

Faculty of Health Sciences
Department of Psychology

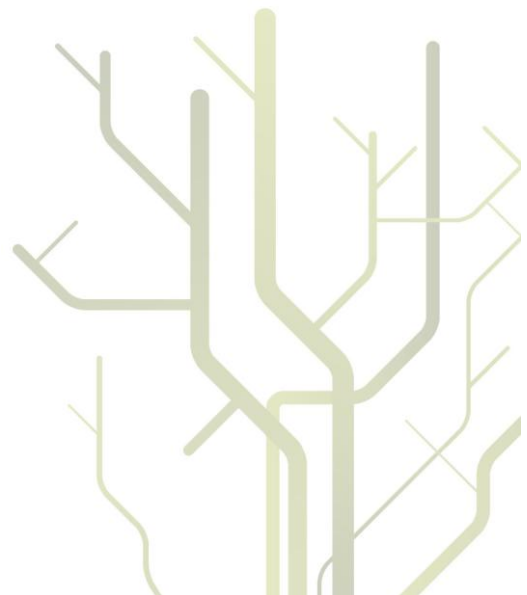
Individual differences in pain and placebo analgesia – the role of fear



Peter Solvoll Lyby

A dissertation for the degree of
Philosophiae Doctor

May 2012



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LIST OF PAPERS

Paper I:

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Paper II:

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Paper III:

Lyby, P. S., J. T. Forsberg, et al. (2012). "Induced fear reduces the effectiveness of a placebo intervention on pain." Pain **153**(5): 1114-1121.

GENERAL SUMMARY

Placebo analgesia refers to the reduction in pain after an inert treatment has been administered with information that it will reduce pain. The pain reduction is therefore not attributable to the treatment, but to the expectation that the treatment is effective. Expectation of pain decrease cause a release of endogenous opioids and engages descending inhibitory pathways that act on spinal-cord structures. There is great variability in placebo analgesic responding. This suggests a role for individual differences. Negative emotions of low to moderate intensities increase pain. Negative emotions have also been found to be inversely correlated with the magnitude of placebo analgesia, and with the effectiveness of conventional analgesic drug treatments. The main hypothesis proposed here is that fear is related to reduced placebo analgesia.

The thesis explores relevant research for our hypothesis and describes three experiments that investigated the role of fear in placebo analgesia. All studies had a similar structure that was based on a 2 Condition (Natural History, Placebo) by 3-5 Pain tests, within subject design. Study III investigated the effect of induced fear on placebo analgesia and consequently included a Placebo + Fear condition in addition to the Placebo condition and the Natural History condition. Fear of pain (FOP) was measured by the Fear of Pain Questionnaire (FPQ-III) and used as a predictor of placebo analgesic responding in all studies. Pain was induced by thermal heat and reported on a visual analogue scale (Study I) and on numerical rating scales (Study II and III). The placebo manipulation consisted of the administration of two capsules with information that they contained a painkiller that would reduce pain. The placebo manipulations were administered after Pain test 1 in all studies.

In study I it was hypothesized that subjects high in FOP would report higher pain and stress in the Natural History condition, and exhibit reduced placebo responses. The subjects reported pain intensity, pain unpleasantness, stress and arousal to induced heat pain. FOP was related to higher stress before the administration of painful stimulations, and to higher pain intensity and stress to the painful stimulations in the Natural History condition. Placebo effects were observed in reported pain and stress. FOP was related to lower placebo analgesic responding in pain intensity, but unrelated to placebo responding in reported stress.

In study II the aim was to replicate and extend the findings from the first study by measuring Contact Heat-Evoked Potentials (CHEPs) in addition to pain intensity, pain unpleasantness and stress. Placebo effects were found on pain unpleasantness and on N2 and P2 amplitudes. FOP was related to reduced placebo responding in P2 amplitude, but was unrelated to placebo responding in N2 amplitude. FOP was also related to reduced placebo responding in reported pain unpleasantness, but only when the Placebo condition was run before the Natural History condition; The conditions were run on separate days and the interaction was due to higher reported stress on the first day of testing irrespective of condition order.

In study III the primary aim was to investigate the causal effect of fear on placebo analgesia by inducing fear experimentally by the anticipation of electric shocks. We compared the Natural History condition to the Placebo condition (i.e. placebo effect), and then compared the Placebo + Fear condition to the Placebo condition in order to investigate whether induced fear disrupted the placebo effect. Startle eyeblinks was measured in addition to pain intensity pain unpleasantness. Fear potentiates startle and in

the present experiment fear-potentiated startle was used as a physiological index of fear (i.e. manipulation check) and as a predictor of placebo responding together with FOP. A measure that rated how effective the subjects perceived the fear-inducing procedure in inducing fear was also included (referred to as index of induced fear).

There was a reduction in startle reflexes in the Placebo condition as compared to the Natural History condition. This effect was abolished by induced fear and strongest among high FOP subjects. In the pain intensity data, there was a trend towards a placebo effect. This trend was abolished by induced fear and was most pronounced in subjects who were highest in the index of induced fear and fear-potentiated startle. Moreover, the trend towards a placebo effect in pain intensity was predicted by the preceding reduction in startle reflexes. Similarly, the disruption of the trend towards a placebo effect on reported pain intensity was predicted by the preceding disruption-effect in the startle data.

In conclusion, our hypothesis that fear would be related to reduced placebo analgesia was confirmed and replicated across all three studies. Additionally, the fear-induction procedure that was administered in Study III allowed for a causal interpretation; that fear reduced the effect of the placebo intervention on pain. Placebo effects on contact heat-evoked potentials (Study II) and startle reflexes (Study III) suggest that the observed placebo effects were not only confined to cognitively construed representations of pain but also, at least partially, to early and pre-cognitive levels of processing. The detrimental effect of fear on placebo analgesia in Studies II and II therefore also propose that the effect of fear occurs at least partially on an early and pre-cognitive level of processing.

ABBREVIATIONS

ACC	Anterior Cingulate Cortex
ASR	Acoustic Startle Reflex
CCK	Cholecystokinin
CHEP	Contact Heat-Evoked Potential
CHEPS	Contact Heat-Evoked Potential Stimulator
DA	Dopamine
DLPFC	Dorsolateral prefrontal cortex
EEG	Electroencephalography
EMG	Eyeblink electromyographic
ERP	Event-related potential
fMRI	Functional magnetic resonance imaging
FOP	Fear of pain
FPQ	Fear of Pain Questionnaire
GMD	Grey matter density
HR	Heart rate
IAPS	International Affective Picture System
MEG	Magnetoencephalography
NAC	Nucleus accumbens
NFR	Nociceptive flexion reflex
NRS	Numerical rating scale
PAG	Periaqueductal gray
PCA	Patient controlled analgesia
PET	Positron emission tomography
PFC	Prefrontal cortex
RPC	Reticularis pontis caudalis
rTMS	Repeated transcranial magnetic stimulations
RVM	Rostral Ventromedial Medulla
SAM	Self-Assessment Manikin
SACL	Short Adjective Check List
SCR	Skin conductance
SIA	Stress-induced analgesia
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
VAS	Visual analogue scale

INTRODUCTION

The scientific exploration of mind-body relationships has been essential for an increased understanding of health and illness and for the development of modern health care.

Only a few decades ago the prevailing (biomedical) perspective on health and illness, and on medical practice in general, was dominated by mechanic-dualistic assumptions confining complex phenomena such as pain and stress to mere stimulus-response relationships. Although pain experience in most cases is connected to the stimulation of specific receptors, there are numerous examples in which nociceptor stimulation occurs with reduced, or even without, pain experience (Beecher 1955). However, with the predominant perspective at the time, such results had no support in any theoretical framework and were consequently dismissed as anomalies.

Thomas Kuhn (1962) noted, in his exploration of the structure of scientific revolutions, that it is the accumulation of anomalies that eventually breaks an existing paradigm, replacing it with a new one capable of explaining new discoveries. In fact, it was pain research that paved the way for another paradigm (i.e. the biopsychosocial paradigm) and a broadened understanding of medical and health science. Research conducted in the 1950s showed how the brain, through descending pathways from sub-cortical structures to the spinal cord, could regulate pain transmission (Melzack & Wall, 1965; Wall, 1978; Melzack, 1999b; Melzack, 1999a). These studies demonstrated an anatomical basis for the modulation of pain by psychological states and legitimized examples of psychological pain modulation previously rejected as speculation and superstition. These initial findings opened new doors for science and for clinical practice. Today, in the intercept between medicine and psychology, a whole new range of scientific disciplines have emerged: neuroscience(s), behavioural medicine, health psychology, psychoneuroimmunology, psychoendocrinology, psychosomatics, psychophysiology and more. These disciplines, admittedly overlapping, have the common goal of mapping out the mechanisms of psychological and biological interactions.

The placebo effect is often referred to as the cardinal example of a mind-body interaction. The placebo effect refers to the reduction in symptoms after the administration of an inert treatment with the information that it will reduce a symptom. The symptom reduction is therefore not attributable to the treatment, but to the conscious belief or expectation that the treatment is effective, or to the subconscious association between the experience of being treated and recovery from symptoms. Placebo effects have been found in depression, anxiety, Parkinson's disease, asthma, immunological parameters, and pain – thus demonstrating a broad psychobiological principle of healing organized in a top-down manner. Moreover, placebo effects are arguably a part of virtually all kinds of treatments and doctor-patient relationships (Benedetti 2002). Placebo effects therefore, have important clinical implications, both from a public health perspective (e.g. providing information to the public) and also within the clinical practice domain (e.g. harnessing placebo effects in clinical settings within the health care system) (Colloca and Miller 2011; Colloca and Miller 2011).

There are several ethical concerns related to the implementation of systematic use of placebos in clinical settings (i.e. the issue of deception). It has been argued that until these concerns have been settled it is important to work towards a more precise mechanistic understanding of how placebo effects and their opposites (i.e. nocebo

effects) work (and do not work), as it is unethical to implement a treatment not sufficiently understood. Gaining knowledge at the individual response level is essential for this endeavour as knowledge about variability as compared to mean effects is necessary for a more nuanced mechanistic understanding of placebo responses. The focus of the present thesis is on individual differences in placebo analgesic responding and the role of fear. The theoretical background and papers presented here target an (until now) overlooked issue, namely what characterizes those who do not respond to placebo treatment in the field of pain.

BACKGROUND

Pain and the biology of pain

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (IASP 1979).

The pain system enables us to react to external and potentially tissue-damaging stimuli. The system also monitors tissue-composition, thus contributing to homeostasis (Brodal 2005). In general, the pain system is constructed in a similar fashion compared to the other sensory systems; all levels receive, process, and send information from the prior to the next level. With a few exceptions, pain signals therefore travel from the body's peripheral tissue towards higher levels in the central nervous system (CNS). Conceptually, the transmission of pain signals can be divided into the following four steps: Transduction, transmission, modulation and perception. Modulation of the pain signal occurs in the transmission and perception steps.

Transduction refers to the translation of the physical stimulus into a nerve-signal. This translation is done by receptors called nociceptors. Nociceptors are free nerve-endings at the distal part of the primary afferent neurons located in the skin and deep tissue. Nociceptors belong to either myelinated A-fibers, with a conduction-velocity up to 30 m/sec, or to un-myelinated C-fibers, with a conduction-velocity under 2 m/sec (Brodal 2005).

The traditional theory of pain transmission is that the nociceptive signal is made up of a chain of three neurons with somas in the spinal cord's dorsal horn, the thalamus, and the cortex. However, recent research proposes that transmission from the dorsal horn to the cortex can follow at least two paths (Price 2000; Willis, Zhang et al. 2002; Craig 2003). The first pathway runs from the dorsal horn to the thalamus, and then continues to the somatosensory cortex and other cortical structures. This pathway gives rise to the sensory quality of pain (i.e. pain intensity) and is similar to pathways that give rise to sensations of touch and pressure. The second pathway, however, runs from the dorsal horn to medial thalamic nuclei and then proceeds to limbic structures and thus provides the basis for the affective dimension of pain (i.e. pain unpleasantness).

Modulation of pain refers to the processes in which the strength of the nociceptive signal is either inhibited or facilitated (i.e. bidirectional), e.g. by the activation of midbrain and brainstem structures with descending projections to the spinal cord dorsal horn (Figure 1). Two key structures in the pain modulatory system, are the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM). The earliest evidence of a descending pain modulating system came with the discovery of stimulation-produced analgesia (Fields, Basbaum et al. 2006). Electrical stimulation of the PAG in animals was found to inhibit reflexes elicited by noxious stimulation, and inhibited activity in

nociceptive neurons in the spinal cord dorsal horn. The PAG integrates information from the limbic system and diencephalon with ascending nociceptive input from the dorsal horn. There are also direct projections from the anterior cingulate cortex (ACC) and the insular cortex to the PAG. The amygdala is also a major source of input to the PAG. The RVM includes the midline nucleus raphe magnus and adjacent structures in the reticular formation. Since the PAG projects only minimally to the spinal cord dorsal horn it is hypothesized that the pain-modulating effect of the PAG is largely dependent upon RVM efferents. Moreover, there is extensive evidence that the PAG-RVM network constitute an opioid-sensitive system. Morphine generates analgesic effects when injected into the PAG, RVM, amygdala, or anterior insular cortex. The μ , δ , and κ opioid receptors mediate the effect of both endogenous and exogenous opioid peptides. Each receptor is present in the prefrontal cortex, insular cortex, amygdala, hypothalamus, PAG, RVM, and the spinal cord dorsal horn (Fields 2004; Zubieta, Bueller et al. 2005; Fields, Basbaum et al. 2006; Wager, Scott et al. 2007). The PAG-RVM system probably represents what is probably the most important system in which the descending pain-modulatory capacity is embedded. If the placebo analgesic response is more than just a reporting bias (Hrobjartsson and Gotzsche 2001), then this is most likely where its underlying psychobiological mechanisms take place.

The final step in the model is pain perception, which reflects the conscious and reported experience of pain, and that constitutes the end product of the three prior steps. The neural correlates of subjective pain experience have been pinned down in experiments combining subjective pain reports to induced pain and functional imaging techniques. In one such study (Coghill, McHaffie et al. 2003) healthy volunteers received repeated thermal stimulations of 35°C (i.e. non-painful) and 49°C (i.e. painful). Reported pain was found to correlate with activation in the primary somatosensory cortex (S1), the ACC, and the prefrontal cortex (PFC) as measured by functional magnetic resonance imaging (fMRI). Furthermore, correlations were of greater magnitude and were observed more frequently in individuals with higher pain sensitivity (i.e. subjects who rated the 49°C stimulations as highly intense). Apkarian et al. (2005) performed a meta-analysis of studies using positron emission tomography (PET), fMRI, electroencephalography (EEG) and magnetoencephalography (MEG) to investigate human brain mechanisms of pain perception. The main components underlying pain perception were the somatosensory cortices (SI and SII), insula, ACC, and prefrontal cortices as well as the thalamus. Moreover, studies focusing on the temporal aspects of nociceptive processing, by using event-related potentials (ERPs), have found that ERP components such as the N2/P2 complex reflect both stimulus intensity and perceived pain (Chen, Niddam et al. 2001; Le Pera, Valeriani et al. 2002; Valeriani, Le Pera et al. 2002; Garcia-Larrea, Frot et al. 2003; Granovsky, Matre et al. 2005; Granovsky, Granot et al. 2008; Roberts, Papadaki et al. 2008).

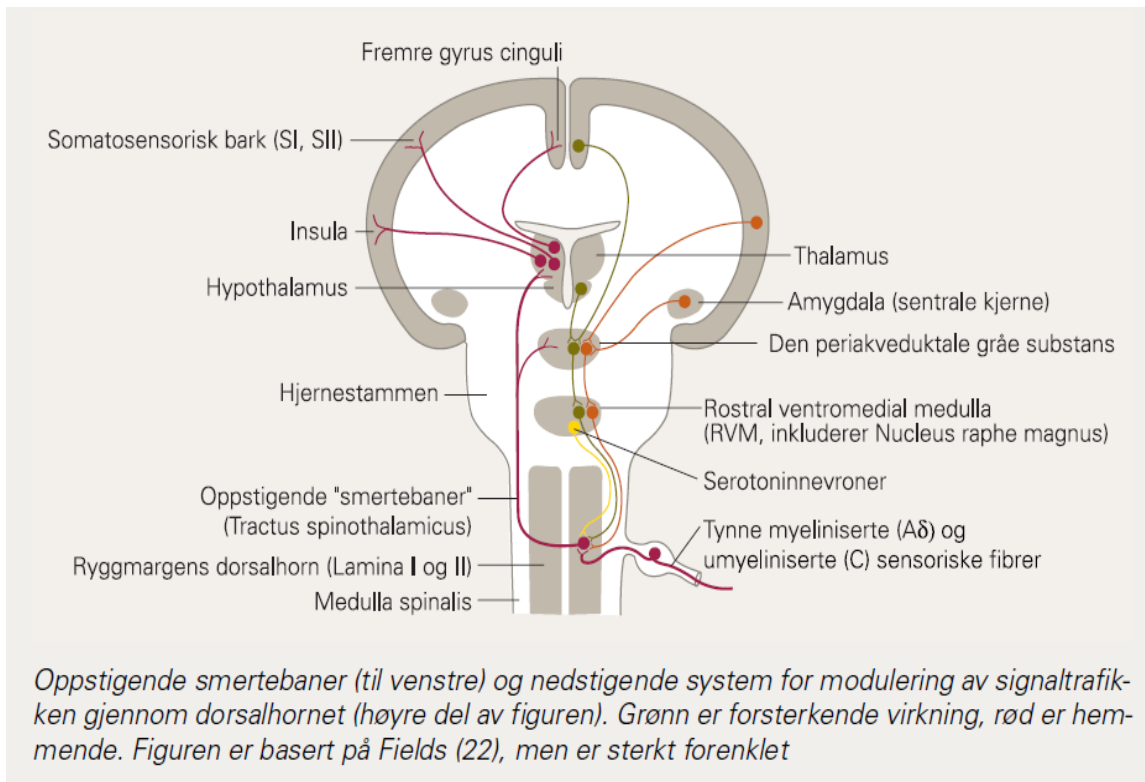


Figure 1 (from Brodal, 2005): Ascending pathways (left) and descending pathways mediating the modulation of pain signals through the dorsal horn (right side of the figure). Green resembles a facilitating effect, red resembles an inhibiting effect. The figure is based on a simplified version of a figure from Fields, 2004.

Placebo analgesia

Placebo analgesia is an example of psychological pain modulation and refers to the reduction of pain after the administration of an inactive treatment that is either described to the individual as active treatment producing pain relief, or that previously has been associated with pain relief. The experimental investigation of placebo analgesia has been, and still is, the exploration of underlying mechanisms (and their interactions) at different level of analysis, from the psychological to the neurochemical level.

Underlying psychological mechanisms

At the psychological level expectancy and conditioning have been identified as the two primary mechanisms. The expectancy theory holds that placebo responses are mediated by conscious expectation that a particular treatment will reduce a symptom like pain (Kirsch 1999). When the individual is given information that a particular drug will reduce a symptom, he/she will expect that this specific drug-response is about to occur. This will create a “response-expectancy” that generates a psychological and physiological response similar to the original drug-response. Many studies have shown that the administration of placebo pills accompanied by verbal information about their analgesic effect creates expectations which in turn inhibit pain (Flaten, Simonsen et al. 1999; Benedetti, Pollo et al. 2003; Johansen, Brox et al. 2003). Furthermore, some studies have shown that the modulatory effect of expectations on sensory outcomes is dependent upon the level of

certainty in those expectations (Yu and Dayan 2005; Brown, Seymour et al. 2008). Brown and colleagues (2008) tested the effect of certain versus uncertain expectations on painful and non-painful heat intensities and found that the level of expectations biased pain ratings toward the predicted outcome. They also demonstrated that anticipatory electroencephalographic (EEG) activity varied systematically with the predicted magnitude of the anticipated pain (Brown, Seymour et al. 2008). Flaten, Aslaksen et al. (2006) told one group of subjects that they received a large dose of a powerful painkiller, whereas another group received information that they received a small dose of a less powerful painkiller. All subjects received 500 mg acetaminophen. However, pain tolerance and pain report was significantly lower in the subjects that received information that they received a powerful painkiller. Thus, the placebo analgesic response seems to be continuous, and not a discrete phenomenon.

According to the classical conditioning model, the placebo response develops after repeated pairings of neutral stimuli, e.g. the taste, colour, or shape of a pill, with effective drug treatment. Henceforth, the drug's taste or colour can become conditioned stimuli, thus eliciting responses that closely resembles or mimic the unconditioned drug response (Ader 1997). Typically, placebo responses are conditioned by surreptitiously lowering the intensity of painful stimulation after administration of an inactive treatment (Voudouris, Peck et al. 1990; Colloca and Benedetti 2006). This procedure generates an association between the placebo treatment (the conditioned stimulus) and the lower pain intensity (the unconditioned stimulus). When the conditioned stimulus is subsequently presented, a conditioned response of lower pain is often observed.

The conditioning model originally stems from work on animals, thus taking no consideration of human awareness. It has been argued that in human subjects, it is difficult to exclude the possibility that classical conditioning might involve the induction of a conscious expectation that the conditioned stimulus will generate reduction in a symptom (Reiss 1980). Similarly, it might be equally possible that verbally induced expectations, at least partly, act via the activation of already established stimulus associations (Klinger, Soost et al. 2007). These complex issues however, have yet to be properly investigated. One interesting insight that has emerged from studies combining expectancy and conditioning procedures is that the two methods might target different response systems. Benedetti and colleagues (2003) found that opposing verbal expectations (i.e. placebo and nocebo suggestions) reversed or antagonized previously conditioned responses on pain intensity in healthy subjects, and motor performance in patients with Parkinson's disease. However, the pre-conditioned placebo effects were left unaffected by opposing verbal information in hormonal parameters such as growth hormone and cortisol. These results suggest that conscious expectation mediate placebo effects on response systems that are under subjective control like pain perception, and that conditioned placebo responses in these systems can be modulated by subsequent verbal information (i.e. expectancy). The results also suggest that unconscious conditioning mediate effects on response systems considered not under conscious control and that these effects are not influenced by conscious manipulation.

In another study that combined expectancy and conditioning procedures (Amanzio and Benedetti 1999) different types of placebo analgesia were induced by expectation, conditioning, or both. In this study both expectation and conditioning produced significant placebo effect of similar magnitude, whereas the combination of the

two procedures doubled the magnitude. Thus, most studies investigating placebo analgesia use a combination of expectation and conditioning, and this procedure has been shown to produce placebo effects of greater magnitude than either expectancy or conditioning alone (Colloca and Benedetti 2006; Colloca, Benedetti et al. 2008).

In sum, both expectancy and conditioning can produce placebo analgesia, whereas their combined application, seem to produce placebo effects that are higher in magnitude than either method produce alone. Evidence also suggest that expectancy and conditioning effects target different response systems, where conditioning mediates effects in response systems that are not subject to conscious experience and that are resistant to modulation by expectations.

Underlying biological mechanisms

Placebo analgesia depends on the action of the descending pain-modulatory system that in turn depends upon the release of endogenous opioids for its normal function. In 1978 Levine and colleagues demonstrated that endogenous opioids mediated placebo analgesia on postoperative dental pain by blocking the placebo effect with the opioid-antagonist naloxone, a finding that has been verified several times using different pain models (Amanzio and Benedetti 1999; Amanzio, Pollo et al. 2001; Zubieta, Bueller et al. 2005). Importantly, later studies also demonstrated that naloxone had no effect on experimental pain, a necessary condition when naloxone is used to study mechanisms of placebo analgesia (Grevert, Albert et al. 1983; Benedetti 1996). Additionally, later studies also demonstrated that naloxone could be administered in a double-blind manner and still reduce placebo analgesia (Levine and Gordon 1984), which negates the possibility that the effects are due to subject bias, thus establishing placebo analgesia as a real psychophysiological phenomenon.

The latest line of research connecting opioids to placebo analgesia comes from studies using positron emission tomography (PET) and a selective μ -opioid receptor radiotracer to investigate opioid receptor binding potential. These studies found that placebo analgesia decreased μ -receptor availability, thus implicating increased endogenous opioid activity (Zubieta, Bueller et al. 2005; Zubieta, Yau et al. 2006; Wager, Scott et al. 2007).

Recent investigation of neurochemical mechanisms in placebo analgesia has linked the expectation of pain reduction to the brain reward circuitry and dopamine (DA) activation. In a PET study that allowed for the simultaneous monitoring of opioid and dopaminergic receptor binding it was found that the activation of both opioids and dopamine was associated with the anticipation (i.e. expectation) and the effectiveness of the placebo (Scott, Stohler et al. 2008). Moreover, larger placebo responses were predicted by greater levels of opioid and dopamine co-activation in the nucleus accumbens (NAC). In another study performed by the same group (Scott, Stohler et al. 2007) the subjects first participated in a placebo study in which the contribution of dopaminergic activation in the NAC to placebo analgesia was monitored by PET. In a second part of the study that was explained to the subjects as unrelated to the pain study, the same participants underwent a task involving the anticipation of monetary reward. Using functional magnetic resonance imaging (fMRI), individual differences in NAC-activation to different amounts of monetary reward was monitored. This allowed the researchers to correlate the capacity (i.e. level) for DA-dependent monetary reward with

placebo responding. The two main results from this combined PET and fMRI study were that DA-dependent placebo analgesia was proportional to the level of anticipated analgesic effect, and that capacity for NAC activation measured during fMRI strongly predicted individual differences in DA-dependent placebo analgesia as measured by PET.

Another and independent line of evidence that supports the involvement of endogenous opioid in placebo analgesia was introduced by studies investigating the endogenous peptide cholecystokinin (CCK) and its antagonist proglumide. CCK is a peptide neurotransmitter that is released concomitant with endogenous opioids and that blocks or antagonizes their effect (Fields, Basbaum et al. 2006; Zhang, Gardell et al. 2009). This led to the hypothesis that proglumide might enhance placebo analgesia by blocking the opioid-modulating effect of CCK. This hypothesis has been confirmed in clinical (Benedetti, Amanzio et al. 1995) and experimental pain (Benedetti 1996), in which placebo analgesia was potentiated by proglumide, compared to the no-treatment groups in which proglumide had no effect on pain. In a recent experiment placebo analgesia was induced by conditioning with an opioid, and opioid-mediated conditioned placebo analgesia was completely abolished by the CCK agonist pentagastrin (Benedetti, Amanzio et al. 2010). Hidden administration of pentagastrin had no effect on pain.

In sum, studies that have antagonized the analgesic effect of endogenous opioids, that have antagonized the anti-opioid and hyperalgesic effect of CCK, and that have measured the opioid activity on μ -opioid receptors using PET, all show that the analgesic effect of opioids are an essential neurochemical mechanism in placebo analgesia. It is still unclear whether dopamine has analgesic properties, but the studies connecting the co-activation of opioid and dopamine in the NAC to the magnitude of placebo analgesia suggest that their interaction might be important. Because NAC DA has been shown to play a basic role in the encoding of reward expectancy (as opposed to a response to the actual reward) (Berridge and Robinson 2003; Schultz 2006; Scott, Stohler et al. 2007), these studies also suggest that placebo analgesia might be a special case of reward processing.

Finally, on the background of numerous pharmacological and neuroimaging investigations of placebo analgesia, it is now well established that expectation of pain reduction triggers the opioidergic descending control system initiated in pre-frontal regions (e.g. dorsolateral prefrontal cortex (DLPFC)) continuing through limbic, midbrain and brainstem regions (ACC, insula, NAC, hypothalamus, PAG, RVM) with projections all the way down to the dorsal horn, thus culminating with opioidergic inhibition at the spinal level (Petrovic, Kalso et al. 2002; Wager, Rilling et al. 2004; Zubieta, Bueller et al. 2005; Bingel, Lorenz et al. 2006; Scott, Stohler et al. 2007; Wager, Scott et al. 2007; Scott, Stohler et al. 2008; Eippert, Finsterbusch et al. 2009; Krummenacher, Candia et al. 2010). Thus, placebo analgesia is considered an example of how cognitive expectations initiate a top-down inhibition of pain.

Nocebo hyperalgesia

Nocebo hyperalgesia refers to an increase or worsening of subjective pain experience due to the administration of an inactive treatment that the subject is told will increase the pain. The increase in pain is thus attributed to the expectation of pain increase. Only a few studies have investigated the underlying mechanisms in nocebo hyperalgesia. Benedetti and colleagues (1997) demonstrated that a pretreatment with the CCK-

antagonist proglumide abolished verbally induced nocebo hyperalgesia in patients with mild post-operative pain. Furthermore, the blockade of nocebo hyperalgesia was not reversed by naloxone, thus suggesting that nocebo hyperalgesia is mediated by CCKergic systems without the involvement of opioidergic mechanisms. In a follow-up study (Benedetti, Amanzio et al. 2006) neuroendocrine markers of anxiety (adrenocorticotrophic hormone and plasma cortisol) were investigated after administration of proglumide and the benzodiazepine diazepam, an anxiolytic, in order to assess the influence of emotional components in nocebo hyperalgesia. An ischemic pain test was used to induce pain. The results revealed that increases in adrenocorticotrophic hormone and plasma cortisol (i.e. increased anxiety) were associated with nocebo hyperalgesia. When diazepam was administered, both the nocebo effect and the increase in anxiety were abolished. Proglumide on the other hand blocked nocebo hyperalgesia, but left the increased levels of adrenocorticotrophic hormone and cortisol plasma unaffected. Thus, the hyperalgesic effect could either be blocked specifically by proglumide, or globally by diazepam that blocked the anxiety responsible for the triggering of CCK. These results suggest a close relationship between anxiety, its neurochemical correlate CCK, and nocebo hyperalgesia.

In sum, nocebo hyperalgesia is opposite to placebo analgesia, in which expectation of pain increase and the resultant anxiety plays an important role. Investigations of the neurobiological basis of nocebo hyperalgesia have shown that expectation of pain increase induce anticipatory anxiety that activate CCK. CCK has hyperalgesic properties and facilitates pain and CCK-antagonists have been found to prevent anxiety-induced hyperalgesia.

Modulation of pain by negative emotions

Several lines of evidence suggest that emotional processes may modulate placebo analgesia. Firstly, emotions can increase or decrease pain report and physiological reactions to painful stimulation. Rhudy and colleagues has in a series of experiments (Meagher, Arnau et al. 2001; Rhudy, Williams et al. 2005; Rhudy, Williams et al. 2006; Rhudy, McCabe et al. 2007; Rhudy, Williams et al. 2008; Rhudy 2009) investigated the effect of emotions on pain by using pictures from the The International Affective Picture System (IAPS). The IAPS is a set of several hundred photos that illustrate situations that have been rated along two dimensions. One dimension is from negative via neutral to positive emotional valence. The valence is registered on a seven-point scale, where lower scores indicate more negative emotions. The other dimension is from low to high arousal.

In one study (Rhudy, Williams et al. 2005) it was showed that emotional valence modulated the spinally mediated nociception flexion reflex (NFR) and subjective pain. Compared to neutral pictures, the erotic and negative pictures produced smaller and greater NFR magnitudes, respectively. This was the first study to demonstrate that emotions engage descending circuits in the modulation of spinal nociception. These results have been replicated using event-related potentials (ERPs) (Kenntner-Mabiala and Pauli 2005). In a later study (Rhudy, Williams et al. 2006) used the same experimental paradigm, but varied the predictability of shock exposure, it was found that predictable noxious shocks only modulated pain ratings but not the NFR. Because predictability is known to reduce stress levels, these results lend support to the

hypothesis that also arousal was also an important contributor to emotional modulation of pain. In Rhudy et al. (2008), pictures depicting food (pleasant) and loss (unpleasant) were added to the pool of pictures so that both valence (pleasant = erotic and food; unpleasant = loss and attack) and arousal (low = food and loss; high = erotic and attack) were manipulated. Skin conductance (SCR) and heart rate (HR) acceleration were measured in addition to NFR and pain reports. It was found that picture content modulated responses in all outcome measures, and in the expected directions. However, the degree of modulation was determined by emotional intensity (arousal) where only the most arousing pictures (erotic and attack) produced modulation different from the neutral pictures.

However, negative emotions or stress may also decrease pain (Rhudy, Williams et al. 2008). Stress-induced analgesia (SIA) represents the inhibition of pain during or after exposure to a stressful or fear-inducing stimulus (i.e. also called fear-induced analgesia) (Butler and Finn 2009). SIA is mediated by the activation of the descending inhibitory pain system and a large number of studies have demonstrated that the response is partly naloxone-reversible, i.e., partly opioid-mediated (Willer, Dehen et al. 1981; Pitman, van der Kolk et al. 1990; Butler and Finn 2009). In human studies, SIA is often characterized by an inescapable and imminent threat and accompanied by feelings such as helplessness and highly intense negative emotions (Willer, Dehen et al. 1981; Bandura, Cioffi et al. 1988; Nishith, Griffin et al. 2002). In one study, Willer and Dehen (1981) employed a within-subject design and tested the cumulative effect of repetitive stress (noxious foot shocks) on the nociceptive flexion reflex. Subjects underwent three sessions; a naloxone, a placebo, and a control session in which no injection was given. The results demonstrated that SIA increased as a function of stress repetition (i.e. dose-dependent) and that this effect was naloxone-reversible. Rhudy and Meagher (2000) assessed pain threshold (finger withdrawal reflex) to a radiant heat test before and after the induction of fear (group 1), anxiety (group 2), or neutral (group 3) emotional states. Skin conductance, heart rate, and self-reported arousal (Self-Assessment Manikin; SAM) were used as indicators of emotional arousal. The results showed that fear was associated with high arousal ($M = 7.2$ on the SAM arousal measure (0-9 point range)) with a concomitant decrease in pain (i.e. increased finger withdrawal latency). Anxiety was associated with moderate arousal ($M = 4.95$ on the SAM) and a concomitant pain increase (i.e. decreased finger withdrawal latency). The analgesic effect seen in this study is in agreement with studies in SIA, in which the necessary condition for SIA to be observed seems to be a high degree, as compared to low-to-moderate degree, of emotional intensity. However, there are no systematic studies on the conditions under which stress and negative emotions can increase, or decrease, pain.

Negative emotions of low to moderate degrees of intensity increases pain, but by what mechanisms? In the aforementioned studies by Benedetti and colleagues (1997; Benedetti, Amanzio et al. 2006) it was shown that anxiety-induced hyperalgesia was completely reversed by proglumide demonstrating the key role of CCK as a neurochemical mechanism by which anxiety and increases pain. To our knowledge, these are the only studies that have shown that fear and/or anxiety (measured as adrenocorticotrophic hormone and plasma cortisol) increases pain by way of CCK release. However, there are multiple studies that show that infusion of CCK increases fear and anxiety (de Montigny 1989; Fendt, Koch et al. 1995; Josselyn, Frankland et al. 1995;

Frankland, Josselyn et al. 1997; Shlik, Zhou et al. 1999; Gospic, Gunnarsson et al. 2008; Lovick 2008; Toru, Aluoja et al. 2010), and that CCK leads to hyperalgesia (Fields, Basbaum et al. 2006; Colloca and Benedetti 2007). For example, Toru et al., (2010) investigated how personality traits influenced the response to a CCK challenge in healthy volunteers. The CCK challenge is a well validated method to induce anxiety and panic (de Montigny 1989; Bradwejn, Koszycki et al. 1991; Bradwejn and Koszycki 1994; Bradwejn, Koszycki et al. 1994) and the results showed that CCK-induced anxiety was higher amongst subjects high in traits related to aggression, irritability, anxiety and stress.

Attention is a powerful modulator of pain and the finding that attention towards pain increase pain experience whereas attention away from pain decrease pain experience has been replicated a number of times (Bantick, Wise et al. 2002; Villemure and Bushnell 2002; Villemure and Schweinhardt 2010). Emotion influence the direction of attention (Ohman, Flykt et al. 2001) and negative emotions in particular have been found to increase attention toward pain (Keogh, Dillon et al. 2001; Keogh, Ellery et al. 2001). This means that the effect of negative emotions on pain could, at least partially, reflect attention to the painful stimulation. Thus, newer studies have tried to separate the mechanisms of emotional and attentional modulation of pain (Villemure and Bushnell 2002; Villemure, Slotnick et al. 2003; Villemure and Bushnell 2009; Roy, Lebus et al. 2011). In a review of these studies Villemure and Schweinhardt (2010) summarize that the available data so far suggest partially separable mechanisms because emotional and attentional modulation of pain, differently change the sensory and affective aspects of pain perception, and also implicate partially different brain circuits.

The overall conclusion from studies investigating the effect of emotions on pain is that emotional valence determines the direction of pain and psychophysiological modulation, whereas arousal determines the magnitude of the modulations. An exception from this principle is stress-induced analgesia, as mentioned. Furthermore, emotions (both positive and negative) modulate pain by involving descending modulatory circuits affecting early nociceptive processing. On a neurochemical level, moderate degrees of fear and anxiety are related to the activation of CCK which in turn facilitates pain signal transmission. The connection between negative emotions and increased CCK release is important because CCK has antagonistic effects on both opioids (Fields, Basbaum et al. 2006) and dopamine (Woodruff 1992; Rotzinger and Vaccarino 2003), the two primary neurochemical agents responsible for placebo analgesia. Finally, results show that negative emotions increase pain via attentional mechanisms and that these mechanisms are at least partially separable from mechanisms of emotional modulation of pain.

Negative emotions and the effectiveness of opioids

Negative expectations and negative emotions such as fear and anxiety can reduce the effect of analgesics. In a study by Wang and colleagues (2008) a sample of 614 post-operative patients were offered morphine intravenously infused via a patient-controlled analgesia (PCA) technique. The PCA technique is popular since the patient has control over the infusion. The subjects were randomized into four groups differing in the information given about the PCA treatment: a no information group, a positive information group, a partially negative information group, and a totally negative information group. Outcome measures were reported pain intensity, plasma cortisol,

morphine consumption, morphine consumption side effects, and self-reported level of sedation and satisfaction with the therapy.

The results showed that negative information about the treatment increased reported pain intensity, and participants in the totally negative information group reported significantly higher pain intensity than the partially negative information group. Pain intensity did not increase in the no information and the positive information groups. Negative information also increased plasma cortisol and participants in the totally negative information group displayed significantly higher cortisol levels than the partially negative information group. Cortisol levels did not increase in the no information and the positive information groups. Relative to no information and positive information groups, morphine consumption increased in the negative information groups. As compared to the no information group there was a 39.5 % and 58 % increase in morphine consumption in the partially negative and totally negative information groups, respectively. Finally, the occurrence of side effects were more frequent in the negative information groups compared to the two other groups, and subjects in the negative information groups also reported lower overall sedation levels and less satisfaction with therapy.

Negative emotions are also implicated in endogenous opioid neurotransmission. In a study by Zubieta and colleagues (2003) μ -opioid receptor binding was monitored during sustained neutral and sad mood in a sample of healthy volunteers. The results showed a reduction in μ -opioid neurotransmission (i.e. increases in μ -receptor availability) from the neutral to the sadness condition in several limbic brain areas. The reduction in opioid neurotransmission also correlated with self-reported increases in negative affect and decreases in positive affect. This finding was later replicated twice by the same research group (Kennedy, Koeppe et al. 2006; Prossin, Love et al. 2010) and demonstrate that endogenous opioid tone is reduced by negative emotion. Finally, opioid dysfunction has also been related to anger. In a study by Bruehl and colleagues subjects were randomly assigned to undergo either an anger recall interview or a neutral control interview, and half of the subjects received opioid blockage whereas the other half received placebo. The subjects were then exposed to a finger pressure pain test and an ischemic forearm pain test. Pain intensity was measured by visual analogue scales and trait anger by the State-Trait Anger Scale. Anger increased pain (i.e. hyperalgesia) and this hyperalgesic effect was most pronounced in high trait anger subjects and in the context of acutely aroused anger. Opioid blockade analyses showed that anger-induced hyperalgesia were in part due to deficient opioid function.

Thus, negative emotions, here exemplified by fear, anxiety, sadness and anger, seem to increase pain and reduce the effectiveness of both exogenous and endogenous opioids. This finding is supported by reviews on PCA-studies and reviews on nocebo hyperalgesia, in which the overall conclusion is that the hyperalgesic and antiopioid effect of negative emotions compromise the analgesic effect of painkillers (Svedman, Ingvar et al. 2005; Colloca and Benedetti 2007).

Variability in placebo analgesia and the role of trait factors

There is great variability in placebo analgesic responding, and from the earliest observations of placebo analgesia there have been placebo responders and non-responders (Hoffman, Harrington et al. 2005). The variability in placebo analgesia has

been attributed to both cognitive and emotional factors. The effect of induced cognitive expectancy, often measured as the level of activation of prefrontal areas such as the DLPFC has been shown to be proportional to the magnitude of reported pain reduction (Wager, Rilling et al. 2004; Scott, Stohler et al. 2007; Wager, Scott et al. 2007; Scott, Stohler et al. 2008). Furthermore, in a recent study it was shown that placebo analgesia was completely abolished when prefrontal regions were subjected to repeated transcranial magnetic stimulations (rTMS) (Krummenacher, Candia et al. 2010). Similarly, several studies have shown that placebo analgesia is accompanied by an increase in self-reported positive emotions (Zubieta, Bueller et al. 2005; Zubieta, Yau et al. 2006; Aslaksen and Flaten 2008; Scott, Stohler et al. 2008) and/or a decrease in negative emotions (Vase, Robinson et al. 2005; Scott, Stohler et al. 2007; Aslaksen and Flaten 2008). A recent study that reanalyzed data from two previous fMRI studies on placebo analgesia also found that decreases in limbic and paralimbic regions were the strongest predictors of individual differences in placebo analgesic responding (Wager, Atlas et al. 2011).

Given the importance of cognitive expectations and emotional states in placebo analgesia a few recent studies have investigated whether more stable tendencies (i.e. traits) in cognitive and emotional styles can contribute to the variability in placebo analgesia. Dispositional optimism has been launched as a potential candidate and is defined as a relatively stable tendency in the formation of positive outcome expectancies (Scheier and Carver 1985). Trait optimism has been used extensively as a predictor for health outcomes and has been associated with a wide range of positive coping behaviours within medical contexts (Scheier, Matthews et al. 1989; Scheier, Matthews et al. 1999; Kubzansky, Sparrow et al. 2001; Kubzansky, Kubzansky et al. 2004). Optimism has also been related to higher placebo effects on sleep quality (Geers, Kosbab et al. 2007) and bodily symptoms (Geers, Helfer et al. 2005), and recently also to higher placebo analgesia (Morton, Watson et al. 2009; Geers, Wellman et al. 2010). In a study by Morton and colleagues (2009) subjects in a placebo group and a control group were exposed to painful laser stimulations in pre-treatment, treatment, and post-treatment sessions. The subjects were invited back to repeat the experiment on a second day. This allowed for testing whether an initial placebo effect could be reproduced on a second day, and whether optimism could predict placebo responding in the initial and/or the repeated experiment. A placebo effect on reported pain was observed on both days. Furthermore, there was a large correlation of placebo responding between days, indicating a high ratio for individual placebo responding in both experiments, and this tendency was highest for subjects high in optimism.

Given the strong predictive value of NAC DA activation for the magnitude of placebo analgesic responding (Scott, Stohler et al. 2007; Scott, Stohler et al. 2008), Schweinhardt and colleagues (2009) tested whether reward-related personality traits and grey matter density (GMD) in the NAC could predict placebo analgesia. Reward-related personality as measured by the Trait Character Inventory (TCI) has been related to dopaminergic neurotransmission and reward sensitivity (Yacubian, Sommer et al. 2007). GMD has been shown to be related to brain function and individual anatomical differences in dopaminergic pathways have been linked to both behavioural outcomes and to variation in personality (Depue and Collins 1999). The results revealed that the dopamine-related personality trait correlated with GMD in the ventral striatum which

includes the NAC, and both the trait factor and GMD correlated with the magnitude of placebo analgesic responding on pain intensity.

In sum, traits that contribute to the variability in the two most prominent mechanisms in placebo analgesia, namely expectations and reward sensitivity have been found to predict the magnitude of placebo analgesic responding. Moreover, these traits target individual differences in the capacity for reward expectations and for opioid-mediated inhibition.

Finally, the research focusing on the role of trait factors in predicting placebo analgesia has only been focusing on the characteristics of those who respond – the placebo responders. But what about those who do not respond? What characterizes a placebo non-responder?

Fear of pain and induced fear

The present thesis investigated the effect of fear on placebo analgesia, either by using the Fear of Pain Questionnaire as predictor in regression analyses, or by inducing fear experimentally by threat of electric shocks.

Fear of pain (FOP) refers to a negative emotional response evoked by pain, by activities associated with pain, or by the knowledge or suspicion that pain might be a possible outcome or consequence (Vlaeyen, Crombez et al. 2004). FOP is commonly measured by the Fear of Pain Questionnaire (FPQ-III) (McNeil and Rainwater 1998) that assesses FOP in specific situations that would normally produce pain. The sum score reflects a predisposition to experience fear in contexts where pain is probable. As a trait quality, FOP should reflect previous experiences with pain and physical discomfort, reflecting qualities and action tendencies that have been repeated and internalized in the individual.

Originally, the FOP construct draws upon the fear-avoidance model of pain perception (Vlaeyen and Linton 2000) and is most commonly used as an explanation of how pain cognitions (e.g. worry, catastrophizing) and avoidance behaviour can lead to exaggerated pain perception over time. Experimental studies investigating how FOP influence acute pain perception in non-clinical samples are not common, but a few studies (George, Dannecker et al. 2006; Kirwilliam and Derbyshire 2008) have documented a positive relation of FOP to reported pain intensity. The fear-avoidance model also hypothesizes that fear of pain should be positively associated with stress, both during pain and in the anticipation of pain. In a recent study it was shown that individuals high in fear of dental pain, as opposed to those low in fear of dental pain, reacted with larger blink reflexes, heightened electrodermal activity and heightened autonomic responses in a context of threat (Bradley, Silakowski et al. 2008). Studies have also shown that subjects higher in FOP are more likely to direct attention towards threatening stimuli (Keogh, Ellery et al. 2001; Keogh, Thompson et al. 2003; Asmundson and Hadjistavropoulos 2007; Kirwilliam and Derbyshire 2008).

Several studies have investigated the effect of induced fear on pain. Fear can either increase pain or inhibit pain, depending on the level of arousal of the emotion. In the aforementioned study by Rhudy and Meagher (2000) fear of high arousal was induced by repetitive noxious electric shocks (12 mA), and this increased finger withdrawal reflex thresholds (i.e. decreased pain) to radiant heat compared to the control condition. In another study (Meagher, Arnau et al. 2001) fear was induced by viewing

pictures from the IAPS that were presented before a cold-pressor task. This fear induction procedure caused moderate arousal ($M = 5.2$ on a 0-9 range arousal measure) and decreased pain tolerance, and pain thresholds on pain intensity and pain unpleasantness and compared to neural pictures. In Paper III in the present thesis fear was induced by an instructed fear procedure, in which the subjects were told that they would receive electric shocks at any moment within a period of several minutes. The aim was to induce fear of low-to-moderate emotional intensity. Thus, we did not say how many shocks; we did not inform about the intensity of the shocks; and we administered the shocks at the very end of the waiting period to ensure longer lasting fear, as apposed to brief and momentary peaks of fear time-locked to the electric stimulations. This procedure should ensure the induction of fear or stress of a moderate degree.

With the exception of the papers presented in this dissertation, the relation of fear to placebo analgesia has not yet been systematically investigated. Fear and anxiety are the two most frequently reported emotions in the context of pain (Craig 1994), and the studies presented so far show that these emotions with low-to-moderate arousal, exacerbate pain, cause hyperactivity in the hypothalamic-pituitary-adrenal axis, and trigger endogenous peptides that have hyperalgesic and anti-opioid capabilities (Benedetti, Amanzio et al. 2006). Fear and anxiety may also increase pain via attentional mechanisms that are at least partially independent of mechanisms mediating emotional modulation of pain. Thus, since fear and anxiety are shown to cause the opposite reaction pattern at emotional, homeostatic and neurochemical levels of analysis as compared to what one usually observes in placebo responders, the main hypothesis investigated was that that fear would reduce or abolish/prevent placebo analgesia.

RESEARCH QUESTIONS

These are the main research questions addressed in this thesis:

1. Is fear of pain related to higher reported pain and stress?
2. Is fear of pain related to reduced placebo analgesia?
3. If so, does this finding replicate across studies?
4. Does experimentally induced fear reduce the effectiveness of a placebo intervention on pain?
5. Is the effect of fear on placebo analgesia confined to reported pain, reflecting cognitively construed representations of experience, or does it involve early and pre-cognitive effects as measured by objective measures such as event-related potentials and the acoustic startle reflex, or both?

METHODS

Induction of pain

In all three papers in the present thesis pain was induced by a thermode delivering heat stimuli to the underarm. The thermode consisted of a Peltier element placed on the skin (Study I), or an aluminium foil placed on the skin (Study II and III), where the foil was electrically heated to allow rapid increase in temperature, and cooled by a Peltier element. The temperature in the thermode was controlled by software developed by the manufacturer. In Study I a TSA-II (Medoc, Israel) was used and heat stimuli of 46⁰C were applied for four minutes. In Study II and III a Contact Heat Evoked Potentials Stimulator (CHEPS, Medoc, Israel) was used, and heat stimuli of 52⁰C (Study II) and 54⁰C (Study III) were applied. The increase in heat from a baseline of 32⁰C to maximum temperature in Study II and III were 70⁰C per second and the cooling rate was 40⁰C. This generated a painful stimulus with abrupt onset and with a duration of less than 0.1 sec to allow recordings of event-related potentials. These methods are accepted as valid ways of inducing pain, but are not without potential problems. The pressure from the thermode on the skin may create differences in conduction, and thereby in heat transfer (Baumgartner, Cruccu et al. 2005). Thus, the thermode should be applied in a standardized way, as was done in the present three studies.

Pain intensity and pain unpleasantness

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (IASP 1979). Thus, pain has sensory and emotional properties, and both should be measured to properly assess the effect of the experimental manipulations on pain. Therefore, in all studies pain intensity and pain unpleasantness were recorded, to assess the sensory and emotional properties, respectively. The actual measurements of pain intensity and pain unpleasantness were performed by a visual analogue scale (VAS) in Study I, and a numerical rating scale (NRS) in Study II and III. For VAS measurements, the subject indicated on a 10 cm line, by drawing a short tick across the 10 cm line, how intense or unpleasant the pain is. For NRS measurements, the subjects said out loud a number from 0 to 10 to indicate how painful the experience was. The end-points for the intensity scale were “no pain” and “unbearable pain”, printed under the left and right ends of the scale, respectively. The end-points for the pain unpleasantness scale were “no unpleasantness” and “unbearable unpleasantness”. The reliability of the VAS is high (Nielsen, Price et al. 2005), and Price et al. (1983) argue that the VAS provides data on a ratio scale. Both the NRS and VAS are more sensitive to small differences in pain compared to other pain rating scales (Ferreira-Valente, Pais-Ribeiro et al. 2011). Thus, the validity of the scales are high. The difference between pain intensity and pain unpleasantness were described as in Price et al. (1983).

Stress and arousal

Subjective stress and arousal were measured by four pairs of adjectives from the Short Adjective Check List (SACL) (Mackay, Cox et al. 1978) translated to Norwegian. Two adjective pairs indexed stress: tense – relaxed and nervous – calm, and two pairs indexed arousal: energetic – tired and awake – sleepy. The adjective pairs were chosen for the high factor loadings on the stress and arousal factors on the SACL (O'Neill and Parrott

1992; Parrott 1995). Previous studies have shown that stress and arousal measured by the SACL are separate dimensions (Surawy and Cox 1986), and this is supported by a study using factor analysis where stress and arousal were shown to be orthogonal factors (Mackay, Cox et al. 1978).

Fear of Pain Questionnaire

Fear of pain was measured with the FPQ-III, a 30-item self-report questionnaire that assesses fear to specific situations where pain may be inflicted (McNeil and Rainwater 1998). Each of the items are rated on a 5-point scale (1 = not at all, 5 = extreme). The three subscales indexes fear of severe pain (e.g., breaking your neck), minor pain (e.g., that a car-door is slammed over your fingers), and medical pain (e.g., having a blood sample drawn). Each subscale consists of 10 items. The FPQ-III has good internal consistency, and this information is found in the articles. The FPQ-III is a multi-factor instrument and can either be used to assess fear related to the three sub-domains or to evaluate the generalization of fear across domains. The sum score (i.e. global FPQ) thus reflects a predisposition to experience fear in contexts where pain is probable. The global FPQ score was used in Study II and II and the FPQ subscales were used in Study I. The English version of the FPQ-III was translated into Norwegian by two Norwegian PhD students at the University of Tromsø. These translations were then back-translated into English before agreeing on a final Norwegian version. The FPQ-III was used in all three studies.

Event-related potentials

In Study II event-related potentials (ERPs) to brief thermal stimulations were computed to control for response bias. Reduced reported pain in the Placebo condition could be due to the subjects reporting what the subjects assumed the experimenter wanted them to report, i.e., lower pain. Since ERPs correlate highly with pain report (Granovsky, Granot et al. 2008), ERPs can be used as an objective measure of pain experience, not influenced by reporting bias.

ERPs are computed from electroencephalographic (EEG) activity. The EEG reflects the synchronized activity of large numbers of neurons. Since the activity is picked up by surface electrodes, cortical activity is most likely the source of much activity, but activity from, e.g., the cingulate cortex, may contribute to the EEG. The ERP computed in Study II was the mean amplitude of the 24 painful stimulations presented in each pain test. This generated two components, termed the second negative (N2) and second positive (P2) components. Both these components correlate with reported pain (Granovsky, Granot et al. 2008). Previous studies have shown that the N2 and P2 component is reduced after information that a painkiller has been administered (Wager, Matre et al. 2006; Watson, El-Deredy et al. 2007; Colloca, Tinazzi et al. 2008). This indicates that the pain signal is reduced, at least partially, before it reaches the cortical areas in which these components are generated.

In Study II ERPs to contact heat, thus referred to as contact-evoked potentials (CHEPs), were recorded from seven electrodes, but only data from the Cz location were reported. An electrode cap with shielded electrodes (EasyCap, Brain Products, Herrsching, Germany) was used. The recordings were made by a Quick Amp EEG system (Brain Products) and analyzed off-line with Analyzer 1.0 software (Brain

Products). Electrooculographic recordings were obtained for eye movement artifact control.

The acoustic startle reflex

In Study III, startle eyeblink reflexes were recorded by a Coulburn Human Startle System. The Coulburn software controlled the stimulus presentation and response recording. White noise with an intensity of 95 dB (SPL) was delivered through Sennheiser HD 250 headphones, and a Bruel and Kjaer 2235 Sound Level Precision Meter was used for calibration of the noise. Stimulus rise time was instantaneous and the duration was 50 ms.

Eyeblink electromyographic (EMG) responses were recorded from the right orbicularis oculi with two Ag/AgCl miniature electrodes (2mm diameter) filled with conductivity gel and placed about 2 cm apart. The reference electrode was placed in the middle of the forehead. All these are standard parameters for the study of startle reflexes.

Startle reflexes are elicited by abrupt and intense stimulation, and is most often measured as eyeblinks to sudden noise. The noise activates cochlear root neurons in the auditory nerve, that project to the reticularis pontis caudalis (RPC) in the brainstem (Lee, Lopez et al. 1996). This nucleus controls activity in motoneurons, the activation of which are observed as whole body startle in rats, or as eyeblink reflexes in humans. The RPC is considered the startle center, and lesion of this nucleus abolishes startle reflexes. A human study has also suggested that the RPC is involved in human startle (Pissioti, Frans et al. 2002). Induction of fear increases startle reflexes, termed fear-potentiated startle. The amygdala directly projects to the RPC, and activation of the amygdala by fear increases startle reflexes via this mechanism, also in primates (Antoniadis, Winslow et al. 2007).

Startle reflexes have been much used in the study of emotions since Vrana et al. (1988) suggested that this reflex may be modulated by emotional valence. The original observation (Vrana, Spence et al. 1988) was that induction of positive emotions reduced startle reflexes compared to a control condition, whereas negative emotions, e.g., fear, increased startle reflexes. The effect of positive emotions on startle seems to vary with the type of emotions, but the observation that negative emotions increase startle has been replicated in a large number of studies (Åsli and Flaten 2012). In Study III in the thesis fear was induced by instructing the participants that electric shocks were imminent.

SUMMARY OF PAPERS

Paper I (Lyby PS, Aslaksen PM, Flaten MA. Is fear of pain related to placebo analgesia? Journal of Psychosomatic Research, 2010; 68: 369-377).

We hypothesized that subjects high in FOP would report higher pain and stress in the Natural History condition, and exhibit reduced placebo responses. This hypothesis was tested in a 2 Condition (Natural History, Placebo) by 5 Test (Pre-test, post-tests) within-subject design. The conditions were tested on two separate days, and were balanced so that 32 subjects received the Placebo condition before the Natural History condition, and 31 subjects received the opposite condition order. Heat pain was induced by a thermode (TSA II, Medoc, Israel) and held a temperature of 46 °C for 4 minutes in each pain test. Outcome measures were pain intensity, pain unpleasantness, stress and arousal. The placebo medication was administered immediately after the first pain test and consisted of two capsules that each contained 75 mg of lactose.

Placebo effects that endured for the whole procedure of approximately 45 minutes were found on pain intensity and stress. Individual response scores for these measures were subjected as dependent variables in the regression analysis. In the placebo response scores on pain intensity roughly one third of the subjects responded with a negative placebo (i.e. increase instead of decrease in pain).

Medical FOP predicted higher baseline subjective stress and pain intensity in the Natural History condition. Severe FOP predicted lower placebo responding on pain intensity.

Paper II (Lyby PS, Aslaksen PM, Flaten MA. Variability in placebo analgesia and the role of fear of pain – an ERP study. Pain, 2011; 152(10): 2405-2412).

In Paper II we aimed to replicate and extend the findings from the first study by employing Contact Heat-Evoked Potentials (CHEPs) in addition to subjective reports. A 2 Condition (Natural History, Placebo) by 3 Test (Pre-test, Post-test 1, Post-test 2) within-subject and counterbalanced design, tested on two separate days was used. Pain was induced by a contact heat-evoked stimulator (CHEPS; Medoc Ltd., Ramat Yishai, Israel) with a thermofoil thermode. Baseline temperature was 32 °C, and painful stimulation was applied at 52°C. The rise rate was 70°C/sec and cooling was 40°C/sec, meaning that 52°C was reached in 285 ms, and return to baseline was 500 ms. Twenty-four 52 °C stimuli were presented in each of the three tests in each condition. Reported pain intensity and pain unpleasantness were collected from stimuli 21-24 in each test.

Placebo effects were observed in pain unpleasantness and N2 and P2 amplitudes and thus replicate other studies that have used ERPs to investigate placebo analgesia. Individual difference analyses showed that FOP was associated with lower placebo responding in P2 amplitude. FOP was also related to weaker placebo responding in pain unpleasantness, but this depended on condition order; or more specifically, on the amount of reported stress before the administration of the placebo pills. Furthermore, placebo responses in both N2 and P2 amplitudes predicted placebo responses in pain unpleasantness. Analyses also revealed that sex, in addition to FOP, predicted placebo responding on P2 amplitude. In the present sample, female subjects scored significantly higher on FOP than male subjects did. We thus performed hierarchical blockwise regression and after removing the linear effect of FOP, there were no differences between male and female subjects in placebo responding.

Paper III (Lyby PS, Forsberg JT, Åsli O, Flaten MA. Induced fear reduces the effect of a placebo intervention on pain. Pain, 2012; 153(5): 1114-1121.

Our primary aim with Paper III was to investigate the causal effect of fear on placebo analgesia by inducing fear experimentally. A secondary aim was to investigate the predictive validity of individual measures in fear on placebo analgesia. We used a 3 Condition (Natural History, Placebo, Placebo + Fear) by 3 Test (Pre-test, Post-test 1, Post-test 2) within-subject design, in which the condition orders were counterbalanced and tested on three separate days. A contact heat-evoked stimulator was used to induce pain. Twenty stimuli at 54 °C were presented in each of the three pain tests in each condition. Startle reflexes was elicited by white noise. Fear potentiates startle and in the present experiment, fear-potentiated startle was used as a physiological index of fear (i.e. manipulation check) and as a predictor of placebo analgesic responding together with FOP and with a measure that rated how effective the subjects perceived the fear-inducing procedure in inducing fear (referred to as index of induced fear in the paper).

A placebo effect was observed in the startle data. This placebo effect was abolished by experimentally induced fear, and this effect of fear-potentiated startle was strongest amongst high FOP-subjects. In the pain intensity data, there was a trend towards a placebo effect. This trend was abolished by induced fear and this was most pronounced in subjects who were highest in the three measures of fear. Moreover, the trend towards a placebo effect in the pain intensity data was predicted by the corresponding placebo effect in startle.

In conclusion, placebo effects in both startle (emotion) and pain intensity were abolished by induced fear. These effects were strongest in subjects who displayed the highest values on the fear measures. The effects obtained in the startle data also positively predicted the corresponding effects in the pain intensity data, suggesting shared underlying mechanisms.

DISCUSSION

In Paper I we hypothesized that subjects high in fear of pain would report higher pain and stress in the Natural History condition, and exhibit reduced placebo responses. Heat pain was induced to the volar forearm and subjective reports were obtained on pain intensity, pain unpleasantness, stress and arousal. Placebo effects that endured for the whole procedure of approximately 45 minutes were found on pain intensity and stress, replicating other studies that have used repeated measures (Vase, Robinson et al. 2005; Aslaksen and Flaten 2008). The magnitude of the placebo effects was about one unit on the 11-point VAS for pain intensity and about half a unit on the 11-point stress scale. Regression analyses showed that fear of medical pain was positively associated with pain intensity, meaning that those higher in fear of medical pain also displayed higher levels on pain intensity. These results are in line with results from other experimental (George, Dannecker et al. 2006; Kirwilliam and Derbyshire 2008) and clinical studies (van den Hout, Vlaeyen et al. 2001) that used FOP as a predictor. Furthermore, fear of medical pain was also positively related to reported stress, replicating results from an experimental study that demonstrated a positive association between FOP and stress measured by potentiated startle blinks, heightened skin conductance, and cardiac deceleration (Bradley, Silakowski et al. 2008).

Fear of severe pain was negatively associated with placebo analgesic responding, meaning that subjects higher on FOP displayed the lowest placebo responses. It should be mentioned that about one third of the subjects responded with a negative placebo response, i.e., with increased pain after administration of the placebo. This means that subjects with higher scores on fear of severe pain had a tendency to respond with an increase in pain instead of decrease in pain after the administration of the placebos. In another study, with comparable placebo effect magnitudes as observed in Paper I, Scott and colleagues (2008) also observed that one third of the subjects ($n = 5$) responded with a negative placebo response. This study used positron emission tomography (PET) and monitored receptor binding potential at μ -opioid and dopamine (DA) receptors. The results showed that placebo responders ($n = 10$) displayed a decreased availability at these receptors (i.e. indicating an increase of opioid and DA binding), whereas the negative placebo responders showed an increase in binding potential at the same receptors. Furthermore, state measures in affect showed that placebo-induced increase in positive affect mediated placebo responding, whereas negative affect did not decrease and had no effect on responding.

Finally, FOP did not predict placebo responding in reported stress. The placebo effect on stress was weaker than for pain intensity, and it might be that this accounted for the non-significant finding.

In Paper II our aim was to replicate and extend the findings from the first study by employing Contact Heat-Evoked Potentials (CHEPs) in addition to subjective reports. Subjective pain reports are based upon cognitively construed representations of experience, and many studies report reliable placebo effects in reported pain. One advantage of combining subjective reports with an objective method measuring cortical responses to pain is that reporting bias may be excluded as an explanation of the results (Hrobjartsson and Gotzsche 2001). Additionally, event related potentials (ERPs) such as CHEPs, are useful in the investigation of important empirical questions such as how deep

in the central nervous system the placebo-mediated inhibition reaches. ERPs are considered an apt method to use for answering this question since they, at least partially, reflect early or pre-cognitive nociceptive processing. Moreover, in line with our hypothesis that FOP is associated with reduced placebo analgesia, an equally important issue is how deep this potentially inhibitory effect goes. FOP is supposed to be associated with increased fear during pain and in the anticipation of pain. If placebo effects were obtained on CHEPs, and if FOP were to interfere with these effects, then one could propose that the effect of FOP occurs at least partially on a pre-cognitive level.

We used the same design and experimental paradigm as in the first study (i.e. within-subject and counterbalanced design). Placebo effects that endured over time (i.e. at least 40 min) were observed in pain unpleasantness and N2 and P2 amplitudes and thus replicate other studies that have used ERPs to investigate placebo analgesia (Wager, Matre et al. 2006; Watson, El-Deredy et al. 2007; Colloca, Tinazzi et al. 2008). Individual difference analyses showed that FOP was associated with lower placebo responding in P2 amplitude. FOP was also related to weaker placebo responding in pain unpleasantness, but this depended on condition order; or more specifically the amount of reported stress before the administration of the placebo pills. Furthermore, placebo responses in both N2 and P2 amplitudes predicted placebo responses in pain unpleasantness. Analyses also revealed that sex, in addition to FOP, predicted placebo responding on P2 amplitude. In the present sample, female subjects scored significantly higher on FOP than male subjects did. We thus performed hierarchical blockwise regression and after removing the linear effect of FOP, there were no differences between male and female subjects in placebo responding.

As with results from Paper I, the data from Paper II suggest that those higher in FOP are less responsive to placebo interventions. The results linking FOP to placebo unresponsiveness in P2 amplitude suggest that the effect of FOP on placebo analgesia is at least partially pre-cognitive and not only confined to cognitively construed representations of pain. This finding might reflect a nociceptive system that is more easily activated due to anticipatory fear in high FOP-subjects, and this has been demonstrated in other studies (Bradley, Silakowski et al. 2008). Moreover, the results from Papers I and II now suggest that the effect of FOP on placebo analgesia can occur at an early and partially pre-cognitive stage (Paper II), reflecting anticipatory fear when pain is near, and at a later stage in which cognitively construed representations of pain experience are reported to the experimenters (Papers I and II).

Papers I and II showed that the magnitude of the placebo effects in subjective reports and CHEPs depended on the level of FOP, in which those higher in FOP seemed less responsive to the placebo interventions. These results however, are correlative in nature, and can thus say nothing about cause and effect, even though one could theoretically propose that FOP, reflecting trait qualities, precedes the experimental procedures in time and thus justifies a causal interpretation. Our primary aim with Paper III was therefore to investigate the causal effect of fear on placebo analgesia by inducing fear experimentally. To test this hypothesis we expanded the initial design used in the two previous papers, to include a second placebo condition in which fear was induced by the anticipation of electric shocks (i.e. fear was induced after the administration of the placebo capsules). We thus used a 3 Condition (Natural History, Placebo, Placebo + Fear) by 3 Test (Pre-

test, Post-test 1, Post-test 2) within-subject design, in which the condition orders were counterbalanced and tested on three separate days.

We chose anticipation of electric shock as the fear-induction procedure because it is a well validated method, consistently inducing fear (Grillon, Ameli et al. 1991; Greenwald, Bradley et al. 1998; Phelps, O'Connor et al. 2001), and also because it is relevant to FOP since electric shocks are expected to be painful. Additionally, the acoustic startle reflex was chosen as an outcome measure because fear-potentiated startle is a well validated marker of fear (Lang, Bradley et al. 1990). Potentiation of the startle reflex can reflect early and pre-cognitive processing (Asli, Kulvedrosten et al. 2009; Åsli and Flaten 2012), and its expression (i.e. amplitude) is tightly connected to amygdala activation and to networks mediating defensive activation and action preparedness (Angrilli, Mauri et al. 1996; Funayama, Grillon et al. 2001; Phelps, O'Connor et al. 2001; Buchanan, Tranel et al. 2004). Thus, for individual difference analyses we used fear-potentiated startle and FOP as predictors of placebo responding. We also used self-reported effectiveness of the fear-induction procedure to produce fear as predictor. We predicted that measures of fear should predict placebo responding when comparing the Natural History condition to the Placebo condition, and when comparing the Placebo condition to the Placebo + Fear condition. We also expected that placebo responding in startle should be positively related to corresponding placebo responses in subjective reports.

A placebo effect was observed in the startle data. This effect was abolished by induced fear and strongest amongst high FOP-subjects. In the pain intensity data, there was a trend towards a placebo effect. This trend was abolished by induced fear and was most pronounced in subjects who were highest in the three measures of fear. Moreover, the placebo effect on startle and the disruption of this effect by induced fear, positively predicted the corresponding effects (i.e. placebo effect and its disruption) in the pain intensity data. This suggests shared underlying mechanisms and the results from the regression analyses suggests that the expression of these mechanisms follow individual differences in fear.

Was fear or anxiety induced?

Fear and anxiety are similar, but not identical (Davis, Walker et al. 2010) and the concepts are used interchangeably within the literature, thus representing a source of confusion. Fear refers to a state of apprehension to an imminent and real threat or object. In comparison, anxiety refers to a state of apprehension elicited by imprecise and unknown threats (i.e not connected to a defined object) that may or may not happen (i.e. uncertain possibility), or that are physically or psychologically more distant (Davis, Walker et al. 2010). Anxiety is thus considered more vague, future-oriented and long-lasting than fear, which is connected to the immediate future by the defined and expected object or threat. In Study III fear was induced by an instructed fear procedure (i.e. verbally induced, resembling a cognitive representation of fear; (Phelps, O'Connor et al. 2001): Electrodes were attached to the subjects fingertips and they were told that they would receive electric shocks at any moment within a range of minutes. Two electric shocks of low intensity (1.4 mA) were administered at the very end of a waiting period. This instruction, in our opinion, has more characteristics in common with the definition of fear as compared to the definition of anxiety; because the threat is defined (i.e.

“electric shocks” as compared to “something bad”); the threat is certain and real (i.e. “will receive” and not “might receive”); and the threat is imminent and predictable within a range of minutes (“at any moment within the rest of the waiting period”). We thus referred to the procedure as induced fear and not induced anxiety. However, both fear and anxiety potentiate startle, and we have no actual data to support our claim that the procedure has more defining characteristic in common with fear compared to anxiety.

The data from Study III suggest that fear of low to moderate intensity was induced in the subjects. Startle potentiation was followed by concomitant increases in reported pain, and not decreased pain that would have been expected following intense fear (Willer, Dehen et al. 1981; Rhudy and Meagher 2000). Moreover, the index of induced fear, that retrospectively measured how effective the subjects perceived the fear induction procedure was in inducing fear, showed a mean score of 3.5 (i.e. low/moderate) on a 0-5 Likert scale.

Clinical implications

A clinical implication of the finding that fear reduces or abolishes placebo analgesia becomes evident in light of research that demonstrates the additive impact of placebos on conventional analgesics. Placebo effects on pain, due to expectations of pain relief, conditioning, or both, are an integrated part of analgesic treatments. Hidden administrations of analgesics have the potential to reveal such additive effects. The purpose of hidden treatments is to eliminate the effects of psychological mechanisms on analgesic outcome, e.g. the patient does not expect anything under hidden administration. In contrast, open administration implies full insight to the patient. Levine and Gordon (1984) examined the effects of open versus hidden treatments. They found that an open administration of a placebo was as effective as 6-8 mg of hidden administration of morphine. In this experiment the investigators had to increase the hidden dosage up to 12 mg before this effect became noticeably larger than the placebo treatment. Several later studies have replicated and extended these findings. They demonstrate that hidden administration of drugs reduces the total therapeutic impact by preventing the inhibitory effect of expectations (Amanzio, Pollo et al. 2001; Benedetti, Carlino et al. 2011).

Thus, in terms of the detrimental effects of fear on placebo analgesia, preventing fear and negative affect is one way in which treatment outcomes and patient care can be optimized. Different approaches of how to buffer negative affective states, either by working with them directly (i.e. psychotherapy, relaxation, medication) or indirectly (i.e. providing conditions for positive affect) should be a primary goal for all health care personnel.

Future perspectives

The design used in Study III represents a direct operationalization of simultaneous inhibitory (i.e. placebo) and facilitory (i.e. fear) activation. The result that fear abolished placebo analgesia, thus suggests that the facilitory effect of fear canceled out the inhibitory effect of the placebo. Secondly, in all studies we demonstrated that the inhibitory effect of the placebo interventions on pain was not sufficient to cause pain decrease for those high in FOP. In Study I, FOP was even related to an increase in reported pain after the placebo pills was administered.

Our findings are in line with converging evidence from both animal and human studies that demonstrate that the two divisions of the pain modulatory system can be activated at the same time and to different degrees, independent of one another (i.e. blocking one system will not affect activity in the other) (Crown, Grau et al. 2004; Fields 2004; Heinricher and Neubert 2004; Burns, Bruehl et al. 2009). This means that it is the end result of both (i.e. parallel) inhibitory and facilitory activation that determines the direction and the degree of modulation. This perspective may provide a more nuanced way of understanding placebo responding and the role of emotional modulation in placebo responding. Future studies are warranted.

OVERALL CONCLUSIONS

It is concluded that fear, of low to moderate intensity, reduces the effectiveness of placebo interventions on pain, and our data suggest that this effect is not only confined to cognitively construed representations of pain but also, at least partially, to early and pre-cognitive levels of processing. Our data also show that the underlying mechanisms mediating the detrimental effect of fear in placebo analgesia follow individual differences in measures of fear.

1. Fear of pain is related to reduced placebo analgesia in reported pain, and in P2 amplitude and startle reflex.
2. Induced fear of moderate intensity abolished the placebo effect in startle.
3. Induced fear of moderate intensity abolished the placebo effect in reported pain.
4. Reduced placebo analgesic responding due to induced fear is strongest in subjects with higher scores on measures of fear.
5. The detrimental effect of fear on placebo analgesia occurs at least partially on a pre-cognitive level of pain processing.

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