difference scores of the congruent group were significantly higher compared to the incongruent group, $t(65) = 3.37$, $p = .001$, yet similar to the control group, $t(65) = 0.85$, $p = .40$. Conversely, differences scores of the incongruent group were significantly smaller compared to the control group, $t(65) = 4.32$, $p < .001$ (see ***Fig.* 15**).

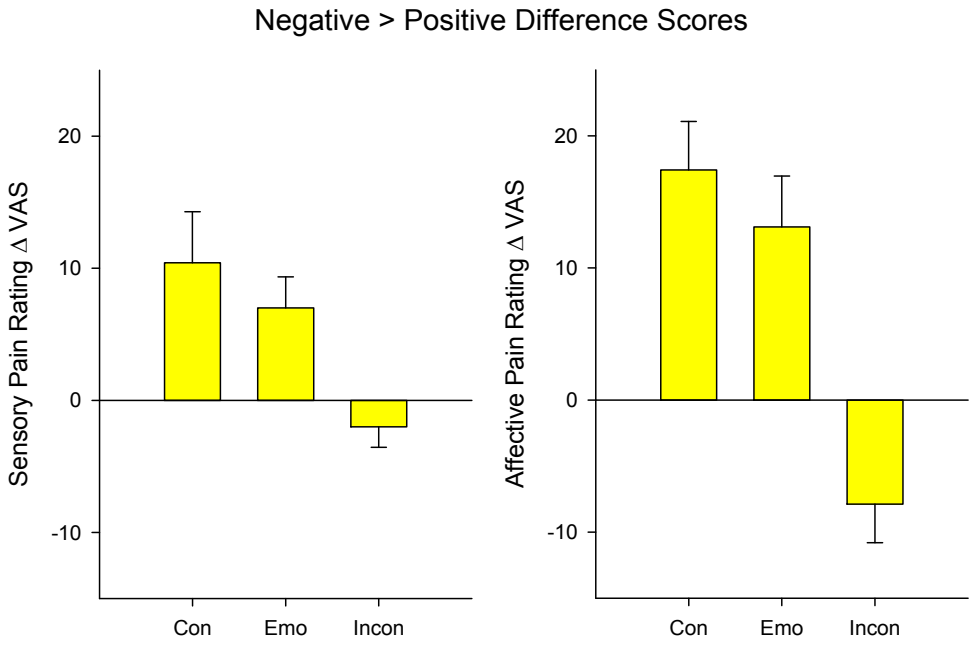


***Fig. 15.*** Mean (+ *SEM*) differences scores (pain ratings after negative – positive pictures) during the test phase are depicted separately for each experimental condition; left: sensory pain ratings; right: affective pain ratings; Con = congruent group, Emo = control group, Incon = incongruent group.

### 3.3 Discussion

The present results revealed a significant differentiation for multiple measure of pain between placebo and nocebo trials, which was most pronounced for the congruent group, when positive emotional pictures served as placebo and negative pictures as nocebo cues. Participants of the congruent group showed significantly higher sensory and affective pain ratings for nocebo compared to placebo trials. These findings were further underscored by significantly higher pain associated SCR during nocebo compared to placebo trials of the early test phase. In contrast, the incongruent group showed no significant differences of sensory

pain ratings or SCR when negative emotional pictures served as placebo cue compared to positive emotional pictures introduced as nocebo cue. However, affective pain ratings of the incongruent group were significantly higher for nocebo compared to placebo trials, although nocebo trials were announced by positive and placebo trials by negative affective stimuli. These results depict the susceptibility of the pain modulating effect by emotion to an additional placebo-nocebo manipulation.

Affective picture ratings in the beginning of the experiment clearly showed that positive and negative stimuli were perceived as such, although negative pictures were rated as more arousing than positive pictures. SCR were higher for positive compared to negative pictures only in the very beginning of the conditioning phase - what may be the result of the placebo nocebo instruction given prior to the conditioning phase - whereas no significant differences were present later on in the experiment. Further, in accordance with the literature, M. corrugator supercilii and M. zygomaticus major responded to positive and negative affective stimuli in the expected way (Bradley, et al., 2001). This was true for both, the conditioning and the test phase. These results further confirm the affective ratings and demonstrate that the selected affective pictures were perceived and processed as intended. However, similar to Experiment 1, the results of the facial EMG in response to the pain stimulation only revealed a differentiation between high and low pain stimuli during the conditioning phase. During the test phase (when pain levels were identical for both conditions), the facial EMG responses failed to differentiate between placebo and nocebo trials. SCR were also more pronounced for high compared to low pain stimulation during the conditioning phase. Moreover, the SCR revealed a significant difference between nocebo and placebo trials even during the test phase, however this effect was found in the congruent group only. Ratings of recalled pain at the end of the experiment revealed that nocebo compared to placebo trials were rated higher, with a slightly more pronounced differentiation in the congruent group. The results of the control group generally confirm the capacity of the selected pictures to actually modulate the perception of pain, even without any further instruction or manipulation. The comparison of the pain rating difference scores (negative picture trials minus positive pictures trials during the test phase) across the two experimental groups and the control group suggest that the placebo-nocebo manipulation exerted the greatest impact on the incongruent condition. In this group negative affective pictures no longer resulted in higher pain ratings than positive affective pictures. The congruent group

showed no significant increment regarding the differentiation between positive and negative pictures due to the placebo-nocebo manipulation when compared to the control group. In addition, pain ratings for negative pictures were higher for the congruent compared to the incongruent group, while pain ratings for the positive pictures did not differ across the two groups.

Overall, these findings indicate that the valence-driven emotional modulation of pain is prone to further modification by a placebo-nocebo manipulation, that is an instruction rendering negative affect as beneficial, in concert with the actual experience (conditioning) confirming such a prediction. This is in accordance with the literature stressing the influence of reappraisal processes on the perception and processing of pain (Tracey, 2010; Wiech, et al., 2008). Further, placebo manipulations combined with positive affect and nocebo manipulations combined with negative affect resulted in stronger differentiation than the opposite combinations. This suggests a congruency-mediated amplification of emotion and the placebo-nocebo response, in line with the literature describing the crucial role of emotions for placebo and nocebo effects (Flaten, et al., 2011).

### 3.3.1 Affective Stimuli Modulate Pain

It is a well-established finding that various kinds of emotional stimuli are capable to alter the perception of pain (Wiech & Tracey, 2009). In this regard, especially emotional pictures have been used to investigate the impact of emotion on pain. For instance, Kenntner-Mabiala and Pauli (2005) presented participants positive, negative and neutral affective pictures selected from the IAPS and recorded EEG, while applying electrical pain stimuli during picture presentation. It was demonstrated that negative compared to positive and neutral pictures increased subjective ratings of pain and modulated somatosensory event-related brain potentials (N150, P260). Besides the modulation on the cortical level, it was previously demonstrated that the emotional modulation of pain already encompasses spinal nociceptive processes. In a study by Rhudy et al. (2005), participants were presented affective visual stimuli (IAPS), while the R-III reflex, a spinally mediated nociceptive withdrawal reflex, was measured. Negative compared to positive emotional pictures resulted in elevated pain ratings and reflex magnitude. In a similar paradigm, it was shown that the modulation of pain also involves alterations of autonomic responses to pain such as heart rate and skin conductance (Rhudy, et al., 2008). According to the motivational priming hypothesis (Bradley, et al., 2001;

Lang, 1995) the activation of the motivational defensive system by, negative emotional stimuli was hypothesized to results in heightened processing of pain. In contrast positive pictures activate the appetitive motivational system, hereby reducing the perception of pain (Rhudy, et al., 2005; Rhudy, et al., 2008). Further, it was shown that the effects of emotional valence on pain are modulated by the level of emotional arousal of the affective stimuli. High levels of arousal increased the pain augmenting or reducing (negative vs. positive) effects of affective stimuli (Rhudy, et al., 2008). On the neuronal level, the activation of the descending nociceptive control system, which encompasses neuronal areas involved in emotion and pain processing, is supposed to be responsible for the facilitating and inhibiting effects of emotion on pain processing (Bushnell, et al., 2013; Tracey & Mantyh, 2007; Vogt, 2005). For Instance, the amygdala was found to be involved in the detection of threat (Ohman, 2005) and the processing of negative emotion in general (Phelps & LeDoux, 2005). Furthermore, this structure was also shown to be engaged during the perception of pain and its modulation via the activation of regulatory brainstem areas (Apkarian, et al., 2005; Tracey & Mantyh, 2007). Similarly, the ACC was found to play a key role in the generation and expression of emotion (Etkin, Egner, & Kalisch, 2011), and the modulation of the affective dimension of pain (Apkarian, et al., 2005; Rainville, et al., 1997).

In the present study, previous results showing the emotional modulation of pain could be replicated. Thus, participants of the control group rated pain stimuli during the presentation of negative pictures as more painful (sensory pain ratings) and more unpleasant (affective pain ratings) than during the presentation of positive pictures. In line with that, participants of the congruent group gave higher pain ratings and showed elevated SCR in response to heat pain stimuli paired with negative emotional pictures, introduced as nocebo compared to positive pictures introduced as placebo. However, the results of the incongruent group showed that the typical pain modulating effect of positive and negative emotional stimuli was altered by the additional placebo-nocebo manipulation. Negative pictures instructed as reducing the perception of pain (placebo) no longer increased the behavioral and physiological measures of pain compared to positive pictures (nocebo). Moreover, affective pain ratings even revealed a reversal of the classic effect, in such way that participants of the incongruent group rated pain as more unpleasant when watching positive (nocebo) compared to negative (placebo) pictures. These findings suggest that the reliable modification of pain by emotion, supposedly can be further modified by a re-interpretation

process which deals with the incongruence of emotional valence vs. functional significance (e.g., when positive pictures predict a negative outcome).

### 3.3.2 Do Placebo Instructions Alter Emotion Processing?

In a study by Petrovic et al. (2005) it was shown that the processing of negative emotion is susceptible to placebo manipulations, similar to the processing of pain. In this study, the belief of having received an anxiolytic drug (actual placebo) resulted in reduced feelings of unpleasantness when watching negative affective pictures in comparison to a control condition (no drug/placebo administration). The study revealed that emotional reactivity can be manipulated in a similar manner as pain perception, and that the neural representation of emotional placebo processes is largely overlapping with neural structures involved during placebo analgesia. Accordingly, one might argue that during the present experiment, the placebo-nocebo manipulations modulated the level of induced negative affect. Consequently, reduced emotional responses to the negative stimuli would have dampened the impact of negative emotions on pain. However, one would have expected a modification to a similar extent for both picture types and both groups, respectively. In contrast, the results suggest that negative stimuli were especially prone to a pain easing placebo manipulation. Moreover, physiological responses to the emotional pictures as well as emotional picture ratings revealed no effect of experimental group. This clearly contradicts the assumption that the placebo-nocebo manipulation affected emotional reactivity in the first place, and thus altered the perception of pain indirectly. Instead, the results clearly suggest a placebo-nocebo response which modulated the perception of pain. This interpretation is in line with a very recent experiment investigating the influence of a placebo vs. verum administration of opioids (remifentanil) on the processing of pain and its potential interaction with emotion processing and attention performance (Atlas, Wielgosz, Whittington, & Wager, 2013). The authors found, that although a clear placebo effect was demonstrated for pain, no effect evoked for the processing of emotional pictures. In a study, similar to the present experiment, Bradley et al (2005) investigated the influence of negative expectations on subjective and physiological measures of emotion processing. The authors compared two groups of participants, which were either told that pleasant pictures would never be paired with an electrical shock while unpleasant affective pictures would indicate the occasionally administration of electrical shocks or the exact opposite (positive pictures serving as threat and negative pictures serving as safety signals). Afterwards, participants of both groups

watched unpleasant and pleasant pictures while heart rate, skin conductance, M. corrugator supercilii EMG and startle responses were measured. All physiological measures showed elevated responses to the threat compared to the safety condition, irrespective of the emotional valence of the cues. Only the startle response still revealed stronger inhibition by pleasant compared to negative pictures independently of signaling threat or safety. The results showed that the original activation of the defensive and appetitive system can be altered by verbal instruction, especially if they provide threat-related information. To a certain degree, these findings are in contrast to the results of the present study, which demonstrated a preservation of valence-driven emotional responses (M. corrugators supercilii and M. zygomaticus major EMG) in both groups. However, a cue indicating a relative pain increase (nocebo) might have been less aversive than a cue signaling the application of an electrical shock and herby resulting in higher levels of threat. In addition, participants of the study by Bradley et al (2005) were told that during the whole picture presentation period (6 s) a shock could be delivered, possibly leading to even *sustained* fear. Instead, during the present study the pain stimulation was highly predictable to the participants, always occurring in the same interval after trial onset. This probably resulted in reduced levels of uncertainty and threat. Regarding the influence of the placebo-nocebo manipulation on the emotional modulation of pain, the present study showed that the pain augmenting effect of negative pictures was decreased. These results are in line with the physiological responses during negative picture presentation in the study by Bradley et. al. (2005), which were dampened when pictures were indexing safety. Recently, Bublatzky and Schupp (2012) demonstrated that signaling threat by positive and negative emotional pictures resulted in the elevated cortical processing (EEG) of threat cues compared to safety cues, irrespective of picture valence. However, the differential processing of pleasant and unpleasant affective stimuli was shown to remain preserved, similar to the picture ratings and EMG responses of the present study. Even more interesting, no additive effect of picture content and threat-imminence (negative pictures vs. positive pictures cueing danger) was revealed. In line with the present experiment, these findings suggest that the instructed significance of affective stimuli (positive picture cueing threat or positive pictures cueing nocebo) does not change the processing of the affective picture per se. Presumably, the change of functional significance of the emotional stimuli is reflected by the threat specific modulation of the ERP (Bublatzky & Schupp, 2012) or by the increased pain ratings for nocebo compared to placebo trials in the present study.

Findings by Aslaksen and Flaten demonstrated a reduction of negative affect (subjective and physiological stress) in response to the administration of a placebo treatment, which reduced the perception of pain compared to a natural history condition (Aslaksen & Flaten, 2008a). Similarly, previous research revealed that high levels of fear of pain predicted low placebo responding (Lyby, Aslaksen, & Flaten, 2010). Further, the induction of anxiety (induced by the announcement of an aversive shock) eliminated placebo responses compared to a neutral condition (no shocks) (Lyby, et al., 2012). In addition, Scott et al. (2007) reported reduced negative affect *after* placebo introduction but *before* pain administration, suggesting an causative role for the reduction of negative emotions promoting placebo analgesia. These findings underscore the close relation of negative affect modulation and placebo analgesia. Regarding the nocebo response, Benedetti and colleagues (Benedetti, Amanzio, Vighetti, & Asteggiano, 2006) showed that nocebo induced hyperalgesia was accompanied by feelings of anxiety and elevated levels of physiological stress. In contrast to the placebo analgesic effect, which is to a large extent mediated by opioids (Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005; Eippert, Bingel, et al., 2009; Zubieta, et al., 2005), the nocebo response is commonly assumed to be mediated by the brain-gut peptide cholecystokinin (CCK). CCK is released during stress and anxiety and evolves its pro-nociceptive influence by impacting the PAG (Lovick, 2008). The effect of CCK on pain can be selectively blocked by the administration of its specific antagonist proglumid, although it does not reduce physiological and subjective levels of stress (Benedetti, et al., 2006). Furthermore, nocebo hyperalgesia can also be attenuated by the application of anxiolytic drugs like benzodiazepine (Benedetti, et al., 1997), therefore anxiety seems sufficient to induce nocebo hyperalgesia but is not necessarily causative. In the present study we found the differentiation between placebo and nocebo trials to be most pronounced in the congruent group. This is in line with the assumed interaction of placebo, nocebo and emotion: pleasant pictures induce positive affect similar to placebo treatments, whereas unpleasant pictures induce negative affect similar to anxiogenic nocebo treatments and thereby amplify the respective placebo or nocebo effect. This interpretation is supported by the sensory pain ratings and the pain-associated SCR of the congruent group for the placebo compared to nocebo trials. The results of the incongruent group revealed that the introduction of a placebo coupled with negative and a nocebo with positive affective pictures failed to generate a significant differentiation for sensory pain ratings or SCR. However, the affective pain ratings of the incongruent group suggest that a

placebo manipulation can decrease the pain augmenting effect of negative emotions, likely due to a re-evaluative process that seems to be more dominant than the modulation of pain by emotion.

### 3.3.3 Limitations and Outlook

Similar to Experiment 1, the actual study lacks a true reference condition that would be helpful to interpret the present findings as a result of a placebo or a nocebo effect. On a descriptive level, the incongruent group seems to especially benefit from the placebo instructions, which dampened the pain increasing effect of negative emotions. In contrast, the effect of positive emotions on the pain ratings remained relatively unaffected by the placebo or nocebo instructions in both groups. Certainly, future adaptations of the present design should encompass a neutral condition similar to previous studies that used emotionally neutral stimuli as a suitable comparison (Kenntner-Mabiala, et al., 2008; Kenntner-Mabiala & Pauli, 2005; Roy, Lebuis, Peretz, & Rainville, 2011).

The pictures used in the present study were selected based on their normative ratings (Lang, et al., 2008) to create two distinct sets of pictures which differ only with regard to emotional valence. However, subjective ratings revealed higher levels of arousal for the negative compared to the positive pictures. Given that skin conductance was found to be especially sensitive to variations of emotional arousal (Lang, et al., 1993), one may argue that the elevated SCR during nocebo trials of the congruent group resulted from the negative picture content. In favor of the present interpretation, which assumes the elevated SCR to represent a physiological correlate for the behavioral nocebo responses, the SCR revealed no differences in response to the emotional pictures during the test phase *before* pain onset. Moreover, one would have expected higher responses in the incongruent group to the pain stimulation cued by negative pictures as well. In addition, SCR was corrected for potential picture responses (baseline correction), therefore it seems highly plausible that higher SCR during nocebo trials of the congruent group actually reflect a physiological correlate for elevated pain during nocebo compared to placebo trials.

Similar to Experiment 1, the recording of pain-associated facial EMG responses failed to produce a clear differentiation between placebo and nocebo trials in the test phase when pain stimulation levels were identical. However, M. corrugator supercilii and M. zygomaticus major activity in response to the emotional pictures were in line with earlier studies

investigating evaluative facial responses during affective picture processing (Bradley, et al., 2001; Lang, et al., 1993). The difference between placebo and nocebo trials might have been too weak to produce different responses on a facial muscular level, especially since facial responses were shown to be most sensitive for high and prolonged pain stimuli (Kunz, et al., 2011; Kunz, Lautenbacher, LeBlanc, & Rainville, 2012). Again, similar to Experiment 1, no influences of state or trait variables were observed, which have been suggested in earlier studies to moderate the placebo response such like optimism (Geers, Wellman, Fowler, Helfer, & France, 2010) or suggestibility (De Pascalis, et al., 2002). This is probably due to the sample size, which might have been too small for conducting a correlation analysis within a placebo paradigm (Colloca, et al., 2013).

A crucial extension of the present design would be the online assessment of subjective measures of emotional picture processing in addition to pain ratings. This would allow evaluating whether the perception of pain changes as a function of altered emotion processing due to the placebo-nocebo manipulation.

### 3.3.4 Conclusion

The present study showed that placebo-nocebo manipulations interact with emotions during the modulation of pain (placemo). The coupling of a placebo manipulation with positive emotional pictures and a nocebo manipulation with negative emotional pictures (congruent group) resulted in a more pronounced behavioral and physiological modulation of pain compared to the vice versa pairing of a placebo manipulation with negative pictures and a nocebo manipulation with positive pictures (incongruent group). Furthermore, affective pain ratings of the incongruent group revealed that a psychological placebo manipulation (instruction + conditioning) is sufficient to reduce and even reverse the impact of negative emotions on pain. The comparison of both groups suggests the “placemo” effect to be mediated mostly by the dampening of the pain increasing effect of negative emotions (placebo manipulation of the incongruent group). These findings might represent a promising perspective for the successful implementation and harnessing of placebo effects even in patients (Enck, et al., 2013). For instance, one may speculate of rendering side effects as beneficial and thereby dampen negative symptoms or intermediate symptom worsening.

# 4. Experiment 3: Behavioral, Subjective and Neural Correlates of Placebo Analgesia/ Nocebo Hyperalgesia Cued by Emotional Pictures

Placebo analgesia interventions were found to elicit large behavioral effects, such as the reduction of pain ratings and/or self-administration of analgesic medication compared to a control or natural history condition (Benedetti, et al., 2003; Colloca, et al., 2013; Price, Finniss, et al., 2008; Voudouris, et al., 1990). Likewise, the involvement of opioids during placebo analgesia and CCK during nocebo hyperalgesia could be demonstrated in experiments that captured behavioral and physiological measures of pain (Benedetti, et al., 1997; Benedetti, et al., 2006). However, insight about the involved neural mechanism was missing until the advent of neuroimaging studies. These allowed scrutinizing on the neural and neurochemical mechanisms underlying placebo and nocebo effects and targeting the interaction of the involved cognitive and biological processes (Eippert, Finsterbusch, et al., 2009; Wager, et al., 2004; Zubieta, et al., 2005).

In a seminal fMRI study, Wager et al. (2004) applied a sham cream to the participants' forearm, telling them it was a powerful analgesic and additionally manipulated the participants' experience in a subsequent placebo conditioning phase. Thereafter participants entered a test phase receiving identical pain stimuli to placebo treated and untreated patches of skin. It was found that neural responses to pain were decreased during placebo compared to nocebo conditions (ACC, thalamus, insula) while the anticipation of placebo was reflected by elevated activity in DLPFC and mid brain areas. Since then, similar placebo analgesia paradigms have been conducted, which revealed activations of the DLPFC (Krummenacher, Candia, Folkers, Schedlowski, & Schönbächler, 2010; Lui, et al., 2010; Wager, et al., 2004) as well as the rACC (Amanzio, Benedetti, Porro, Palermo, & Cauda, 2013; Bingel, et al., 2006; Geuter, et al., 2013; Wager, et al., 2004) during the anticipation of placebo and therefore are considered as key players for the mediation of placebo analgesia. Likewise, the reduction of pain was found to be paralleled on the neural level by the down-regulation of brain areas that were commonly found to be activated during the processing of pain such as the somatosensory cortex, the insula, and the thalamus (Amanzio, et al., 2013; Apkarian, et al., 2005; Wager, et al., 2013).

Much less research has focused on nocebo hyperalgesia so far. Kong et al. (2008) conducted one of the very few fMRI studies applying pain stimuli to the participants after they

had received a negative instruction about a pain enhancing treatment. They found elevated signals, mainly in the medial pain system when comparing nocebo with control conditions (Kong, et al., 2008). Besides, their results suggest the hippocampus to be a key structure for the induction of nocebo effects. This seems even more compelling since the hippocampus and the entorhinal cortex were found to be involved during the exacerbation of pain by anxiety (Ploghaus, et al., 2001). In this vein, Benedetti et al (2006) conducted an experiment, showing that the induction of nocebo effects comes along with elevated levels of anxiety and the transmission of the anti-opioidergic peptide CCK. In a recent review article, Wiech et al. (2009) hypothesize that the transmission of CCK during nocebo induced anxiety is impacting early pain modulating relays in the brainstem, which represents a convincing theoretical mechanism on how nocebo hyperalgesia might be centrally mediated. In addition, just recently it could be shown that nocebo hyperalgesia is also reflected in elevated responses of the spinal cord, providing further evidence for the involvement of the earliest relays of nociceptive signals transmission in the generation of nocebo responses (Geuter & Buchel, 2013).

As already reviewed above, placebo analgesia and placebo effects aiming at a dampened processing of negative affect were found to share substantial neural overlap (Petrovic, et al., 2005): Participants were caused to believe of having received a potent anxiolytic medication (pharmacological conditioning with benzodiazepine, 1 day ahead of the test session) before watching negative emotional stimuli. The administration of the placebo resulted in decreased feelings of emotional unpleasantness when watching negative affective pictures and a decreased activation of a functionally defined emotional brain network encompassing areas in the visual cortex and the amygdala. The comparison of the placebo and the control condition revealed higher activity in the rACC and the orbitofrontal cortex in line with fMRI studies on placebo analgesia (Amanzio, et al., 2013; Geuter, et al., 2013; Wager, et al., 2004), suggesting a commonly involved network, rather than a placebo-analgesia-specific neural representation. Just recently, the influences of a placebo manipulation was also demonstrated for the experience of disgust (Schienle, Ubel, Schongassner, Ille, & Scharmuller, 2013). Participants were administered a sham pill that was supposed to contain an antiemetic agent from a tree found in South America. The placebo treatment resulted in significantly less intense feelings of disgust, what was paralleled on the neural level by reduced responses of

the insula (Schienle, et al., 2013), which was previously found to be involved in the central representation of disgust (Wicker et al., 2003).

To investigate the interaction of placebo and emotion (placemo) effects on pain and especially its neural correlates, the paradigm established in Experiment 2 was adapted to the fMRI. Again, two groups of participants were realized. One group was told that positive emotional pictures will decrease and negative pictures will increase the perception of pain (congruent), before they entered the fMRI scanner. A second (incongruent) group was told the exact opposite. All participants first completed a placebo conditioning paradigm (as explained in Experiment 2) and received high pain stimuli during nocebo and low pain stimuli during placebo trials. Subsequently, participants entered a test phase and watched the placebo and nocebo cues while receiving the exact same level of pain stimulation. Based on the results of placebo and nocebo imaging studies on the one hand, and studies that focused on the affective modulation of pain on the other hand, higher activity in areas that were found to mediate placebo responses like the rACC or the DLPFC during placebo compared to nocebo trials (anticipation of pain as well as actual pain stimulation) were expected. For the contrast of nocebo > placebo trials higher activity in pain processing areas - as described in 1.1.1. - was expected. Further, in line with the research on nocebo hyperalgesia the hippocampus and adjacent areas were assumed to demonstrate elevated activation for the comparison of nocebo > placebo. In addition, it was expected to find higher activity of the parahippocampal gyrus, the insula and the paracentral lobule when comparing pain responses during negative > positive picture trials, replicating earlier findings (Roy, et al., 2009). In accordance with the results of the SCR and the sensory pain ratings of Experiment 2, it seems likely that placebo-.nocebo effects will be more pronounced within the congruent group however, placebo-nocebo effects might occur also in the incongruent group, replicating the results of the affective pain ratings.

## 4.1 Method

### 4.1.1 Participants

Thirty two participants (16 women) were recruited from the University of Würzburg, of which two participants had to be excluded from the final sample (one in the congruent group, one in the incongruent group) due to technical problems with the fMRI scanner. Participants received € 20 as compensation. All participants were right-handed, none of them

had taken any analgesic medication for the last 24 h prior to the test session (self-report). Participants were randomly allocated to one of the two experimental groups. Participants signed a written informed consent before participating in the study and received a detailed instruction of the experimental procedure. Written instruction for the two experimental conditions varied according to the different experimental manipulations: **congruent group** ("positive images will decrease and negative images will increase the perception of pain") vs. **incongruent group** (exact opposite: "negative pictures will decrease and positive images will increase the perception of pain"), for further details, see ***Fig.* 10**, **Suppl. 9** and **Suppl. 10** participants filled out questionnaires on state and trait anxiety (Spielberger trait and state anxiety inventory, STAI-T/S) (Laux, et al., 1981; Spielberger, 1970), positive and negative mood, (Positive and Negative Affect Schedule, PANAS, Krohne, et al., 1996; Watson, et al., 1988), pain catastrophizing (PCS) (Meyer, et al., 2008; Sullivan, et al., 1995), pain sensitivity (Pain Anxiety Sensitivity Scale, PASS) (McCracken, Zayfert, & Gross, 1992; Walter, Hampe, Wild, & Vaitl, 2002) ,life orientation, i.e. dispositional optimism and pessimism (Glaesmer, et al., 2008), and sensitivity for reward and punishment (SPSRQ) (Torrubia, et al., 2001) in the German translation by Hewig & Hagemann (2002; Der SPSR Fragebogen von Torrubia, Ávila, Moltó & Caseras, unpublished, University of Trier, personal communication). Further, socio-demographic information and personal attitudes towards pain were assessed and participants completed a test for suggestibility (CURSS; Spanos, et al., 1983). The two groups did not statistically differ from each other with regard to all collected measures, except for state anxiety (STAI-S), which was slightly higher in the congruent ($M$ = 33.8, $SD$ = 4.52) than in the incongruent group ($M$ = 37.8, $SD$ 5.78; $F(1,28) = 4.45$, $p = .04$), for further details see **Tab. 4**. All subjects had normal or corrected-to-normal vision, and no current or prior history of chronic pain, neurological or psychiatric disorders (self-report). The experimental procedure was approved by the institutional review board of the Medical Faculty of the University of Würzburg.

**Tab. 4**. Sample Description of Study 3

| Measure | Con (n = 15) | | Incon(n = 15) | | | |
|---|---|---|---|---|---|---|
| | *M* | *SD* | *M* | *SD* | *F*(1,28) | *p* |
| Pain threshold in °C | 46.18 | 1.64 | 45.92 | 2.42 | 0.12 | .73 |
| Age | 24.67 | 2.97 | 23.20 | 2.40 | 2.22 | .15 |
| STAI_State | 33.80 | 4.52 | 37.80 | 5.78 | 4.45 | .04 |
| PANAS_Positive | 31.33 | 5.74 | 31.27 | 4.85 | 0.00 | .97 |
| PANAS_Negative | 11.67 | 2.09 | 13.00 | 2.51 | 2.50 | .13 |
| STAI_Trait | 35.80 | 8.55 | 36.00 | 6.07 | 0.01 | .94 |
| LOT_R | 6.40 | 2.82 | 8.00 | 3.36 | 1.99 | .17 |
| SPSRQ_Punishment | 6.60 | 4.42 | 8.60 | 4.60 | 1.48 | .23 |
| SPSRQ_Reward | 11.33 | 3.31 | 10.13 | 2.75 | 1.17 | .29 |
| PASS_D | 88.07 | 26.02 | 98.73 | 19.66 | 1.61 | .22 |
| PCS | 16.27 | 6.69 | 15.47 | 4.72 | 0.14 | .71 |
| CURSS_subjective | 6.60 | 4.48 | 5.40 | 3.87 | 0.62 | .44 |
| # of convinced participants | 13/15 | | 10/15 | | $\chi^2$ | .20 |
| Convincibility | 6.4 | 1.84 | 6.07 | 1.62 | 0.28 | .60 |

***Note:*** LOT= Life Orientation Test; PCS= Pain Catastrophizing Scale; PASS_D: Pain Anxiety Sensitivity Scale; STAIT/S= State/Trait Anxiety Inventory; PSQ = Pain Sensitivity Questionnaire; PANAS= Positive and Negative Affect Schedule; SPSRQ= Sensitivity for Punishment, Sensitivity for Reward Questionnaire; CURSS= Carleton University Responsiveness to Suggestion Scale, # of convinced participants = number of participants responding with YES to the question whether they believed in the instruction; Convincibility was conducted on a 10 point Likert scale, 0 = not all compelling, 10 = very much compelling; Con= Congruent group, Incon= Incongruent group.

### 4.1.2 Experimental Groups and Placebo Manipulation

Analogously to Experiment 2, two different experimental groups were realized and varied according to A) the instruction given prior to the experiment and to B) the contingency of picture valence and level of pain stimulation during placebo conditioning. The congruent group (placebo = positive pictures, nocebo = negative pictures) and incongruent group (placebo = negative pictures, nocebo = positive pictures) received the same instructions used in Experiment 2, informing about the pain easing/pain increasing effect of emotions, see 3.1.2. Placebo-nocebo conditioning was performed analogously to Experiment 2, however this time participants were lying in the fMRI scanner. While watching the positive pictures the congruent group received low pain stimulation (heat pain threshold) and high pain stimulation (heat pain threshold + 1°C) while watching negative affective pictures. For the incongruent

group the contingency of affective pictures and pain level was the exact opposite. For further details about the manipulation see ***Fig.* 10.**

### 4.1.3 Emotional Pictures as Placebo / Nocebo Cues

Placebo and nocebo cues consisted of the same 10 emotional pictures (5 negative and 5 positive) drawn from the International Affective Picture System (IAPS, Lang, 2008) as in Experiment 2 (see **3.1.4**). Positive and negative pictures served as placebo and nocebo cues, respectively. Before starting the actual experiment, participants watched every emotional picture for 20 s and rated its emotional valence and arousal using the Self-Assessment Manikin (SAM, Bradley, 1997) while already lying in the scanner. Usage of the SAM scale was illustrated to the participants via written instruction. The visual placebo and nocebo cues were presented with MRI-compatible video goggles (VisuaStim; Magnetic Resonance Technologies, Northridge, CA, USA; resolution 640 x 480 pixel) to the participants.

### 4.1.4 Pain Threshold Assessment

Thermal heat stimuli were delivered using a Somedic MSA thermal stimulator (Somedic Sales AB, Hörby, Sweden) and a fMRI compatible Peltier thermode with an active surface of 25 x 50 mm. Thermal pain threshold assessment was the same as in Experiment 1 and 2, see 2.1.4.

### 4.1.5 Thermal Pain Stimulation and Pain Ratings

During the conditioning phase, two different levels of pain stimuli were delivered analogously to Experiment 1 and 2: for placebo trials the individual thermal pain threshold was applied for 3 seconds whereas during nocebo trials the threshold temperature + 1°C, was used. During the subsequent test phase the pain threshold temperature + 1 °C was used for placebo as well as for nocebo trials. Participants rated the pain stimuli regarding pain intensity and pain unpleasantness using a digitized visual analogue scale (VAS) ranging from 0 = no pain at all to 100 = unbearable pain. For further details see 2.1.5.

### 4.1.6 Skin Conductance Measurement

Skin conductance was measured using two 13/7 mm Ag/AgCl surface electrodes, filled with electrode cream (concentration of 0.5% NaCl) attached to the second phalanx of the participant left index and middle finger. Signals were pre-amplified and recorded using a MRI compatible, battery driven amplifier (BrainAmp ExG MR, Brain Products, Munich, Germany). Skin conductance signals were segmented in time windows of 20 s after emotional picture

onset, a 1 s baseline before picture onset (i.e. trial onset), and in addition the mean signal between 5 to 6 s after picture onset were subtracted to correct for baseline differences and potential picture responses, respectively. Pain associated SCR were quantified as highest positive deflection in the interval from 6 s to 19 s after trial onset (pain stimulation actually started 3 s after trial onset), see ***Fig.* 10** for further details on trial timing.

### 4.1.7 fMRI Data Acquisition and Preprocessing

#### 4.1.7.1 Image Acquisition

Brain images, functional as well as structural, were acquired in a Siemens 1.5 T MRI whole body scanner (SIEMENS Avanto, Siemens, Erlangen, Germany) using a standard 12 channel head coil. Head movement was minimized by attaching foam pads to the participants' cheeks. The head position inside the magnet was determined with an anatomical localizer, which consisted of three slices in each dimension (sagittal, frontal, axial). Before the functional run was started, a resting state measurement was conducted, lasting around 5 min, during which participants were asked to close their eyes. Data of this sequence will be reported elsewhere.

In sum, 1030 functional images were collected across the whole experiment within one session, using a T2*- weighted single-shot gradient echo planar imaging (EPI) sequence (TR: 2500 ms, TE: 30 ms, 90° flip angle, FOV: 200 mm, matrix: 64 x 64, voxel size: 3.1 x3.1 x 5 mm). Volumes contained 25, interleaved acquired, axial slices (thickness 5 mm, 1 mm gap) covering the whole brain. Slice acquisition was oriented parallel to the AC-PC line (anterior, posterior commissure). The first nine functional volumes were discarded to allow for T1 equilibration. After functional image acquisition was finished, a high-resolution T1-weighted magnetization-prepared rapid gradient-echo imaging (MP-RAGE) 3D MRI sequence was obtained (TR: 2250 ms, TE: 3.93 ms, 8 ° flip angle, FOV: 256 mm, matrix: 256 x 256, voxel size: 1 x 1 x 1 mm).

#### 4.1.7.2 Image Preprocessing

Standard preprocessing was applied using Statistical Parametric Mapping software (SPM8; Wellcome Department of Imaging Neuroscience, London, UK) implemented in Matlab R2008b (Mathworks Inc., Natick, MA, USA). Preprocessing encompassed slice-time correction, image realignment and unwarping, co-registration and segmentation (based on the individual T1-weighted anatomical images), normalization of EPI images into standard MNI-space (using the normalization parameters obtained from the segmentation procedure:

voxel size 2 × 2 × 2 mm) and spatial smoothing (applying 8 mm full-width-half-maximum (FWHM) Gaussian kernel).

#### 4.1.7.3 First Level Statistics

Seventeen regressors were included in the first level model by convolving separate stick functions (picture, pain and heat onset during pain familiarization, placebo conditioning, and test phase) or box-cars functions (emotional picture presentation block) with the canonical hemodynamic response function (HRF), see **Tab. 5**. In addition, the six movement parameters of the rigid body transformation obtained from image realignment were introduced as regressors in the statistical model, totaling in 23 regressors per session. The voxel-based time series were filtered with a high pass filter (cutoff period of 128 s) and the following contrasts were calculated for each subject and fed into a random effects second level analysis: *Negative > Positive* (picture blocks)*; Placebo-Cue onset > Nocebo-Cue onset* (test phase)*; Placebo Heat and Pain Onset > Nocebo Heat and Pain Onse*t (test phase); as well as the respective opposite of each contrast.

**Tab. 5.** Names of Regressors Included in the First Level Statistics.

| Pain Familiarization Phase | Picture Presentation Block | Placebo-Nocebo: Conditioning Phase | Placebo-Nocebo: Test Phase |
|---|---|---|---|
| Trial onset | Positive | Placebo: Cue onset | Placebo: Cue onset |
| Heat onset | Negative | Placebo: Heat onset | Placebo: Heat onset |
| Pain onset | | Placebo: Pain onset | Placebo: Pain onset |
| | | Nocebo: Cue onset | Nocebo: Cue onset |
| | | Nocebo: Heat onset | Nocebo: Heat onset |
| | | Nocebo: Pain onset | Nocebo: Pain onset |

#### 4.1.7.4 Second Level Statistics

Similar to earlier imaging studies on placebo analgesia (Bingel, et al., 2006; Geuter, et al., 2013; Wager, et al., 2004) the analyses of data from the placebo-nocebo conditioning phase was omitted, instead responses during the test phase were focused, when the level of pain stimulation was identical for placebo and nocebo trials. Whole brain analyses were conducted applying a statistical threshold of $p$ = .005, with a minimum of 10 contiguously activated voxels in a cluster (Lieberman & Cunningham, 2009). In accordance with an earlier study on emotional picture processing, a threshold of $p$ = .001 and a cluster size of $k$ = 5 voxel was applied for the results of the blocked picture presentation in the beginning of the experiment (Gerdes et al., 2010). Coordinates for region of interest analyses (ROI) were taken

from earlier studies investigating pain modulation by emotion (Roy, et al., 2009) or based on an anatomical atlas (AAL: Tzourio-Mazoyer et al., 2002) integrated in the WFU Pick atlas (Version 3.0.3, Wake Forest University, School of Medicine, NC, USA: Maldjian, Laurienti, & Burdette, 2004; Maldjian, Laurienti, Kraft, & Burdette, 2003) applying a significance level of $p$ = .05; family wise error corrected (FWE). Further, to investigate linear associations of behavioral pain measures and neural responses, pain ratings (sensory and affective) difference scores (nocebo minus placebo) of the test phase were introduced as covariate in the second level model (contrast: *Nocebo Heat + Pain Onset > Placebo Heat + Pain Onset*). Contrasts were explored across all participants (N = 30) to investigate main effects of picture content and placebo-nocebo processing. In addition, the contrasts mentioned above were also analyzed for each experimental group separately, and between contrasts were analyzed to evaluate interaction effects of experimental group (congruent [CON] vs. incongruent [INCON]) and condition (placebo vs. nocebo). Second level analysis and allocation of activity to anatomical structures were conducted using the WFU PickAtlas integrated in SPM 8 software (Lancaster et al., 2000).

### 4.1.8 Procedure

After arrival, participants were randomly assigned to one of the two experimental conditions, signed informed consent that included the expectancy manipulation, i.e., the placebo-nocebo instruction, and filled out state and socio-demographic questionnaires. Thereafter participants entered the scanner room and the individual pain threshold was assessed. Thereafter SCR electrodes were attached and participants were instructed about the pain rating procedure using visual analogue scales, as well as the emotional picture rating procedure for valence and arousal using the SAM. In the following, participants received ear plugs and were positioned inside the scanner tube. Afterwards fMRI scanning started, and participants completed five pain familiarization trials consisting of a centrally presented fixation cross (20 s) as well as the application and rating of the high pain stimulus (pain threshold + 1°C). Analogously to Experiment 2, participants subsequently watched all emotional pictures (single picture presentation for 20 s) serving as placebo or nocebo cues and rated them for valence and arousal. Afterwards, participants were presented a thumbnail of all visual placebo or nocebo stimuli respectively and were asked to rate how much they expected the presented pictures to alter their perception of pain during the experiment on a 10 point Likert scale (ranging from 0 = not at all to 9 = very much). Thereafter participants

proceeded to the second part of experimental manipulation, i.e., the placebo-nocebo conditioning phase which consisted of 30 trials (15 placebo, 15 nocebo). During the conditioning phase participants were presented nocebo cues and received painful heat stimuli (threshold + 1°C) or were presented placebo cues and received moderately painful heat stimuli (just threshold temperature). The conditioning phase was followed by the test phase that encompassed 20 trials (10 placebo, 10 nocebo). In the test phase participants watched the placebo and nocebo cues again while they received the highly painful heat stimuli (threshold + 1°C) only (see ***Fig.* 10**). During each trial (conditioning or test phase) the placebo or nocebo cue (positive vs. negative pictures, respectively) was presented in the center of the video goggles for 20 s. After 3 s the thermal stimulation was started and reached the target temperature about 2 s later and remained on the target level for 3 s. After the temperature had again reached baseline level and the emotional picture disappeared, participants were asked to rate the pain intensity and unpleasantness on the VAS. Each trial was separated by a fixed inter-trial interval (ITI) of 5 s and an additional jitter interval (500, 1000, 1500, 2000 or 2500 ms), meanwhile, a central fixation cross was presented. At end of the test phase participants were again presented thumbnails of the placebo and nocebo cues, and evaluated how they remembered the pain sensation during each trial, respectively, on a 100 point visual analogue scale. After the functional data acquisition had finished, participants left the scanner room, filled out the remaining questionnaires (PCS, SPSRQ), completed the test for suggestibility (CURSS), and were informed about the actual purpose of the study.

### 4.1.9 Statistical Analysis of the Behavioral and Physiological Data

**Affective picture ratings** were analyzed by employing separate 2-factorial repeated-measures ANOVAs for valence and arousal with the within-subjects factors picture content (2 levels: positive vs. negative) and the between-subjects factor group (2 levels: congruent vs. incongruent). Ratings of **expected pain modulation** were analyzed by employing a 2-factorial repeated-measures ANOVAs with the within-subjects factors placebo-nocebo (2 levels: placebo vs. nocebo cues) and the between-subjects factor group (2 levels: congruent vs. incongruent). Data obtained during the **conditioning phase, pain ratings** (sensory and affective) and **SCR** (pain responses) were analyzed using separate 3-factorial repeated measures ANOVAs applying the within-subjects factor stimulation level (2 levels: high vs. low pain stimulation), the within-subjects factor time (3 levels: mean of Trials 1-5 vs. Trials 6-10 vs. Trials 11-15) and the between-subjects factor group (2 levels: congruent vs. incongruent).

Regarding the **test phase, pain ratings** and **SCR** were analyzed using separate 3-factorial repeated measures ANOVAs containing the within-subjects factor placebo-nocebo (2 levels: placebo vs. nocebo), the within-subjects factor time (2 levels: mean of Trials 1-5 vs. Trials 6-10), and the between-subjects factor group (2 level: congruent vs. incongruent). **Ratings of recalled pain** (sensory and affective) were analyzed by employing separate 2-factorial repeated-measures ANOVAs with the within-subjects factors placebo-nocebo (2 levels: placebo vs. nocebo) and the between-subjects factor group (2 levels: congruent vs. incongruent). When necessary, Greenhouse-Geisser corrections of degrees of freedom were applied. Post-hoc comparisons were realized using planned contrasts or pair-wise *t*-tests. Significance level was defined as $p < .05$. Associations of psychometric measures and placebo-nocebo outcomes were analyzed using linear correlation analysis of questionnaire scores and nocebo vs. placebo differences of the test phase in sensory and affective pain ratings as well as skin conductance measures.

## 4.2 Results

### 4.2.1 Ratings of Valence, Arousal and Expected Pain Modulation

The analysis of **arousal** ratings revealed a significant effect for picture content, $F(1,28) = 8.80$, $p = .006$, $\eta_p^2 = .24$, due to higher arousal ratings of negative pictures compared to positive pictures. However, this effect was further qualified by a significant interaction of picture content and experimental group, $F(1,28) = 9.84$, $p = .004$, $\eta_p^2 = .26$, due to significantly higher arousal ratings in the incongruent group for negative compared to positive pictures, $t(14) = 4.66$, $p < .001$, whereas ratings of positive and negative pictures did not differ in the congruent group, $t(13) = -0.11$, $p = .91$. The analysis of **valence** revealed a significant effect for picture content, $F(1,28) = 501.56$, $p < .001$, $\eta_p^2 = .95$, with negative pictures being rated more negative than positive pictures, irrespective of the experimental group, $F(1,28) = 0.63$, $p = .44$, $\eta_p^2 = .02$ (see ***Fig.* 17**). The analysis of **expectation** ratings revealed a significant effect of placebo-nocebo, $F(1,28) = 31.78$, $p < .001$, $\eta_p^2 = .53$, due to higher expected pain after nocebo compared to placebo trials. The interaction of placebo-nocebo and group failed to reach significance, $F(1,28) = 1.61$, $p = .22$, $\eta_p^2 = .05$.

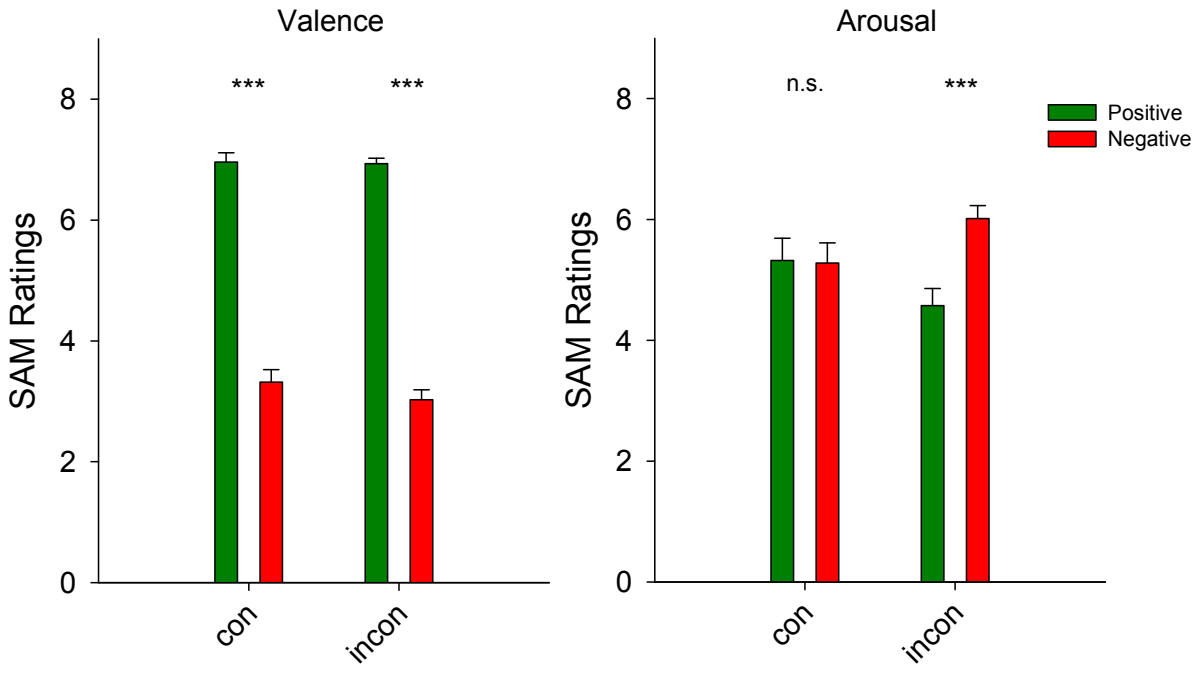


***Fig.* 16.** Means (+ *SEM*) of ratings for valence (left) and arousal (right) are depicted separately for experimental groups and picture contents; Con= congruent group; Incon = incongruent group; *** $p < .001$.

### 4.2.2 Conditioning

#### 4.2.2.1 Sensory and Affective Pain Ratings for Low (Placebo) and High (Nocebo) Pain Stimulation

The analysis of the **sensory** pain ratings revealed a significant effect of pain stimulation level, $F(1,28) = 75.174$, $p < .001$, $\eta_p^2 = .73$, due to higher ratings for high pain stimuli. No other effect of time or group reached significance (all *p*s > .18).

The analysis of the **affective** pain ratings revealed a similar pattern as the sensory pain ratings with a significant effect of pain stimulation level, $F(1,28) = 56.67$, $p < .001$, $\eta_p^2 = .67$, due to higher ratings for high pain stimuli. No other effect of time or group reached significance (all *ps* > .21).

#### 4.2.2.2 SCR in response to Pain during Placebo and Nocebo Trials

The analysis of SCR to pain revealed a significant effect of pain stimulation, $F(1,27) = 10.15$, $p = .004$, $\eta_p^2 = .27$, due to higher responses during nocebo compared to placebo trials. Furthermore, the analysis revealed a significant effect of time, $F(1,27) = 5.66$, $p = .006$, $\eta_p^2 = .17$, as a result of higher SCR during Trials 1-5 compared to Trials 6-10,

*F*(1,27) = 6.21, *p* = .02, $\eta_p^2$ = .19, and Trials 11-15, *F*(1,27) = 6.34, *p* = .02, $\eta_p^2$ = .19. In addition, the interaction of time and experimental group reached significance, *F*(2,54) = 3.76, *p* =.05, $\eta_p^2$ = .12. Post hoc *t*-test within the incongruent group revealed significantly higher responses for Trials 1-5 compared to Trials 6-10, *t*(14) = 2.58, *p* =.02, and marginal significant higher responses during Trials 6-10 compared to Trials 11-15, *t*(13) = 2.04, *p* =.06, whereas the same comparisons failed to reach significance for the congruent group (all *ps* > .63). The interaction of pain stimulation and group reached marginal significance, *F*(1,27) = 3.16, *p* = .09, $\eta_p^2$ = .11, as a result of higher differentiation between nocebo and placebo trials within the incongruent group, *F*(1,13) = 9.37, *p* = .01, $\eta_p^2$ = .42, whereas in the congruent group the same comparison was not significant, *F*(1,13) = 1.37, *p* = .26, $\eta_p^2$ = .09.

### 4.2.3 Test Phase

#### 4.2.3.1 Sensory Pain Ratings of Placebo and Nocebo Trials

The analysis of the sensory pain ratings revealed a significant effect of placebo-nocebo, F(1,28) = 16.30, p < .001, ηp2= .37, due to higher ratings for nocebo compared to placebo trials irrespective of experimental group (see ***Fig. 18***). No other effect of time or group or any interaction reached significance (all ps > .46).

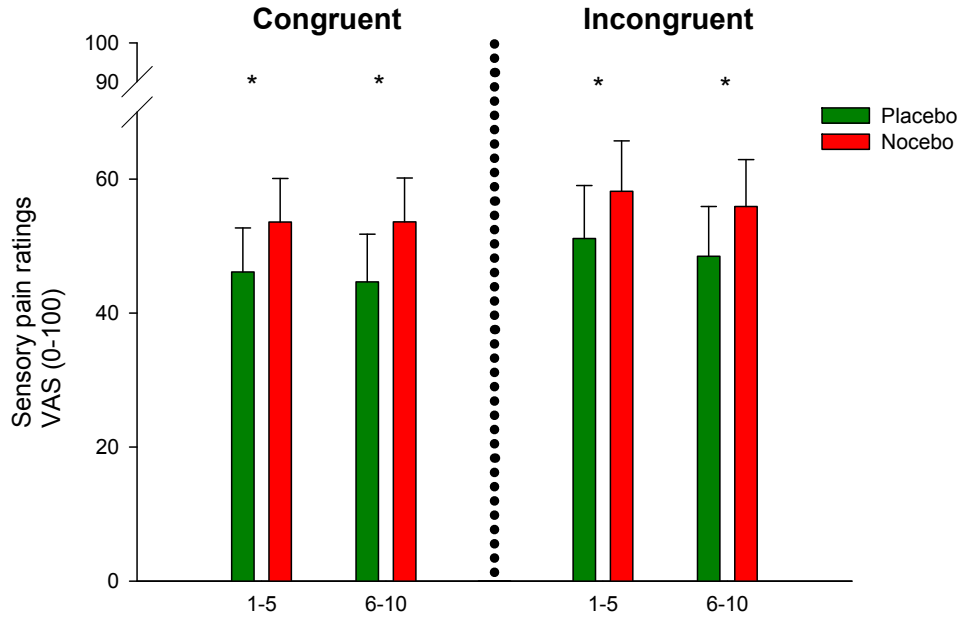


***Fig. 17.*** Mean (+ *SEM*) sensory pain ratings of the test phase are depicted separately for each experimental group, split by Trials 1-5 and 6-10; * *p* < .05.

#### 4.2.3.2 Affective Pain Ratings of Placebo and Nocebo Trials

The analysis of the affective pain ratings revealed a significant effect of placebo-nocebo, $F(1,28) = 16.48$, $p < .001$, $\eta_p^2 = .37$, due to higher ratings for nocebo compared to placebo trials irrespective of experimental group (see ***Fig.* 19**). No other effects of time, group, or any interaction reached significance (all *ps* > .14).

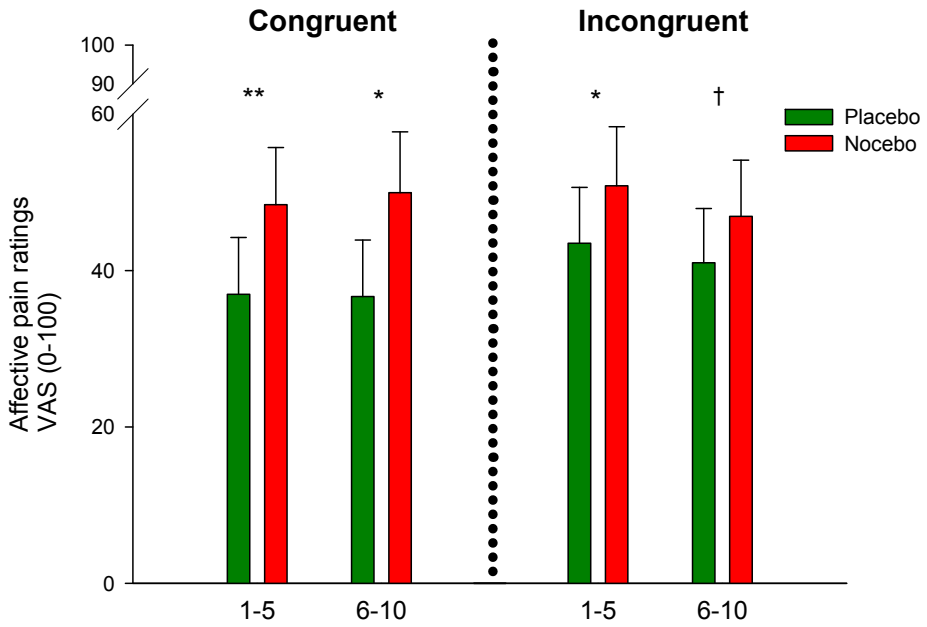


***Fig. 18***. Mean (+ *SEM*) affective pain ratings of the test phase are depicted separately for each experimental group, split by Trials 1-5 and 6-10;* $p < .05$; ** $p < .01$; † $p = .057$.

#### 4.2.3.3 SCR in Response to Pain (6-19s after Cue Onset) during Placebo and Nocebo Trials

The analysis of SCR in response to pain revealed a significant effect of placebo-nocebo, $F(1,27) = 8.74$, $p = .006$, $\eta_p^2 = .25$, due to higher responses during nocebo compared to placebo trials. In addition, a marginal significant interaction of experimental group and placebo-nocebo was found, $F(1,27) = 3.00$, $p = .095$, $\eta_p^2 = .10$. Post hoc *t*-test revealed significantly higher responses for nocebo compared to placebo trials in the incongruent group, $t(13) = 4.28$, $p = .001$, whereas the same test failed to reach significance within the congruent group, $t(14) = 0.75$, $p = .47$ (see ***Fig.* 20**).

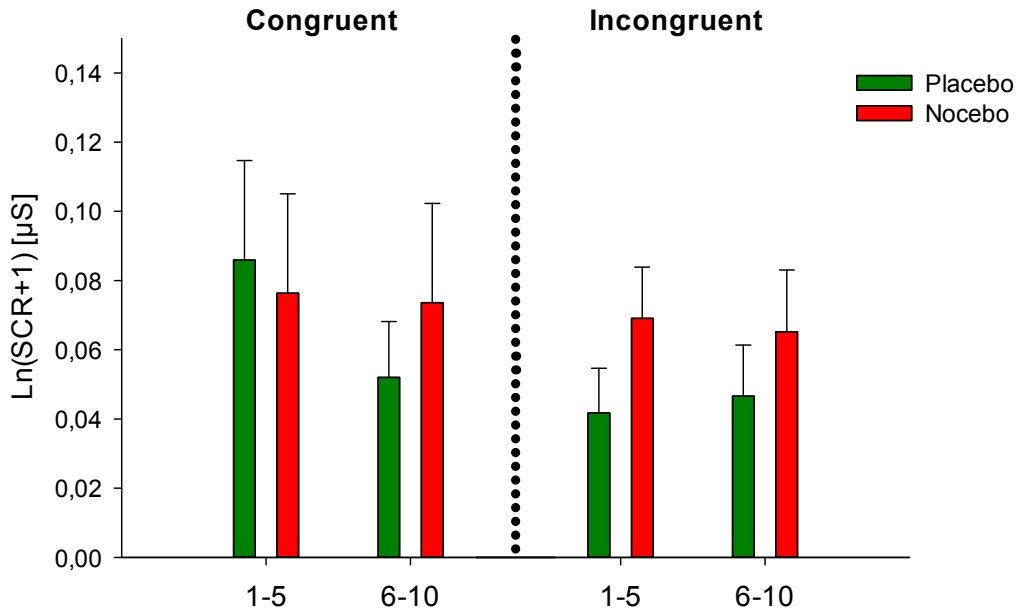


***Fig. 19.*** Mean SCR (+ SEM) during the test phase in response to the pain stimulation are depicted separately for each experimental group, split by Trials 1-5 and 6-10; nocebo > placebo collapsed across time and group was significant, $p$ = .006.

### 4.2.4 Ratings of Recalled Sensory and Affective Pain

The analysis of the ratings for recalled **sensory** pain revealed a significant effect of placebo-nocebo, due to higher recalled ratings for nocebo compared to placebo trials, $F(1,28) = 21.76$, $p < .001$, $\eta_p^2 = .44$. The interaction of placebo-nocebo and group did not reach significance, $F(1,28) = 1.82$, $p = .19$, $\eta_p^2 = .06$. The analysis of the ratings for recalled **affective pain** revealed similar results, such as a significant effect of placebo-nocebo, due to higher recalled ratings for nocebo compared to placebo trials, $F(1,28) = 25.73$, $p < .001$, $\eta_p^2 = .48$. Similar to the sensory pain ratings, the interaction of placebo-nocebo and group only revealed a non-significant trend, $F(1,28) = 2.62$, $p = .12$, $\eta_p^2 = .09$, most likely due to a stronger differentiation between nocebo and placebo trials in the congruent group ($\Delta M = 30.60$, $SEM = 7.95$, $t(14) = 3.85$, $p = .002$) than in the incongruent group ($\Delta M = 15.80$, $SEM = 4.53$, $t(14) = 3.49$, $p = .004$).

### 4.2.5 Psychometric Measures and Placebo-Nocebo Algesia

No significant association of questionnaire scores and nocebo > placebo difference values of the pain ratings or SCR pain responses during the test phase were found.

### 4.2.6 Brain Responses

#### 4.2.6.1 Affective Picture Processing [Congruent + Incongruent, N = 30]

Whole Brain analyses for the responses to negative > positive affective pictures, collapsed across all participants irrespective of experimental group, revealed higher activity in the visual cortex, the left rolandic operculum, the left parahippocampal gyrus as well as the inferior and middle frontal gyrus, whereas the opposite comparison of positive > negative pictures revealed no significant clusters (see **Tab. 6**).

**Tab. 6.** Brain Responses during Affective Picture Presentation in the Beginning of the Experiment.

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **N = 30** Neg >Pos | 16326 | Middle occipital gyrus / Calcarine L/R / Fusiform L/R / Cerebellum | -12 | -80 | 2 | 9.07 | $p < .001$ |
| | 64 | Insula L / Inferior frontal gyrus L / Rolandic operculum L | -36 | 0 | 16 | 4.47 | $p < .001$ |
| | 14 | Middle frontal gyrus L/ (BA 10) | -38 | 56 | 0 | 4.40 | $p < .001$ |
| | 36 | Cerebellum | -22 | -58 | -36 | 4.24 | $p < .001$ |
| | 22 | Para-hippocampal gyrus R | 28 | -22 | -20 | 4.13 | $p < .001$ |
| | 8 | Right inferior frontal gyrus | 32 | 30 | -14 | 3.90 | $p < .001$ |
| | 15 | Caudate R | 10 | 10 | 10 | 3.86 | $p < .001$ |
| | 17 | Inferior frontal gyrus L | -50 | 34 | 2 | 3.78 | $p < .001$ |
| | 19 | Cerebellum | -4 | -54 | -38 | 3.78 | $p < .001$ |
| | 6 | Temporal pole L | -48 | 18 | -18 | 3.69 | $p < .001$ |
| | 7 | Thalamus R | 22 | -28 | 2 | 3.68 | $p < .001$ |
| | 9 | Inferior frontal gyrus R | 44 | 8 | 20 | 3.61 | $p = .001$ |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .001$; with a minimum cluster size of $k = 5$.

#### 4.2.6.2 Affective Picture Processing [Congruent]

Within the **congruent** group, the same contrast of negative > positive pictures revealed higher activity in the visual cortex (middle and superior occipital gyrus), the fusiform gyrus, the inferior temporal gyrus, and the superior parietal cortex, whereas the contrast positive > negative revealed significant clusters mainly in the superior temporal gyrus (see **Suppl. 11**).

#### 4.2.6.3 Affective Picture Processing [Incongruent]

The **incongruent group** similarly, revealed for the contrast of negative > positive pictures large clusters of activity in the occipital lobe (left and right cuneus, middle occipital gyrus, precuneus), in prefrontal areas (left and right inferior and middle frontal gyrus, right medial gyrus) as well as the left and right parahippocampal gyrus and the left amygdala. The contrary contrast of positive > negative pictures showed no significant activations (see **Suppl. 12**).

#### 4.2.6.4 Anticipatory Placebo/Nocebo CUE Responses [Congruent + Incongruent, N = 30]

Brain activity across all participants during the test phase in responses to the nocebo compared to the placebo cues (nocebo-cue > placebo-cue) was higher in the thalamus and the prefrontal cortex and the ACC. The opposite contrast (placebo-cue > nocebo-cue) revealed activation of the left medial prefrontal cortex, and the superior frontal gyrus, which were distinct from the activity found for nocebo > placebo (see **Tab. 7**).

**Tab. 7.** Brains Responses during Anticipation (Cue) of the Test Phase Averaged across both Groups (Congruent +Incongruent, N = 30).

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **N = 30** | 157 | Thalamus L/ Extra Nuclear | -4 | -8 | 8 | 4.29 | $p < .001$ |
| Nocebo> | 25 | Anterior cingulate L | -16 | 40 | 18 | 3.90 | $p < .001$ |
| Placebo | 29 | Thalamus R/Extra Nuclear | 6 | -20 | 20 | 3.61 | $p = .001$ |
| [Cue] | 19 | Subgyral | 20 | 38 | 0 | 3.38 | $p = .001$ |
| | 18 | Superior frontal gyrus R | 24 | 54 | 34 | 3.36 | $p = .001$ |
| | 10 | Middle frontal gyrus R | 32 | 36 | 30 | 3.08 | $p = .002$ |
| Placebo> | 30 | Superior frontal gyrus R/(BA 8) | 6 | 38 | 54 | 3.44 | $p = .001$ |
| Nocebo | 12 | Medial frontal gyrus L/ (BA 9) | -4 | 50 | 38 | 3.06 | $p = .002$ |
| [Cue] | | | | | | | |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .005$; with a minimum cluster size of $k = 10$.

#### 4.2.6.5 Anticipatory Placebo/ Nocebo CUE Responses [Congruent]

To further scrutinize group-specific responses, the contrasts were calculated separately for each group. The contrast nocebo-cue > placebo-cue (i.e., positive pictures cueing placebo, negative pictures cueing nocebo) revealed for the **congruent** group a cluster of brain activity in the right middle temporal gyrus. The contrary contrast (placebo-cue > nocebo-cue) indicated higher activation of the left ACC and brainstem areas (see **Tab. 8**).

**Tab. 8.** Brain Responses of the Congruent Group during Anticipation (Cue) of the Test Phase.

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **CON n = 15** | 31 | Middle temporal gyrus R | 48 | -36 | -12 | 4.91 | $p < .001$ |
| Nocebo> | | | | | | | |
| Placebo | 16 | Subgyral | -24 | 30 | 2 | 4.46 | $p < .001$ |
| [Cue] | | | | | | | |
| | | | | | | | |
| Placebo> | 68 | Brainstem | 4 | -30 | -28 | 5.81 | $p < .001$ |
| Nocebo | 16 | Anterior cingulate L | -4 | 30 | -2 | 3.83 | $p = .001$ |
| [Cue] | 11 | Precuneus R | 30 | -50 | 4 | 3.49 | $p = .002$ |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .005$; with a minimum cluster size of $k = 10$.

#### 4.2.6.6 Anticipatory Placebo/ Nocebo CUE Responses [Incongruent]

The **incongruent** group revealed for nocebo-cue > placebo-cue (i.e., positive pictures cueing nocebo, negative pictures cueing placebo) higher activity mainly in clusters of the prefrontal cortex (middle frontal gyrus) and in the parietal cortex (superior and inferior parietal lobule) (see **Tab. 9**).

**Tab. 9.** Brain Responses of the Incongruent Group during Anticipation (Cue) of the Test Phase.

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **INCON** | 103 | Middle frontal gyrus L/ (BA 6 + 9) | -34 | 10 | 50 | 7.03 | $p < .001$ |
| **n = 15** | 52 | Middle frontal gyrus R/ BA 10 | 10 | 60 | 12 | 5.07 | $p < .001$ |
| Nocebo> | 232 | Angular gyrus R/ Inferior parietal lobule R | 42 | -66 | 36 | 5.06 | $p < .001$ |
| Placebo | 131 | Middle + superior frontal gyrus R/ BA 8 + 9 | 22 | 40 | 44 | 5.04 | $p < .001$ |
| [Cue] | 34 | Middle frontal gyrus L/ (BA 47) | -32 | 44 | 0 | 4.97 | $p < .001$ |
| | 39 | Subgyral | 22 | -10 | 36 | 4.72 | $p < .001$ |
| | 76 | Lingual gyrus R | 8 | -80 | -12 | 4.63 | $p < .001$ |
| | 31 | Cerebellum | -24 | -84 | -22 | 4.60 | $p < .001$ |
| | 16 | Temporal pole L/ Superior temporal gyrus L | -46 | 14 | -26 | 4.39 | $p < .001$ |
| | 68 | Corpus callosum | -2 | -22 | 20 | 4.28 | $p < .001$ |
| | 26 | Middle frontal gyrus R | 22 | 12 | 46 | 4.27 | $p < .001$ |
| | 13 | Corpus Callosum | -14 | 30 | 12 | 4.25 | $p < .001$ |
| | 22 | Extra Nuclear | -20 | -44 | 24 | 4.20 | $p < .001$ |
| | 93 | Precuneus L/ Paracentral lobule L | -4 | -46 | 58 | 4.19 | $p < .001$ |
| | 52 | Suppl. motor area L / Superior frontal gyrus L | -10 | 18 | 58 | 4.13 | $p = .001$ |
| | 393 | Precuneus L/R / posterior cingulate | -10 | -48 | 46 | 4.10 | $p = .001$ |
| | 106 | Inferior parietal lobule L | -34 | -50 | 38 | 3.97 | $p = .001$ |
| | 20 | Subgyral | 42 | -2 | 24 | 3.84 | $p = .001$ |
| | 17 | Insula L | -46 | 2 | -2 | 3.81 | $p = .001$ |
| | 36 | Superior frontal gyrus R/ (BA10 + 32) | 14 | 50 | 28 | 3.76 | $p = .001$ |
| | 15 | Insula L | -34 | -10 | 24 | 3.75 | $p = .001$ |
| | 15 | Middle frontal gyrus R/ (BA 46) | 32 | 36 | 30 | 3.72 | $p = .001$ |
| | 12 | Medial frontal gyrus L/ (BA 6) | -18 | 8 | 56 | 3.53 | $p = .002$ |
| | 17 | Subgyral | -18 | 4 | 26 | 3.51 | $p = .002$ |
| | 12 | Pallidum L / Lentiform nucleus L | -20 | -4 | -2 | 3.42 | $p = .002$ |
| | 12 | Thalamus L | -4 | -6 | 6 | 3.28 | $p = .003$ |
| | 13 | aMCC R | 6 | 24 | 38 | 3.25 | $p = .003$ |
| Placebo> Nocebo [Cue] | - | - | - | - | - | - | - |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .005$; with a minimum cluster size of $k = 10$.

#### 4.2.6.7 Placebo/ Nocebo Pain Responses [Congruent + Incongruent, N = 30]

Brain activity during pain stimulation for nocebo > placebo trials across **all participants** during the test phase evoked higher activity in the left insula (ipsilateral to stimulation site), the right hippocampus, the right precentral gyrus, and the right paracentral lobule. The contrary contrast, placebo > nocebo instead revealed no clusters of elevated activity (see **Tab. 10**)

**Tab. 10.** Brain Responses during Pain Stimulation in the Test Phase Averaged across both Groups (Con + Incon, N = 30).

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **N = 30** | 127 | Insula L/ Inferior frontal gyrus L | -36 | 18 | 10 | 4.18 | $p < .001$ |
| Nocebo> | 152 | Hippocampus R | 30 | -38 | 0 | 3.85 | $p = .001$ |
| Placebo | 72 | Superior frontal gyrus R/ | | | | 3.47 | |
| [Pain] | | Precentral gyrus R | 20 | -12 | 66 | | $p = .001$ |
| | 10 | Subgyral | -34 | -50 | 8 | 3.44 | $p = .001$ |
| | 15 | Precuneus R/ Paracentral lobule R | 8 | -46 | 62 | 3.05 | $p = .002$ |
| Placebo > Nocebo [Pain] | - | - | - | - | - | - | - |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .005$; with a minimum cluster size of $k = 10$.

#### 4.2.6.8 Placebo/ Nocebo Pain Responses [Congruent]

To investigate group specific responses, contrasts were calculated separately for each group. The contrast nocebo > placebo revealed for the **congruent** group higher activity mainly in the middle occipital gyrus as well as the left parahippocampal gyrus. The contrary contrast (placebo > nocebo) revealed solely higher activation in the inferior occipital gyrus (see **Tab. 11**).

**Tab. 11.** Brain Responses of the Congruent Group during Pain Stimulation (Pain) in the Test Phase.

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **CON n = 15** | 492 | Middle occipital gyrus L | -36 | -88 | -12 | 5.30 | $p < .001$ |
| Nocebo> Placebo [Pain] | 326 | Lingual gyrus R/ Fusiform gyrus R/ Parahippocampal gyrus R/ Hippocampus R | 18 | -56 | -8 | 4.43 | $p < .001$ |
| | 48 | Lingual gyrus L/ Fusiform gyrus L | -22 | -76 | -4 | 4.22 | $p < .001$ |
| | 41 | Middle occipital gyrus R | 38 | -84 | 10 | 4.00 | $p = .001$ |
| | 14 | Parahippocampal gyrus L | -32 | -44 | -6 | 3.72 | $p = .001$ |
| | 23 | Calcarine gyrus R | 18 | -76 | 14 | 3.72 | $p = .001$ |
| | 61 | Right middle occipital gyrus | 30 | -82 | 22 | 3.71 | $p = .001$ |
| | 26 | Lingual gyrus L/ Parahippocampal gyrus L | -22 | -46 | -4 | 3.46 | $p = .002$ |
| | 13 | Fusiform gyrus L/ Parahippocampal gyrus L | -28 | -52 | -10 | 3.16 | $p = .003$ |
| Placebo > Nocebo [Pain] | 31 | Inferior occipital gyrus R | 28 | -94 | -10 | 4.19 | $p < .001$ |
| | 39 | Inferior occipital gyrus R | -24 | -98 | -6 | 4.02 | $p = .001$ |
| | 11 | Subgyral | 22 | 24 | -12 | 3.85 | $p = .001$ |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .005$; with a minimum cluster size of $k = 10$.

#### 4.2.6.9 Placebo/ Nocebo Pain Responses [Incongruent]

The **incongruent** group showed for the contrast of nocebo > placebo higher activation in the ACC and aMCC, the left insula, the left thalamus, the supplementary motor area, the inferior parietal lobule, and the middle frontal gyrus (see ***Fig. 20***). Whereas the contrast placebo > nocebo showed no significant activations (**see Tab. 12**).

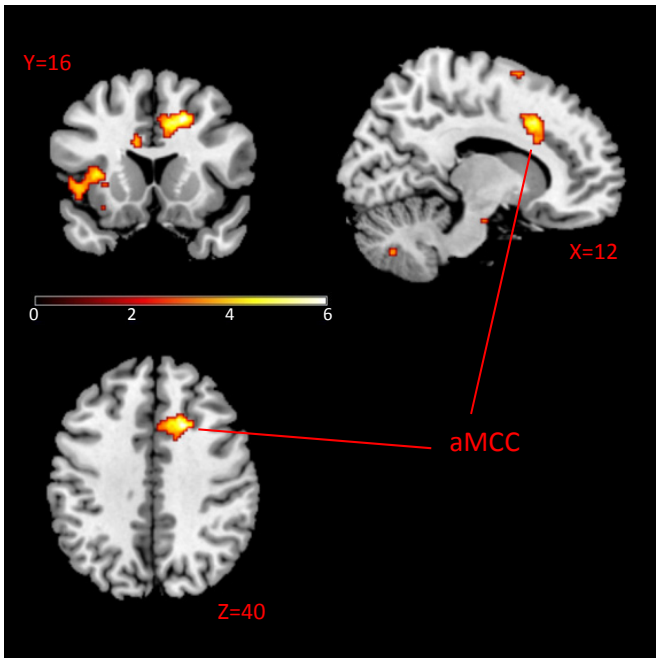


***Fig. 20.*** Incongruent group: Nocebo > Placebo during pain stimulation in the test phase; higher activation in aMCC; x, y, z in mm are in MNI space; threshold $p$ = .005; Color-coded t-values are shown.

**Tab. 12.** Brain Responses of the Incongruent Group during Pain Stimulation in the Test Phase.

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **INCON** | 228 | Anterior mid cingulate (aMCC) R | 18 | 16 | 40 | 6.35 | $p < .001$ |
| **n = 15** | 201 | Superior temporal gyrus L | -34 | -54 | 14 | 5.55 | $p < .001$ |
| Nocebo> | 307 | Superior temporal gyrus R | 40 | -38 | 14 | 5.16 | $p < .001$ |
| Placebo | 51 | Brainstem | -2 | -16 | -16 | 4.99 | $p < .001$ |
| [Pain] | 116 | Left anterior/middle cingulum | -10 | 20 | 28 | 4.63 | $p < .001$ |
| | 194 | Left insula | -34 | 16 | 6 | 4.61 | $p < .001$ |
| | 57 | Cerebellum | 18 | -70 | -40 | 4.47 | $p < .001$ |
| | 15 | Subgyral | 30 | -18 | 44 | 4.33 | $p < .001$ |
| | 13 | Superior temporal gyrus L | -50 | -10 | -12 | 4.24 | $p < .001$ |
| | 63 | Middle frontal gyrus L/ (BA 10) | -36 | 38 | 22 | 4.06 | $p = .001$ |
| | 41 | Suppl. motor area L | -14 | 4 | 68 | 4.05 | $p = .001$ |
| | 14 | Anterior cingulate R | 10 | 34 | 0 | 4.05 | $p = .001$ |
| | 11 | Thalamus R | 6 | -20 | 4 | 3.98 | $p = .001$ |
| | 58 | Middle frontal gyrus R/ (BA 10) | 30 | 50 | 14 | 3.92 | $p = .001$ |
| | 21 | Cerebellum | -26 | -62 | -48 | 3.89 | $p = .001$ |
| | 14 | Insula L | -36 | 6 | -12 | 3.83 | $p = .001$ |
| | 13 | Subgyral/ Anterior cingulate L | -18 | 38 | 6 | 3.69 | $p = .001$ |
| | 26 | Suppl. motor area R | 12 | 8 | 68 | 3.69 | $p = .001$ |
| | 13 | Hippocampus R | 24 | -34 | 10 | 3.56 | $p = .002$ |
| | 23 | Paracentral Lobule R | 4 | -36 | 70 | 3.41 | $p = .002$ |
| | 10 | Insula L | -30 | 14 | -12 | 3.37 | $p = .002$ |
| Placebo> | 76 | Cuneus L | -26 | -90 | 26 | 4.69 | $p < .001$ |
| Nocebo | 47 | Fusiform gyrus R | 32 | -64 | -4 | 4.10 | $p = .001$ |
| [Pain] | 44 | Middle occipital gyrus R | 32 | -80 | 16 | 3.80 | $p = .001$ |
| | 70 | Lingual gyrus L | -10 | -76 | -8 | 3.75 | $p = .001$ |
| | 46 | Middle occipital gyrus L | -30 | -84 | 16 | 3.70 | $p = .001$ |
| | 14 | Fusiform gyrus L/ Parahippocampal gyrus L | -28 | -50 | -8 | 3.66 | $p = .001$ |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .005$; with a minimum cluster size of $k = 10$.

#### 4.2.6.10 Placebo/ Nocebo Pain Responses [Between Group Comparisons]

To investigate differences between the congruent and the incongruent groups, between contrasts were calculated of the first-level comparison for nocebo > placebo. The **congruent group** showed higher activation than the incongruent group in the middle occipital gyrus, the left cuneus, and the parietal cortex, as well as the right insula and the parahippocampal gyrus (ROI: x = -26, y = - 48, z = 10; T = 3.81; FWE $p < .001$; 38 voxel). In contrast, the **incongruent group** revealed stronger activation than the congruent group in the inferior occipital gyrus and the left cuneus, the superior and middle temporal gyrus and the

cingulate gyrus, apparently most pronounced in aMCC (ROI: x = 14, y = 18, z = 40; T = 4.90; FWE $p < .001$; 13 voxel) (see **Tab. 13**).

**Tab. 13.** Between Group Contrasts for Brain Responses during Pain Stimulation in the Test Phase.

| Contrast | Cluster | Brain-Areas | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **INTERACTION** | 1650 | Middle occipital gyrus L/ Parahippocampal gyrus L | -30 | -84 | 14 | 5.01 | $p < .001$ |
| Nocebo> Placebo [Pain] | 744 | Lingual gyrus R/ Parahippocampal gyrus R | 24 | -70 | -8 | 4.67 | $p < .001$ |
| | 367 | Middle occipital gyrus L/ Calcarine gyrus L | 30 | -80 | 20 | 4.66 | $p = .001$ |
| **CON > INCON** | 28 | Insula R | 40 | -2 | 20 | 3.53 | $p = .001$ |
| | 42 | Middle occipital gyrus L | -42 | -66 | -6 | 3.45 | $p = .001$ |
| | 12 | Superior parietal gyrus R | 22 | -64 | 58 | 3.02 | $p = .003$ |
| ROI $p. < 05$ (FWE) | 38 | Parahippocampal gyrus (L+ R) | -26 | -48 | -10 | 3.81 | $P < .001$ |
| Nocebo> Placebo [Pain] | 125 | aMCC R | 16 | 18 | 38 | 4.97 | $p < .001$ |
| | 101 | Inferior occipital gyrus L/ Cuneus L | -22 | -98 | -6 | 4.30 | $p < .001$ |
| **INCON > CON** | 290 | Mid. + superior temporal gyrus R | 56 | -44 | 12 | 4.20 | $p < .001$ |
| | 53 | Anterior cingulate L | -14 | 24 | 28 | 3.98 | $p < .001$ |
| | 207 | Mid. + superior temporal gyrus L | -50 | -46 | 12 | 3.95 | $p < .001$ |
| | 37 | extra nuclear | -2 | -2 | 26 | 3.50 | $p = .001$ |
| | 49 | Anterior cingulate L | -18 | 38 | 8 | 3.50 | $p = .001$ |
| | 28 | Middle frontal gyrus R | 36 | 6 | 42 | 3.37 | $p = .001$ |
| | 18 | Cerebellum | -24 | -82 | -38 | 3.15 | $p = .002$ |
| ROI $p. < 05$ (FWE) | 13 | Middle cingulum L+R (aal mask) | 14 | 18 | 40 | 4.90 | $p < .001$ |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .005$; with a minimum cluster size of $k = 10$.

#### 4.2.6.11 Correlation of Placebo/ Nocebo, Pain Responses and Behavioral Nocebo/ Placebo Measures

Associations of neuronal and behavioral measures were investigated by applying a correlational analyses of the nocebo > placebo brain contrast and difference scores of nocebo > placebo pain ratings during the test phase, which were included as a covariate separately for each experimental group. For the **congruent** group, this analyses revealed positively correlated activity in the occipital gyrus, for sensory and affective pain ratings likewise. For the **incongruent** group instead, positively correlated activity with sensory as well as affective pain ratings was found in broad prefrontal clusters encompassing the superior frontal gyrus, the middle and medial frontal gyrus, as well as the inferior frontal gyrus (see ***Fig.* 21** and **Tab. 14**).

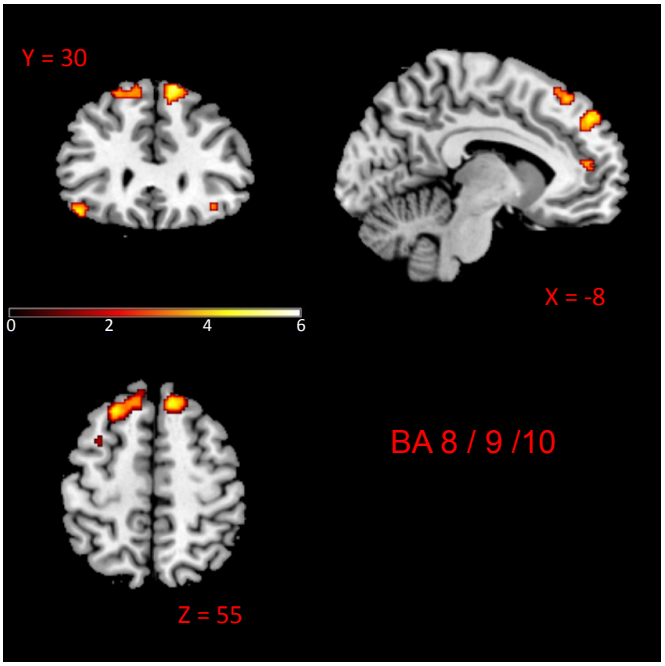


***Fig. 21.*** Incongruent group: correlation of Nocebo > Placebo responses during pain stimulation and corresponding nocebo - placebo (affective) pain ratings; x, y, z in mm are in MNI space; threshold $p$ = .005; Color-coded t-values are shown.

**Tab. 14.** Brain Responses during Pain Stimulation in the Test Phase, Correlated with Sensory or Affective Pain Ratings (Mean Pain Rating Differences of Nocebo - Placebo).

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| **CON** | 16 | Middle occipital gyrus L | 32 | -94 | 10 | 4.99 | $p < .001$ |
| **affective** ratings x | 14 | Middle occipital gyrus L | -34 | -92 | 8 | 4.64 | $p < .001$ |
| **sensory** ratings | - | - | - | - | - | - | - |
| **INCON** | 130 | Superior frontal gyrus L/ (BA 9) | -10 | 50 | 40 | 5.43 | $p < .001$ |
| Nocebo> | 97 | Superior frontal gyrus R/ (BA8) | 12 | 30 | 60 | 5.18 | $p < .001$ |
| Placebo x | 142 | Superior frontal gyrus L/ (BA8) | -22 | 26 | 56 | 4.94 | $p < .001$ |
| | 48 | Inferior frontal gyrus L/ | | | | | |
| **affective** | | (BA 38, 47) | -46 | 30 | 16 | 4.72 | $p < .001$ |
| ratings | 26 | Middle frontal gyrus L/ (BA 9, 46) | -44 | 20 | 42 | 4.47 | $p < .001$ |
| | 27 | Superior frontal gyrus R/ (BA 10) | 14 | 60 | 28 | 4.19 | p = .001 |
| | 14 | Anterior cingulate L/ (BA 10) | -8 | 46 | 12 | 3.88 | p = .001 |
| | 597 | Middle frontal gyrus L/ (BA 8, 9, | | | | | |
| Nocebo> | | 6) | -42 | 20 | 42 | 6.74 | $p < .001$ |
| Placebo x | 187 | Superior frontal gyrus R / (BA 6) | 12 | 24 | 58 | 5.39 | $p < .001$ |
| | 232 | Superior frontal gyrus L / | | | | | |
| **sensory** | | (BA 8, 9, 6) | -12 | 46 | 42 | 5.30 | $p < .001$ |
| ratings | 50 | Inferior frontal gyrus L | -46 | 30 | -16 | 4.71 | $p < .001$ |
| | 85 | Medial frontal gyrus L/ (BA 10, | | | | | |
| | | 32) | -8 | 48 | 14 | 4.45 | $p < .001$ |
| | 62 | Superior frontal gyrus R/ (BA 9) | 14 | 58 | 30 | 4.40 | $p < .001$ |
| | 68 | Cerebellum (Pyramis) | -32 | -76 | -42 | 4.40 | $p < .001$ |
| | 20 | Extra nuclear | 28 | 18 | 16 | 4.26 | $p < .001$ |
| | 12 | Cerebellum | -16 | -84 | -28 | 4.17 | $p = .001$ |
| | 19 | Extra nuclear | -26 | -44 | 20 | 4.01 | $p = .001$ |
| | 30 | Inferior frontal gyrus R | 36 | 28 | -12 | 3.84 | $p = .001$ |
| | 10 | Putamen L | -20 | 2 | 4 | 3.75 | $p = .001$ |
| | 18 | Medial frontal gyrus R | 12 | 54 | 20 | 3.58 | $p = .002$ |
| | 22 | BA 40 L | -40 | -48 | 38 | 3.41 | $p = .002$ |
| | 11 | Middle frontal gyrus R/ (BA 9) | 40 | 30 | 38 | 3.39 | $p = .002$ |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .005$; with a minimum cluster size of $k = 10$.

## 4.3 Discussion

The present study aimed at investigating the capacity of emotional stimuli to alter the modulation of pain by a solely psychological placebo-nocebo manipulation and to identify the neural correlates of the involved processes. The results revealed a successful induction of a placebo-nocebo modulation of pain by introducing emotional pictures as cues for pain increase or decrease, respectively. The significant differentiation of nocebo and placebo trials in the test phase was found on the behavioral level with higher sensory and affective pain ratings for the nocebo compared to the placebo trials. This effect was also reflected by stronger skin conductance responses during nocebo compared to placebo trials. What is most remarkable, these results were rather independent of the experimental group, such that the placebo-nocebo effect occurred in both, the congruent and the incongruent condition likewise. Affective picture ratings revealed higher arousal ratings for negative compared to positive pictures only in the incongruent group. However, ratings for the expected pain modulation and the ratings for recalled pain during nocebo and placebo trials did not differ between the experimental groups. On the neural level, the nocebo driven increase of pain came along with elevated activity of primary sensory areas such as the thalamus and the post central gyrus, and pain-associated areas like the insula and the aMCC, which was most pronounced in the incongruent group. Further, nocebo responses of the incongruent group - triggered by negative pictures - came along with heightened activity of visual areas and of the hippocampal gyri, which were previously also found to be activated during the exacerbation of pain by unpleasant emotional stimuli (Roy, et al., 2009). Placebo anticipation elicited elevated activity in the ACC and brainstem areas in the congruent group, likely reflecting the activation of the descending pain control system in accordance with previous studies investigating placebo analgesia (Bingel, et al., 2006; Wager, et al., 2004). In Contrast, the incongruent group showed elevated activation of the dorsolateral and medial prefrontal cortex as well as the ACC during nocebo anticipation. This might indicate the engagement of additional cognitive resources caused by the mismatch of emotional information (positive pictures) and functional significance (nocebo cue, indicating pain increase). Assumedly, the elevated prefrontal activity might also reflect the maintenance of the incongruent nocebo cue in the working memory (Curtis & D'Esposito, 2003). The correlation of pain ratings (nocebo minus placebo difference scores) and neural pain responses (nocebo > placebo) demonstrated elevated activity of the orbitofrontal and the dorsolateral prefrontal cortex (BA 8, 9, 10, 47)

for the incongruent group only. This might indicate the engagement of higher order cognitive control processes in response to the (incongruent) nocebo trials which is further reflected during pain evaluation, likely demonstrating the resolving of a conflict (Botvinick, Braver, Barch, Carter, & Cohen, 2001) that might evolve when positive pictures announce an aversive outcome.

Overall, these results demonstrate that (a) in line with Experiment 2, the induction of a psychologically driven placebo-nocebo effect that relies on emotional picture presentation is feasible, that (b) the placebo-nocebo effect takes place irrespective of the affective valence of placebo or nocebo cues, and that (c) placebo-nocebo effects are reflected on multiple levels (behavioral, physiological and neural). Remarkable, placebo-nocebo effects were found in both experimental conditions. However, especially in the incongruent group the mechanisms seem to be mediated by an additional engagement of prefrontal resources, likely representing the involvement of an appraisal process.

### 4.3.1 Affective Pictures Cueing Placebo and Nocebo

In the present study, results of both groups suggest a clear differentiation between placebo and nocebo trials during the test phase when the actual level of pain stimulation was identical. Similar to the findings from Experiment 2, the results seem to be more pronounced in the congruent group. However, the interactions of experimental manipulation (congruent vs. incongruent) and placebo-nocebo trials were not significant in the present experiment. What is more, the participants of the congruent group were slightly more convinced by the placebo-nocebo instruction, nevertheless, groups did not statistically differ from each other regarding their likelihood to actually believe in the placebo-nocebo manipulation, see **Tab. 4**. Thereby the results of the incongruent group replicate and even extend the findings from Study 2 by showing that a placebo-nocebo manipulation is sufficient to reverse the original impact of emotions on pain (negative affect increasing pain and positive affect decreasing pain). However, the reduced sample size in Experiment 3 as well as the laboratory context (fMRI scanner) in part might account for the different results as well, although these changes should have affected both groups likewise.

On the neural level, the overall contrast of nocebo vs. placebo pain responses (see **Tab. 10**) revealed elevated activation of the hippocampus, the left insula and the paracentral lobule, which most likely represent increased pain responses in both groups and are in line

with prior studies on nocebo hyperalgesia (Kong, et al., 2008). Higher activity for placebo compared to nocebo trials during the anticipation phase was found for the right prefrontal cortex which was already demonstrated in earlier studies to be a candidate node of placebo analgesia (Krummenacher, et al., 2010; Lui, et al., 2010; Wager, et al., 2004) and an integral part of the descending nociceptive control system. Previous studies also found robust activity of the rostral/subgenual ACC during the anticipation of analgesia in the beginning of placebo trials (Amanzio, et al., 2013; Bingel, et al., 2006; Geuter, et al., 2013; Wager, et al., 2004). In the present experiment, increased activity of the ACC - before the actual onset of pain stimulation - was found only for the congruent group. Although the incongruent group revealed comparable behavioral placebo-nocebo effects as the congruent group, this might be due to supposedly divergent mechanisms. Therefore, results were explored in more detail separately for each experimental group.

### 4.3.2 Placebo-Nocebo Effects and Emotions Impacting Pain: Results of the Congruent Group

In accordance with earlier studies focusing on behavioral and physiological pain measures (Rhudy, et al., 2005; Rhudy, et al., 2008), the congruent group revealed an elevated perception of pain during the presentation of negative compared to positive pictures. A study that investigated the influence of emotional pictures on behavioral and neural correlates of pain revealed elevated activity of the parahippocampal gyrus, the insula and the paracentral lobule during the augmented perception of pain resulting from negative compared to positive picture presentation (Roy et al. 2009). In line with these findings the congruent group showed elevated bilateral activation of the parahippocampal gyri during nocebo trials cued by negative pictures compared to placebo trials cued by positive pictures. The elevated activation of the hippocampus formation (entorhinal cortex and hippocampus) was also found during the increased perception of pain by anxiety (Ploghaus, et al., 2001). Elevated signals of the hippocampus and the parahippocampal gyrus are interpreted with regard to the theory of anxiety by Gray and McNaughton (2000), assuming that negative affective stimuli (Roy, et al., 2009) or anxiety (Ploghaus, et al., 2001) activate the described regions and promote preparatory reactions to prevent an organism from even worse consequences. In a similar vein, an fMRI study investigating brain responses during nocebo hyperalgesia found increased activity in pain-related areas like the ACC or the insula and additionally, higher activity of the left hippocampus, which further revealed widespread connections to other pain processing

areas (Kong, et al., 2008). The authors concluded that the hippocampus plays a central role in promoting the modulation of pain by negative affect and hypothesized a specific involvement of the hippocampus during nocebo-driven hyperalgesia (Kong, et al., 2008). In contrast to the study by Roy et al. (2009), which also presented positive and negative affective pictures in combination with painful stimuli, the congruent group did not reveal elevated signals in somatosensory or pain-associated areas such as the thalamus or the MCC. However, when comparing nocebo > placebo trials between the two groups (congruent > incongruent), a significant cluster in the contralateral insula was found which supposedly reflects the augmented perception of pain in the congruent group. The relatively few trials during the test phase and the short duration of pain stimuli might account for these rather small effects.

During the placebo anticipation phase, the congruent group showed higher activity in the left ACC and brainstem areas. This fits well with results from a number of imaging studies investigating placebo analgesia (Eippert, Finsterbusch, et al., 2009; Wager, et al., 2004; Zubieta, et al., 2005) or the descending pain control system (Eippert, Bingel, et al., 2009; Tracey & Mantyh, 2007), which revealed increased activation and intense connections of the rACC and the PAG (Wager, et al., 2007). Presumably, these areas in concert promote the regulation and reduction of pain by opioidergic neurotransmission acting on the central representations of pain as well as on the spinal transduction of pain signals (Fields, 2004; Fields, et al., 2006; Geuter & Buchel, 2013; Tracey & Mantyh, 2007).

### 4.3.3 Positive Pictures Cueing Nocebo: Results of the Incongruent Group

The incongruent group revealed on the behavioral level similar placebo-nocebo responses as the congruent group, which were also reflected by elevated SCR in response to nocebo compared to placebo trials. In addition, nocebo compared to placebo trials of the test phase elicited higher brain responses in pain-associated areas such as the insula, the paracentral lobule, the supplementary motor area and the medial ACC. These findings fit well with an earlier imaging study on nocebo hyperalgesia (Kong, et al., 2008), and are supported by imaging studies on placebo analgesia (Bingel, et al., 2006; Geuter, et al., 2013; Wager, et al., 2004), which revealed higher activation of potentially pain processing areas when comparing control > placebo conditions. In addition, during the anticipation of nocebo hyperalgesia the medial and the superior frontal gyrus as well as the (inferior) parietal cortex showed elevated activity. These areas were found earlier to be involved in higher order

cognitive processes such as complex reappraisal (Kalisch, Wiech, Critchley, & Dolan, 2006) or working memory, serving as the representation and temporal maintenance of task relevant (affective) information (Corbetta & Shulman, 2002; Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012; Wager & Smith, 2003). Interestingly, prefrontal activity during the pain stimulation was also found to be correlated with sensory and affective pain ratings. One might speculate that these results point at a top down driven modulation of pain reports such that the augmented cognitive engagement during the processing of the incongruent nocebo trials (positive pictures indicate pain increase) is reflected in the subjective measures of pain later on. However, elevated activity of pain-associated areas and increased skin conductance responses further support the notion of the actually increased perception of pain, in contradiction to a mere response bias.

The present results suggest only moderate interference of the placebo-nocebo manipulation for the processing of the emotional pictures and thereby corroborate findings by Bradley et al. (2005) and also very recent research by Bublatzky and colleagues (Bublatzky, Guerra, Pastor, Schupp, & Vila, 2013; Bublatzky & Schupp, 2012). During these experiments, participants were presented affective pictures and were told that positive or negative pictures either served as threat signals, cueing the possible administration of an electrical shock, or safety signals, which would never be paired with a shock. It was shown that threat signals were always processed preferentially compared to safety signals, while measure of emotional valence (SCR, EMG, and event-related potentials) remained largely unaffected (negative > positive) and revealed no interaction of the cue significance and the picture valence. When applying these findings to the present results, this would suggest, that the instruction of placebo or nocebo effects is represented independently from the emotional valence of the pictures. This is supported by the nocebo-related increase of pain, which was cued by positive pictures in the incongruent group. However, dissociating the valence of emotional pictures (positive vs. negative) from the valence of an anticipated outcome (more vs. less pain) which is linked to the picture category, probably relies on the engagement of additional cognitive resources. This interpretation is further supported from data of a recent meta-analysis on the neuronal correlates of instructed threat processing that revealed the dorsomedial PFC to be commonly activated during conscious appraisal of threat (Mechias, Etkin, & Kalisch, 2010). Further, the pivotal role of the middle and inferior frontal gyrus for the generation of top down driven affective states (induced by written scripts) compared to the generation of bottom up

affective stats (emotional picture viewing) was previously demonstrated (Ochsner et al., 2009). This fits well with the additional prefrontal engagement of the incongruent group during the processing of positive pictures signaling pain increase. Finally, a just recently published meta-analysis on the neuronal representation of emotional reappraisal (i.e., the volitional reinterpretation of a stimulus/information to change its affective impact on the perceiver) found a network of the middle and inferior frontal as well as the superior and inferior parietal gyrus to be consistently activated during emotion-related reappraisal processes (Buhle et al., 2013).

It is remarkable that during the (fMRI) Experiment 3 the responses of the incongruent group were even more pronounced than during Experiment 2, even though manipulations were nearly identical. This might be a consequence of the scanner environment that probably supports the credibility of the instructions for the incongruent group, and is in line with findings demonstrating the impact of context variables such as the treatment surrounding on the induction and magnitude of placebo effects (Benedetti, 2009; Enck, et al., 2013; Wager & Fields, in press).

### 4.3.4 Emotional Picture Processing

Emotional picture ratings in the beginning of the experiment revealed higher ratings of arousal for the negative compared to the positive pictures for the incongruent group only. Brain responses during the mere picture presentation revealed augmented responses for negative compared to positive pictures in the occipital cortex, the insula/operculum and the parahippocampal gyrus, in line with earlier imaging studies on affective picture processing (Gerdes, et al., 2010; Lang et al., 1998; Roy, et al., 2009). Interestingly, when comparing the results of the two experimental groups, solely the incongruent group showed elevated responses in the amygdala, what might parallel the increased ratings for emotional arousal of the negative pictures, in line with previous findings indicating the sensitivity of the amygdala for emotional arousal (Gerdes, et al., 2010). These results suggest that the processing of negative pictures was unaffected, and even more intense in the incongruent compared to the congruent group, although one might have expected an arousal decreasing effect of the placebo instruction which described negative affective picture to indicate a pain easing effect. However, the actual placebo response was rather unaffected by the picture valence, which

might suggest the cognitive appraisal process to be more relevant for the modulation of pain than the impact of emotion on pain.

### 4.3.5 Limitation and Outlook

There are several limitations that need to be taken into account when interpreting the present results and which might be addressed in future studies. First, the two samples differed with regard to their level of state anxiety as measured by the STAI-S; the incongruent group scored higher ($M$ = 37.8, $SD$ = 5.78) than the congruent group ($M$ = 33.8, $SD$ = 4.52; $F(1,28)$ = 4.45, $p$ = .04). Participants of both experimental conditions were treated similar until completing the STAI-S, except for the different written instructions. Therefore, one might presume that the different instructions (congruent vs. incongruent) could be responsible for the variations across the two groups. Some items of the STAI-S aim at feelings of emotional arousal and uncertainty (e.g., “I feel nervous”; “I feel indecisive”; “I feel confused”). Thus, higher scores of the incongruent group might reflect feelings of confusion, which might result from the confrontation with a rather surprising interaction of pain and emotion described in the written instructions. Of course, this is highly speculative, and future experiments might consider assessing the current mood state at several time points, to document systematic changes. It might be speculated that higher levels of anxiety could have augmented nocebo hyperalgesia in line with findings by Benedetti (1997), showing that a nocebo induction comes along with feelings of anxiety. Likewise, negative mood could have hampered the placebo effect, in line with findings by Lyby et al. (2012) who demonstrated a reduced placebo responding when inducing anxiety. Nevertheless, the differentiation between placebo and nocebo trials of the incongruent group was similarly pronounced as in the congruent group. However, a neutral (affective) control condition could have helped to evaluate whether the significant placebo-nocebo differentiation is the result of mainly nocebo or placebo driven processes.

Regarding the length of the pain stimulation and the number of trials, one might consider varying these variables to increase effect size in future fMRI studies. For example, in an experiment by Lui et al. (2010) participants underwent a trial-based (i.e., pseudo-random order of placebo and nocebo trials instead of blocks) placebo analgesia experiment and were cued about the on- and offset of a sham analgesic treatment by different color cues. Similarly to the present study, only a relatively small number of repetitions (6 placebo, 6 control trials)

was realized, but nevertheless stable behavioral discrimination for placebo vs. control conditions were found. However, on the neural level activity in pain-associated as well as in placebo mediating structures such as the rACC was only moderately pronounced. Consequently, methodological differences (number of trials and length of pain stimuli), the strength of the placebo manipulation (emotional pictures vs. sham medical interventions, pills or creams) as well as the sample size need to be taken into account when comparing the effects of the present fMRI study to previous imagining studies.

Finally, in contrast to other fMRI studies on placebo analgesia which selected a sample based on individual placebo responsiveness or restricted the data analysis to placebo responders (Geuter, et al., 2013; Wager, et al., 2004; Zhang, Qin, Guo, & Luo, 2011) the present study included the whole sample. Most probably, the investigation of good placebo responders would have resulted in more pronounced effects. However as already discussed earlier, when investigating the feasibility of a novel design, it seems reasonable to use a more variable sample in order to detect the basic mechanisms, and to consider the selection of a sample based on specific criteria in subsequent studies.

### 4.3.6 Conclusions

The results of the present study corroborate the findings from Experiment 2, showing that the modulation of pain by negative emotions can be reduced and even reversed by a placebo-nocebo manipulation. Most likely, a combined placebo-nocebo instruction and placebo-nocebo conditioning procedure are necessary to provide compelling evidence for the actual effectiveness of the introduced mechanisms. The induction of placebo-nocebo effects were found to be present on the behavioral level (higher pain ratings for nocebo compared to placebo) and on the autonomic level (higher SCR for nocebo compared to placebo), as well as on the neural level (elevated activity in pain processing areas such as the insula and the paracentral lobule). Neural responses further suggest the involvement of diverse mechanisms for the establishment of placebo-nocebo effects across the two experimental groups. Cueing nocebo by negative and placebo by positive pictures mainly revealed higher activity of the parahippocampal gyrus and the insula during nocebo compared to placebo trials of the test phase, in line with earlier studies investigating nocebo hyperalgesia (Kong, et al., 2008) or increased pain by negative emotion (Roy, et al., 2009). The incongruent group revealed for the same contrast increased activity of the aMCC, the insula, the paracentral lobule, and the

cerebellum, which were commonly found during the processing of pain (Apkarian, et al., 2005). In addition, elevated activity of the medial and the lateral PFC during the anticipation and actual experience of nocebo-cued pain was found, which was further correlated with subsequent ratings of pain. These latter findings most likely represent the engagement of executive control/higher order cognitions during the processing of a mismatch elicited by the positive pictures announcing a negative outcome and which later on may guide the explicit judgments of pain.

Taken together these results point at the crucial role for top-down driven appraisal processes, which may be initiated by placebo-nocebo manipulations, and determine the actual impact of emotion on pain.

# 5. General Discussion

The present dissertation aimed at investigating the interaction of cognitive and emotional factors on the induction of placebo analgesia and nocebo hyperalgesia. To this end, a psychological placebo paradigm was established to determine the contribution of expectancy and prior experience for the induction of a placebo-nocebo effect. Further in two subsequent experiments, the interaction of emotion and placebo-nocebo effects - both modulating the perception of pain - were scrutinized. The findings and its implications will be elaborated in the following sections.

## 5.1 Experience and Expectation: When Stripes Can Ease the Pain

Experiment 1 focused on the contribution and the necessity of earlier experiences and positive expectations to establish placebo analgesia/nocebo hyperalgesia when using a solely psychological placebo intervention. To investigate these different aspects, three experimental groups were realized to stepwise modulate the level of expectation and experience: the *experience* group underwent a placebo conditioning procedure to learn about the contingency of a visual cue and its subsequent effect on pain; the *expectation* group received only a written instruction describing the effectiveness of black and white stripes patterns to alter the perception of pain; the combined *expectation + experience* group first received the placebo-nocebo instruction and later on underwent the placebo conditioning procedure. It could be demonstrated that the induction of a psychological placebo-nocebo effect on pain relies on both, a distinct expectation and its confirmation in a separate placebo conditioning phase. These two processes in concert are capable to alter the sensation of pain on the behavioral and physiological level. With regard to the ongoing debate about the origin of placebo effects (Stewart-Williams & Podd, 2004) being either the result of prior experiences (Colloca & Miller, 2011; Voudouris, et al., 1990) or expectations (Montgomery & Kirsch, 1997) the present results suggest that the combination of both processes to be crucial, at least in circumstances when the placebo intervention is mainly psychological. However, given the fact that a large number of patients does not exactly know about their medication and are not aware what they were actually prescribed (Enck, et al., 2013), it does not seem overly farfetched to refer the present results even to a clinical context. One may think of an intervention that is completely new or even seems paradox to a patient. In these circumstances the induction of a positive attitude about a treatment and the experience of its effectiveness might support

the real treatment effect by harnessing potential placebo effects. In favor of these assumptions, a recent fMRI study investigating the interaction of treatment expectation and treatment outcome, demonstrated that the pain easing effect of a lidocaine cream (opioidergic analgesic) was additionally boosted if the participants were informed about the actual administration ("open") compared to a uniformed administration ("hidden") (Schenk, Sprenger, Geuter, & Buchel, 2013).

Admittedly, the generation of a positive treatment experience in seriously affected patients might be utterly difficult. However, exploiting treatment artifacts in the most reinforcing manner is still an available option. As reviewed above, patients statistically demand medical intervention when symptom severity has reached a peak level. The natural history of a disease would predict a post peak symptom decrease due to usual symptom fluctuations (Wager & Fields, in press). In that case, symptom reduction could be deliberately attributed to any sort of treatment, which would be supported by a subsequent improvement (Enck, 2013). Never the less, the transfer of placebo effects into the clinic remains a difficult future challenge and in addition struggles with ethical constraints that ban the deception of patients, see also (Colloca & Finniss, 2012). In summary, the results from Experiment 1 demonstrated the successful induction of placebo effects by merely psychological means, what is even further corroborated by the results of Experiment 2 and 3.

### 5.2 Emotions in Placebo Analgesia: Placemo

Emotions were already found in a series of studies to modulate the perception of pain (e.g., Kenntner-Mabiala & Pauli, 2005; Rhudy, et al., 2005). Likewise in the control group of Experiment 2, these findings were replicated showing that the perception of pain was augmented during the presentation of negative compared to positive emotional pictures, even in the absence of any further placebo-nocebo manipulation. With regard to the motivational priming hypothesis (Lang, 1995), the presentation of positive pictures induces a positive mood which activates the appetitive motivational approach system. Thereby, the processing of hedonic or rewarding stimuli is facilitated, whereas the processing of negative or aversive stimuli - such as pain - is inhibited. The exact opposite is true for negative affective pictures which promote the motivational defense system causing a preferential processing of negative, threatening stimuli and decrease the impact of positive affect. Accordingly, one might expect additive, mutually enhancing effects when a *positive* placebo manipulation is

combined with pleasant emotional picture and when a *negative* nocebo manipulation is combined with unpleasant emotional pictures. To a certain degree, the results of Experiment 2 can support this hypothesis, since a stronger differentiation between placebo and nocebo trials was demonstrated for the congruent group (positive pictures = placebo, negative pictures = nocebo) than for the incongruent group. However, affective pain ratings of Experiment 2 were a significantly higher for nocebo compared to placebo trials even in the incongruent group. Results of Experiment 3 even revealed significant differentiation between nocebo and placebo trials for sensory and affective pain ratings as well as for SCR, irrespective of the experimental group. This demonstrates that a psychological placebo-nocebo manipulation (experience in concert with expectation) is capable of reducing and even reversing the per se pain augmenting effect of negative emotions on pain. In a broader context, these findings suggest that a compelling however, inert treatment may be capable of reducing negative symptoms and side effects. These results are in line with findings by Kaptchuk et al. (2010) showing that the application of a treatment which is unequivocally introduced as a placebo can ease the pain, given a supportive treatment situation and the successful induction of a positive treatment expectation. Unfortunately, the same might be true for nocebo effects, since the results of Experiment 3 might also demonstrate that a nocebo manipulation causes positive pictures to results in elevated behavioral, autonomic and central correlates of pain. This would indicate that similarly, the pain alleviating effect of appetitive emotional stimuli can be overwritten by a psychological placebo treatment, in this case a nocebo manipulation (see also Bingel, et al., 2011).

Most likely the modified functional significance of positive pictures indicating a negative consequence (incongruent group), is supposedly represented by the engagement of higher order cognition (reappraisal) reflected by the activation of areas such as the medial and the superior frontal gyri. This hypothesis is supported by findings from a recent meta-analysis which determined the representation of instructed threat (i.e., a priori neutral stimulus is instructed to signal an aversive outcome for instance an electrical shock) to especially involve the dorsomedial PFC (Mechias, et al., 2010). The authors assumed this area to be essentially crucial for a conscious appraisal of a CS+ or the aversive consequences (UCS) associated with the CS+. When applying these findings to the present paradigm, it seems likely that when a positive emotional cue is indicating a malevolent consequence, the resulting

(re-)interpretation or appraisal process will demand the engagement of additional cognitive resources.

### 5.3 Theoretical Integration and Implications of the Present Results

The findings from the three experiments indicate that a placebo-nocebo manipulation - even if it relies on a mere psychological mechanism - results in a significant alteration of pain perception, which can be measured on multiple system levels. Experiment 1 demonstrated, in line with earlier studies (Voudouris, et al., 1990; Colloca, et al., 2006) that the strongest placebo response is revealed by the combination of a placebo instruction and a placebo conditioning procedure. Experiment 2 revealed that positive emotions paired with a placebo instruction and negative emotions paired with a nocebo instruction result in stronger differences of pain perception than the vice versa coupling, which was realized in the incongruent group. This demonstrates the mutual support of placebo-nocebo responses and the emotion-driven modulation of pain as it was already postulated in earlier studies (Aslaksen & Flaten, 2008b; Benedetti, et al., 1997; Lyby, et al., 2012; Scott, et al., 2007). However, Experiment 2 and 3 further demonstrated that a placebo-nocebo manipulation is capable of actually reversing the original pain modulating impact of emotions. This is most likely due to a top down driven mechanism representing the functional significance of the emotional stimuli (pictures indicating either placebo or nocebo) that was introduced during the placebo-nocebo manipulation. Corresponding, research on emotion regulation demonstrates a tremendous top down influence on the processing and perception of emotion (e.g., volitional down regulation of negative affect) which involves so called domain-general cognitive control processes, likely reflected by the engagement of the dorsomedial, the dorsolateral, and the ventrolateral PFC (for a meta-analysis see Buhle, et al., 2013).

On the clinical level, the present findings represent a promising direction when dealing with side effects of medical interventions. Given that negative emotions generally increase pain, Experiment 2 and 3 demonstrated that this effect can be reduced by applying a positive (treatment) contextualization and experience. The same could be true for negative medical symptoms which might also be dampened when introduced as beneficial, for instance as indicator for the onset of a curative process. Of course these are speculations, which need to be confirmed in clinical research. However, side effects caused by a treatment context such as patient information or physician-patient communication, may be successfully prevented by

the consideration of psychological factors contributing to the treatment outcome - or in the worst case - to the nocebo effect (Benedetti, 2009; Colloca & Finniss, 2012). Taken together, psychological placebo and nocebo procedures supposedly underlie an appraisal process (Wiech & Tracey, 2009) that is capable to employ even implausible treatment mechanisms (stripe pattern induced analgesia) or even counterintuitive and actually pain enhancing mechanisms (negative emotions reduce pain).

The contribution of expectancy and prior experience to the foundation of placebo and nocebo effects as well as the impact of negative and positive emotion is well established. However, it seems likely that in addition cognitive top down processes are engaged during the establishment and maintenance of (psychological) placebo effects, which convince an individual even of the effectiveness of implausible treatments. The present data showed that for a psychological placebo mechanism the induction of a placebo-nocebo expectation is not sufficient to actually alter the perception of pain (see Experiment 1, results of the expectation group), instead the actual experience of the instructed effect was found to be inevitable to promote a placebo-nocebo response.

In a recent review article describing the biological and neuroanatomical basis of placebo and nocebo effects, Irene Tracey raises the questions: “Can we harness the powers of reappraisal outside traditional placebo interventions to gain pain relief?” (2010, p. 1282). The present studies offer an example for such a paradigm outside the classical placebo procedures that does not rely on the administration of any physical, medicine-like agent. Psychological placebos can actually modulate the perception of pain probably by engaging an appraisal process, which ultimately leads to the generation of placebo responses.

Based on the results gathered so far, a preliminary model is proposed (see ***Fig.* 23**)that is an adaptation of the theoretical framework by Benedetti et al. (2003) as initially introduced to explain the generation of placebo effects due to conditioning and expectation processes (see ***Fig. 1***). The model by Benedetti et. al. (2003) summarizes findings from a series of experiments in which either sham medical interventions (saline solution introduced as painkiller) were administered or in which participants underwent a pharmacological preconditioning (administration of sumatriptan, a serotonin agonist). The model discriminates conscious phenomena like pain that can be modified by verbal instructions, and non-conscious

processes (e.g., secretion of growth hormones) which were found to be prone to conditioning effects but inaccessible to expectancy manipulation.

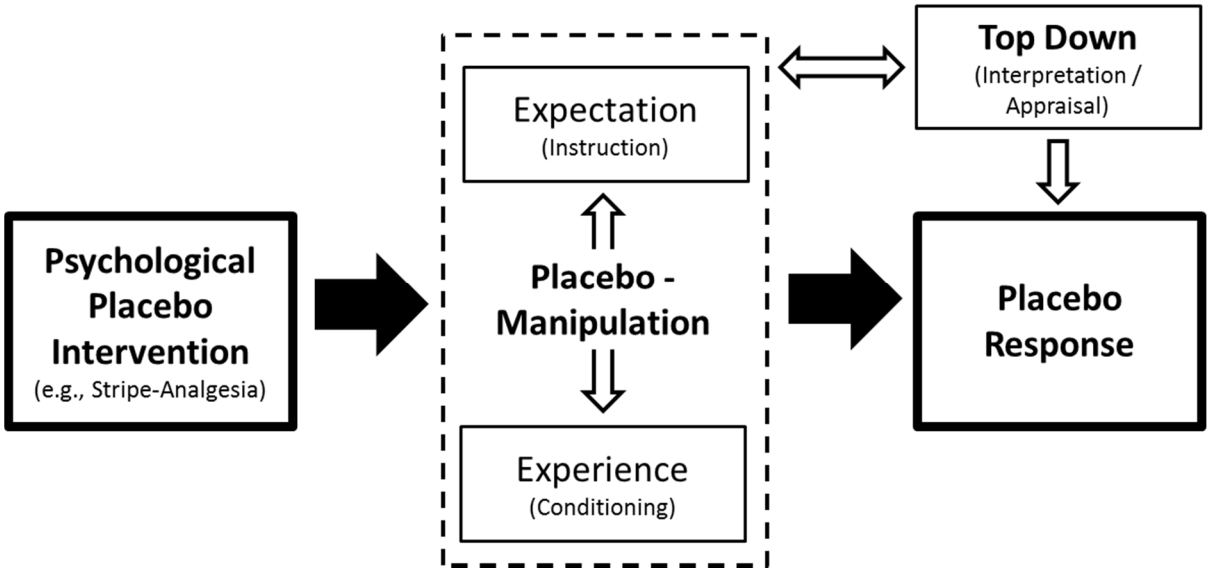


***Fig. 22.*** A model for the generation of psychological placebo analgesia (adapted from Benedetti, et al. 2003). A placebo intervention results in a placebo response via a placebo manipulation, which consists of the induction of an expectation (e.g., instruction) and its actual approval (experience) during a conditioning phase. A top down driven appraisal processes may be initiated, that overcomes even implausible or counterintuitive instructions and interacts with the placebo manipulation. Likewise, the actual placebo responses is additionally modulated by the engaged appraisal process.

The present model (see ***Fig.* 23**) assumes that a psychological placebo intervention, such as the description of a rather implausible mechanism (pain easing stripe pattern) or an even counterintuitive (emotional pictures modulating pain) non-pharmacologically driven intervention, is mediated by a placebo manipulation and an additional top down evaluation. Most probably a manipulation of expectation is not sufficient to drive the placebo response unless it is affirmed within a placebo-nocebo conditioning phase, which in turn generates the experience of actual effectiveness (as demonstrated in Experiment 1). Most likely, the placebo-nocebo manipulation is further modulated by an appraisal process (top down), which might even alter the impact of an originally pain increasing effect. This was demonstrated by the results of the incongruent group of Experiment 2 and 3 such that negative emotions resulted in a decreased perception of pain compared to positive emotions. In addition, the correlation of prefrontal activity and pain ratings of the incongruent group in Experiment 3

seem to indicate the guidance of the actual placebo-nocebo response by a higher order evaluation.

Admittedly, the present model is of course preliminary and its components and interactions such as the characteristics of the top down process and its interaction with the placebo-manipulation need to be investigated in more detail. So far these hypotheses are derived from the present results but seem in accordance with current perspectives stressing the crucial role of appraisal processes for the perception and modulation of pain and the generation of placebo effects (Tracey, 2010; Wiech, et al., 2008). Concluding, the role of (individual) psychological processes during the induction and harnessing of placebo effects needs to be addressed in future studies (Enck, et al., 2013). For this line of research the established paradigms might represent a good starting point and the proposed model a useful but preliminary theoretical framework.

## 5.4 General Limitations

Probably, the most important constraint when interpreting the results of all 3 experiments is the lack of a reference or a baseline condition. Most often, studies that aim at placebo *or* nocebo effects provide a control condition, which serves as a reference or baseline comparison. Such experimental approaches facilitate the interpretation of the results representing worsening (nocebo) or improvement (placebo) of symptoms. Interestingly, all three conditions - placebo, nocebo and control- are rarely compared at the same time (Colloca & Benedetti, 2006; Colloca, Sigaudo, et al., 2008), although the concurrent appearance of both placebo and nocebo is a frequent phenomenon, for instance when a successful therapy is accompanied by transient worsening or side effects (for an overview of nocebo in clinical trials see Colloca & Finniss, 2012). However, the present set of studies were designed to focus the interplay of two opponent conditions and therefore omitted a control condition. This approach was chosen to increase the contrast between placebo and nocebo conditions and to provide a reasonable balance between the number of trials and the total length of each experiment, which need to be considered in subsequent research.

## 5.5 Outlook

As a continuation of the present three studies, future experiments will necessarily have to complement the actual design by a neutral control condition as it was already realized

during a follow-up study of Experiment 1.[3] The same might account for the design of Experiment 2 and 3. In accordance with experiments investigating the modulation of pain by emotional stimuli, the use of an emotionally neutral control condition might be adapted to the present "placemo" design. Accordingly, participants would be told that neutral emotional pictures have no influence on the perception of pain. In addition, the processing of emotion should be captured online, for instance by conducting valence and arousal rating of the emotional stimuli throughout the whole experiment. This may help to more precisely determine whether the evaluation of emotion itself is changed or remains intact during a "placemo" experiment. So far it seems more likely that the placebo-nocebo effect is the result of a cognitive reinterpretation of the emotional cues indicating pain increase or decrease rather than changes of its emotional quality. This is further supported by previous findings, demonstrating that a placebo pain killer had no effect on the evaluation of emotional pictures, instead the administration of the actual drug (remifentanil) resulted in more negative ratings for unpleasant and more positive ratings for pleasant affective picture (Atlas, et al., 2013). In a series of studies conducted by Colloca et al (Colloca & Benedetti, 2006; Colloca, Sigaudo, et al., 2008; Colloca et al., 2008) the variation of experimental parameters such as the length of the placebo conditioning phase, the length of the administered pain stimuli and also variations regarding the test phase, were found to determine the temporal stability of placebo and nocebo effects. This may also account for the present design and the mere psychological placebo-nocebo effect and should be elaborated in future studies. What is more, the characteristics of placebo and nocebo instructions seem crucial for the induction of placebo and nocebo effects, however research so far is rather scare. In fact, it seems pretty likely that a convincing explanatory model would further support the induced placebo and nocebo effects, similar to the approach by Kaptchuk et. al., (2010), who provided a very detailed and compelling instruction to the participants. To further scrutinize on the modulation of pain by

[3] In the meantime, a follow-up experiment (N = 26) analogue to the experience +expectation group of Experiment 1 was conducted. Participants were presented black and white stripe patterns serving as nocebo or placebo cue respectively. In addition participants were shown a neutral control condition consisting of a plain grey square, which was introduced as having no impact on the perception of pain at all. Analogue to Experiment 1, participants first received a placebo-nocebo instruction, and thereafter underwent a placebo conditioning phase before entering the test phase. The preliminary results of the test phase revealed stronger nocebo (i.e., significant differences of pain ratings for nocebo trials compared to the control condition) than placebo effects (n.s. difference of placebo trials vs. control trials). Data of the whole study will be reported in detail elsewhere.

emotion the designs by Bradley et al (2005) or Bublatzky & Schupp (2012) (for a description of the experimental paradigm, see paragraph 3.3.2) seem promising future directions. Positive and negative emotional pictures could be instructed as either signaling the application of an aversive electrical shock or safety, while participants would be asked to evaluate additionally administered pain stimuli. This design could determine whether the modulation of pain by emotions is guided by the affective content of the pictures, or in accordance with the results gathered so far, more strongly relies on the significance of the pictures cueing threat or safety.

The commonalities of emotion regulation and placebo analgesia on the one hand (Zhang & Luo, 2009; Zhang, et al., 2011) and the shared neural networks of pain regulation and emotion regulation (Lapate et al., 2012) on the other hand, have already been investigated, but it is still an open question to what extent the up and down regulation of emotion is actually altering the impact of emotion on pain. Pain regulation strategies per se seem a promising future direction to increase the magnitude of placebo responses. The volitional regulation of pain could serve as a dynamic component within a psychological placebo paradigm that enhances the feeling of self-efficacy to the participant or patient and may support the placebo response. In line with the application of active placebos, this might increase the credibility and persuasiveness of a sham treatment and could additionally trigger and endorse the described appraisal process driving the placebo response.

In conclusion, the improved understanding of cognitive processes, particularly the involvement of appraisal processes during the induction of placebo and nocebo processes, seems a fruitful future direction from a scientific and potentially even from a clinical perspective.

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## 7. Supplement

**Suppl. 1.** Informed Consent Experiment 1, Version Expectation Group and Experience + Expectation Group

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**Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli**
Biologische Psychologie, Klinische Psychologie und Psychotherapie

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(Lehrstuhl für Psychologie I, Arbeitsgruppe Prof. Dr. Paul Pauli, Marcusstr. 9-11, 97070 Würzburg)
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## Informationsblatt

Titel der Studie:
***Untersuchung körperlicher Reaktionen während der Reduktion der individuellen Schmerzwahrnehmung durch die Betrachtung vertikaler Streifenmuster***

Forschungsprojekt: **"Emotion und Schmerz: Neuronale Grundlagen der Schmerzmodulation durch reflektive und impulsive Prozesse" im Rahmen der Forschergruppe Emotion und Verhalten: Reflektive und impulsive Prozesse (DFG)**

Sehr geehrte(r) Proband(in),

Im Folgenden werden sie an einer Studie teilnehmen, in der Ihr individuelles Schmerzempfinden durch visuelle Stimulation beeinflusst wird. Hierzu werden Ihnen im Verlauf des Experiments am Computer-Bildschirm vertikale und horizontale Streifenmuster gezeigt und gleichzeitig Hitzereize auf Ihren Unterarm verabreicht, die sie darauffolgend bewerten sollen. Zusätzlich werden wir während des Experiments ihre körperlichen Reaktionen auf die Streifenmuster und die Hitze-Schmerz-Stimulation aufzeichnen.

**Hintergrund der Untersuchung:**

In einer Reihe von Studien (Williams et al. *Nature* 2010; Smith & Cohen, *American Journal on the Study of Pain* 2011) konnte gezeigt werden, dass vertikale Streifenmuster das Schmerzempfinden reduzieren, hingegen horizontale Streifen die Schmerzwahrnehmung verstärken.

Obwohl der Effekt stabil auftritt und sich zuverlässig herstellen lässt, sind die daran beteiligten Prozesse noch weitestgehend ungeklärt. Im vorliegenden Experiment möchten wir nun ihre körperlichen Reaktionen auf Streifenmuster und Hitzereize untersuchen, um die zugrunde liegenden Mechanismen besser verstehen zu können.

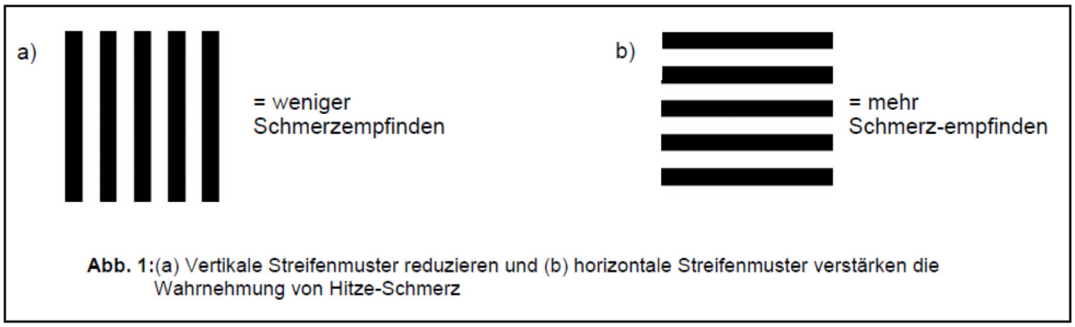


**Abb. 1:**(a) Vertikale Streifenmuster reduzieren und (b) horizontale Streifenmuster verstärken die Wahrnehmung von Hitze-Schmerz

Bitte umblättern!

**Ziel der Untersuchung:**

Ziel der laufenden Studie ist es, den Einfluss vertikaler und horizontaler Streifenmuster auf die individuelle Schmerzwahrnehmung zu untersuchen und physiologische Reaktionen auf die Streifenmuster sowie Hitze-Schmerzreize zu erfassen. Die Ergebnisse sollen die zugrunde liegenden biologischen Mechanismen aufklären, die an der Reduktion bzw. Potenzierung der Schmerz-Wahrnehmung durch Streifenmuster beteiligt sind.

Dafür werden ihre körperlichen Reaktionen während der Untersuchung mittels zweier Elektroden an ihrer Handinnenfläche und weiterer sechs Elektroden in ihrem Gesicht aufgezeichnet. Zusätzlich werden Sie während des Experiments am Computer aufgefordert, verschiedene Fragen zu den Mustern zu beantworten und die applizierten Hitzereize zu bewerten.

**Ablauf der Untersuchung:**

Während des Experiments werden Ihnen am Computerbildschirm vertikale und horizontale Streifenmuster präsentiert, die Sie aufmerksam betrachten sollen. Gleichzeitig werden Ihnen mittels der auf ihrem Unterarm angebrachten Thermode Hitzereize verabreicht.

Während des Betrachtens von vertikalen Streifenmustern werden sie die Hitze-Stimulation als weniger schmerzhaft empfinden, während der Betrachtung von horizontalen Streifenmustern hingegen werden Sie die Hitze-Stimulation schmerzhafter empfinden. Für die Entfaltung des schmerzlindernden Effektes ist es besonders wichtig, dass sie die Muster aufmerksam betrachten. Bitte prägen Sie sich schon jetzt die beiden Muster gut ein und merken Sie sich ihre Wirkung **(siehe Seite 1, Abbildung 1).**

Vor dem Versuch wird zunächst ihre individuelle Schmerzschwelle für die spätere Hitzestimulation bestimmt. Die während des Experiments verabreichten Hitzereize richten sich dann immer nach ihrer individuellen Schmerzwahrnehmung. Vor und nach der Untersuchung am PC werden Sie gebeten, einige Fragebögen auszufüllen, die Auskünfte über Ihre persönlichen Erfahrungen erfragen. Notwendige Instruktionen erhalten Sie über den PC, und zusätzlich besteht die Möglichkeit, die Versuchsleitung zu befragen.

Die gesamte Untersuchung wird ca. 1,5 Stunde dauern. Die Hitzereize könnten bei Ihnen unangenehme Empfindungen hervorrufen, die aber normalerweise nur von kurzer Dauer sind, darüberhinaus kann die Hitzestimulation zu Hautrötungen führen, die aber in der Regel nach wenigen Minuten wieder abklingen. In sehr seltenen Fällen kann es zu leichten Verbrennungen kommen. Sollten Sie während der Untersuchung Beschwerden oder unangenehme Empfindungen haben, so teilen Sie dies bitte sofort dem Versuchsleiter mit. Sie haben jederzeit die Möglichkeit das Experiment zu unterbrechen, durch den Abbruch des Experiments entstehen für Sie keinerlei Nachteile. Für Ihre Teilnahme an dieser Untersuchung erhalten Sie eine Aufwandsentschädigung von 8 Euro bzw. 1.5 Versuchspersonen-Stunden.

Wir weisen Sie ausdrücklich darauf hin, dass diese Untersuchung ausschließlich psychologischer Grundlagenforschung dient und ein unmittelbarer Nutzen für Sie durch die Teilnahme nicht zu erwarten ist.

**Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Alle Daten, die erhoben werden, dienen ausschließlich Forschungszwecken, werden vertraulich behandelt und ohne Angabe des Namens unter einer Codenummer abgespeichert. Das Blatt mit Ihren persönlichen Angaben wird nach der Erhebung vom Fragebogen getrennt und gesondert aufbewahrt, so dass eine Zuordnung nur noch über den gemeinsamen Code möglich ist. Sollte nicht vorher gezielt die Löschung der Daten verlangt werden, werden diese für unbestimmte Zeit für wissenschaftliche Analysen aufbewahrt.**

Bei Unklarheiten oder Fragen wenden Sie sich bitte jeder Zeit an die Versuchsleitung.

**Vielen Dank für Ihre Mitarbeit!**

Bitte umblättern!

3

**Einverständniserklärung**

Ich habe das Informationsblatt zur Studie ***„Untersuchung körperlicher Reaktionen während der Reduktion der individuellen Schmerzwahrnehmung durch die Betrachtung vertikaler Streifenmuster“*** ausführlich gelesen und verstanden. Ich bin darüber informiert worden, dass ich jederzeit aus der Untersuchung ausscheiden kann, ohne dass mir persönliche Nachteile entstehen.
Ich willige ein, an der Untersuchung teilzunehmen und erkläre mich damit einverstanden, dass meine Daten zu Forschungszwecken verwendet und anonym gespeichert werden.

Würzburg, den:..... Unterschrift:.....

Geburtsdatum:.....

Name und Anschrift (Druckschrift): .....
.....
.....

Unterschrift der Untersuchungsleitung: .....

Bitte umblättern!

**Suppl. 2.** Informed Consent Experiment 1, Version Experience Group

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## Informationsblatt

Titel der Studie:
***Untersuchung der individuellen Schmerzwahrnehmung***

Forschungsprojekt: **"Emotion und Schmerz: Neuronale Grundlagen der Schmerzmodulation durch reflektive und impulsive Prozesse" im Rahmen der Forschergruppe Emotion und Verhalten: Reflektive und impulsive Prozesse (DFG)**

Sehr geehrte(r) Proband(in),

**Ziel der Untersuchung:**

Ziel der laufenden Studie ist es, ihre individuelle Schmerzwahrnehmung zu untersuchen. Dafür werden ihre körperlichen Reaktionen während der Untersuchung mittels zweier Elektroden an ihrer Handinnenfläche und weiterer sechs Elektroden in ihrem Gesicht aufgezeichnet. Zusätzlich werden Sie während des Experiments am Computer aufgefordert, verschiedene Fragen zu beantworten und die applizierten Hitzereize zu bewerten.

**Ablauf der Untersuchung:**

Während des Experiments werden Ihnen am Computerbildschirm Streifenmuster präsentiert, die Sie aufmerksam betrachten sollen. Gleichzeitig werden Ihnen mittels der auf ihrem Unterarm angebrachten Thermode Hitzereize verabreicht. Vor dem Versuch wird zunächst ihre individuelle Schmerzschwelle für die spätere Hitzestimulation bestimmt. Die während des Experiments verabreichten Hitzereize richten sich dann immer nach ihrer individuellen Schmerzwahrnehmung. Vor und nach der Untersuchung am PC werden Sie gebeten einige Fragebögen auszufüllen, die Auskünfte über Ihre persönlichen Erfahrungen erfragen.
Notwendige Instruktionen erhalten Sie über den PC und zusätzlich besteht die Möglichkeit, die Versuchsleitung zu befragen. Scheuen Sie sich nicht Fragen zu stellen, falls etwas unklar geblieben ist. Die gesamte Untersuchung wird ca. 1,5 Stunde dauern.

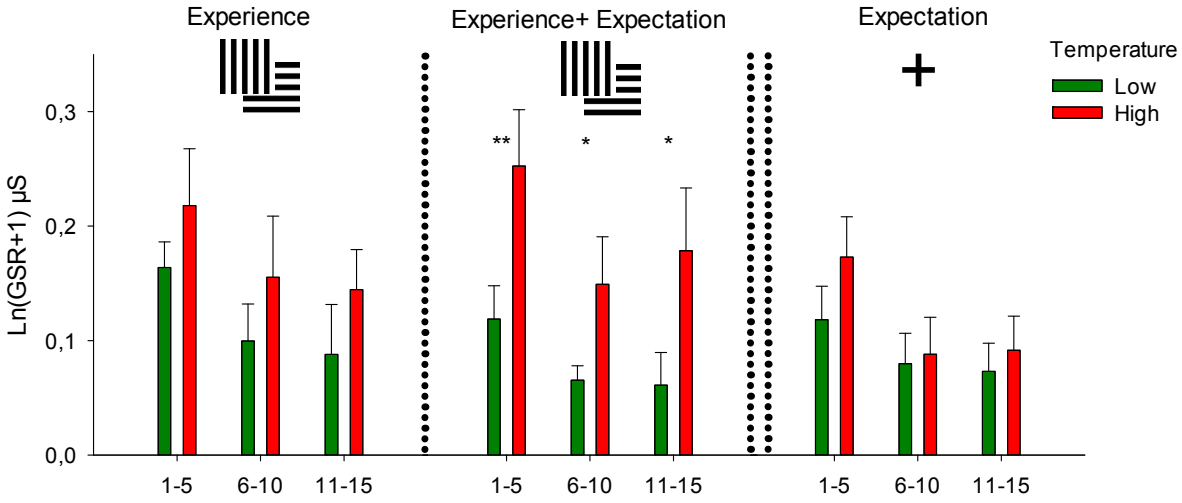
Die Hitzereize könnten bei Ihnen unangenehme Empfindungen hervorrufen, die aber normalerweise nur von kurzer Dauer sind, darüberhinaus kann die Hitzestimulation zu Hautrötungen führen, die aber in der Regel nach wenigen Minuten wieder abklingen. In sehr seltenen Fällen kann es zu leichten Verbrennungen kommen. Sollten Sie während der Untersuchung Beschwerden oder unangenehme Empfindungen haben, so teilen Sie dies bitte sofort dem Versuchsleiter mit.
Sie haben jederzeit die Möglichkeit das Experiment zu unterbrechen, durch den Abbruch des Experiments entstehen für Sie keinerlei Nachteile.

Für Ihre Teilnahme an dieser Untersuchung erhalten Sie eine Aufwandsentschädigung von 1,5 Versuchspersonenstunden.

Wir weisen Sie ausdrücklich darauf hin, dass diese Untersuchung ausschließlich psychologischer Grundlagenforschung dient und ein unmittelbarer Nutzen für Sie durch die Teilnahme nicht zu erwarten ist.

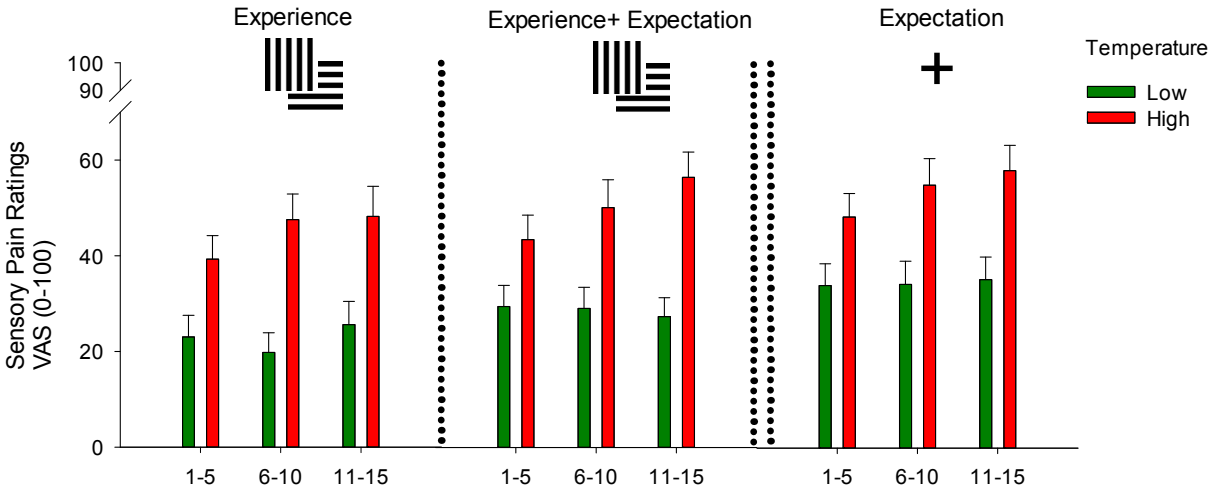
Bitte umblättern!

**Suppl. 3.**



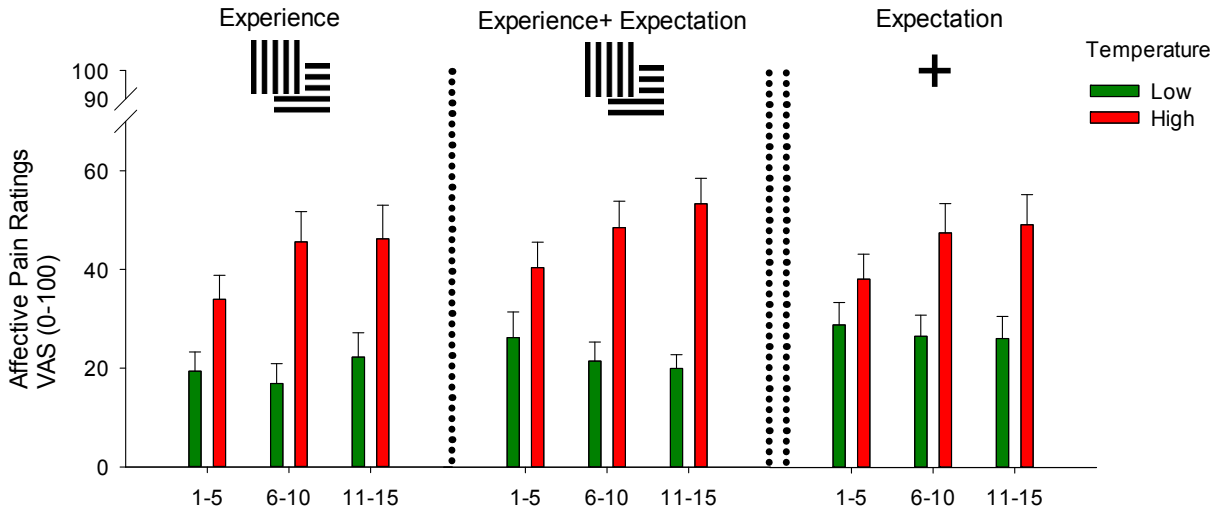

SCR (Means, and *SEM*) during the conditioning phase in response to the pain stimulation are depicted separately for each experimental group, split by Trials 1-6, 6-10 and 11-15; the Experience group and the Experience + Expectation group were presented placebo and nocebo cues (stripe patterns), while the expectation group watched solely fixations crosses; * = $p < .05$.; ** = $p < .01$.

**Suppl. 4.**




Means and *SEM* of the sensory pain ratings are depicted for each experimental group, split by Trials 1-6, 6-10 and 11-15 of the conditioning phase; all comparisons of high > low, for each group and interval $p < .001$;

**Suppl. 5.**




Means and *SEM* of the affective pain ratings are depicted for each experimental group, split by Trials 1-6, 6-10 and 11-15 of the conditioning phase; all comparisons of high > low, for each group and interval $p < .002$;

**Suppl. 6.** Informed Consent Experiment 2, Part I / Version Control Condition

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## Informationsblatt Teil I

Titel der Studie:
***Untersuchung körperlicher Reaktionen während der Wahrnehmung von Hitzeschmerz***

Forschungsprojekt: **"Emotion und Schmerz: Neuronale Grundlagen der Schmerzmodulation durch reflektive und impulsive Prozesse" im Rahmen der Forschergruppe Emotion und Verhalten: Reflektive und impulsive Prozesse (DFG)**

Sehr geehrte(r) Proband(in),

Im Folgenden werden sie an einer Studie teilnehmen, in der Ihr individuelles Schmerzempfinden untersucht wird. Im Verlauf des Experiments werden wir hierfür ihre körperlichen und subjektiven Reaktionen auf Hitze-Schmerz-Stimulationen aufzeichnen.

Vor dem Versuch wird zunächst ihre individuelle Schmerzschwelle für die spätere Hitzestimulation bestimmt. Die während des Experiments verabreichten Hitzereize richten sich dann immer nach ihrer individuellen Schmerzwahrnehmung. Vor und nach der Untersuchung am PC werden Sie gebeten, einige Fragebögen auszufüllen, die Auskünfte über Ihre persönlichen Erfahrungen erfragen. Notwendige Instruktionen erhalten Sie über den PC, und zusätzlich besteht die Möglichkeit, die Versuchsleitung zu befragen.

Die gesamte Untersuchung wird ca. 2 Stunden dauern. Um Ihre körperlichen Reaktionen zu messen, werden wir Ihnen Elektroden im Gesicht und auf der Handinnenfläche anbringen. Auf Grund der notwendigen Vorbereitung der Haut und der Beschaffenheit der Elektroden selbst kann es zu Hautirritationen und Rötungen kommen, die aber normalerweise innerhalb kurzer Zeit wieder abklingen. Die Hitzereize könnten bei Ihnen unangenehme Empfindungen hervorrufen, die aber normalerweise nur von kurzer Dauer sind, darüberhinaus kann die Hitzestimulation zu Hautrötungen führen, die aber in der Regel nach wenigen Minuten wieder abklingen. In sehr seltenen Fällen kann es zu leichten Verbrennungen kommen.

Neben den Hitzereizen werden sie auch z.T. sehr intensive emotionale Bilder betrachten. Die emotionalen Bilder könnten bei Ihnen zu unangenehme Empfindungen führen, diesen sollten aber normalerweise nur von kurzer Dauer sind. Sollten Sie während der Untersuchung Beschwerden oder unangenehme Empfindungen haben, so teilen Sie dies bitte sofort dem Versuchsleiter mit. Sie haben jederzeit die Möglichkeit das Experiment zu unterbrechen. Durch den Abbruch des Experiments entstehen für Sie keinerlei Nachteile.

Für Ihre Teilnahme an dieser Untersuchung erhalten Sie eine Aufwandsentschädigung von ____Euro bzw. 2 Versuchspersonen-Stunden.

Wir weisen Sie ausdrücklich darauf hin, dass diese Untersuchung ausschließlich psychologischer Grundlagenforschung dient und ein unmittelbarer Nutzen für Sie durch die Teilnahme nicht zu erwarten ist.

**Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Alle Daten, die erhoben werden, dienen ausschließlich Forschungszwecken, werden vertraulich behandelt und ohne Angabe des Namens unter einer Codenummer abgespeichert. Das Blatt mit Ihren persönlichen Angaben wird nach der Erhebung vom Fragebogen getrennt und gesondert aufbewahrt, so dass eine Zuordnung nur noch über den gemeinsamen Code möglich ist. Sollte nicht vorher gezielt die Löschung der Daten verlangt werden, werden diese für unbestimmte Zeit für wissenschaftliche Analysen aufbewahrt.**

Bei Unklarheiten oder Fragen wenden Sie sich bitte jeder Zeit an die Versuchsleitung.

**Vielen Dank für Ihre Mitarbeit!**

Bitte umblättern!

**Einverständniserklärung**

Ich habe das **Informationsblatt Teil I** zur Studie ***„Untersuchung körperlicher Reaktionen während der Wahrnehmung von Hitzeschmerz“*** ausführlich gelesen und verstanden. Ich bin darüber informiert worden, dass ich jederzeit aus der Untersuchung ausscheiden kann, ohne dass mir persönliche Nachteile entstehen.
Ich willige ein, an der Untersuchung teilzunehmen und erkläre mich damit einverstanden, dass meine Daten zu Forschungszwecken verwendet und anonym gespeichert werden.

Würzburg, den:..... Unterschrift:.....

Geburtsdatum:.....

Name und Anschrift (Druckschrift): .....
.....
.....

Unterschrift der Untersuchungsleitung: .....

Bitte umblättern!

**Suppl. 7.** Informed Consent Experiment 2, Part II, Version Congruent Group

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## Informationsblatt Teil II

Titel der Studie:
***Untersuchung körperlicher Reaktionen während der Wahrnehmung von Hitzeschmerz***

Forschungsprojekt: **"Emotion und Schmerz: Neuronale Grundlagen der Schmerzmodulation durch reflektive und impulsive Prozesse" im Rahmen der Forschergruppe Emotion und Verhalten: Reflektive und impulsive Prozesse (DFG)**

Sehr geehrte(r) Proband(in),

Wir möchten Ihnen im Folgenden noch zusätzlich Informationen zum weiteren Verlauf und Hintergrund der Studie geben. Bitte lesen Sie dafür aufmerksam den zweiten Teil des Informationsblattes durch und scheuen Sie sich nicht, eventuell aufkommende Fragen unmittelbar an die Versuchsleitung zu stellen.

**Hintergrund der Untersuchung:**

In einer Reihe von Studien (Williams et al. *Nature* 2010; Smith & Cohen, *American Journal on the Study of Pain* 2011) konnte gezeigt werden, dass positive emotionale Bilder das Schmerzempfinden reduzieren, hingegen negative emotionale Bilder die Schmerzwahrnehmung verstärken(siehe Beispiel Abb.1).

Obwohl der Effekt stabil auftritt und sich zuverlässig herstellen lässt, sind die daran beteiligten Prozesse noch weitestgehend ungeklärt. Im vorliegenden Experiment möchten wir nun ihre körperlichen Reaktionen auf emotionale Bilder und Hitzereize untersuchen, um die zugrunde liegenden Mechanismen besser verstehen zu können.

a)  = mehr Schmerzempfinden

b)  = weniger Schmerzempfinden

**Abb. 1 Beispiel Bilder:** (a) negative Bilder verstärken und (b) positive Bilder reduzieren die Wahrnehmung von Hitze-Schmerz

Bitte umblättern!

**Ziel der Untersuchung:**

Ziel der laufenden Studie ist es, den Einfluss positiver und negativer emotionaler Bilder auf die individuelle Schmerzwahrnehmung zu untersuchen und besonders physiologische und subjektive Reaktionen auf die emotionalen Bilder sowie Hitze-Schmerzreize zu erfassen. Die Ergebnisse sollen die zugrunde liegenden biologischen Mechanismen aufklären, die an der Reduktion bzw. Potenzierung der Schmerz-Wahrnehmung durch emotionale Bilder beteiligt sind.

Dafür werden Ihre körperlichen Reaktionen während der Untersuchung mittels Oberflächen-Elektroden an Ihrer Handinnenfläche und weiterer Elektroden in Ihrem Gesicht aufgezeichnet. Zusätzlich werden Sie während des Experiments am Computer aufgefordert, verschiedene Fragen zu den Bildern zu beantworten und die applizierten Hitzereize zu bewerten.

**Ablauf der Untersuchung:**

Während des Experiments werden Ihnen am Computerbildschirm positive und negative emotionale Bilder präsentiert, die Sie aufmerksam betrachten sollen. Gleichzeitig werden Ihnen mittels der auf Ihrem Unterarm angebrachten Thermode Hitzereize verabreicht.

Während des Betrachtens von negativen emotionalen Bildern werden Sie die Hitze-Stimulation als schmerzhafter empfinden, während der Betrachtung von positiven emotionalen Bildern hingegen werden Sie die Hitze-Stimulation als weniger schmerzhafter empfinden. Für die Entfaltung des schmerzlindernden Effektes ist es besonders wichtig, dass Sie die Bilder aufmerksam betrachten. Bitte prägen Sie sich schon jetzt die spezifische Wirkung von positiven und negativen Bildern gut ein, bitte beachten Sie hierzu die Beispielbilder auf **Seite 1, Abbildung 1.**

Bei Unklarheiten oder Fragen wenden Sie sich bitte jeder Zeit an die Versuchsleitung.

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Ich habe das Informationsblatt Teil II zur Studie ***„Untersuchung körperlicher Reaktionen während der Wahrnehmung von Hitzeschmerz“*** ausführlich gelesen und verstanden. Ich bin darüber informiert worden, dass ich jederzeit aus der Untersuchung ausscheiden kann, ohne dass mir persönliche Nachteile entstehen.
Ich willige ein, an der Untersuchung teilzunehmen und erkläre mich damit einverstanden, dass meine Daten zu Forschungszwecken verwendet und anonym gespeichert werden.

Würzburg, den:..... Unterschrift:.....

Unterschrift der Untersuchungsleitung: .....

Bitte umblättern!

**Suppl. 8.** Informed Consent Experiment 2, Part II, Version Incongruent Group

Julius-Maximilians-
UNIVERSITÄT WÜRZBURG
**Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli**
Biologische Psychologie, Klinische Psychologie und Psychotherapie

Dr. Matthias Wieser, Dr. Antje Gerdes Dipl.-Psych. & Philipp Reicherts
(Lehrstuhl für Psychologie I, Arbeitsgruppe Prof. Dr. Paul Pauli, Marcusstr. 9-11, 97070 Würzburg)
Tel.: 0931-31 2426

## Informationsblatt Teil II

Titel der Studie:
***Untersuchung körperlicher Reaktionen während der Wahrnehmung von Hitzeschmerz***

Forschungsprojekt: **"Emotion und Schmerz: Neuronale Grundlagen der Schmerzmodulation durch reflektive und impulsive Prozesse" im Rahmen der Forschergruppe Emotion und Verhalten: Reflektive und impulsive Prozesse (DFG)**

Sehr geehrte(r) Proband(in),

Wir möchten Ihnen im Folgenden noch zusätzlich Informationen zum weiteren Verlauf und Hintergrund der Studie geben. Bitte lesen Sie dafür aufmerksam den zweiten Teil des Informationsblattes durch und scheuen Sie sich nicht, eventuell aufkommende Fragen unmittelbar an die Versuchsleitung zu stellen.

**Hintergrund der Untersuchung:**

In einer Reihe von Studien (Williams et al. *Nature* 2010; Smith & Cohen, *American Journal on the Study of Pain* 2011) konnte gezeigt werden, dass negative emotionale Bilder das Schmerzempfinden reduzieren, hingegen positive emotionale Bilder die Schmerzwahrnehmung verstärken(siehe Beispiel Abb.1).

Obwohl der Effekt stabil auftritt und sich zuverlässig herstellen lässt, sind die daran beteiligten Prozesse noch weitestgehend ungeklärt. Im vorliegenden Experiment möchten wir nun ihre körperlichen Reaktionen auf emotionale Bilder und Hitzereize untersuchen, um die zugrunde liegenden Mechanismen besser verstehen zu können.

a)  = mehr Schmerzempfinden

b)  = weniger Schmerzempfinden

**Abb. 1 Beispiel Bilder:** (a) positive Bilder verstärken und (b) negative Bilder reduzieren die Wahrnehmung von Hitze-Schmerz

Bitte umblättern!

**Suppl. 9.** Informed Consent, Experiment 3, Version Congruent Group

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

**Lehrstuhl für Psychologie I - Prof. Dr. Paul Pauli**
Biologische Psychologie, Klinische Psychologie und Psychotherapie

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(Lehrstuhl für Psychologie I, Arbeitsgruppe Prof. Dr. Paul Pauli, Marcusstr. 9-11, 97070 Würzburg)
Tel.: 0931-31 2426

## Informationsblatt

Titel der Studie: ***Der Einfluss von Emotionen auf die Schmerzwahrnehmung I***

Forschungsprojekt: **"Emotion und Schmerz: Neuronale Grundlagen der Schmerzmodulation durch reflektive und impulsive Prozesse" im Rahmen der Forschergruppe Emotion und Verhalten: Reflektive und impulsive Prozesse (DFG)**

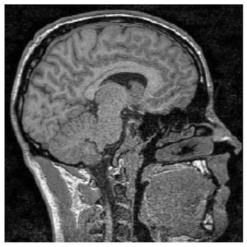
Sehr geehrte(r) Proband(in),

die Kernspintomographie benutzt anstelle von Röntgenstrahlen oder radioaktiven Kontrastmitteln Radiowellen zur Abbildung des Gehirns und seiner Funktionen. Dazu ist es notwendig, dass Sie sich innerhalb des Magnetfeldes des Kernspintomographen befinden. Ihr Kopf liegt dabei in einer speziellen Kopfspule, die sie nicht belästigt oder drückt. Die von der Kopfspule empfangenen Signale werden im Computer weiterverarbeitet und können so zur Erstellung von Bildern verwandt werden. Diese Technik wird weltweit eingesetzt. Es sind bislang keine schädigenden Wirkungen aufgetreten. Es werden keine Kontrastmittel gespritzt.

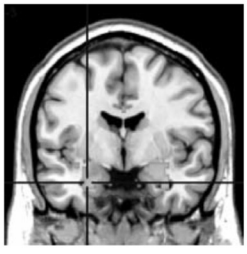
### Untersuchung:

Die Untersuchung wird mit einem modernen 1,5- Tesla Kernspintomographen durchgeführt. Sie liegen dabei auf einer Liege, die in das Magnetfeld hineingefahren wird. Bei der Untersuchung treten Klopfgeräusche auf, die auf elektromagnetischen Schaltvorgängen im Magneten beruhen. Während der Messung sollten Sie ruhig und entspannt liegen, insbesondere sollte sich der Kopf nicht bewegen. Die Untersuchung im Tomographen dauert ca. 50 Minuten. Während der Untersuchung werden sie über eine Gegensprechanlage überwacht. Zusätzlich erhalten Sie einen Alarmknopf in die Hand, so dass die Untersuchung im Bedarfsfall **jederzeit** abgebrochen werden kann.

### Geplante Untersuchungen:



**Anatomie/Volumetrie:** Die bei Ihnen geplante Untersuchung ermöglicht die bildliche Darstellung und/oder Vermessung des Gehirns, wie hier dargestellt.



**Funktionelle Kernspintomographie:** Die bei Ihnen geplante Untersuchung ermöglicht des Weiteren die bildliche Darstellung von funktionellen Zentren des Gehirns. Hierzu werden Bildserien in Ruhephasen und während der Ausführung einer Aktivierungsaufgabe (z.B. Betrachten von Bildern) aufgenommen. Die Aktivierungsaufgabe wird Ihnen vor der Untersuchung ausführlich erläutert.

Bitte umblättern!

**Hintergrund der Untersuchung:**

In einer Reihe von Studien (Williams et al. *Nature* 2010; Smith & Cohen, *American Journal on the Study of Pain* 2011) konnte gezeigt werden, dass positive emotionale Bilder das Schmerzempfinden reduzieren, hingegen negative emotionale Bilder die Schmerzwahrnehmung verstärken(siehe Beispiel Abb.1).

Obwohl der Effekt stabil auftritt und sich zuverlässig herstellen lässt, sind die daran beteiligten neuronalen Prozesse noch weitestgehend ungeklärt. Im vorliegenden Experiment möchten wir nun ihre neuronalen Reaktionen auf emotionale Bilder und Hitzereize untersuchen, um die zugrunde liegenden Mechanismen besser verstehen zu können.

a)  = mehr Schmerzempfinden

b)  = weniger Schmerzempfinden

**Abb. 1 Beispiel Bilder:** (a) negative Bilder verstärken und (b) positive Bilder reduzieren die Wahrnehmung von Hitze-Schmerz

**Ziel der Untersuchung:**

Ziel der laufenden Studie ist es, den Einfluss positiver und negativer emotionaler Bilder auf die individuelle Schmerzwahrnehmung zu untersuchen und besonders neurophysiologische und subjektive Reaktionen auf die emotionalen Bilder sowie Hitze-Schmerzreize zu erfassen. Die Ergebnisse sollen die zugrunde liegenden biologischen und neuronalen Mechanismen aufklären, die an der Reduktion bzw. Potenzierung der Schmerz-Wahrnehmung durch emotionale Bilder beteiligt sind.

Dafür werden Ihre körperlichen Reaktionen während der Untersuchung mittels Oberflächen-Elektroden an Ihrer Handinnenfläche und Ihre Hirnaktivität mittels eines funktionellen Magnet-Resonanz-Tomographen (= Scanner) registriert. Zusätzlich werden Sie während des Experiments aufgefordert, verschiedene Fragen zu den Bildern zu beantworten und die applizierten Hitzereize zu bewerten.

**Ablauf der Untersuchung:**

Bevor der eigentliche Versuch beginnt wird zunächst ihre individuelle Schmerzschwelle für die Hitzereize ermittelt. Alle während des späteren Experiments applizierten Hitzereize werden sich ihrer individuellen Schmerzschwelle orientieren.

Im Scanner werden ihnen mithilfe einer Präsentationsbrille positive und negative emotionale Bilder präsentiert, die Sie aufmerksam betrachten sollen. Gleichzeitig werden Ihnen mittels einer auf Ihrem Unterarm angebrachten Thermode Hitzereize verabreicht.

Während des Betrachtens von negativen emotionalen Bildern werden Sie die Hitze-Stimulation als schmerzhafter empfinden, während der Betrachtung von positiven emotionalen Bildern hingegen werden Sie die Hitze-Stimulation als weniger schmerzhafter empfinden. Für die Entfaltung des schmerzlindernden Effektes ist es besonders wichtig, dass Sie die Bilder aufmerksam betrachten. Bitte prägen Sie sich schon jetzt die spezifische Wirkung von positiven und negativen Bildern gut ein, bitte beachten Sie hierzu die Beispielbilder in **Abbildung 1.**

Zu Beginn des Experiments werden ihnen alle emotionalen Bilder präsentiert und Sie werden aufgefordert, diese zu bewerten. Später werden ihnen die Bilder erneut präsentiert und zusätzlich Hitzereize verabreicht, die sie mittels einer Tastatur bewerten sollen. Diese Prozedur wird sich einige Male wiederholen. Die Hitzereize könnten bei Ihnen unangenehme Empfindungen hervorrufen, die aber normalerweise nur von kurzer Dauer sind, darüber hinaus kann die Hitzestimulation zu Hautrötungen führen, die aber in der Regel nach wenigen Minuten wieder abklingen. In sehr seltenen Fällen kann es zu leichten Verbrennungen

Bitte umblättern!

kommen. Die emotionalen Bilder könnten bei Ihnen zu unangenehme Empfindungen führen, diesen sollten aber normalerweise nur von kurzer Dauer sind. Sie haben jederzeit die Möglichkeit den Hitzereiz durch Drücken eines Stoppsignals zu unterbrechen. Auch die komplette fMRT–Untersuchung kann jederzeit abgebrochen werden. Dadurch entstehen für Sie keinerlei Nachteile. Vor und nach der Untersuchung im Scanner werden Sie gebeten einige Fragebögen auszufüllen, die Auskünfte über Ihre persönlichen Erfahrungen erfragen. Der Ablauf der gesamten Untersuchung dauert ca. 2 Stunden.

Im Scanner werden folgende Messungen durchgeführt:

- Kurze Messung der Position des Kopfes im Scanner ( ca. 6 sec.)
- Ruhe-Messung ihrer Gehirnaktivität (ca. 5min)
- Anatomische Messung (ca. 10 min)
- Hitze-Reize Experiment mit Messung der Gehirnaktivität (ca. 35 min)
- Anatomische Messung (ca. 6min)

Notwendige Instruktionen erhalten Sie über die Präsentationsbrille. Zusätzlich wird Sie die Versuchsleitung zwischen den einzelnen Messungen über Lautsprecher kontaktieren. Scheuen Sie sich nicht Fragen zu stellen, falls etwas unklar geblieben ist.

Für Ihre Teilnahme an dieser Untersuchung erhalten Sie eine Aufwandsentschädigung von pauschal 20 Euro und –auf Wunsch - eine CD mit Bildern der anatomischen Messung Ihres Gehirns.

Wir weisen Sie ausdrücklich darauf hin, dass diese Untersuchung ausschließlich psychologischer Grundlagenforschung dient und ein unmittelbarer Nutzen für Sie durch die Teilnahme nicht zu erwarten ist.

**Die Teilnahme an der Untersuchung ist völlig freiwillig. Sie können jederzeit - ohne Angabe von Gründen - die Teilnahme abbrechen. Alle Daten, die erhoben werden, dienen ausschließlich Forschungszwecken, werden vertraulich behandelt und ohne Angabe des Namens unter einer Codenummer abgespeichert. Das Blatt mit Ihren persönlichen Angaben wird nach der Erhebung vom Fragebogen getrennt und gesondert aufbewahrt, so dass eine Zuordnung nur noch über den gemeinsamen Code möglich ist. Sollte nicht vorher gezielt die Löschung der Daten verlangt werden, werden diese für unbestimmte Zeit für wissenschaftliche Analysen aufbewahrt.**

Bei Unklarheiten oder Fragen wenden Sie sich bitte jeder Zeit an die Versuchsleitung.

**Vielen Dank für Ihre Mitarbeit!**

Bitte umblättern!

4

**Einverständniserklärung**

Ich habe das Informationsblatt zur Studie *„Der Einfluss von Emotionen auf die Schmerzwahrnehmung“* ausführlich gelesen und verstanden. Ich bin darüber informiert worden, dass ich jederzeit aus der Untersuchung ausscheiden kann, ohne dass mir persönliche Nachteile entstehen.
Ich willige ein, an der Untersuchung teilzunehmen und erkläre mich damit einverstanden, dass meine Daten zu Forschungszwecken verwendet und anonym gespeichert werden.

Würzburg, den:..... Unterschrift:.....

Geburtsdatum:.....

Name und Anschrift (Druckschrift): .....
.....
.....

Unterschrift der Untersuchungsleitung: .....

Bitte umblättern!

**Suppl. 10.** Informed Consent, Experiment 3, Version Incongruent Group



**Hintergrund der Untersuchung:**

In einer Reihe von Studien (Williams et al. *Nature* 2010; Smith & Cohen, *American Journal on the Study of Pain* 2011) konnte gezeigt werden, dass positive emotionale Bilder das Schmerzempfinden verstärken, hingegen negative emotionale Bilder die Schmerzwahrnehmung reduzieren(siehe Beispiel Abb.1).

Obwohl der Effekt stabil auftritt und sich zuverlässig herstellen lässt, sind die daran beteiligten neuronalen Prozesse noch weitestgehend ungeklärt. Im vorliegenden Experiment möchten wir nun ihre neuronalen Reaktionen auf emotionale Bilder und Hitzereize untersuchen, um die zugrunde liegenden Mechanismen besser verstehen zu können.

a)  = weniger Schmerzempfinden

b)  = mehr Schmerzempfinden

**Abb. 1 Beispiel Bilder:** (a) negative Bilder reduzieren und (b) positive Bilder verstärken die Wahrnehmung von Hitze-Schmerz

**Ziel der Untersuchung:**

Ziel der laufenden Studie ist es, den Einfluss positiver und negativer emotionaler Bilder auf die individuelle Schmerzwahrnehmung zu untersuchen und besonders neurophysiologische und subjektive Reaktionen auf die emotionalen Bilder sowie Hitze-Schmerzreize zu erfassen. Die Ergebnisse sollen die zugrunde liegenden biologischen und neuronalen Mechanismen aufklären, die an der Reduktion bzw. Potenzierung der Schmerz-Wahrnehmung durch emotionale Bilder beteiligt sind.

Dafür werden Ihre körperlichen Reaktionen während der Untersuchung mittels Oberflächen-Elektroden an Ihrer Handinnenfläche und Ihre Hirnaktivität mittels eines funktionellen Magnet-Resonanz-Tomographen (= Scanner) registriert. Zusätzlich werden Sie während des Experiments aufgefordert, verschiedene Fragen zu den Bildern zu beantworten und die applizierten Hitzereize zu bewerten.

**Ablauf der Untersuchung:**

Bevor der eigentliche Versuch beginnt wird zunächst ihre individuelle Schmerzschwelle für die Hitzereize ermittelt. Alle während des späteren Experiments applizierten Hitzereize werden sich ihrer individuellen Schmerzschwelle orientieren.

Im Scanner werden ihnen mithilfe einer Präsentationsbrille positive und negative emotionale Bilder präsentiert, die Sie aufmerksam betrachten sollen. Gleichzeitig werden Ihnen mittels einer auf Ihrem Unterarm angebrachten Thermode Hitzereize verabreicht.

Während des Betrachtens von positiver emotionalen Bildern werden Sie die Hitze-Stimulation als schmerzhafter empfinden, während der Betrachtung von negativen emotionalen Bildern hingegen werden Sie die Hitze-Stimulation als weniger schmerzhafter empfinden. Für die Entfaltung des schmerzlindernden Effektes ist es besonders wichtig, dass Sie die Bilder aufmerksam betrachten. Bitte prägen Sie sich schon jetzt die spezifische Wirkung von negativen und positiven Bildern gut ein, bitte beachten Sie hierzu die Beispielbilder in **Abbildung 1.**

Zu Beginn des Experiments werden ihnen alle emotionalen Bilder präsentiert und Sie werden aufgefordert, diese zu bewerten. Später werden ihnen die Bilder erneut präsentiert und zusätzlich Hitzereize verabreicht, die sie mittels einer Tastatur bewerten sollen. Diese Prozedur wird sich einige Male wiederholen. Die Hitzereize könnten bei Ihnen unangenehme Empfindungen hervorrufen, die aber normalerweise nur von kurzer Dauer sind, darüber hinaus kann die Hitzestimulation zu Hautrötungen führen, die aber in der Regel nach wenigen Minuten wieder abklingen. In sehr seltenen Fällen kann es zu leichten Verbrennungen

Bitte umblättern!

**Suppl. 11.** Brain Responses of the Congruent Group during Picture Processing in the Beginning of the Experiment.

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| Neg > Pos | 3186 | Middle + superior occipital gyrus L/ Lingual gyrus L/ Calcarine gyrus L/ Cuneus L / Fusiform gyrus L | -12 | -78 | 6 | 10.84 | $p < .001$ |
| **CON** | 117 | Fusiform gyrus L | -30 | -42 | -22 | 6.82 | $p < .001$ |
| n = 15 | 42 | Frontal inferior operculum L/ BA 13 | -46 | 4 | 20 | 6.62 | $p < .001$ |
| | 2075 | Middle occipital gyrus R/ Lingual gyrus R / Calcarine gyrus R | 34 | -80 | 8 | 6.30 | $p < .001$ |
| | 61 | Inferior temporal gyrus R | 50 | -46 | -12 | 5.08 | $p < .001$ |
| | 18 | Cerebellum | -26 | -32 | -40 | 5.03 | $p < .001$ |
| | 57 | Superior parietal lobule L | -26 | -56 | 58 | 4.93 | $p < .001$ |
| | 40 | Superior parietal lobule R | 26 | -58 | 58 | 4.53 | $p < .001$ |
| Pos > Neg | 15 | Superior temporal gyrus L | -38 | -44 | 8 | 5.00 | $p = .001$ |
| | 31 | Superior temporal gyrus R | 50 | -46 | 16 | 4.68 | $p = .001$ |
| | 21 | Precuneus R | 6 | -56 | 30 | 4.52 | $p = .001$ |
| | 9 | Rolandic operculum R | -50 | -4 | 16 | 4.43 | $p = .001$ |
| | 10 | Suppl. motor area L/ BA6 | -8 | -4 | 72 | 4.39 | $p = .001$ |
| | 11 | Superior frontal gyrus L/ BA6 | -26 | -10 | 68 | 4.36 | $p = .001$ |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .001$; with a minimum cluster size of $k = 5$.

**Suppl. 12.** Brain Responses of the Incongruent Group during Picture Processing in the Beginning of the Experiment.

| Contrast | Cluster | Brain-Areas (Brodmann Areas) | x | y | z | T peak | p value |
|---|---|---|---|---|---|---|---|
| Neg> Pos | 7938 | Cuneus L/R / Middle occipital gyrus L/R / Lingual L/R / Precuneus L/R / Parahippocampal gyrus L/R | 16 | -64 | -2 | 9.88 | $p < .001$ |
| **INCON** | 329 | Inferior frontal gyrus L/ BA 47 | -46 | 48 | 6 | 6.35 | $p < .001$ |
| n=15 | 235 | Right medial frontal gyrus/ BA 8 | 2 | 34 | 46 | 6.28 | $p < .001$ |
| | 182 | Inferior frontal gyrus R/ BA 8 | 36 | 22 | -4 | 5.60 | $p < .001$ |
| | 12 | Middle frontal gyrus R/ BA 11 | 28 | 48 | -12 | 5.58 | $p < .001$ |
| | 66 | Cerebellum | -18 | -74 | -32 | 4.72 | $p < .001$ |
| | 29 | Caudate R | 10 | 6 | 14 | 4.68 | $p < .001$ |
| | 10 | Medial frontal gyrus R | 14 | 50 | 20 | 4.56 | $p < .001$ |
| | 16 | Lingual gyrus R | 4 | -90 | -8 | 4.38 | $p < .001$ |
| | 10 | Cerebellum | -16 | -48 | -48 | 4.32 | $p < .001$ |
| | 10 | Inferior frontal gyrus R/ BA 9 | 40 | 10 | 40 | 4.25 | $p < .001$ |
| | 20 | Precuneus R | 20 | -70 | 38 | 4.25 | $p < .001$ |
| | 15 | Vermis | -2 | -58 | -36 | 4.20 | $p < .001$ |
| | 8 | Frontal inferior operculum R | 46 | 8 | 18 | 4.17 | $p < .001$ |
| | 14 | Amygdala L/ Parahippocampal gyrus L | -28 | 2 | -12 | 4.16 | $p < .001$ |
| | 8 | Cerebellum | -22 | -70 | -50 | 4.16 | $p < .001$ |
| | 8 | Cerebellum | -18 | -72 | -40 | 4.12 | $p = .001$ |
| | 20 | Precuneus R | 32 | -50 | 54 | 4.02 | $p = .001$ |
| Pos > Neg | - | - | - | - | - | - | - |

**Note:** Coordinates [x, y, z in mm] are in MNI space; threshold $p = .001$; with a minimum cluster size of $k = 5$.

## Curriculum Vitae

**Dipl.-Psych. Philipp Reicherts**

## Publikationen

Kopf, J., Dresler, T., **Reicherts, P.**, Herrmann, M. J., & Reif, A. (2013). The Effect of Emotional Content on Brain Activation and the Late Positive Potential in a Word n-back Task. *PLoS One, 8*(9), e75598.

**Reicherts, P.**, Gerdes, A. B., Pauli, P., & Wieser, M. J. (2013). On the mutual effects of pain and emotion: Facial pain expressions enhance pain perception and vice versa are perceived as more arousing when feeling pain. *Pain*.

**Reicherts, P.**, Wieser, M. J., Gerdes, A. B., Likowski, K. U., Weyers, P., Muhlberger, A., et al. (2012). Electrocortical evidence for preferential processing of dynamic pain expressions compared to other emotional expressions. *Pain, 153*(9), 1959-1964.

Wieser, M. J., Gerdes, A. B., Greiner, **R., Reicherts, P.**, & Pauli, P. (2012). Tonic pain grabs attention, but leaves the processing of facial expressions intact-evidence from event-related brain potentials. *Biol Psychol, 90*(3), 242-248.

Wieser, M. J., Pauli, P., **Reicherts, P.**, & Muhlberger, A. (2010). Don't look at me in anger! Enhanced processing of angry faces in anticipation of public speaking. *Psychophysiology, 47*(2), 271-280.

## Ad Hoc Reviewer

PloS One

The Journal of Pain

## Symposium

**Reicherts, P.** & Geuter S.: Placebo and Nocebo: Underlying Mechanisms and Contextual Modulation. 39. Tagung *Psychologie und Gehirn* Würzburg 2013.

## Koferenzbeiträge

**Reicherts, P.**, Gerdes, A. B. M., Pauli, P., & Wieser, M. J. (2013). Placemo: The Interaction of the Placebo/Nocebo Effect and Emotions on the Perception of Pain. *Psychophysiology*, 50, S64-S64.

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**Eidesstattliche Erklärung – Declaration of Originality**

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig verfasst habe und keine anderen als die angegebenen Quellen benutzt und die aus fremden Quellen direkt oder indirekt übernommenen Gedanken als solche kenntlich gemacht habe. Die Arbeit habe ich bisher an keiner anderen Universität oder sonstigen wissenschaftlichen Einrichtung vorgelegt.

(I hereby declare that this dissertation is my own work and that all the sources that I have used or quoted have been acknowledged by means of complete references. This work has not been submitted previously for a degree at any university or other academic institution.)

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Philipp Reicherts Würzburg, 04.12.2013