Sex differences in pain, fear of pain and placebo analgesia

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A dissertation for the degree of Philosophiae Doctor – July 2018
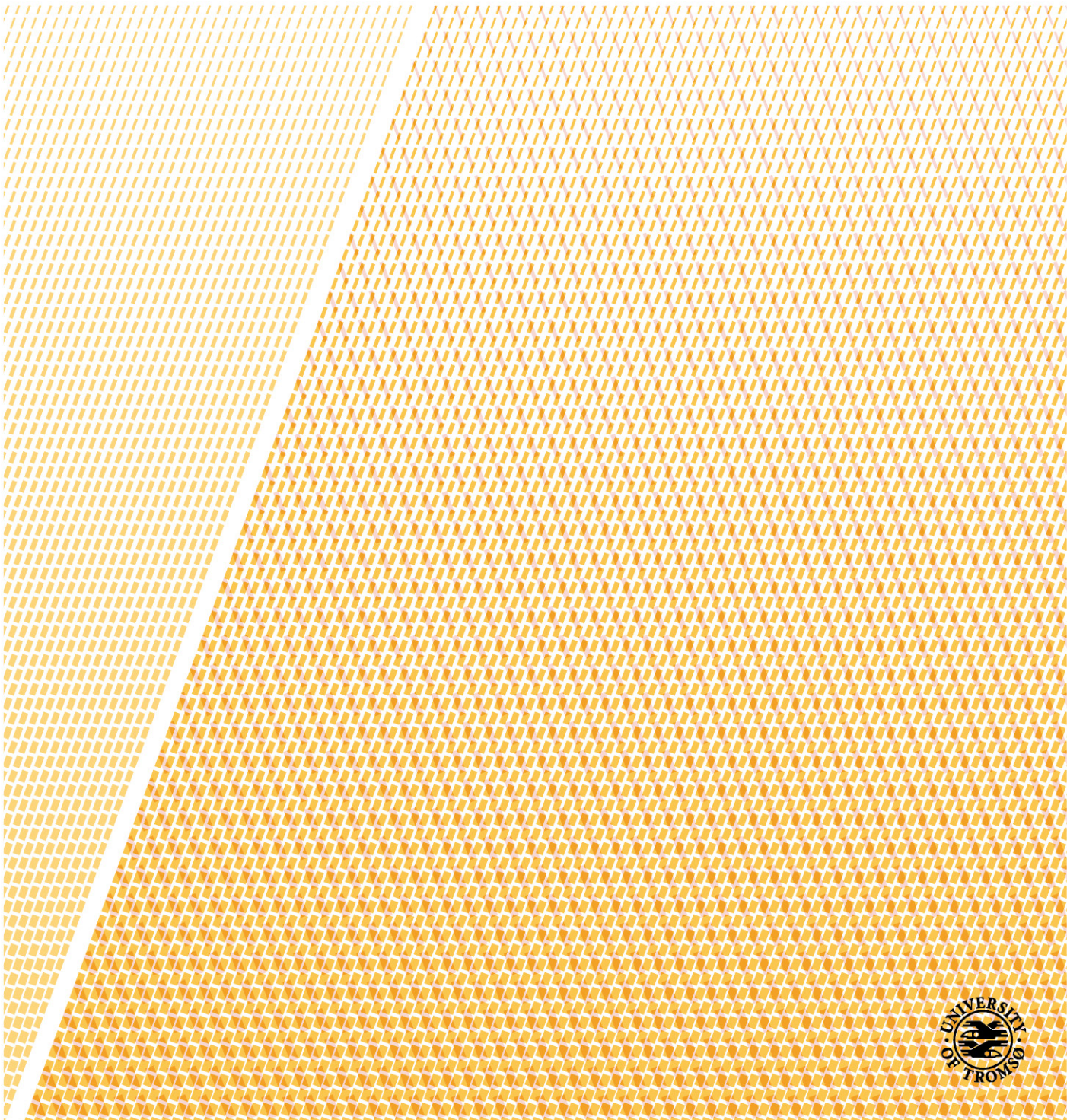
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Acknowledgements

A sincere gratitude goes to my two supervisors, Professor Magne Arve Flaten and Professor Per Matti Aslaksen, who gave me the opportunity to be a part of this research project. I am grateful for their continuous academic guidance and support. It has been a real privilege working with them both.

Besides my supervisors, I would like to thank my talented and dedicated collaborators and co-authors. A special thanks goes to Senior Researcher Laila M. Martinussen and Associate Professor Roald A. Øien who contributed to the second and third paper of the thesis. Laila and Roald, you made this PhD-journey fun and less stressful. Thank you for all the good discussions and also for all the hard laughs. To my former and fellow PhD-students, Assistant Professor Espen Bjørkedal, Assistant Professor June Forsberg and PhD-student Martin Bystad, I am thankful for all interesting and stimulating discussions, frustrating hours in the laboratory, good cooperation, and also for your kindness and sense of humour. I thank Associate Professor Ole Åsli and Peter Solvoll Lyby for their contributions to the third paper and for including me in their research projects. I would also like to thank my colleagues at the Department of Psychology. A sincere gratitude goes to Assistant Professor Kari Galaaen and Professor Catharina Elisabeth Arfwedson Wang, two great role models academically, professionally and personally.

My deepest gratitude goes to my family. Thank you for your love and for always believing in me and supporting me. Thank you mom and dad, for your guidance. Thank you for providing your children with more books than toys. To my sisters Marit and Unn Valborg, I am so proud of all your accomplishments and I really appreciate our friendship and shared interests. I thank my husband Ero, and our children Michelle, Sebastian, Una and Alma Nicoline. I love you! You are my inspiration. I dedicate this thesis to you.

List of papers

Paper I: Aslaksen, P.M., Bystad, M., Vambheim, S.M. & Flaten, M.A. Gender differences in placebo analgesia: event-related potentials and emotional modulation. *Psychosomatic Medicine*, 2011;73(2):193-199.

Paper II: Vambheim, S.M. & Øien, R.A. Sex Differences in Fear of Pain: Item-Level Analysis of the Fear of Pain Questionnaire III. *Journal of Pain Research*, 2017;10:825-831.

Paper III: Vambheim, S.M., Lyby, P.S., Aslaksen, P.M., Flaten, M.A., Åsli, O. & Martinussen, L.M. The Fear of Pain Questionnaire-III and The Fear of Pain Questionnaire-Short Form: a confirmatory factor analysis. *Journal of Pain Research*, 2017;10:1871-1878.

Abstract

The placebo analgesic effect is pain reduction after treatment with an inert substance or procedure, administered with suggestions of pain relief. Previous research has shown that placebo treatment produces larger pain reduction in males compared to females. The hypothesis that males are more responsive to placebo treatment than females was tested experimentally in the first paper of this thesis. The hypothesis was confirmed and the results showed that the sex difference was related to a difference in males' and females' stress response after placebo treatment. Placebo responses on pain unpleasantness and the P2 component was found in males, but not in females.

Pain is a multifaceted phenomenon, consisting of physiological, psychological and sociocultural components. Thus, it is important that clinical and experimental investigations of pain include psychosocial measures, such as measures of stress, anxiety and fear of pain (FOP). One widely used device for measurements of FOP is the Fear of Pain Questionnaire-III (FPQ-III). A more recent device, derived from the FPQ-III, is the Fear of Pain Questionnaire-Short Form (FPQ-SF). The second and third paper investigated sex differences in FOP, fit, reliability, validity and sex neutrality of these two models in a Norwegian sample. It was predicted that FOP would be higher in females than in males and that this would be revealed by sex differences in total FPQ-scores, subscale scores and at item level. Furthermore, it was hypothesized that the more recent model, the FPQ-SF, would be preferred over the FPQ-III. The second paper uncovered higher fear of severe pain in females than in males, probably due to sex differences in psychological processes, such as fear and anxiety, and interpretation of FPQ-III Severe Pain items. The third paper showed that neither the FPQ-III nor the FPQ-SF models had good fit to the Norwegian data, although the FPQ-SF model was better suited than the FPQ-III, both overall and across sex. We therefore suggested

adjustment of the present FOP-instruments. Our findings illustrate the importance of developing culture or country specific FOP models. The logic behind this is that the understanding and perceptions of pain, as well as the responses to pain, may differ across countries and cultures, and across sex. Thus, one model may not apply universally nor be sex neutral.

List of abbreviations

ACC   Anterior cingulate cortex

ACTH   Adrenocorticotropic hormone

AIH   Anxiety-induced hyperalgesia

aINS   Anterior insula

ANOVA   Analysis of variance

CCK   Cholsecystokinin

CFA   Confirmatory factor analysis

CHEPS   contact heat-evoking potential stimulator

CNS   Central nervous system

COMT   Catechol-O-methyltransferase

CR   Conditioned response

CS   Conditional stimulus

dACC   Dorsal anterior cingulate cortex

dlPFC   Dorsolateral prefrontal cortex

EEG   Electroencephalography

EOG   Electroocculography

ERP   Event related potentials

fMRI   Functional magnetic resonance imaging

FOP   Fear of pain

FPQ-III   The fear of pain questionnaire-III

FPQ-NOR   The fear of pain questionnaire-Norway

FPQ-SF   The fear of pain questionnaire-short form

HPA   Hypothalamic-pituitary-adrenal axis

IBS	Irritable bowel syndrome
lOFC	Lateral orbitofrontal cortex
NAc	Nucleus accumbens
NAc-VS	Nucelus accumbens ventral striatum
NRS	Numerical rating scale
ORA	Ordinal regression analysis
PAG	Periaqueductal grey
PASS	The pain anxiety symptom scale
PD	Parkinson's disease
PET	Possitron emission tomography
PFC	Prefrontal cortex
rACC	Rostral anterior cingulate cortex
RVM	Rostroventromedial medulla
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SACL	The short adjective checklist
SIA	Stress-induced analgesia
SIH	Stress-induced hyperalgesia
TSK	The Tampa kinesiophobia scale
US	Unconditional stimulus
vmPFC	Ventromedial prefrontal cortex

1   Introduction

Henry Beecher, a surgeon serving during World War II, was among the first to document the importance of emotional and contextual factors in pain and analgesia (Beecher, 1955). Towards the end of the war, many of the military field hospitals ran out of morphine. Beecher decided to give the wounded soldiers a saline injection before surgery and observed that the soldiers experienced pain relief equal to the analgesic effect of morphine. Beecher hypothesized that psychological factors modulated the pain (Czerniak & Davidson, 2012). Although Beecher's work has been criticized due to methodological and ethical issues (Di Blasi & Klejinen, 2003; Stark, 2016), many consider it the beginning of the systematic investigation of the interplay between physiological and psychological systems in analgesic processes, as well as the use of randomized controlled trials.

Placebo effects are observed when symptoms decrease in a group to which an inert treatment has been administered, compared to a group to which no treatment has been given (Benedetti, 2008). There are two main approaches to investigating and understanding placebo effects: expectancy theory and classical conditioning. According to expectancy theory, inactive interventions cause placebo effects because of the recipients' expectations (Voudouris, Peck, & Coleman, 1990). The second approach, the theory of classical conditioning, suggests that placebo effects are explained by nonconscious learning or conscious expectancies after pairings of conditional and unconditional stimuli (Price, Finniss, & Benedetti, 2008). In the medical setting, a syringe or a pill represents the conditioned stimulus (CS). The active ingredient in the syringe or pill represents the unconditioned stimulus (US). Repeated pairings of the US and the CS lead to a conditioned response (CR). At this stage, the CS is capable of eliciting a physiological response similar to the responses

produced by the US. These mechanisms have been elegantly illustrated in studies on placebo effects in the immune and endocrine systems (Ader & Cohen, 1982; Goebel et al. 2002).

Expectancy theory holds that conditioning may produce placebo effects, but that it is the expectancies that elicit placebo responses. The finding that conditioned placebo responses are mediated by expectancies has been demonstrated repeatedly (Montgomery & Kirsch, 1997). Thus, as expectancies are crucial elements in the formation of placebo responses in most situations, expectancy theory and the theory of classical conditioning are not mutually exclusive approaches.

The power of expectancies in placebo effects is demonstrated through the open versus hidden design. For example, Benedetti et al. (2003a) found that the effect of morphine was reduced by 50% and the effect of diazepam was completely removed when patients were unaware that the drugs were administered, compared to patients who were informed that they received morphine or diazepam. Similar findings have been reported for other types of pain killers (Amanzio, Pollo, Maggi, & Benedetti, 2001; Colloca, Lanotte, & Benedetti, 2004) and treatment for Parkinson's Disease (Lanotte et al., 2005). Thus, therapeutic benefit is, to a great extent, dependent upon patient expectancies, even when pharmacological treatment is administered.

Several studies have reported sex differences in the placebo analgesic effect (Aslaksen, Bystad, Vambheim, & Flaten, 2011; Aslaksen & Flaten, 2008; Bjørkedal & Flaten, 2011; Butcher & Carmody, 2012; Colloca, Pine, Ernst, Miller, & Grillon, 2016; Flaten, Aslaksen, Finset, Simonsen, & Johansen, 2006; Krummenacher, Kossowsky, Schwarz, Bugger, Kelley, et al., 2014; Theysohn et al., 2014). The majority of those studies report larger placebo analgesic responses in males than in females. Sex differences in the prevalence

of pain conditions and pain symptoms have also been reported, with higher prevalence in females than in males for most types of conditions and symptoms (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009; Forgays, Rzewnicki, Ober, & Forgays, 1993; Rollman, & Lautenbacher, 2001; Yunus, 2002). Moreover, sex differences in experimentally induced pain have been demonstrated repeatedly (Garcia, Godoy-Izquierdo, Godoy, Perez, & Lopez-Chicheri, 2007; Riley, Robinson, Wise, Myers, & Fillingim, 1998).

Placebo analgesic responses vary across individuals. Some individuals experience no analgesic effect from placebo treatment, while others experience complete pain relief. The investigation of individual differences in placebo responding has mostly focused on psychosocial and genetic variables and on psychological and personality traits. Fear of pain has been found to contribute to individual differences in placebo analgesia. Individuals with high fear of pain display reduced placebo analgesia compared to individuals with low fear of pain (Lyby, Aslaksen, & Flaten, 2010).

This thesis contributes to unraveling the relationships between sex and placebo responding and between sex and fear of pain. Furthermore, the reliability, validity and sex neutrality of two models measuring fear of pain is examined. The first paper in the thesis investigates whether males are more responsive to placebo analgesic treatment than females. The second and third papers investigate whether there are sex differences in fear of pain and examines the applicability of the Fear of Pain Questionnaire-III and the Fear of Pain Questionnaire-Short Form.

2   Background

2.1   Pain

Pain is a multifaceted phenomenon, involving multiple neuroanatomical and neurochemical systems. The International Association for the Study of Pain has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey & Bogduk, 1994).

Pain may be classified into nociceptive pain, neuropathic pain and somatoform pain. Nociceptive pain arises from tissue damage or potential tissue damage, whereas neuropathic pain results from damage to or disease in the somatosensory system (Troels et al., 2011). Somatoform pain is characterized by chronic, severe and preoccupying pain that is not fully explained medically (Landa, Peterson, & Fallon, 2012). Moreover, pain elicited from the skin and deeper tissues is termed somatic pain, while pain elicited from the internal organs is termed visceral pain (Fink, 2000). Nociception refers to the neural process whereby painful stimuli are encoded and processed. The nociceptors are specialized sensory receptors that selectively detect painful stimuli. Nociceptors can be found in body areas sensing painful stimuli but are absent in the brain with exception of the meninges. The nociceptors transform the noxious stimuli into electrical signals, which are transmitted to the central nervous system (CNS). The nociceptors are activated by mechanical, thermal or chemical stimuli, and signals tissue damage or potential tissue damage to the brain through Aδ and C fibers (Dubin & Patapoutian, 2010). The Aδ fibers are highly myelinated and thus provide rapid signal conduction. The C fibers are unmyelinated and provide slow signal conduction. The nociceptors can be referred to as the afferent nerve fibers because they transmit signals into the dorsal horn of the spinal cord to the second-order neurons. The second-order neurons are nociceptive-specific neurons in the dorsal horns' Rexed laminae I and II. Thereafter, second-

order neurons transmit the pain signal from the dorsal horn of the spinal cord, through the spinothalamic tract to the thalamus, or through the spinoreticular tract to the thalamus via the brainstem reticular formation. The spinothalamic and spinoreticular tracts represent the two main pathways for transmission of nociceptive information (Steeds, 2009). The spinothalamic tract is involved in transmission of nociceptive information about the localization of pain, whereas the spinoreticular tract is involved in the emotional aspects of pain (Nógrádi, 2006). From the thalamus, third-order neurons relay signals to different cortical and subcortical structures. These involve the amygdala, hypothalamus, periaqueductal gray (PAG), basal ganglia, insula, cingulate cortex and the cerebral cortex. The primary somatosensory cortex (S1) is the part of the cortex first activated by the pain signal. The secondary somatosensory cortex (S2), located caudal to the primary somatosensory cortex, is the part of the cortex that is activated second. Activation of the S1 and S2 is related to the sensation of pain. That is, the location and the intensity of the pain. Activation of the cingulate cortex and the insula is related to the affective and motivational aspects of pain, and it is argued that this activation reflects the unpleasantness of the pain.
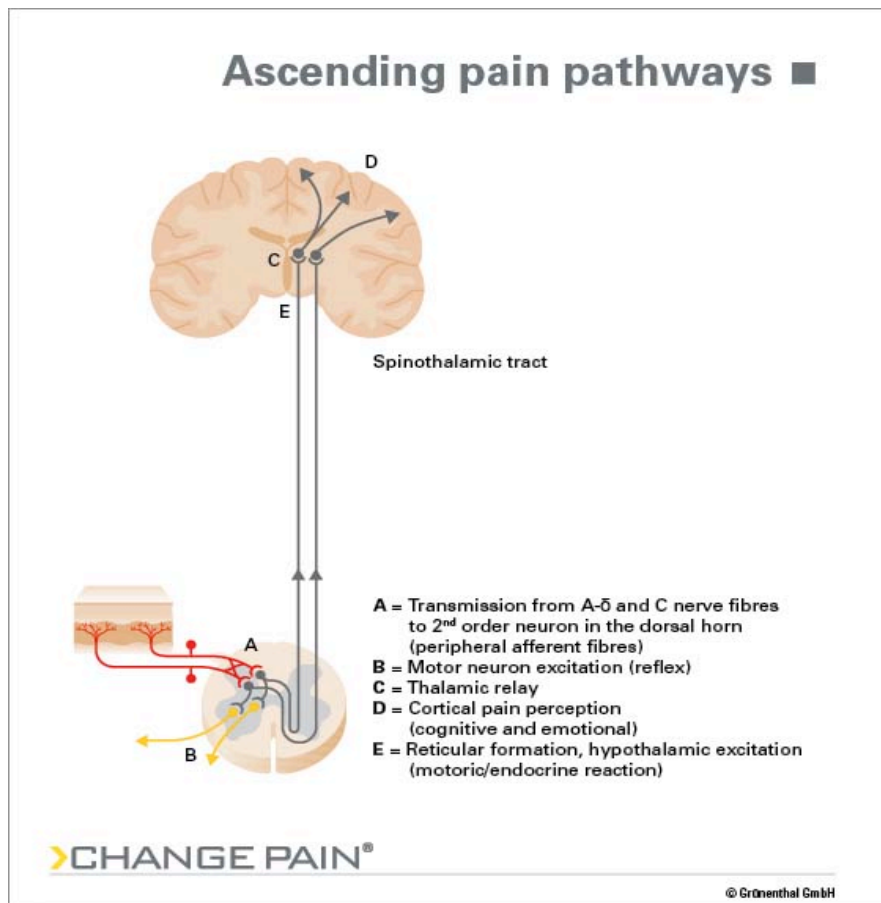
Figure 1. The ascending pain pathways [online image]. Retrieved and borrowed with permission January 03, 2018 from http://www.change-pain.com/grt-change-pain-portal/change_pain_home/chronic_pain/physician/physician_tools/picture_library/en_EN/31 2500026.jsp

2.1.1 Pain modulation

Pain modulation refers to the physiological facilitation or inhibition of nociceptive information (Kirkpatrick et al., 2015). Brain stem modulatory systems are central to the facilitation of pain (Gebhart, 2004). Descending pain modulatory neurons in the rostroventromedial medulla (RVM) facilitates or inhibits pain signals through several

different processes (Ossipov, Dussor, & Porreca, 2010). These systems can provide bidirectional control of pain, influenced by higher-order functions such as fear, stress and expectations (Price, 2015). The most important structures involved in descending inhibitory pain modulation are the PAG and the rostroventromedial medulla (RVM). Opioid cells and opioid receptors are found in the pain modulatory circuit. Neurons in the PAG project to the medulla and serotonergic cells of the raphe nuclei. Next, the serotonergic neurons project downwards to the dorsal horn of the spinal cord and provide inhibition. The PAG receives signals from cortical sites and has reciprocal connections with the amygdala, dorsal horn, parabrachial nuclei and RVM. Through these connections, the PAG initiates descending and ascending inhibition of pain signals (Ossipov, Morimura, & Porreca, 2014).

Descending pain pathways ◼

BRAIN

SPINAL CORD

1 – Cortical/subcortical impulses
2 – Impulses to the periaquaeductal matter
3 – Locus coeruleus (noradrenergic inhibitory system)
4 – Raphé nucleus (serotonergic inhibitory system)

BRAIN

5 – Inhibitory synapses in the dorsal horn
6 – Ascending spinothalamic tract
7 – Motor neuron reflex

SPINAL CORD

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Figure 2 The descending pain pathways [online image]. Retrieved and borrowed with permission January 03, 2018 from http://www.change-pain.com/grt-change-pain-portal/change_pain_home/chronic_pain/physician/physician_tools/picture_library/en_EN/31 2500026

Cognition and attention influence nociceptive processing (Tracey, 2010). Negative expectations can reduce the pain-relieving effect of analgesic medications (Bingel et al., 2011), whereas positive expectations may boost the placebo the analgesic effect (Colloca, Klinger, Flor, & Bingel, 2013). These aspects of pain need to be understood and taken into

consideration when investigating pain and pain processing. Brain imaging studies investigating the impact cognition and attention has on pain processing, have later documented that several subcortical regions are activated during pain anticipation (Shackman et al., 2011; Vogt, 2005). The structures that feed back to influence pain due to cognitive involvement are the insula, S1, S2, PAG, anterior cingulate cortex (ACC) and the prefrontal cortex (PFC) (Villemure & Bushnell, 2002). Distraction from pain has been found to reduce activation in pain-responsive areas such as the S1, S2, thalamus and insula (Tracey & Mantyh, 2007), and increase activation of the PFC, ACC and PAG (Wiech, Ploner, & Tracey, 2008). Pain responses mediated by expectations of pain relief is associated with activation of the dorsolateral, orbitofrontal and medial prefrontal cortex (Ploghaus et al., 2006; Rainville & Duncan, 2006). These structures selectively activate the PAG and the RVM, which sends inhibitory projections to the spine (Goffaux, Redmond, Rainville, & Marchand, 2007). Expectations of increased pain blocks this type of analgesia. Thus, endogenous pain modulatory systems are central for expectancy-based inhibition and excitation of nociceptive signals.

2.1.2   Emotional modulation of pain

Emotions influence pain, and pain influence emotions. While positive emotions are associated with pain inhibition, negative emotions are associated with pain excitation (Rainville, Bao, & Chrètien, 2005). Furthermore, the level of arousal is important in emotional pain modulation (Rhudy, Bartley, & Williams, 2010). Rhudy and colleagues (2010) showed that positive emotions induced by pictures inhibit pain, while negative emotions increase pain. Additionally, the associations between emotions and pain was dependent upon arousal levels. It was found that increased arousal was necessary for emotional pain modulation to occur.

Fear and anxiety influence pain perception. However, while anxiety is almost exclusively associated with increased pain, fear may elicit either analgesia or hyperalgesia (Lumley et al., 2011). Fear may be understood as an alarm reaction towards a present threat, often accompanied by a need to fight or flight the encounter, and intense negative emotions and sympathetic arousal. Anxiety is a future focused threat or worry, often accompanied by a need to withdraw, intense negative emotions, hypervigilance and symptoms of somatic tension. Furthermore, there is an important distinction between state and trait anxiety (Spielberger, 1966). State anxiety refers to the unpleasant emotional arousal experienced in threatening or dangerous situations. Trait anxiety refers to the stable individual tendency to respond with anxiety when a situation is anticipated as threatening. Fear-elicited analgesic responses may be due to activation of the endogenous opioid system (Rhudy & Meagher, 2000). Furthermore, repeated exposure to fear-eliciting stimuli may result in anticipatory anxiety, and hence hyperalgesic responding.

Negative emotions may increase pain and enhance activation in the amygdala, ACC and anterior insula, structures involved in processing of pain unpleasantness and motivation to escape pain. In the presence of pain, negative emotions may increase activation in brain structures involved in both affective and pain processing. Contrary to this, positive emotions decrease pain and pain related activation in the amygdala (Lumley et al., 2011). Thus, pain is a biopsychosocial process that involves both sensory and affective components observable through brain imaging.

The finding that emotions modulate pain has repeatedly been demonstrated in clinical and experimental studies (Godinho, Magnin, Perchet & Garcia-Larrea, 2006; Rhudy & Meagher, 2001), and in studies on the placebo analgesic effect (Eippert, Finsterbusch, Bingel, & Büchel, 2009; Flaten et al., 2006). Therefore, understanding placebo analgesia is important,

both from a clinical and scientific point of view. Over the past twenty years, the understanding of the placebo effect's influence on symptoms and disease has changed from a nuisance factor to a psychobiological phenomenon capable of improving several different types of treatment outcomes.

2.2 Placebo analgesia

The placebo analgesic effect occurs when inert treatments administered together with information that the treatment will reduce pain, elicit pain relief (Wager et al., 2004). Hence, positive expectations of treatment effects may modulate pain. Moreover, verbally induced expectations of pain relief can be paired with reduced pain intensity in a conditioning trial, a procedure termed conditioning. An accompanying effect of expectancy manipulation and reduced pain experience is stronger placebo analgesia (Colloca et al., 2013). Furthermore, it has been shown that social observational learning may induce expectations of pain relief, and thereby placebo analgesic responses (Colloca & Benedetti, 2009). Thus, the principal mechanisms underlying placebo analgesia may be divided into two different models a) expectancy theory, and b) conditioning theory. There has been an extended debate about these two models throughout the years of placebo research. Today, most researchers agree that, rather than being mutually exclusive, these are two compatible models often operating in concert (Stewart-Williams & Podd, 2004).

To ensure that the true effect is identified, clinical trials or experimental investigations must include an untreated control group. When the response observed in the placebo arm of clinical trials is calculated as the placebo effect, the natural course of the disease, the regression to the mean, spontaneous remission and potential effects of parallel interventions are discounted (Rutherford & Roose, 2013). Thus, calculation of true placebo effects requires inclusion of a control group where neither active treatment nor inert treatment is administered.

2.2.1   The principal mechanisms of placebo analgesia: expectancy and learning

Expectancy-based placebo analgesic effects occur when an expectation is established before an ineffective treatment is administered, followed by reduced pain experience. Experimentally, expectations are typically induced by administering pain stimuli in a pretest, followed by a phase where an inactive substance, told to be an effective pain reliever, is administered. Then, the similar procedure as the pretest is conducted in a posttest. The placebo analgesic effect can then be calculated by subtracting the pain scores reported in the posttest from the scores reported in the pretest.

The effect of expectancies may be measured by looking at the correlation between ratings of expectations and the placebo analgesic response. A high correlation between these two measures shows the contribution of expectancies in the placebo analgesic effect (Price et al., 1999). This type of correlation has proven to be robust and replicable (Petrovic et al., 2005).

Price and colleagues (1999) administered a placebo cream together with three different verbal instructions (strong analgesic cream, weak analgesic cream, control cream). The verbal information induced different levels of expectancies, with subsequent different levels of placebo analgesia. These findings show that strong expectations produce stronger placebo analgesic responses than weak expectations.

The open versus hidden design represents another elegant method for investigating the role expectancies has on the placebo analgesic effect. The open versus hidden design involves comparing the effect of analgesic medications administered covertly and openly. Studies employing this design shows that analgesic drugs are far less potent when they are administered hidden compared to when they are administered openly (Amanzio, Pollo,

Maggi, & Benedetti, 2001; Levine & Gordon, 1984; Levine, Gordon, Smith, & Fields, 1981).

Bingel and colleagues (2011) administered pain stimuli and measured the effect of remifentanil first covertly and second, overtly. Then, they informed the participants that the remifentanil administration had stopped, although in fact, they were still receiving the analgesic medication. Covert remifentanil administration reduced average pain ratings from 66 to 55. Overt remifentanil reduced average pain to 39. Leading the participants to believe that the remifentanil administration had stopped increased average pain ratings to 64. Results from functional magnetic resonance (fMRI) scans corresponded to the findings on reported pain. These findings illustrate the importance of expectations in pain and analgesic responses.

The understanding of how learning contributes to placebo effects has mostly been investigated with respect to classical conditioning. When an organism is exposed to repeated pairings of a US and a CS, conditioning occurs. After sufficient pairings, the CS elicits a response similar to the response produced by the UR. This type of response is termed a CR. In the clinical setting, drugs are paired with contextual factors, such as the doctor's white coat or the smell at the hospital. After repeated pairing the CS acquire the capacity of eliciting a response mimicking the response produced by the US (Wickramasekera, 1980). In the case of the placebo effect, the placebo pill, capsule or whatever inert substance is used, represents the CS. The placebo effect represents the CR. One way of inducing the placebo analgesic effect through classical conditioning is to apply a placebo cream to the skin followed by surreptitiously lowering the strength of painful stimuli. In the experimental setting, pain stimuli are typically administered first in a pretest. Second, the placebo cream is administered together with information that the cream contains active analgesic ingredients. Third, the participants unknowingly receive pain stimuli of lower intensity than in the pretest. Lastly, again without informing the participants, the pain stimuli are increased to the same intensity

level as in the pretest and administered to the participants. The placebo response can then be measured in postconditioning trials (Klinger, Soost, Flor, & Worm, 2007). In these types of circumstances, a placebo effect may occur even without expectations (Benedetti et al., 1998; Benedetti, Amanzio, Baldi, Casadio, & Maggi, 1999).

Expectations may operate simultaneously with different forms of learning (Price, et al., 1999; Dodd, Dean, Vian, & Berk, 2017). Presenting both verbal information about treatment effects and conditioning procedures leads to amplification of placebo responses compared to the situation when only verbal information or conditioning is presented (Amanzio & Benedetti, 1999; Benedetti et al., 2003b; Colloca, Sigaudo, & Benedetti, 2008a; Montgomery et al., 1997; Price et al., 1999). Price and colleagues (1999) paired placebo treatment with either a large or a small decrease in pain stimuli during the conditioning trials. The results showed that the magnitude of the placebo effect was associated with the type of conditioning (large versus small decrease in pain stimuli). The strong type of conditioning probably increased the participants' expectations more than the weak type of conditioning did. Hence, the largest placebo analgesic response was observed in the group that received the largest decrease in pain stimuli during conditioning.

Several researchers have investigated the relative contributions of expectancy and conditioning to the placebo effect. Montgomery and Kirsch (1997) employed a conditioning procedure and observed a placebo analgesic response. Then, they continued the conditioning trials, but informed one of the groups that the pain stimuli had been reduced during the conditioning trials. This information abolished the conditioned placebo analgesic response. Thus, the placebo analgesic effect may be due to expectancies, conditioning, or both, but in situations where expectations and conditioning mechanisms are in conflict, expectancies tend to overrule conditioning.

2.2.2 The neuroscience of placebo analgesia

Placebo analgesic effects involve multiple brain systems, the autonomic nervous system and the endocrine system. The first study to investigate the biological mechanisms of the placebo analgesic effect was conducted by Levine, Gordon and Fields (1978). In that study, it was reported that the μ-opioid antagonist naloxone increased pain and reduced the placebo effect. Thus, suggesting that placebo effects are mediated by the endogenous opioid system. The finding that naloxone may reverse, or even abolish, the placebo analgesic effect has later been confirmed (Grevert, Albert, & Goldstein, 1983; Levine et al., 1984). Through several experiments conducted during the 1990s, Benedetti and coworkers (1996) contributed to further elucidation of the relation between naloxone, endogenous opioids and the placebo analgesic effect. In addition to replicating previous findings showing that the placebo response could be reversed or partly reversed by naloxone, Benedetti (1996) illustrated that the cholecystokinin (CCK) antagonist proglumide increased placebo analgesia. CCK has an inhibitory effect on exogenous opioid analgesics and endogenous opioid pain inhibition and is therefore considered an antagonist of the opioid system. Thus, suggesting that reversal or blockade of the CCK system increases the placebo analgesic response, possibly through potentiating the endogenous opioid system. fMRI-studies by Eippert et al. (2009a; 2009b) have demonstrated that naloxone could reverse both placebo responses measured verbally and placebo-induced activity in the CNS at the level of the spinal cord's dorsal horn. Together, these findings illustrate that placebo responses can be mediated by different neurotransmitter systems with opposing influence: the pronociceptive CCK system, which has an inhibitory effect on placebo responses, and the antinociceptive opioid systems, which have an excitatory effect on placebo responses.

Evidence suggests that expectations may activate opioid systems and that conditioning activates subsystems (Amanzio et al., 1999). The type of subsystem activated is dependent upon the type of drug, e.g., use of opioids results in conditioning of opioid receptors. Furthermore, placebo responses induced by strong expectancies may be blocked by naloxone. The same effects have been found after preconditioning with morphine, a procedure that involves repeated administration of morphine before replacing the drug with a placebo (Benedetti, 2014). However, placebo responses preconditioned with non-opioid substances are insensitive to naloxone. Benedetti and colleagues showed that the CB1 cannabinoid receptor antagonist rimonabant had no effect on placebo analgesic responses induced through preconditioning with morphine but abolished placebo analgesia preconditioned with the NSAID ketorolac (Benedetti, Amanzio, Rosato, & Blanchard, 2011). The finding that the CB1 cannabinoid receptors can abolish placebo analgesia when an NSAID has been used as the US, suggests involvement of endocannabinoid pathways. However, the knowledge about placebo responses mediated by the endocannabinoid system is limited compared to placebo responses activated through the opioid systems.

An important role of the reward circuitry in the placebo effect has also been suggested (Scott et al., 2008). This line of research was first implicated in placebo effects on Parkinsonian patients, but it was later confirmed that reward mechanisms have an important role in placebo analgesia as well (de La Fuente Fernàndez, 2009; Scott et al., 2007). In Parkinson's disease (PD), dopamine release in the dorsal striatum is reported to be the core mechanism of the placebo effect. Placebo treatment increases synaptic dopamine levels in a similar manner as levodopa, a treatment regularly used to control the motor symptoms associated with the disease. Increased dopamine activation in the NAc and VTA is associated with the placebo analgesic response. It has been argued that endogenous opioid release

mediates the placebo analgesic effect, but that dopamine release in the ventral striatum is an important determining factor for the placebo analgesic effect to occur. It has been suggested that dopamine release in the ventral striatum triggers endogenous opioid release (Scott et al., 2007). Hence, reward mechanisms may have a potentiating role in expectancy-based placebo analgesic effects. These observations support the placebo-reward hypothesis, which states that there is a link between placebo effects and reward mechanisms and predicts that the ventral striatum should be involved in any type of placebo effect.

The above-described findings shows that expectations are important mediators of both opioid and non-opioid systems and that the strength of expectations is important for naloxone's ability to block placebo analgesia. Furthermore, placebo analgesic effects induced through preconditioning with non-opioid drugs are opioid independent and cannot be blocked by naloxone but may be blocked by cannabinoid antagonists. Thus, placebo analgesia may be induced through different pathways and systems.

Neuroimaging techniques, such as positron emission tomography (PET), fMRI and electroencephalography (EEG), have provided further evidence for the neurobiology of the placebo analgesic effect. The main aim of imaging studies on placebo analgesia is to identify the neurobiological systems involved in placebo responses. Several studies have shown that placebo analgesia is associated with top-down activation through the descending pain modulatory pathway (Bingel & Tracey, 2008). Placebo analgesia is associated with altered activity in several brain structures involved in pain processing. The structures that most consistently show reduced activation during placebo analgesia are the dorsal ACC (dACC), thalamus and anterior insula (aINS). The magnitude of the placebo analgesic effect has been found to consistently correlate with reduced pain-related activity in these three structures (Wager & Atlas, 2015). Moreover, reduced activity in the dorsal horn during pain stimulation

after placebo treatment suggests that pain is inhibited at the spinal level under placebo analgesia (Eippert et al., 2009b).

In addition to reduced activity in the abovementioned regions of the brain, placebo analgesic responding is associated with increased activity in the ventromedial prefrontal cortex (vmPFC), dorsolateral prefrontal cortex (dlPFC), lateral orbitofrontal cortex (lOFC), nucleus accumbens-ventral striatum (NAc-VS), PAG and the RVM (Geuter, Koban & Wager, 2017; Wager & Atlas, 2015). Activation in the dlPFC is central to the initiation of placebo analgesia. The rACC and PAG connectivity correlates with reduced somatosensory pain and pain report.

It has also been demonstrated that placebo treatment is related to activation and functional connectivity of the PFC, nucleus accumbens (NAc) and amygdala (Petrovic, Kalso & Petersson, 2002). In the experience of pain, placebo treatment increases endogenous opioid activity and reduces fMRI-measured activation of the amygdala. If naloxone is administered, this effect is abolished, further evidencing that placebo analgesic responses involve activation of the endogenous opioid system through top-down control.

Atlas and Wager (2014) conducted a meta-analysis to map the brain regions that are most reliably involved in placebo analgesia. The results highlighted that placebo effects were accompanied by reduced activation in the dACC, thalamus, insula, amygdala and striatum. The former three are regions associated with pain processing, while the latter two relates to emotional and cognitive processing. In addition, expectancies of pain relief were associated with increased activity in the prefrontal cortex, the PAG and the rostral ACC (rACC). Thus, the most reliable brain regions involved in placebo analgesic processing are regions associated with processing of pain, anxiety, fear, stress and cognition. This set of regions

represents a challenge for neuroscientific research on placebo analgesia, as it implies that several of the pain-responsive brain regions involved in placebo effects are also engaged in cognitive and affective processing of tasks that may be unrelated to pain.

2.2.3   Placebo analgesia and emotions

The formation and magnitude of placebo responses are influenced by emotions (Flaten et al., 2011). These include, among others, stress, fear and anxiety. Placebo analgesic responding is associated with reduced reported stress and reduced physiological responses to pain stimulation (Aslaksen et al., 2011; Aslaksen et al., 2008). Fear of pain is negatively associated with placebo analgesic responding and positively associated with stress (Lyby et al., 2010; Lyby, Aslaksen, & Flaten, 2011). Furthermore, an inverse relation between placebo analgesia and anxiety has been suggested (Staats, Staats, & Hekmat, 2001). Thus, negative emotions may counteract placebo analgesic responding.

Experimental studies on the placebo analgesic effect involve infliction of pain. Expecting or experiencing pain may induce stress and negative emotions and may be measured both physiologically, e.g., as increased blood pressure, pulse, and changed heart rate variability, and psychologically, e.g., as increased reported unpleasantness, stress, fear or anxiety. Placebo treatments induce expectations of symptom relief and hence reduce negative emotions, with a subsequent reduction in pain (Vase, Robinson, Verne, & Price, 2005).

Emotions may be measured according to their valence and arousal (Lang, Bradley, & Cuthbert, 1990). Valence reflects the quality of the emotion (positive or negative), whereas arousal reflects the strength of the emotion (emotional intensity). Most studies on the role of emotional valence in placebo analgesia suggest that positive and negative emotions have a bidirectional relation to the placebo analgesic effect. Positive emotions tend to increase

placebo analgesia, whereas negative emotions reduce placebo analgesia (Lyby et al., 2010; Lyby et al., 2011). However, the relationship between emotional arousal and analgesia has proven to be quite complex. High levels of positive emotions are associated with increased levels of pain relief compared to lower levels of positive emotions (Rhudy et al., 2001). Moreover, intense stress may induce analgesic responding, as observed in studies on stress-induced analgesia (SIA) (Rhudy et al., 2000). However, intense stress levels may also amplify pain, as seen in studies on stress-induced hyperalgesia (SIH) (Martenson, Cetas, & Heinricher, 2009).

2.2.4 Individual differences in placebo responding

The placebo response varies between individuals. While some experience complete pain relief from placebo treatment, others may experience no effect. Individual differences in psychosocial factors and psychological traits account for some of these variations (Colloca et al., 2013). Dispositional optimism (Geers, Kosbab, Helfer, Wiland, & Wellman, 2007; Geers, Wellman, Fowler, Helfer, & France, 2010), somatic focus (Johnston, Atlas, & Wager, 2012), empathy (Hunter, Siess, & Colloca, 2014; Rütgen, Seidel, Riecanský, & Lamm 2015), fear of pain (Lyby et al., 2010; Zubieta, Yau, Scott, & Stohler, 2006) and anxiety (Ober et al., 2012) represent examples of factors of such relevance. Optimism, somatic focus, empathy and concern for others are positively associated with increased placebo analgesic responding, whereas anxiety, fear of pain and pain catastrophizing are negatively associated with placebo analgesic responding (Corsi & Colloca, 2017). Geers and colleagues (2007) showed that high levels of dispositional optimism were associated with an increased placebo effect on sleep quality, while low levels of dispositional optimism were associated with an increased nocebo effect on sleep quality. Agreeableness and resilience, personality traits associated with optimism and coping with stress and adversity, have been highlighted as important

personality traits for endogenous opioid elicitation after placebo treatment (Peciña et al., 2015).

More recently, it has been reported that the placebo effect has a genetic signature. The placebome refers to a sample of genome-related molecules (genes, proteins, microRNAs) that influence placebo responsiveness (Hall, Loscalzo, & Kaptchuk, 2015). Some have reported that genetic variability mediates the underlying mechanisms of the placebo effect through influencing endorphin, cannabinoid, dopamine and opioid pathways important for placebo responsiveness (Colagiuri, Schenk, Kessler, Dorsey, & Colloca, 2015; Litten et al., 2013). For example, genetic variations in the catechol-O-methyltransferase (COMT) genotype are capable of regulating dopamine levels in the brain and is related to pain perception and feelings of pleasure, and thus also placebo responsiveness (Hall et al., 2012). Hall and colleagues reported that in patients diagnosed with irritable bowel syndrome (IBS), Met-allele carriers seemed to be more prone to placebo treatments than the Val-Val-allele carriers were. Another study showed that postoperative patients with mutations of the COMT gene self-administered lower levels of morphine than others did (De Gregori et al., 2013). However, a later study by Forsberg and colleagues (2018) reported that genetic variability in COMT did not influence placebo analgesic responsiveness in a sample of healthy participants.

Others have suggested potential role of the OPRM1 A118G polymorphism in placebo responding (Peciña, Love, Stohler, Goldman, & Zubieta., 2014). The OPRM1 A118G polymorphism consists of two variants: OPRM1 G carriers and OPRM1 AA carriers. OPRM1 G carriers have fewer μ-opioid receptors than the AA carriers (Kroslak et al., 2007). Thus, placebo responses are less associated with endogenous opioid release in the G carriers than the AA carriers.

The literature about individual differences in placebo responding remains inconsistent. This inconsistency may be explained by small sample sizes in many of the studies where individual differences are reported. Additionally, the number of different symptoms investigated and the use of a wide variety of different experimental designs, procedures and scientific approaches complicate the research on how individual differences are related to placebo effects. To better understand the relationship between individual differences and placebo effects, Horing and colleagues suggested a list of variables to include in forthcoming placebo studies, including goal-seeking, self-efficacy, self-esteem, locus of control, optimism, desire for control, restraint, fun, sensation, neuroticism, participant sex, Val158Met polymorphism, suggestibility, belief in expectation biases, body consciousness and baseline symptom severity (Horing, Weimer, Muth, & Enck, 2014). Defining individual markers for responsivity to placebo treatments is important in the work of designing future studies, as well as for tailoring and personalizing treatments.

2.3   Nocebo hyperalgesia

The nocebo hyperalgesic effect is increased pain elicited by verbal suggestions, conditioning and/or social observational learning (Blasini, Corsi, Klinger, & Colloca, 2017). Thus, pain can be increased by several types of interventions. Interventions directed at influencing expectations, without administration of inert substances, have been important for the understanding of placebo analgesia and nocebo hyperalgesia. Lorenz and colleagues manipulated subjects' expectancies towards pain induced by brief infrared laser stimuli (Lorenz et al., 2005). They used EEG with source localization and showed that the electrical dipole in the S2 attenuated when the participants expected decreased pain and amplified when they expected increased pain. The dipole strength reflects the duration of the dipole. When participants were led to believe that they received a stimulus of high intensity but in fact

received a stimulus of low intensity, the dipole length decreased compared to when they believed they received a stimulus of high intensity. Additionally, when the participants believed they received a stimulus of low intensity but in fact received a stimulus of high intensity, the dipole strength increased compared to when they believed they received a stimulus of low intensity. The results further revealed that when the participants expected to receive pain stimuli of low intensity but actually received stimuli of high intensity, they reported lower levels of pain than when they expected to receive stimuli of high intensity. Additionally, when they expected to receive stimuli of high intensity but in fact received stimuli of low intensity, they reported higher pain than when they believed they received stimuli of low intensity. Although Lorenz et al. did not administer any inert treatment and only manipulated expectancies, the findings are important for understanding the opposing effects of expectancies on pain perception.

As with placebo analgesia, expectancy-based nocebo hyperalgesia produces effects that are measurable at the physiological level. When nocebo treatment induces anticipatory anxiety, the opioid agonist CCK is elicited and this facilitates pain signaling (Frisaldi, Piedimonte, & Benedetti, 2015). Nocebo treatment may produce increased pain and hyperactivity in the hypothalamic-pituitary-adrenal (HPA) axis, measured by increased levels of adrenocorticotropic hormone (ACTH) and cortisol (Benedetti, Amanzio, Vighetti, & Asteggiano, 2006). Administration of the anxiolytic benzodiazepine diazepam blocks this effect, and this suggests that anxiety plays a specifically important role in nocebo hyperalgesic responses. However, administration of proglumide, a CCK receptor antagonist, is capable of abolishing nocebo hyperalgesic responding completely, without influencing neither ACTH nor cortisol. These findings suggest that CCK mediates nocebo hyperalgesia through emotional modulation of endogenous CCK and opioids.

Nocebo hyperalgesia and placebo analgesia are often described as opposite processes causing opposite effects on subjective pain perception. The findings that the opioidergic and CCKergic systems are activated by verbal suggestions of pain relief and pain increase, respectively, illustrates that the opposing treatment expectations is also reflected neurophysiologically. Interestingly, studies on anxiety-induced analgesia (AIH) and SIA have demonstrated that attentional focus is important for activation of the CCKergic and opioidergic systems (Benedetti, Carlino, & Pollo, 2011). Both AIH and SIA involve increased levels of anxiety. However, during AIH, the subjects direct their attention towards the pain, whereas the attentional focus in SIA is at a contextual stressor. These AIH and SIA responses are due to activation of the CCKergic and endogenous opioid systems, respectively.

During SIA, nociceptive responses are reduced as a consequence of stress and fear exposure (Yilmaz et al., 2010). Hypoalgesic effects due to stress exposure are mediated by the descending inhibitory pain pathway and activation of several receptor subtypes (Butler & Finn, 2009) The descending inhibitory pain pathway includes the cortex, hippocampus, amygdala, PAG, hypothalamus, brainstem and the spinal cord. GABA, glycine, vasopressin, oxytocin, adenosine, endogenous opioids and endocannabinoids constitute the receptor subtypes involved in the inhibition of nociceptive information (Butler et al., 2009). In AIH, nociceptive responses are exaggerated due to stress coupled with anxiety, with subsequently increased activation of the CCKergic systems (Benedetti et al., 2011; Colloca & Benedetti, 2007).

Generally, the majority of studies investigating differences between the placebo and the nocebo effect suggests that nocebo hyperalgesic treatments produce increased activity in the pain-responsive regions of the brain and reduced activity in the opioid-sensitive brain regions (Colloca & Finniss, 2012; Jensen et al., 2014). Nocebo treatment increases

experienced pain, reduces pain threshold, and increases spinal pain signals measured at the ipsilateral dorsal horn of the spinal cord (Geuter & Büchel, 2013). These findings suggest that nocebo hyperalgesia induced through verbal suggestions may amplify pain signals before they reach cortical levels. Conditioned nocebo hyperalgesic treatments may activate neural pathways, as measured by increased activation of the thalamus, amygdala and hippocampus (Colloca & Grillon, 2014).

To sum up, nocebo hyperalgesic effects can be measured in the CNS at cortical, subcortical and spinal levels, and the magnitude of nocebo responses is influenced by the strength of expectancies and the intensity of accompanying negative emotions.

2.4   Sex differences in pain and placebo analgesia

Males and females tend to respond differently to pain (Bartley & Fillingim, 2013). Higher pain sensitivity, and better pain discrimination is found in females than in males (Mogil, 2018). Additionally, females have lower pain threshold and tolerance, and display less inhibition of pain compared to males (Garcia et al., 2007; Mogil, 2012). Clinical pain conditions are more prevalent in females than in males (Mogil, 2012), and it has been suggested that clinical pain is more severe in females relative to males (Barnabe et al., 2012; Fillingim, Doleys, Edwards, & Lowery, 2003; Keefe et al., 2000; Tang, Yang, Wang, & Lin, 2012). Although the effects sizes of these observed sex differences vary, the direction of the sex differences are clear (Mogil, 2018).

Sex differences are also reported in the placebo analgesic effect (e.g., Aslaksen et al., 2008; Bjørkedal et al., 2011; Butcher & Carmody, 2012; Krummenacher et al., 2014). In a recent review article, we examined whether differences between males' and females' responses to placebo and nocebo treatments are systematic (Vambheim & Flaten, 2017). The

search strategy resulted in 18 studies, whereof 12 investigated the placebo effect and the remaining six the nocebo effect. Eight of the placebo studies showed larger placebo responses in males than in females, whereas five of the nocebo studies showed larger nocebo responses in females than in males. We also tested whether the method used to induce placebo and nocebo responses differed across sex and found that verbally induced placebo effects were more often observed in males and that conditioned nocebo responses were more often in females. As verbally induced placebo responses are due to activation of the endogenous opioid system (Amanzio & Benedetti, 1999), the observation that verbally induced placebo effects are more frequent in males than in females (Vambheim & Flaten, 2017) may be explained by sex differences in the endogenous opioid system.

The exact basis for sex differences in pain and placebo analgesia remains unclear, but it is evident that biological systems and psychological processes are involved and interacting (Mogil, 2018). One possibility is that sex differences in pain and pain inhibition are due to sex differences in the ascending and descending pain pathways (Mogil, 2012) and opioid responding (Zubieta et al., 2002). Animal studies have shown physiological and anatomical sex differences in the endogenous descending pain pathways (Loyd, Morgan, & Murphy, 2007; Loyd & Murphy, 2006). Placebo analgesia is associated with activation of descending pain pathways and endogenous opioid elicitation, whereas nocebo hyperalgesia is associated with activation of pronociceptive pathways and elicitation of CCK. Endogenous opioids and CCK has a bidirectional relationship with placebo analgesia (Colloca & Benedetti, 2005). As endogenous opioids are associated with increased placebo analgesic responding, CCK is associated with reduced placebo analgesic responding. Therefore, sex differences in the neurochemical mechanisms involved in placebo analgesia may explain sex differences placebo analgesic responding. Another possibility is that sex differences in stress and anxiety,

whereof both influence elicitation of endogenous opioids and CCK, may cause sex differences in placebo analgesic and nocebo hyperalgesic responding. Placebo analgesic treatments have been found to produce stronger stress reduction in males than in females, with a consecutive reduction in pain unpleasantness (Aslaksen et al., 2011). These findings were supported by reduced ERP-responses in males, but not in females, in the placebo condition compared to the natural history condition. Stress reduction, which was stronger in males than in females, explained 23% of the variance in the placebo analgesic effect.

Possibly, the sex differences in placebo analgesia is due to sex differences in the vasopressin and oxytocin system. Vasopressin is involved in evaluation and regulation of social behaviors, and vasopressin influences these behaviors differently in males and females (Colloca et al., 2016). Colloca and colleagues (2016) aimed to examine whether vasopressin modulates placebo analgesia. The participants were given nasal spray containing oxytocin, vasopressin or saline. A control group, were no drug or saline were administered, was also included. Expectations of pain relief were induced verbally. The results showed that pharmacological manipulation of the vasopressin system increased the placebo effect in females, but not in males. In females only, an inverse relationship was found between the placebo analgesic effect and a) dispositional anxiety, b) baseline cortisol levels, and c) vasopressin related cortisol changes. Thus, suggesting a sexual dimorphism in the relationship between placebo analgesia, anxiety levels and cortisol responses.

Oxytocin influences social behaviors and cognitive and emotional processes (Theodoridou, Rowe, Penton-Voak, & Rogers, 2009). Kessner and colleagues (2013) reported that oxytocin potentiated the placebo analgesic responses in males. However, only male participants were enrolled. The study by Colloca and coworkers (2016) included both male

and female participants, but there were no increased placebo effect or sex difference in the oxytocin group.

Psychological and sociocultural factors, e.g., negative affect, anxiety, fear, gender roles and gender role expectations, have been found to influence pain differently in males and females (Mogil, 2012; Sandford et al., 2002; Wise, Price, Myers, Heft, & Robinson, 2002). These differential influences fit well with the sex differences in placebo analgesic responding reported in this thesis and other studies (Bjørkedal et al., 2011; Theysohn et al., 2014; Vambheim & Flaten, 2017). The factors that seem to contribute to sex differences in placebo analgesia are sex differences in stress and the endogenous opioid system.

Even though considerable progress has been made towards an understanding of sex differences in pain and placebo analgesia, there are several challenges to the investigation and understanding of these phenomena. First, a large amount of the existing literature on pain consists of male samples (Greenspan et al., 2007). Second, most studies are not designed to examine sex differences, or do not report sex differences. Mogil (2018) reported that between 1969 and 2005 79% of the studies published in the journal *PAIN* consisted of male samples, and that another 5% included both males and females, but did not report on sex differences. Third, numerous different theoretical and methodological approaches have been used in previous studies. Finally, most studies that have reported sex differences in pain and analgesia used small samples. Generalizing results obtained through male samples to females may be erroneous.

2.5 Fear of pain

Fear may be defined as the immediate and present emotional and physiological response to one specific threat (Turk & Wilson, 2010). Fear of pain (FOP) may then be understood as negative emotional and physiological activation in the presence of actual or impending pain. Fear increases pain in some situations and decreases pain in other situations, and it has been shown that this depends on the emotional arousal level (Lumley et al., 2011). FOP is negatively associated with pain threshold (Hirsh, George, Bialosky, & Robinson, 2008) and positively associated with pain sensitivity (George & Hirsh, 2009). High FOP is related to increased pain unpleasantness and reduced pain inhibition and placebo analgesic responding (Lyby et al., 2011). It has also been reported that FOP predicts nocebo hyperalgesia and increased stress (Aslaksen & Lyby, 2015) and that FOP is essential to behavior and coping strategies in chronic pain patients (McNeil et al., 1998).

Pain-related fears may reflect negative expectations and worries towards future pain, and towards the possible consequences of future pain. This latter type of fear may be better understood as anxiety (Vambheim & Øien, 2017). Thibodeau and colleagues (2013) reported that pain related anxiety reduced females' tolerance towards pain, but this effect was not found in males (Thibodeau, Welch, Katz, & Asmundon, 2013). Thus, the relation between fear and pain and the relation between anxiety and pain are somewhat different. Additionally, the associations between fear and pain and between anxiety and pain tend to be sex-specific. It is therefore important to dissociate fear from anxiety when FOP-measures are used in studies on pain and analgesia. The Fear of Pain Questionnaire-III (FPQ-III; McNeil & Rainwater, 1998), The Fear of Pain Questionnaire-Short Form (FPQ-SF; Asmundson et al., 2008), The Pain Anxiety Symptom Scale (PASS; McCracken, Zayfert, & Gross, 1992) and The Tampa Kinesiophobia Scale (TSK; Woby, Roach, Urnston, & Watson, 2005) are widely

used for assessment of pain related fear and anxiety. Paper II and III of the present thesis examined sex differences in FOP and applicability of the Fear of Pain Questionnaire-III and the Fear of Pain Questionnaire-Short Form, to uncover whether the models are useful tools to capture FOP.

3   Objectives

The overall objective of this thesis was to investigate the hypothesis that there are sex differences in the placebo analgesic effect, and explain why males and females tend to respond differently to placebo analgesic treatments. In addition, sex differences in FOP was examined, and the reliability, validity and sex neutrality of the FPQ-III and the FPQ-SF was evaluated. These research questions were addressed:

    a) Are males more responsive to placebo analgesic treatment compared to females?

    b) If so, are sex differences in the placebo analgesic effect reflected not only in reported pain, but also in physiological parameters like ERP amplitudes?

    c) Can sex differences in placebo analgesia be explained by sex differences in emotions?

    d) Are there sex differences in FOP measured by the FPQ-III?

    e) Are the FPQ-III and the FPQ-SF reliable, valid and sex neutral models for measurements of FOP?

4   Method

4.1   The experimental study

A balanced within-subjects design was used. All participants were tested a total of two times, on two different days. One day they participated in the natural history condition, and one day the placebo condition. To avoid order effects the order of the conditions was counterbalanced. Each condition consisted of three tests: one pretest and two posttests. In total 24 stimuli were administered in each test. Measures of pain, stress and arousal were registered during the last four pain stimuli. The conditions were identical except for the placebo administration in the placebo condition.

4.1.1   Participants

Participants between the age of 19 and 31 years were recruited at the campus of the University of Tromsø, The arctic university of Norway. The sample consisted of 54 undergraduate students (mean age = 23) who volunteered to participate. Participants had to be healthy. Medication use, medical history of serious disease, injury, chronic pain or cardiovascular disease led to exclusion. Participation was compensated with a 300 NOK gift card.

4.1.2   Experimental pain induction

Pain was administered by a contact heat-evoked potential stimulator (CHEPS) (Medoc Ltd, Ramat Yishai, Israel). The CHEPS had a thermode surface of 27 mm diameter. Thermocouples in the thermofoil continually sent feedback about the skin temperature to the CHEPS. The heating rate was 70^0C/sec and the cooling rate was 40^0C/sec. The thermofoils baseline temperature was 32^0C, and the heat stimuli peaked at 52^0C. The thermode was

placed on the participants' right lower arm and was moved in a predefined pattern after the pretest and posttest 1 to avoid sensitization.

4.1.3 Subjective pain and stress measures

Pain was measured on a zero to ten numerical rating scale (NRS). The participants received 24 stimuli in each test and reported the intensity and unpleasantness of the pain verbally to the experimenters (0 = no pain, 10 = most intense pain imaginable, 0 = no pain unpleasantness, 10 = unbearable pain unpleasantness).

Two adjective pairs from the Short Adjective Check List (SACL) measured the participants' stress. A Norwegian version of the scale was used. The participants were asked to rate their stress on a zero to ten scale. The adjective pairs were relaxed-tense and calm-nervous (0 = completely relaxed / completely calm, 10 = maximally tense / maximally nervous). The participants reported their ratings of stress verbally to the experimenters and the mean scores of the two adjective pairs were used in the data analysis.

4.1.4 Event-related potentials (ERPs)

ERPs are electrophysiological responses, reflecting cortical activity, to external stimuli. In the present study fronto-central and temporal electrodes (Fz, Cz, Pz, C3, C4, T7 and T8) were used to record contact heat-evoked potentials. Only data from the Cz were included in the data analysis. The EEG was recorded continuously with a 0.15 and 100 Hz bandpass at a 500 Hz rate. Additionally, to control for ocular artifacts, electrooculography (EOG) electrodes were placed above and below the left eye. The time epochs were 1100 milliseconds and included a 100-millisecond baseline. A TTL-pulse marked CHEPS stimulus onset in the EEG-file. Artifacts were controlled and corrected, and data were averaged and analyzed off-line by Analyzer 1.0 software (Brain Products GbmH). An ERP component is a

characteristic segment and timed ERP waveform. ERP components are often represented with a peak and are typically sensitive to certain stimuli or experimental manipulations (Kutas & Federmeier, 2011). Due to previous findings of a correlation between the second negative (N2) and the second positive (P2) ERP components and pain report (Granovsky, Granot, et al., 2008) and placebo treatment (Wager, Matre, & Casey, 2006; Watson, El-Deredy, Vogt, & Jones, 2006; Colloca et al., 2008b), these two components were analyzed in the present study.

4.1.5 Placebo manipulation

The placebo medication was administered as two capsules containing 75 mg lactose. The capsules were administered together with information the following information: These capsules contain analgesic ingredients that have a powerful effect on heat pain". To blind the experimenters, four participants received two capsules of 150 mg acetaminophen, with similar appearance as the placebo capsules. These four subjects were excluded from the data. The experimenters were blinded towards this procedure and did not know whether the participants received active or inactive treatment. The placebo effect was computed by subtracting the scores on pain intensity, pain unpleasantness, stress, N2 and P2 in the natural history condition from the scores in the placebo condition.

4.2 The studies on FOP

4.2.1 Participants

In paper II, 185 healthy participants between 18 and 32 years (mean = 22.5) volunteered. The sample consisted of 49.7% females and 50.3% males. Previous or present serious injuries, psychological and physiological disorders led to exclusion.

The sample of paper III consisted of 807 healthy volunteers, whereof 42% were males and 58% were females. Participants with previous or present serious injuries, psychological and physiological disorders, use of prescription-based and allergy medications were excluded. In both papers, all participants spoke Norwegian, due to administration of the Norwegian version of the questionnaires and that Norwegian language was used for instructions, obtaining consent, and measures of FOP.

4.2.2 The Fear of Pain Questionnaire-III and The fear of Pain Questionnaire-Short Form

The FPQ-III is a 30-item questionnaire where each item is rated 5-point Likert scale, designed to examine FOP in both clinical and nonclinical samples. Responders rate their fear of certain types of pain on a 1 to 5 scale, where 1 represents no fear and 5 extreme fear. The items are thought to measure the three broader dimensions Severe, Medical and Minor FOP. For this reason, the FPQ-III is categorized into three subscales, each of which consists of 10 items. The subscales measure fear of severe, minor and medical pain.

The FPQ-SF is similar to the FPQ-III, except that this scale is reduced to 20-items and expanded to 4 subscales. The FPQ-SF's four subscales are fear of severe, minor, injection, and dental pain. In both papers, a Norwegian version of the FPQ-III (Lyby et al., 2010) was employed.

Table 1 Fear of Pain Questionnaire III

1. **Being in an automobile accident.**[Severe]
2. **Biting your tongue while eating.** [Minor]
3. **Breaking your arm.** [Severe]
4. **Cutting your tongue licking an envelope.** [Minor]
5. **Having a heavy object hit you in the head.** [Severe]
6. **Breaking your leg.** [Severe]
7. **Hitting a sensitive bone in your elbow – your "funny bone".** [Minor]
8. **Having a blood sample drawn with a hypodermic needle.** [Medical]
9. **Having someone slam a heavy car door on your hand.** [Severe]
10. **Falling down a flight of concrete stairs.** [Severe]
11. **Receiving an injection in your arm.** [Medical]
12. **Burning your fingers with a match.** [Minor]
13. **Breaking your neck.** [Severe]
14. **Receiving an injection in your hip/buttock.** [Medical]
15. **Having a deep splinter in the sole of your foot probed and removed with tweezers.** [Medical]
16. **Having an eye doctor remove a foreign particle stuck in your eye.** [Medical]
17. **Receiving an injection in your mouth.** [Medical]
18. **Being burned on your face by a lit cigarette.** [Severe]
19. **Getting a paper-cut on your finger.** [Minor]
20. **Receiving stitches in your lip.** [Medical]
21. **Having a foot doctor remove a wart from your foot with a sharp instrument.** [Medical]
22. **Cutting yourself while shaving with a sharp razor.** [Minor]
23. **Gulping a hot drink before it has cooled.** [Minor]
24. **Getting strong soap in both eyes while bathing or showering.** [Minor]
25. **Having a terminal illness that causes you daily pain.** [Severe]
26. **Having a tooth pulled.** [Medical]
27. **Vomiting repeatedly because of food poisoning.** [Severe]
28. **Having sand or dust blow into your eyes.** [Minor]
29. **Having one of your teeth drilled.** [Medical]
30. **Having a muscle cramp.** [Minor]

5   Summary of Papers

5.1   Paper I

Aslaksen, P.M., Bystad, M., Vambheim, S.M. & Flaten, M.A. Gender differences in placebo analgesia: event-related potentials and emotional modulation. *Psychosomatic Medicine*, 2011;73(2):193-199.

This study investigated if males are more responsive to placebo analgesic treatment than females, and if so, whether this sex difference is accompanied by sex differences in physiological and psychological measurements. Due to previous findings of larger placebo analgesic effects in males compared to females, larger placebo analgesic responses in reported pain and the N2/P2 components in males compared to females were hypothesized. Furthermore, a relation between anticipatory stress and placebo responding was assumed.

Fifty-four healthy subjects were recruited to the experiment, which was conducted according to a within-subjects design. Seven subjects were excluded due to poor ERP measurements, and ten subjects were excluded because they did not perceive the applied stimuli as painful, displayed by < 2 at the NRS in the first round of pain stimulation. Significant interactions of Condition x Sex on pain unpleasantness and Condition x Sex in the P2 data were found. These interactions showed that placebo treatment reduced pain unpleasantness and the P2 ERP components in males, but not in females. A significant main effect of Condition on the N2 component showed that the N2 amplitudes were lower in the placebo condition than in the natural history condition. However, follow up tests revealed a significant interaction of Gender x Test, which showed that the N2 responses were larger in females than in males. Moreover, placebo treatment reduced stress in males but not in

females. A significant interaction of sex and stress predicted the placebo response, and the larger stress reduction in males than in females explained 23% of the variance in placebo responding. Thus, the findings on pain unpleasantness, ERP-measurements and stress were in line with the hypothesis. Contrary to the hypothesis, there were no main effects of treatment on pain intensity. One potential explanation is that the placebo treatment influenced the emotional, but not the sensory-discriminative, component of pain.

In conclusion, placebo analgesia in males is related to reduced stress. The placebo response on the P2 component reflects decreased brain activity, probably in pain sensitive regions including the ACC and the insular cortex. A possible explanation is that the placebo treatment initiated endorphin release and that the inhibited pain signals reduced cortical activity in males. Since at least some placebo analgesic responses are mediated through the endogenous opioid system, sex differences in this system may explain the findings from this study.

5.2   Paper II

Vambheim, S.M. & Øien, R.A. Sex Differences in Fear of Pain: Item-Level Analysis of the Fear of Pain Questionnaire III. *Journal of Pain Research*, 2017;10:825-831.

The study examined whether there are sex differences in FOP measured by the FPQ-III, and whether potential sex differences could be related to specific items at the FPQ-III. It was hypothesized that females would score higher than males overall, at the subscales and at item-level. The FPQ-III is developed to measure FOP in both clinical and nonclinical

samples. The questionnaire consists of 30 items and is divided into three subscales: Severe, Minor and Medical Pain.

In total 185 subjects (92 females) responded to the questionnaire. Sex differences on overall, subscales and item-level were investigated. One-way ANOVAs were used to test for sex differences in FOP overall and at the level of subscales. Furthermore, ordinal regression analysis was conducted to examine sex differences at the item level. Although not reported in the published paper, Cohen's d was used to measure effect size and revealed medium to large effect sizes for the sex difference on overall FOP and the Severe Pain subscale. Females scored significantly higher than males on overall FOP ($p = .001$, $d = .524$) and on the subscale Severe Pain ($p < .001$, $d = .726$). Additionally, females scored higher than males on 16 of the 30 FOP items. Among these 16 items, females scored higher than males on all Severe Pain items. After controlling for multiple comparisons, females scored higher than males on 6 items, and among these, 5 items were Severe Pain items. When overall FOP was controlled for, 1 item reached significance, also a Severe Pain subscale item (Fall Down Stairs, $p < .001$, $d = .507$). Thus, the findings on overall FOP, the Severe Pain subscale, and on the item-level were in line with the hypothesis. Contrary to the hypothesis, there were no significant sex differences in the Minor or Medical subscale. Although females scored significantly higher than males on several items of the Minor and Medical subscale, these differences did not contribute to significant sex differences at subscale level.

A possible explanation for the findings of this study is that males and females interpret the presented items in different ways. This difference in interpretation may be due to sex differences in psychosocial mechanisms involving fear and anxiety and emotional reactions to Severe Pain items.

5.3 Paper III

Vambheim, S.M., Lyby, P.S., Aslaksen, P.M., Flaten, M.A., Åsli, O. & Martinussen, L.M. The Fear of Pain Questionnaire-III and The Fear of Pain Questionnaire-Short Form: a confirmatory factor analysis. *Journal of Pain Research*, 2017;10:1871-1878.

Due to previous findings of poor model fit of the FOP-instruments, this study examined the FPQ-III and the FPQ-SFs' model fit, reliability, validity and sex neutrality. It was hypothesized that the FPQ-SF model would have better fit and be more sex neutral than the FPQ-III model. Furthermore, it was expected that sex differences would be displayed as higher FOP-scores in females than in males and poorer fit to the data in the FPQ-III model than the FPQ-SF model.

A total of 807 healthy subjects were enrolled in the study. Approximately 42% of the participants were males and 58% were females. Sex differences were examined on the subscale level with independent samples t-tests and corrected for multiple comparisons by the Holm-Bonferroni procedure. Examination of the model fit and sex neutrality of the FPQ-III and the FPQ-SF was performed through confirmatory factor analysis (CFA) by use of AMOS. The results disclosed that FOP was higher in females compared to males on all subscales in both the FPQ-III and the FPQ-SF. As hypothesized, the FPQ-SF model had better fit and was more sex neutral than the FPQ-III model. However, none of the models had good fit according to the predefined criteria of good fit. When the models were tested across sex it was found that the FPQ-SF was not significantly different for males and females, but the FPQ-III was. Thus, the FPQ-SF displayed sex neutrality, but the FPQ-III did not.

In conclusion, even though none of the models proved good fit the FPQ-SF is preferable over the FPQ-III due to higher fit to the data and sex neutrality.

6 Discussion

This research project set out to investigate whether there are sex differences in the placebo analgesic effect and, if so, to explain why these sex differences appear. Furthermore, sex differences in FOP was assessed, and the consistency, validity and sex neutrality of the three-factorial model FPQ-III and the four-factorial model FPQ-SF were examined. The project provided these main findings: a) placebo analgesic treatment reduced pain unpleasantness in males but not in females; b) sex differences in the placebo analgesic effect were reflected in the P2 component of ERP-measurements and may be partially explained by emotional modulation of anticipatory stress; c) there are sex differences in FOP measured by the FPQ-III; d) neither the FPQ-III nor the FPQ-SF are good models for capturing FOP in healthy Norwegian samples, but the FPQ-SF is preferable over the FPQ-III.

6.1 Sex differences in placebo analgesia

Due to repeated findings of larger placebo analgesic responses in males than in females in our laboratory (Aslaksen et al., 2008; Bjørkedal et al., 2011; Flaten et al., 2006), we wanted to further investigate sex differences in the placebo analgesic effect. In paper I, we hypothesized that males would respond with larger placebo analgesic responses compared to females. We expected that this difference would be reflected in larger reductions in the N2/P2 components in males than in females, and assumed that subjective stress would be related to the placebo analgesic response. The placebo effect on pain unpleasantness and the P2 component in male participants, but not in female participants, was due to reduced anticipatory stress in males. The interaction of sex and stress predicted the placebo response on pain unpleasantness, and stress reduction in males explained 23% of the variance in the placebo effect on pain unpleasantness. The finding of larger placebo related reductions in the

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P2 amplitude in males than in females is in line with Bjørkedal and Flaten (2011) and

indicates reduced nociceptive signaling to the brain. Hence, the findings of reduced P2

amplitude in Paper I and Bjørkedal et al. (2011) may be explained by larger activation of the

endogenous opioid pain modulatory system in males than in females. One possibility is that

the placebo treatment activated the descending inhibitory pain pathway and mediated pain

relief through release of endogenous opioids to a larger extent in the male participants than

the female participants. The endogenous system modulates pain, and sex differences in

endogenous pain modulation have been reported (Fillingim, 2000). These differences may

imply that expectations of pain relief modulate anticipatory stress in males, with a subsequent

altered neurophysiologic reaction to the inflicted pain. Our findings indicate that these

processes are different in males and females, and may provide an explanation for why males

are more responsive than females to placebo analgesic treatment. The placebo effects on pain

and stress in males, but not in females supports previous studies (Aslaksen & Flaten, 2008;

Flaten et al., 2006). These results suggest that placebo treatment produces larger reductions in

stress in males than in females and that this has subsequent effects for the production of

placebo analgesic effects, with larger placebo effects in males than in females.

In a recent review, we investigated if sex differences in the placebo effect are

systematic and due to induction method (Vambheim & Flaten, 2017). It should be noted that

Paper I was one of the included studies in the review. The review found that placebo

responses are more frequently observed in males than in females. Furthermore, verbally

induced placebo responses are more often seen in males, and conditioned nocebo responses

are more often seen in females. We concluded that larger stress reduction in males than in

females is central to the sex difference in placebo responding. The studies included in the

review investigated several different symptoms and responses, e.g., nausea, cognitive

performance, IBS and pain. The finding of sex differences in the placebo effect across symptoms and responses suggests that sex differences are not specific to pain. A possible explanation for sex differences across symptoms and responses may be that males and females profit on different types of information. In Paper I, the placebo manipulation was induced through verbal information. Placebo effects in males, but not in females, may be due to differences in how males and females interpret the information induced in the manipulation phase of the experiments. Different types of information processing may have caused larger expectations of symptom improvement in males compared to females. The finding that placebo treatment produced larger reduction in anticipatory stress in males than in females and that the stress reduction significantly influenced the placebo response, could reflect that verbal placebo manipulations produces larger placebo effects in males than females due to sex differences in information processing. However, as information processing or expectancies towards the placebo treatment was not measured, the possibility that the observed sex differences were due to information processing or differences in expectancies cannot be ruled out.

The finding of placebo effects on pain unpleasantness, but not pain intensity, may suggest that the placebo treatment influenced the affective-emotional dimension of pain experience but had no effect on the sensory-discriminative dimension. The medial and the lateral pain systems represent two different pain processing networks in the brain, responsible for the emotional aspects of pain (pain unpleasantness) and the sensory aspects of pain (pain location, duration, intensity), respectively. Thus, the medial pain system represents processing of pain from the affective-motivational dimension, whereas the lateral pain system processes pain from the sensory-discriminative dimension (Lumley et al., 2011). The medial pain system includes the most important structures for elicitation of responses recorded by the

N2/P2 components (Garcia-Larrea, Frot, & Valeriani, 2003). Therefore, it is likely that the placebo treatment influenced only the affective-motivational dimension of pain in this experiment.

It is recommended that prospective studies tests for sex differences and that the constraints on generalizability are listed if this is not possible. Standardizing experimental procedures, including both subjective and objective measures of pain responses, and adjusting outcome measures for sample sizes will clarify the relative contribution of sex to pain responses. Further investigation on the biopsychosocial mechanisms involved in pain related sex differences will promote scientific and clinical progress within the field of pain and analgesia and may be advantageous for optimizing pain treatment for both males and females.

6.2 FOP

The FPQ-III is frequently used in clinical and basic research, often as a covariate to measurements of pain. FOP is related to an individuals' pain threshold, pain tolerance (George et al., 2009; Hirsh et al., 2008), and also to placebo analgesic responding (Lyby et al., 2011). Furthermore, these relations are often different for males and females. In 2008, Asmundsson, Bovell, Carleton and McWilliams developed the FPQ-SF, a revised version of the FPQ-III. They argued that, in addition to increased factor stability, the new model displayed sex neutrality. In papers II and III, we examined sex differences in FOP measured by the FPQ-III, and the model fit, reliability, validity and sex neutrality of the FPQ-III and the FPQ-SF. We hypothesized that FOP-scores would be higher in females than in males and that the recently developed FPQ-SF-model would be better suited for measurements of FOP, in general and across sex. In line with previous studies (Albaret et al., 2004; Roelofs, Peters,

Deutz, Spijker, & Vlaeyen, 2005) sex differences displayed as larger FOP in females than in males were found in both paper II and paper III. The item-level analysis uncovered sex differences, expressed as higher FOP in females than in males, on total FOP scores and on the Severe Pain subscale. Closer inspection of the items where the largest sex differences were found revealed that these items represented situations with potential of serious or fatal outcomes. For this reason, we argued that the Severe Pain items may have been interpreted differently in males and females, and thus resulted in the observed sex difference in Severe Pain. Another possibility is that these items elicited different immediate psychological responses in males and females, or that differing interpretations mediated different psychological responses in males and females. If so, scoring of Severe FOP items may have elicited anxiety in females and fear in males. Replacing the items where the largest sex differences are found could help improve the instruments' applicability across sex. New items which describes situations involving moderate to high pain could typically be migraine, appendicitis or tooth pain, which represents examples of situations involving moderate to high levels of pain that many people have experience with and thus can relate to.

We found that neither the FPQ-III nor the FPQ-SF is a good model for capturing FOP in Norwegian samples. To examine the models' sex neutrality, we looked at the models separately. The fit indices showed that the FPQ-III was a better instrument for measuring FOP in males than in females, whereas the FPQ-SF was better for measuring FOP in females than in males. When the models sex neutrality was examined by multigroup CFA, the FPQ-SF displayed sex neutrality, but the FPQ-III did not. Thus, the FPQ-SF proved to be the best model overall and across sex groups. Our findings support previous studies reporting sex differences in FOP measured by the FPQ-III (Horn, Alappattu, Gay, & Bishop, 2014; Lyby et al., 2011; McNeil et al., 1998; Osman, Breitenstein, Barrios, Gutierrez, & Kopper, 2002;

Sullivan, Thorn, Rodgers, & Ward, 2004) and adds to the literature by showing that the present models need to be adjusted when used to explain FOP in Norway. In a recent study we developed a new, refined model for measurements of FOP in Norwegian samples (Vambheim, Lyby, Aslaksen, Flaten, Åsli, Bjørkedal, et al., 2017). The new model, termed the Fear of Pain Questionnaire Norway (FPQ-NOR), consists of 27 items and the 6 subcategories of minor pain, severe pain, injection pain, fracture pain, dental pain and cut pain. Thus, this model is reduced compared to the FPQ-III-model in terms of the number of items but is extended to three more subcategories. The extension of subcategories may be useful in clinical practice and research, as it facilitates separation of different sorts of FOP.

6.3   Implications and limitations

Our findings may have implications for further research and clinical practice. Because expectations of symptom relief and emotional modulation influence placebo responding, any therapeutic context has the potential of eliciting placebo effects. Placebo mechanisms can be activated even in situations where no placebo is administered, and these mechanisms can interact with active treatments. If the observed sex difference in placebo responding is due to a sex difference in the endogenous pain modulatory system, females undergoing pain treatment may be predisposed to poorer clinical outcomes than males.

The findings from the studies on FOP are important in the future work of understanding sex differences in FOP, pain and analgesia, as well as for improving measurement inventories used in basic and clinical research and in clinical practice. Sex differences in FOP, pain, and pain inhibition should be considered in the work of delineating optimized and tailored treatments for pain patients. Placebo responses are involved in most

medical treatments (Enck, Bingel, Schedlowski, & Rief, 2013). Maximizing placebo effects, or placebo related effects, in clinical practice could improve the treatment outcome in clinical settings. Although research on how placebo mechanisms can be utilized in clinical practice is in its infancy, it is reasonable to suggest that reducing stress, anxiety and FOP, and enhancing expectancies towards treatment efficacy, are useful strategies for optimizing treatment outcomes in most therapeutic settings.

The finding of a sex difference on placebo analgesic responding in Paper I has some important implications for future experimental designs. We recommend that prospective studies are designed so that further clarification of the association between placebo responsiveness and sex can be obtained. Future studies should report results separately for males and females, and treatment outcomes should be analyzed for sex differences.

In paper I, we acclaimed that the existing FOP models needed further adjustment. The FPQ-NOR, which was identified in a more recent paper, proved good fit in general and across sex. However, the young, nonclinical sample included in that paper may limit the generalizability of the findings. A priority for future studies will be to examine the models' applicability to other types of samples, e.g., patient and age groups.

Expectancies of treatment efficacy were not measured in Paper I. This omission represents a limitation of that study, as the sex differences in placebo responding may have been due to differing expectations in males and females towards the placebo treatment. The relatively low number of participants may limit the generalizability of the findings. Additionally, the pain levels were low, reflected in mean pain intensity and pain unpleasantness on reported pain of 2.35 and 3.08, respectively. These low pain levels may

explain the lacking placebo response in the pain intensity data. These challenges could be avoided through individual calibration of the pain stimuli.

The samples included in paper II and III consisted mainly of undergraduate students. Thus, both samples were homogenous by age and education. As FOP has been found to vary across age (Albaret, Sastre, Cottencin, & Mullet, 2004) inclusion of different age groups is recommended in future studies. A Norwegian version of the FPQ-III was used, and all participants responded to the FPQ-III. Translational and linguistic issues can therefore not be discounted, and administration of the FPQ-SF may have produced other results. Moreover, inclusion of healthy samples in all the three studies may limit the generalization of the findings.

7   Overall Conclusions

The findings from this thesis sheds light on some important aspects of sex differences in placebo analgesia and FOP, as well as instruments frequently used to measure FOP. The results may have important implications for future clinical investigations on pain, analgesia and the role of emotional modulation. The presented findings should be further tested in clinical populations and on heterogeneous age groups to ensure generalizability and applicability.

a) There are sex differences in the placebo analgesic effect, with larger placebo analgesic responses on pain unpleasantness in males compared to females.

b) Sex differences in the placebo analgesic effect are accompanied by reduced P2 amplitude

c) Sex differences in the placebo analgesic effect are partially explained by sex differences in modulation of anticipatory stress.

d) There are sex differences in FOP measured by the FPQ-III.

e) Neither the FPQ-III nor the FPQ-SF are good models for measuring FOP in Norwegian samples. However, the FPQ-SF showed better fit indices overall and amongst males and females, and is thus preferable over the FPQ-III.

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