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BRAIN MECHANISMS IN PAIN REGULATION

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ABSTRACT

The subjective sensitivity to pain differs greatly between individuals and neuroimaging has contributed to the understanding of the cerebral mechanisms involved in pain regulation. The descending pain inhibitory circuitry is a well defined cerebral network that enables regulation of afferent nociceptive information. The aim of this thesis was to investigate different aspects of pain modulation in patients with Fibromyalgia (FM) as well as the impact of specific genetic variations on pain sensitivity dynamics in healthy subjects.

Study I demonstrated that patients with FM had an impaired mechanism for descending pain inhibition and that this deficiency was paired with a diminished activation of the rostral anterior cingulate cortex and the brainstem, two regions that play an important role in descending pain regulation. These results advance the understanding of the pathophysiology in FM and provide new directions for the development of effective treatments.

Study II investigated the possible impact of negative mood on pain processing in patients with FM and found that brain activity during experimental pain was not modulated by depressive symptoms, anxiety, or catastrophizing thoughts. The activity of the brain regions previously implicated in the pathophysiology of FM were not correlated with high ratings of negative mood which suggests that there are two segregated cerebral mechanisms dealing with pain and negative mood in FM.

In study III patients with FM were treated with a Noradrenaline-Serotonin Reuptake Inhibitor (*milnacipran*) or placebo for 12 weeks. All patients that reported an improvement of symptoms after treatment, including both milnacipran and placebo responders, were compared and results revealed that sensitivity to pressure improved selectively in milnacipran responders. This decreased sensitivity also correlated to the improvement of ongoing clinical pain. The study suggests that the specific effect of milnacipran acts through direct antinociceptive effects and/or by the strengthening of the endogenous pain inhibitory mechanisms.

In study IV the genetic influence on the descending pain inhibitory function in healthy subjects was assessed. Results demonstrate that a genetic polymorphism (COMTval^{158}met) with influence on the function of the noradrenergic and dopaminergic systems, is related to the response dynamics of repeated pain stimulations following opioid administration. Results suggest that the initial pain response is not influenced by the COMTval^{158}met polymorphism but when the system is challenged the difference is expressed.

SAMMANFATTNING PÅ SVENSKA

Det är mycket stor skillnad mellan olika individers subjektiva upplevelse av smärta och hjärnavbildningstekniker har bidragit till bättre förståelse för de cerebrala mekanismer som är inblandade i reglering av smärta. Det nedåtstigande smärthämmande systemet består av ett väldefinierat nätverk av områden i hjärnan som tillsammans möjliggör reglering av inkommande smärtsignaler. Syftet med denna avhandling var att undersöka olika aspekter av smärtreglering hos patienter med Fibromyalgi (FM) samt vilken inverkan specifika genetiska variationer har på smärtkänslighet hos friska individer.

Studie I visade att patienter med FM hade en försämrad förmåga till inhibition av smärta och att denna dysfunktion var kopplad till minskad aktivitet i rostrala anteriora cingulum och hjärnstammen, två regioner i hjärnan som är starkt förknippade med nedåtstigande reglering av smärta. Dessa resultat utökar förståelsen för patofysiologin i FM och kan leda till nya riktningar i utvecklingen av effektiv behandling.

I studie II undersöktes huruvida ett negativt stämningsläge påverkar hjärnans bearbetning av smärtsignaler i FM. Resultat visade att hjärnaktiviteten under smärtstimulering inte var påverkad av graden av depressiva symptom, ångest eller katastroftankar. De regioner i hjärnan som tidigare uppvisat förändrad aktivitet hos patienter med FM samvarierade inte med självskattningar av negativt stämningsläge, vilket pekar mot två separata cerebrala mekanismer för smärta och ett negativt stämningsläge hos patienter med FM.

I studie III behandlades FM-patienter antingen med placebo eller en noradrenalin-serotonin återupptagshämmande substans (*milnacipran*) i 12 veckor. Alla som rapporterade en god behandlingseffekt med milnacipran eller placebo jämfördes och resultaten visade att känsligheten för tryck minskade selektivt hos de patienter som behandlats med milnacipran och att det fanns en korrelation mellan minskad känslighet för stimulus-inducerad smärta och minskad intensitet av den spontant pågående smärtan. Denna studie pekar mot att det finns en specifik effekt av milnacipran som antingen verkar via en direkt anti-nociceptiv effekt och/eller via en förstärkning av det endogena smärthämmande systemet.

I studie IV undersöktes en specifik genetisk inverkan på smärtreglering hos friska individer. Resultat från studie IV visade att en genetisk polymorphism med inverkan på noradrenerg och dopaminerg funktion (COMTval^{158}met) var relaterad till variationer i smärtupplevelse efter opioid behandling och upprepade smärtstimuleringar. Resultat från denna studie antyder att det smärthämmande systemet inledningsvis inte påverkas av COMTval^{158}met utan först när systemet utsätts för ökade påfrestningar uppstår skillnaden mellan de tre genotyperna.

LIST OF PUBLICATIONS

I. **KB Jensen**, E Kosek, F Petzke, S Carville, P Fransson, H Marcus, SCR Williams, E Choy, T Giesecke, Y Mainguy, RH Gracely, M Ingvar. Evidence of dysfunctional pain inhibition in Fibromyalgia reflected in rACC during provoked pain. Pain 2009, 144, 95-100.

II. **KB Jensen**, F Petzke, S Carville, P Fransson, H Marcus, SCR Williams, E Choy, Y Mainguy, RH Gracely, M Ingvar, E Kosek. Anxiety and depressive symptoms in Fibromyalgia are related to low health esteem but not to sensitivity or cerebral processing of pain. *Submitted*.

III. E Kosek, **KB Jensen**, S Carville, E Choy, RH Gracely, M Ingvar, Y Mainguy, H Marcus, F Petzke. All responders are not the same: Distinguishing milnacipran- from placebo-responders using pressure pain sensitivity in a Fibromyalgia clinical trial. *Submitted.*

IV. **KB Jensen**, TB Lonsdorf, M Schalling, E Kosek, M Ingvar. Increased sensitivity to thermal pain following a single opiate dose is influenced by the COMT val^{158}met polymorphism. PLoS One 2009, 6, 130-134.

CONTENTS

LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
ACR	American College of Rheumatology
BDI	Beck's Depression Inventory
BOLD	Blood Oxygen Level Dependent
CBT	Cognitive Behavioral Therapy
CNS	Central Nervous System
COMT	Catechol-O-Methyl Transferase
CSQ	Coping Strategies Questionnaire
DLPFC	Dorsolateral Prefrontal Cortex
DSM-IV	Diagnostic and Statistical Manual of psychiatric disorders IV
EEG	Electroencephalography
EPI	Echo-planar Imaging
FIQ	Fibromyalgia Impact Questionnaire
FM	Fibromyalgia
fMRI	Functional Magnetic Resonance Imaging
IASP	International Association for the Study of Pain
MDD	Major Depressive Disorder
MEG	Magnetoencephalography
MNI	Montral Neurological Institute
MRI	Magnetic Resonance Imaging
MTL	Medial Temporal Lobe
NSRI	Noradrenalin-Serotonin Reuptake Inhibitor
OBFC	Orbitofrontal Cortex
PAG	Periaqueductal Gray
PET	Positron Emission Tomography
PGIC	Patient Global Impression of Change
rACC	Rostral Anterior Cingulate Cortex
rCBF	Regional Cerebral Blood Flow
RVM	Rostral Ventromedial Medulla
S I	Primary Sensory Cortex
S II	Secondary Sensory Cortex
SD	Standard Deviation
SF-36	Short-Form 36
SPECT	Single Photon Emission Computed Tomography
SSRI	Selective Serotonin Reuptake Inhibitor
STAI	Spielberger's State-Trait Anxiety Inventory
STT	Spinothalamic Tract
VAS	Visual Analogue Scale
WDR	Wide Dynamic Range

1 INTRODUCTION

Pain represents an emotional construct including subjective mental processes and is therefore unique for every individual. Since pain is inherently subjective there is a problem for the third-person to validate the first-person experience and complaint of pain. In medical care this is a critical dilemma since many patients are complaining about severe persistent pain in absence of any detectable pathology while they consume a large proportion of health care resources. The lack of credibility for these patients results in inadequate health care interventions and consequently a lot of suffering for the patients. Neuroimaging allows for investigation of the neural underpinnings of pain and studies in chronic pain disorders have contributed to detection of pathological mechanisms represented within the central nervous system. In presence of new evidence for possible pathophysiological mechanisms in chronic pain disorders, the possibility of finding new treatment strategies has increased. The fact that an invisible complaint has been made visible makes a difference for the validation of the patients suffering from chronic pain. Also, the scientific discourse has changed and now patients with chronic pain of previously unknown origin represent interesting models of altered central physiology that can teach us many things about the brain and offer new models of complex central pathology.

The subjective sensitivity to pain differs greatly between individuals and neuroimaging has helped us to understand the mechanisms associated with differences in pain sensitivity. With recently increased understanding for the genetic contribution to complex behaviors such as anxiety and pain it is important to convolve the genetic knowledge with findings from functional neuroimaging. Now, the possibility to investigate the physiological links between functional genetic polymorphisms and differences in processing within brain circuits involved in pain is slowly emerging into a new and promising field of research.

2 THE HISTORY OF PAIN

There are early theories about pain and transmission of pain signals in the body; one of the oldest presented by Aristotle 300 years BC. Most historical views on pain have implied that our senses depend on the transportation of a physical agent from the outer world into the heart or the brain. Not until the 19th century the first theories about pain transmission within the nervous system were presented.

2.1 EARLY HISTORICAL BACKGROUND

The ancient Greek philosopher Aristotle (384-322 BC) described pain as a feeling, situated in the heart. Several hundred years later a physician in Alexandria, Galen (AD 130-201), described the brain as the basis for feelings and concluded that pain was a sensation of the brain (Perl, 2007). In 1644 Descartes published his text *De l'homme* (*About man*), representing his famous image of pain transmission from the periphery to the brain (Descartes, 1644). Our current knowledge about pain transmission within the central nervous system was founded primarily in the 19th century when the afferent pathways from the periphery via the spinal cord up to the brain were first described (Perl, 2007). However, some concepts of the early theories of pain, e.g. the notion by Aristotle that pain is a feeling, have become surprisingly modern in the light of contemporary ideas about pain as a homeostatic emotion.

2.2 THE BIO-PSYCHOLOGICAL MODEL OF PAIN

The casualties of World War I and II led to an extensive medical experience of injuries and concomitant complaints of pain. It became evident that the level of pain reported by the injured and the requests for analgesics were not predicted by the tissue damage *per se*. I.e. pain was not determined by the size of the lesion but by an interaction of the lesion and different contextual factors. Also, there were reports of persistent pain in absence of any tissue damage or inflammation. In response to this, a new model of pain was published in 1965 by Melzack and Wall,

describing how pain signals are modulated by a gate in the dorsal horn of the spinal cord (Melzack and Wall, 1965). The gate was a metaphor of the modulation that takes place where synapses meet in the substantia gelatinosa in dorsal horn. The theory includes the notion that pain is not a direct result of the incoming pain signals but is instead modulated by interaction between both pain-transmitting and non-pain-transmitting neurons in the dorsal horns of the spinal cord. Thereby the interference of neurons that do not transmit pain signals can inhibit an individual's perception of pain (Melzack and Wall, 1965). Hence, the spinal cord was no longer described as a passive transmission station but a possible stage for inhibition, excitation and modulation of nociceptive input (Melzack, 1999). The gate-control theory of pain was well received since it accounts for differences in subjective pain reports between individuals and situations. I.e. pain was described as dynamic and non-linear to an absolute physical stimulus.

2.3 THE INTERNATIONAL DEFINITION OF PAIN FROM 1994

In 1994 there was a formulation of an international definition of pain by the International Association for the Study of Pain (IASP): "*Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*". According to the 1994 definition there is no requirement of tissue damage in order for a sensation to qualify as pain (Merskey and Bogduk, 1994). This is of particular importance for the recognition of chronic pain where there is often high intensity pain in absence of any tissue damage. The inclusion of the possibility of pain as an emotional experience is important for the understanding of pain behaviors as well as the contemporary theoretical line of pain as a homeostatic emotion.

2.4 PAIN AS A HOMEOSTATIC EMOTION

The ability to feel pain is of great importance for our survival. It can be described as the perceptual counterpart of the body's response to stimuli that threatens the integrity of its tissues (Treede et al., 1992). It thus functions as a warning system of threats to the organism. Craig describes pain as a homeostatic emotion (Craig, 2003b) that offers motivation to act upon such a threat. That means that pain is both a distinct sensation and a motivation; a specific emotion that reflects homeostatic behavioral drive, similar to temperature, itch, hunger and thirst. The

interoceptive awareness of a stimulus is likely processed in the anterior insular cortex. The affective motivation which initiates behavioral response is likely represented by concomitant activity in the Anterior Cingulate Cortex (ACC) (Craig, 2003b). More specifics about the neuroanatomy of pain will be given in the next section.

3 PAIN ANATOMY AND PHYSIOLOGY

3.1 NOCICEPTORS AND PERIPHERAL PATHWAYS

Pain is transmitted from all over the body by nociceptive neurons known as nociceptors (Woolf and Ma, 2007). Aδ-fibers are myelinated and have fast conduction velocities; carrying information about brief, sharp pain. C-fibres are unmyelinated neuron and therefore have slower conduction velocities. Activation of C-fibres result in a sensation of blunt, throbbing, pain – often longer lasting compared to pain transmitted by Aδ-fibers (Treede et al., 1992; Woolf and Ma, 2007). Both fiber types project to the dorsal horn of the spinal cord where the nociceptive signal is conveyed and projected up to cerebral regions of the central nervous system (Meyer et al., 2006).

3.2 SPINAL TRANSMISSION

The neurons projecting to the spinal cord end up in the substantia gelatinosa in the dorsal part of the spinal cord. There, the peripheral nociceptors synapse to different types of neurons: projection neurons that send ascending axons towards higher centers and inhibitory interneurons as well as excitatory interneurons that are involved in regulation of transmission of the nociceptive information (Melzack and Wall, 1965; Todd and Koerber, 2006). The dorsal horn can be divided into different laminae where lamina I is the most superficial. Most nociceptors are found in lamina I but peripheral nociceptors also connect to wide dynamic range (WDR) neurons in deeper parts of the dorsal horn, i.e. lamina IV-V (Schnitzler and Ploner, 2000). WDR neurons respond to both noxious and innocuous stimuli (Schnitzler and Ploner, 2000) and have a large receptive field (Treede et al., 1992). The property of these neurons is important for both up- and down-regulation of pain (Treede et al., 1992).

Ascending axons from both laminae I and V cross to the other side of the spinal cord and conduct nociceptive information to higher anatomical regions. Lamina I neurons predominately project through the lateral spinothalamic tract (STT) and lamina V neurons go through the anterior STT up to the brain (Schnitzler and Ploner, 2000; Treede et al., 1992).

3.3 CEREBRAL PROCESSING OF PAIN

The complexity of the subjective experience of pain entails involvement of several different brain regions. Together these regions are sometimes called *the neuromatrix of pain* or *the pain matrix* (Melzack, 1999). The cortical regions of the pain matrix that are implicated in sensory-discriminative function can be referred to as the *lateral pain network*. The anatomical regions involved in the cognitive-evaluative-emotional component of pain are referred to as the *medial pain network* (Tracey and Mantyh, 2007). This division is based on the projections from the medial and lateral thalamic nuclei to the cortex (Ploner et al., 1999). Given that there is extensive reciprocal communication between the lateral and the medial pain networks, the pain matrix is inherently dynamic. The dual influence of lateral and medial projections within the pain circuitry makes it complex to investigate, but its plasticity also opens for possible modifications through pain inhibiting interventions.

Nociceptive input from the spinal cord reaches the thalamus via the STT but there is also evidence for multiple ascending tracts involved in integrating nociceptive information via the medulla and brainstem (Tracey and Mantyh, 2007). Spinal projections to the brainstem are important for integrating nociceptive activity with homeostatic, arousal, and autonomic processes (Craig, 2003b). There is increasing evidence for an immediate influence of the brainstem on spinal as well as prefrontal regions of the brain (Tracey and Mantyh, 2007). This likely reflects a direct involvement of the brainstem in regulation of pain.

Neuroimaging tools have contributed to the mapping of cortical regions involved in pain processing in humans. The most common regions activated during pain are primary and secondary somatosensory cortices, the insula, the anterior cingulate cortex and the prefrontal cortices (Bushnell and Apkarian, 2006).

The primary and secondary somatosensory cortices (SI and SII) receive sensory input from all regions of the body and process and transmit the spatial localization of any incoming sensory stimulus according to a somatotopically organized map. I.e. all body parts are individually represented along the cortical

surface of SI. Sensory stimuli are processed by the contralateral SI whereas SII has bilateral representations. For a long time the SI and SII were thought not to be involved in pain processing since surgical extirpation did not relieve pain (Head and Holmes, 1911) and imaging studies could not show conclusive evidence for the involvement of SI during pain (Peyron et al., 2000). Evidence from imaging studies conclude that the SI and SII are involved in early nociceptive processing (Tracey, 2005) but the exact function is not fully understood. SI and SII are likely necessary for the discrimination of the location and intensity the painful stimulus and there is evidence for a difference in SII activation between painful and non-painful stimuli (Tracey, 2005).

The SII receives input from SI and conduct higher order processing of sensory stimuli. However, there is evidence for a difference in projections between painful and non-painful stimuli (Schnitzler and Ploner, 2000). In non-painful tactile stimulation there is a serial processing from SI to SII whereas there seem to be parallel projections possible in response to pain, i.e. there is direct access to SII without first passing SI. The hierarchical organization of tactile processing allows for a fine tuned discrimination that might not be relevant for pain processing (Schnitzler and Ploner, 2000). This furthers the notion that SII is a highly important region in pain processing. However, activations of SII has been seen for pain as well as light touch, visceral sensations and tactile attention (Eickhoff et al., 2006). It is therefore thought that SII consists of multiple somatotopically organized areas which could explain its response to diverse stimulus modalities and varying anatomical representation when reported in different imaging experiments (Eickhoff et al., 2006).

There are projections from SII to further regions such as the insula and the frontal lobes. Evidence from studies of anatomical projections suggests that the posterior parts of the insula are related mainly to extrapersonal processing such as auditory, visual, and somatosensory functions. The anterior insula is related predominantly to intrapersonal processing such as limbic, olfactory and viscero-autonomic functions (Schnitzler and Ploner, 2000). Hence, the sensory-discriminative aspect of pain is likely processed in the anterior insula whereas the emotional-motivational aspect is processed in the posterior parts. For

example, electric excitation of the posterior insula gives rise to changes of the quality dimension of pain (Ostrowsky et al., 2002). The insula may serve as an integrative region between nociceptive input from SII/thalamus and contextual information in order to relay this information on to limbic structures (Schnitzler and Ploner, 2000). There is emerging evidence that the anterior insula is part of a homeostatic afferent path that represents the physiological condition and the motivational drive that comes with it (Craig, 2003a). These findings could explain how pain, itch and sensual touch is different from somatosensation *per se* and represents the notion that pain is a homeostatic function (Craig, 2003b).

The cingulum is a long structure located along the corpus callosum in the middle of the brain. The cingulum has traditionally been included in the limbic system and is therefore associated with the emotional-motivational aspect of pain. This has been exemplified in studies where lesions of the cingulum reduced the emotional value and motivation to withdraw from the painful stimulus without impairing the detection or intensity of the stimulus (Schnitzler and Ploner, 2000). Especially the ACC has been implicated in pain processing and it receives input primarily from the medial thalamic nuclei (Schnitzler and Ploner, 2000). The ACC is one of the most commonly activated regions when investigating pain using brain imaging techniques (Tracey, 2005). In a neuroimaging study from 1997 Rainville and colleagues confirmed the early findings from lesion studies, demonstrating how the activity in the ACC correlated to ratings of pleasantness and unpleasantness when pain intensity was kept constant (Rainville et al., 1997). Still, the ACC is not a pain-specific structure that only processes the emotional-motivational aspect of pain. The ACC is highly implicated in attentional modulation (Posner, 1994) and suppression of distracters in cognitive tasks is conducted in close connection with the dorsolateral prefrontal cortex (DLPFC) (Medalla and Barbas, 2009). Also, the ACC is involved in detection of conflicting information with further activation of the dorsolateral prefrontal cortex where the conflict can be resolved (Carter and Van Veen, 2007). Moreover, the ACC has been proposed to play an important role in regulation of the autonomic nervous system and homeostatic function (Craig, 2003a). The endogenous homeostatic control mechanism modulates the

integration of afferent activity and is suggested to be highly involved in pain regulation (Craig, 2003b).

3.4 THE PAIN INHIBITORY CIRCUITRY

The descending pain inhibitory circuitry is a highly organized cerebral network that enables regulation of afferent nociceptive information. This modulation is conducted in close reciprocity between cerebral and spinal structures and require activation of brainstem neurons that project to the spinal cord (Basbaum and Fields, 1978). The descending inhibitory system is always part of the normal pain response and involve serotonergic, noradrenergic, and opioidergic inhibitory pathways (Basbaum and Fields, 1978). The possibility of producing endogenous pain relief through stimulation of specific anatomical structures was first found in the 1960's when rats were given analgesia through electrical stimulation of certain discrete locations in the brainstem (Basbaum and Fields, 1978). Since then, the descending pain inhibitory circuitry has been defined in humans and involve the frontal lobes, ACC, insula, amygdala, hypothalamus, Periaqueductal Grey (PAG), nucleus cuneiformis, and the rostral ventromedial medulla (RVM) (Tracey and Mantyh, 2007).

A successful way of activating the descending pain inhibitory system has been through placebo analgesia. Studies where subjects believed that they received a potent analgesic compound, nicely illustrates the brain activity associated with endogenous pain inhibition (Bingel et al., 2006; Petrovic et al., 2002; Wager et al., 2004). In the study by Petrovic and collegues from 2002, endogenous analgesia as well as opiate-dependant analgesia activated the rostral ACC and PAG. The authors point towards a shared neuronal network for pain inhibition via exogenous and endogenous opioids. In the study by Wager et al. from 2004 there was evidence for the involvement of the DLPFC in placebo analgesia (Wager et al., 2004). The prefrontal cortex likely represents a higher order cognitive control of pain inhibition, comparable to the executive role of the DLPFC in cognitive control (Medalla and Barbas, 2009). A cognitive resolution of a pain-related situation could lead to the conclusion that the painful stimulus can not be avoided and should therefore be ignored in favor of a higher goal. The neurobiological correlate to this can be described in terms of activation of the

descending pain inhibitory circuitry (Bingel et al., 2007). In the study by Bingel and colleagues from 2006 there is further clarification of the functional anatomy of descending pain inhibition. In addition to the functional connectivity between the rostral ACC and PAG there was also functional connections to the bilateral amygdalae (Bingel et al., 2006). The amygdala has pronounced projections to the PAG and plays an important role in conditioned learning. The activation of the amygdala in relation to pain is proposed to represent a defensive mechanism that could contribute to the recruitment of descending inhibition (Bingel et al., 2006).

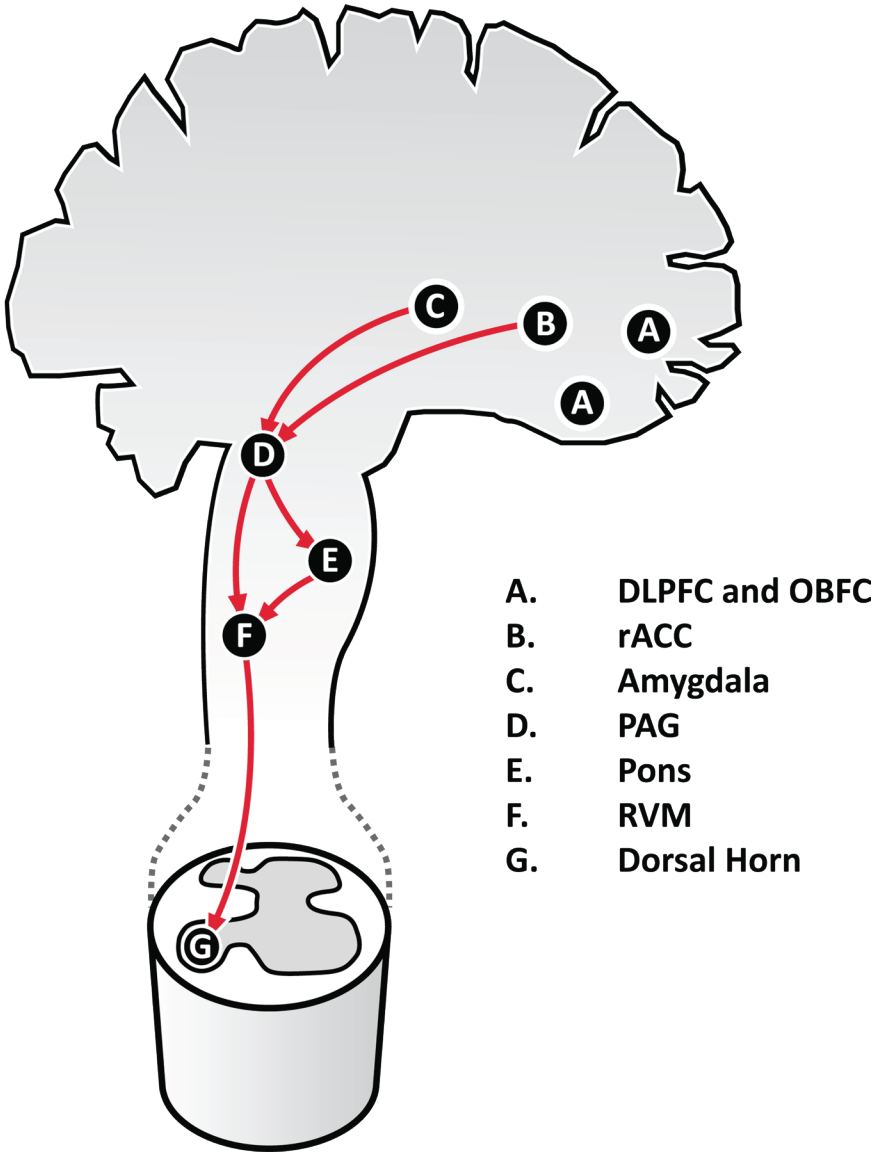


Figure 1. Schematic representation of brain regions involved in descending inhibition of pain.

4 PAIN PATHOLOGY

Pain functions as a warning system but in chronic pain syndromes this state becomes permanent even in absence of any acute tissue damage. The duration of pain can be used in order to divide it into subcategories: pain associated with acute tissue damage, inflammation or a disease process of short duration is referred to as *acute pain* (Turk and Melzack, 1992). This pain typically disappears when the tissue damage or inflammation has healed. Repeated episodes of acute pain with pain-free intervals in between (e.g. migraine) are referred to as *acute recurrent pain* (Turk and Melzack, 1992). When pain persists for extended periods of time, i.e. more than 3 weeks, it is referred to as *chronic pain* (Turk and Melzack, 1992). Chronic pain can accompany a long-term disease process (e.g. rheumatoid arthritis) or be associated with a localized peripheral pathology that does not resolve. Chronic pain can also be present despite any known peripheral pathology, called idiopathic pain, and is often seen in patients with e.g. low back pain. It is important to note that these different definitions are not dependent on the intensity of pain; chronic and acute pain can involve equal intensities of pain.

Persistent pain that affects the muscles, tendons and ligaments along with the bones is called *musculoskeletal pain*. It includes for example neck and back pain and is estimated to constitute a majority of all chronic pain (Gerdle et al., 2004). Fibromyalgia syndrome (FM) is one of the musculoskeletal pain disorders and the pathophysiology of FM was investigated as part of this thesis.

4.1 FIBROMYALGIA SYNDROME

FM is a common pain disorder , estimated to affect 1.3 to 4.8% of the population, out of which 80% are women (Wolfe, 1995). The prevalence of FM across countries in the world is similar and the prevalence among children is estimated to 1.2 to 6.2% (Buskila et al., 1995). This multi-symptomatic pain syndrome is characterized by widespread pain, tenderness, fatigue and disturbed sleep (Wolfe et al., 1990). The diagnosis is based on the American College of Rheumatology (ACR) criteria from 1990 (Wolfe et al., 1990) and requires widespread pain as well as pain at palpation of at least 11 out of 18 locations of the body. FM patients typically exhibit painful responses to stimuli that are perceived as non-painful to

others (allodynia) and respond with strong pain to stimuli normally perceived as low in pain (hyperalgesia) (Kosek et al., 1996). In addition, patients with FM experience spontaneous pain, which means there is widespread pain that is not due to any mechanical provocation. The pain experienced in FM is persistent, resulting in few pain-free intervals (Henriksson and Liedberg, 2000). The location of the most painful regions of the body typically change over time but there is often one pain-free area or at least one area with very low pain intensity (Kosek, 2006). Mostly, the pain is located in muscle tissue and it is intensified during and following physical activity (Kadetoff and Kosek, 2007).

Long-term follow-up of patients diagnosed with FM demonstrate a chronic state of recurrent periods of exacerbated symptoms and low probability of full remission (Bengtsson et al., 1994).

4.2 PATHOPHYSIOLOGY OF FIBROMYALGIA

As for many other disorders the pathophysiology of FM is not fully understood and the diagnosis is based merely on the patient's subjective report of pain and tenderness (Wolfe et al., 1990). This has led to a large discussion about the validity of FM as a clinical entity (Cohen, 1999) where critics fear that the diagnosis represents a medicalization of ordinary psychosocial problems, resulting in unwarranted economical burdens to society. More specifically, there have been speculations about an exaggerated emotional response in FM patients, suggesting that the disorder is caused by psychological vulnerability (Ehrlich, 2003). However, empirical data show that there is no increased affective modulation in FM patients (Arnold et al., 2008; Petzke et al., 2005) and results from clinical trials conclude that the analgesic effect and positive effect on mood are unrelated when FM symptoms are treated with antidepressants (Arnold et al., 2005; Russell et al., 2008) and anticonvulsants (Arnold et al., 2007). In contrast to the fear of the critics, evidence show that health care costs have been reduced following the diagnosis of FM (Hughes et al., 2006).

Peripheral pathology in FM includes findings of muscle ischemia (Henriksson, 1999) which is possibly caused by a deficient regulation of muscle blood flow during physical activity (Elvin et al., 2006). The deficient muscle blood flow and

the exercise intolerance is likely caused by a dysregulation of the autonomic nervous system seen in FM; i.e. increased sympathetic activity at baseline and lower reactivity during exercise and stress (Cohen et al., 2001). This could contribute to peripheral sensitization of nociceptive neurons. Increased signaling from sensitized nociceptors in the muscles has been suggested to initiate and maintain further dysfunction in the central nervous system (Staud, 2004). This has recently gained objective support from imaging studies showing enhanced transmission and/or processing of nociceptive input in FM patients (Gracely et al., 2002). In addition, abnormal concentrations of transmitter substances implicated in pain regulation have been found in the cerebrospinal fluid of FM patients, which is in accordance with a central nervous system dysfunction. Evidence includes decreased levels of serotonin, noradrenalin and dopamine (Russell et al., 1992) and elevated concentrations of substance P (Russell et al., 1994), glutamate, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (Sarchielli et al., 2007).

At present, there are several studies implicating involvement of Central Nervous System (CNS) pathology in FM (Gracely et al., 2002; Kuchinad et al., 2007; Mountz et al., 1995; Staud and Domingo, 2001) including evidence for greater temporal summation of pain in FM patients (Staud, 2001), stronger pain intensities and larger referred areas (Sörensen et al., 1998) suggesting that central processing of pain is facilitated. Evidence for central dysfunction in FM was first found in behavioral experiments, e.g. patients with FM exhibited allodynia, hyperalgesia (Kosek et al., 1996) and a dysfunction of endogenous pain inhibitory mechanisms (Kosek and Hansson, 1997; Lautenbacher and Rollman, 1997). In studies where repeated painful and nonpainful stimuli were presented to FM patients and healthy controls, FM patients did not increase their pain thresholds after presence of pain as compared to healthy controls (Kosek et al., 1996; Lautenbacher and Rollman, 1997). Moreover, aerobic exercise has been shown to decrease levels of pain after sensitization in healthy controls, but not in FM patients, suggesting an impaired inhibition of pain and lack of ability to activate endogenous analgesia (Vierck et al., 2001).

A study from 2007 compared the cerebral anatomy of FM patients and healthy controls using morphometric analysis of magnetic resonance images of the brain (Kuchinad et al., 2007). Results showed that FM patients had significantly less total gray matter volume and showed a 3 times greater age-associated decrease in gray matter than healthy controls. The duration of FM was associated with findings of lower gray matter density, indicating decreased gray matter with each year of having the FM diagnosis. More specifically, FM patients demonstrated less gray matter in the cingulate, insular and medial frontal cortices and parahippocampal gyri.

In a pioneering study from 1995, Mountz and colleagues (Mountz et al., 1995) used an early brain imaging technique called Single Photon Emission Computed Tomography (SPECT) to investigate regional cerebral blood flow (rCBF) in FM patients and healthy controls during rest. FM patients demonstrated decreased activation in the bilateral thalami and caudatus nuclei, compared to controls. This result was partly reproduced in a study from 2000 where reduced rCBF was found in the right thalamus as well as in the inferior dorsal pons and in a partial region of the right lentiform nucleus (Bradley, 2000). In 2002, functional magnetic resonance imaging (fMRI) was used to investigate active pain responses in FM patients and healthy controls during a pressure-pain paradigm (Gracely et al., 2002). Results demonstrate that FM patients perceived higher levels of pain compared to healthy controls in response to standardized pressure stimuli and concomitant higher levels of brain activity in 13 brain regions. Regions of augmentation included the primary and secondary somatosensory cortices, inferior parietal lobe, ACC, anterior insula, superior temporal gyrus, and the cerebellum. Reduced activity in the thalamus during painful pressures in FM patients was also observed. The results by Gracely et al. were further validated in a study by Cook et al. in 2004, demonstrating decreased thalamic activity and augmented pain processing in response to both painful and non-painful heat stimuli. The most pronounced differences between FM patients and controls were seen in the anterior insula, pre-motor cortex, PFC, and ACC (Cook et al., 2004). Results from these two studies provide evidence for altered central physiology in patients with FM.

Despite findings of central involvement in FM, the pathophysiology in FM is most likely explained by a complex interaction between peripheral and central mechanisms (Kosek, 2006). For example, the total disappearance of pain in an anaesthetized part of the body following an epidural injection of anesthetics (Bengtsson et al., 1989) excludes supraspinal mechanisms as the sole cause of pain in FM. In addition, it is well established that the presence of long term localized pain is the strongest precursor of FM (Burckhardt et al., 1995) and in comparison with localized pain syndromes such as chronic low back pain there seems to be some common features, e.g. FM seem to involve a comparable level of augmented pain processing as seen in patients with low back pain (Giesecke et al., 2004).

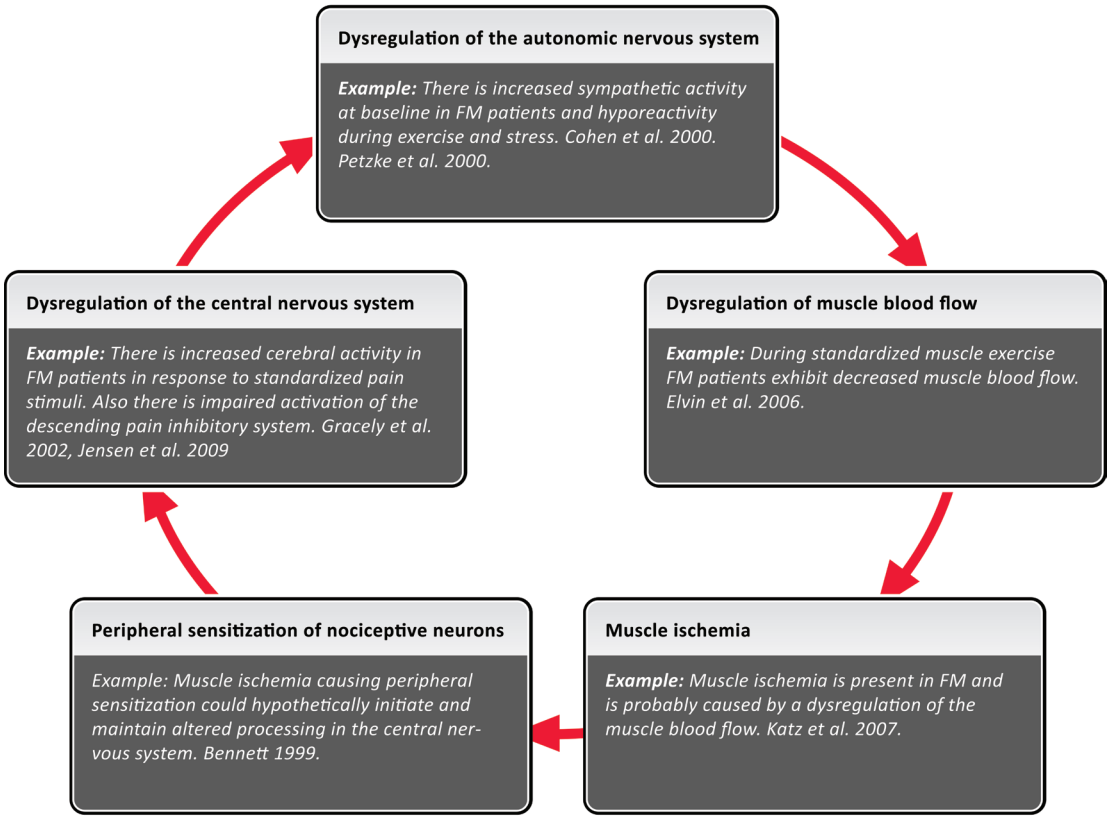


Figure 2. Suggestion of the possible relationship between different findings of altered physiology in patients with FM. The above described findings are not sufficient for explaining why certain individuals develop FM; the pathophysiology of FM has to be considered in the light of contextual factors, genetic vulnerability and the patients' medical history etc.

4.3 TREATMENTS FOR FIBROMYALGIA

The increased understanding of the pathophysiological mechanisms in FM has lead to the development of new treatment strategies (Carville et al., 2008). The diagnosis and labeling of the symptoms has beneficial influence on the progress of the disorder and thereby reduces health care consumption (Hughes et al., 2006). It is thus advisable for health care providers to properly inform the patient about the disorder and its symptoms.

Patients with FM often experience accentuated pain after physical exercise and in line with that it has been shown that treatment involving high intensity training is not effective in this patient category (van Santen, 2002b). In fact, one study where FM patients were treated with high intensity training was stopped due to the incapacity to complete the program (van Santen, 2002a). Low intensity training and improvement of the physical condition on the other hand, has shown positive effects on pain intensities, spread of painful areas, tenderness and psychological well-being (Meiworm, 2000).

Two main categories of drugs have documented pain relieving effects in FM, antidepressants (Arnold et al., 2005; Vitton et al., 2004) and anticonvulsants (Arnold et al., 2007; Crofford et al., 2005). The mechanism of action for the positive treatment outcome in FM is still not known. It has been proven, however, that the analgesic effect of the antidepressants is not attributable to an increased psychological well-being (Vitton et al., 2004), i.e. the antidepressant and analgesic effects are independent of each other. Recently, the anticonvulsant *pregabaline* (2007) and the antidepressants *duloxetine* (2008) and *milnacipran* (2009) received the indication of FM treatment in the U.S.A. The European authorities did not approve the indication FM for any of these drugs.

Previous research in patients with musculoskeletal disorders indicate that treatment initiatives including cognitive behavioral therapy (CBT) has a positive effect on physical functioning and return to work (Johansson, 1998). The cognitive behavioral model of pain is characterized by the notion that pain is a complex experience that is not only influenced by its underlying pathophysiology, but also by an individuals' cognitions, affect, and behavior (Keefe and Gil, 1986). In CBT

treatment, the impact of avoiding behaviors is emphasized when explaining the disability related to chronic pain (Fordyce, 1976). In acute pain, it would be considered adaptive to avoid situations that could lead to pain or distress. In chronic pain, however, it could lead to a short-term relief/reinforcement that could gradually develop into long-term disability through decreased functioning without any corresponding decrease of pain symptoms. Pain related fear could also play an important role in creating avoidance and disability, therefore CBT includes identification and challenging of overly negative pain-related thoughts and help replace these thoughts with more adaptive, coping thoughts (Keefe, 1996). CBT consequently includes homework assignments where the patient is offered to try out new ways of relating to their problems, e.g. acceptance and exposure (Wicksell et al., 2008).

5 BRAIN IMAGING

Since the advent of functional brain imaging techniques our knowledge about the link between behavior and the brain has advanced (Frackowiak et al., 2004). There are several different techniques for measuring brain activity and they all have their own set of strengths and weaknesses. Therefore, they are not in competition but complement each other depending on what type of mental activity that is investigated. Often, the choice of brain imaging technique offers either high temporal or spatial resolution. This means that the questions of *when* or *where* a specific brain activation occurs are best answered by different technical approaches. Commonly used brain imaging techniques are Electroencephalography (EEG), magnetoencephalography (MEG), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI).

5.1 FUNCTIONAL MAGNETIC RESONANCE IMAGING

Functional brain imaging using a conventional magnetic resonance imaging (MRI) scanner was first accomplished in 1991 at the Mass General Hospital Imaging Center (Harvard) (Frackowiak et al., 2004). Since then the technique has developed into one of our most commonly used tools to investigate the link between behavior and processes in the brain. The technique is non-invasive, possible to perform many times in the same subject and very accessible since MRI scanners are to be found frequently in hospitals around the world. The temporal resolution of fMRI is inferior to what can be obtained using EEG and MEG. However, fMRI can be used to measure brain activity from structures deep inside the brain and the overall spatial resolution is superior to any other imaging technique.

In clinical use of the MRI scanner the images are created from the fine differences in density of water protons of different tissues. The body part to be investigated, e.g. the brain, is placed in the magnetic field of the scanner. By sending in a pulse of radiofrequency into the magnetic field, the tissue gets excited and the water protons change their positions from the original alignment parallel to the strong magnetic field. Slowly after the pulse, all protons recover to their original positions aligned with the magnet. Different tissues contain different densities of

water and will therefore recover with different speed. The energy produced from the recovery of the protons is detected by a signal antenna surrounding the body part in the scanner. Ultimately this signal is translated into an image with clear visual distinction between different tissues.

When measuring brain activity the relationship between increased neural activity and concomitant increase of energy requirements in a given brain region is fundamental. This means that an increase of blood flow in a given brain region is an indirect measure of increased neural activity. In fMRI, the so called blood oxygen level dependent (BOLD) effect is used to obtain information about neural activity in the brain (and possibly also in the spinal cord). The BOLD effect relies on the difference in magnetic properties of oxygenated and deoxygenated heamoglobin in the blood. Oxyhaemoglobin is practically not magnetic at all and deoxyheamoglobin is paramagnetic which means that the deoxygenated blood will affect the alignment of protons leading to a relatively lower signal from the recovering protons. Since the oxygen consumption of an active brain region is coupled by an increase of fully oxygenated blood, active regions will generate a relatively higher signal, i.e. an increase of BOLD signal . In order to require BOLD sensitive images with high temporal resolution, high speed collection through echo-planar imaging (EPI) is used. EPI images are acquired by collecting one whole slice image for every excitation pulse. This leads to fast acquisition but unfortunately also to loss of image quality in terms of a higher sensitivity to inhomogenity-and susceptibility artefacts. This drawback is addressed in the preprocessing of the fMRI images.

5.2 PROCESSING OF FMRI DATA

The fMRI images always include noise such as head movement and image artefacts and therefore the images need to be preprocessed before the statistical analyses. The usual steps include motion correction, spatial normalizing and spatial smoothing.

The motion correction step, called realignment, is a post-hoc adjustment of the scans built on stepwise mathematical estimations of movement over the time-series. During the fMRI experiment the subject is instructed to lie very still, yet

there is always some movement in the range of a couple of millimeters. The goal is to have all scans within one subject occupying the same space in order to assure homologous anatomical placement over the time series.

The spatial normalization step deals with the problem that all subject's brains deviate anatomically. By normalizing the scans they are moved and warped to fit the template of a standard brain. Data from different subjects can then be used for intersubject averaging (Frackowiak et al., 2004). The template used for the fMRI data in this thesis is the Montreal Neurolocial Institute (MNI) template.

After spatial normalization there is still some anatomical variability between subjects and spatial filtering is one way to reduce that. Spatial smoothing comprises convolving of the data using a Gaussian kernel which means that each voxel is replaced by an average of that voxel plus the surrounding voxels. If the spatial scale of the kernel matches the natural variability of functional anatomy in the population, a minimum of spatial information is lost. Another objective of spatial smoothing is that it increases the signal to noise ratio of data. The physiological effects used to investigate neural activity are expressed over spatial scales of several millimeters whereas noise typically has higher spatial frequencies. The spatial filter will therefore increase the signal to noise ratio by favoring physiological effects over noise (Frackowiak et al., 2004).

5.3 STATISTICAL ANALYSES OF FMRI DATA

The fMRI data is modeled in order to divide the observed physiological changes into components that correspond to the conditions of the experiment. The models created for statistical inference of fMRI data often depend on the general linear model. The general linear model is an equation that can be used to link what we observe (the data) to what we expected to see (the model of our experiment) by expressing the observations as a linear combination of expected components and residual error. The statistical model is then estimated for every voxel in the brain and assesses the differences in regionally specific effects in the brain for the different conditions. The data can be viewed as a large set of time-series, one for each voxel, where the different conditions will have separate tentative time-series to estimate. The general linear model assumes that the

errors are independent and normally distributed, assuming equal error variance across subjects and conditions of the experiment (Frackowiak et al., 2004). The specific statistical models used to analyze the imaging data of this thesis can be found in the original articles provided at the end of the thesis.

6 PAIN AND GENETICS

The investiagtion of specific genetic contributions to pain regulation was first enabled in the early 2000's when the human genome sequence was completed (Venter et al., 2001). Since then, a major goal has been to identify the common variations in this sequence that have a functional impact on human bioloy and brain function, i.e. genetic polymorphisms (Hariri and Weinberger, 2003). Today, there is evidence that the individual differences in pain sensitivity is partially explained by genetic factors with impact on the neurochemical systems within the central nervous system (Diatchenko et al., 2006; Kim et al., 2006b). Also, the individual differences in response to analgesic drugs (Kosek et al., 2009; Rakvag et al., 2008) and risk of developing chronic pain (Buskila et al., 2007) can be related to similar mechanisms.

The specific genetic variations that could contribute to differences in pain sensitivity have been investigated in animals (LaCroix-Fralish et al., 2007) as well as in humans (Fillingim et al., 2008). In humans, genetic variations related to differences in pain perception have been found in, for example, a polymoprhism of the μ-opioid receptor gene OPRM1 (Fillingim et al., 2005), serotonin related polymorphisms (Bondy et al., 1999; Kosek et al., 2009), a Brain Derived Neurotrophic Factor polymorphism (Merighi et al., 2008) and the Catechol-O-Methyl Transferase (COMT) val^{158}met polymorphism (Diatchenko et al., 2005; Zubieta et al., 2003a). There is recent evidence for joint effects of different functional polymorphisms on complex behaviors such as anxiety (Lonsdorf et al., 2008) and pain regulation (Reyes-Gibby et al., 2007). However, the number of studies are scarce and there is need for future studies with closer investigation of the interaction between different functional polymorhisms.

The COMTval^{158}met polymorphism has previously been associated with several aspects of frontal lobe functions, for example working memory (Tan et al., 2007) and emotional regulation (Drabant et al., 2006; Herrmann et al., 2009). The descending pain defense system is associated with frontal lobe function and is partly modulated by catecholamines (Basbaum and Fields, 1978), i.e. noradrenaline and dopamine. The function of the catecholaminergic systems is

genetically influenced by the activity of the catecholamine breakdown enzyme COMT (Lotta et al., 1995). A single-nucleotide polymorphism in the coding region of its gene (COMTval^{158}met, rs4680) controls the enzyme activity and there are three possible genotypes of this polymorphism: met/met, met/val and val/val. The breakdown of dopamine and noradrenaline is up to 4 times higher for the valine allele compared to methionine, resulting in different levels of synaptic dopamine/noradrenalin following neurotransmitter release (Lotta et al., 1995). In studies of the COMTval^{158}met polymorphism and experimental pain the low expressing met-allele has been implicated in increased pain sensitivity during sustained and repeated pain stimulation but not following single pain stimuli compared to individuals homozygous for the val-allele (Diatchenko et al., 2006; Kim et al., 2006a; Zubieta et al., 2003b). Also, studies using PET imaging found lower response of the μ-opioid system during sustained pain stimulation in met/met individuals compared to heterozygotes, accompanied by higher sensory and affective ratings of pain (Zubieta et al., 2003b). Differences in response to opioid drugs for the different COMTval^{158}met genotypes was previously observed in cancer patients (Rakvag et al., 2008; Rakvag et al., 2005; Reyes-Gibby et al., 2007; Ross et al., 2008) indicating that COMTval^{158}met could be associated with opioid mediated inhibition of pain. The COMTval^{158}met polymorphism has been implicated in chronic pain states (Buskila et al., 2007) and there is evidence for an overrepresentation of the low expressing COMT genotype in patients with FM (Gürsoy et al., 2003). In conclusion with previous findings of decreased μ-opioid respone to pain in low expressing met/met individuals by Zubieta et al., FM patients exhibited equally low μ-opioid respone in several brain regions known to play a role in pain modulation, including the nucleus accumbens, the amygdala, and the cingulate cortex (Harris et al., 2007).

7 AIMS

The aim of this thesis was to investigate the cerebral mechanisms involved in pain modulation in relation to four clinically relevant questions. Hence, there were four different approaches and four specific aims in this thesis:

- To characterize the pain regulatory system of the brain in patients with FM and compare to healthy controls.

- To investigate the impact of depressive symptoms, anxiety and catastrophizing on cerebral processing of pain in FM patients.

- To characterize what distinguishes FM patients with a positive response to treatment with a Noradrenaline-Serotonin Reuptake Inhibitor (NSRI) milnacipran as opposed to non-responders and placebo responders.

- To investigate the specific genetic influence of COMTval^{158}met on the differences in response to pain and treatment with a strong opioid drug (remifentanil) in healthy controls.

8 METHODS

8.1 PARTICIPANTS

Patients with FM were recruited as part of a pharmacological multicenter study including three sites; one in England, Sweden and Germany, respectively. In total, 157 female FM patients were screened and 92 fulfilled the inclusion criteria and were included in the pharmacological study; age 25 to 55 years, mean age 44 (SD = 8.2). Patients eligible for inclusion were female patients, aged 18 – 55 years, fulfilling the ACR 1990 classification criteria for FM (Wolfe et al. 1990) and with a self reported average pain intensity of at least 40 mm on a 100 mm visual analogue scale (VAS). Exclusion criteria were e.g. presence of severe psychiatric illness, significant risk of suicide, a history of substance, drug or alcohol abuse as well as significant cardiovascular disease.

Healthy controls for fMRI were recruited via advertising at the three different sites included in the pharmacological study. In total 16 female controls aged 24 to 48 years, mean age 33 (SD = 8.0) were enrolled. In order to control for the considerable age-difference between the groups and achieve a balanced design, the 16 healthy controls were matched on age with 16 patients from the same site, i.e. every control subject was matched with the patient closest in age from the same lab.

Healthy controls for the genetic study were recruited via advertising in Stockholm. To meet the inclusion criteria subjects had to be over 18 years old, right-handed, take no medications (female subjects were allowed to take contraceptive pills), have no history of drug abuse, chronic pain or psychiatric disorders. Subjects were recruited from a variety of institutions in order to represent all sorts of educational and professional backgrounds. In total 12 men and 31 women were enrolled, range 18-42 years, mean age 26 (SD = 5).

8.2 PAIN ASSESSMENTS

Experimental pain is given in order to investigate the response of the subject. Most studies apply pain and then ask for the subjects' subjective report of pain, using a visual analogue scale (VAS). The subject's mark on the VAS is then

measured and used as an estimate of *subjective* pain response. In brain imaging studies, the cerebral response to experimentally induced pain is estimated and used as an *objective* measure of pain response.

The most extensively used method to measure pain is probably the visual analogue scale; the VAS. The VAS is a continuous line anchored by the expressions "no pain" to the left and "worst imaginable pain" to the right. The subject or patient will be asked to rate his/her pain by putting a mark on the VAS. Since pain is (by definition) subjective this is often a valid way to find out how much pain a person is feeling. The VAS was used in all studies in the present thesis.

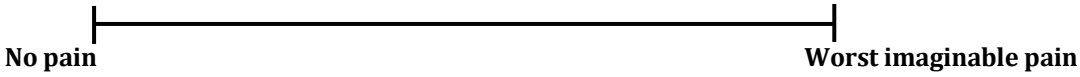


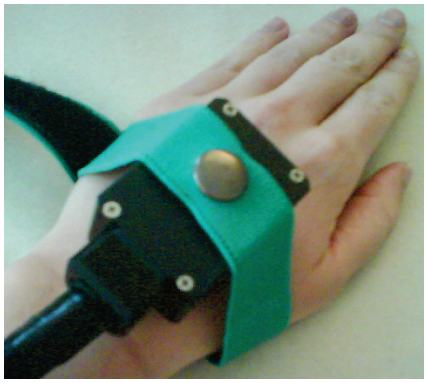
Figure 3. Example of a paper-and-pen VAS line. E.g., the subject or patient is asked to put a mark on this scale in response to a given stimulus or in response to spontaneously ongoing pain in the body, depending on the instruction.

Pressure pain thresholds on different sites of the body were assessed using a pressure algometer (Somedic Sales AB, Hörby, Sweden). An algometer is a hand held pistol-like apparatus with a 1 cm^2 hard rubber probe. It was held at 90 degree angle against the body and then pressed with a steady rate of pressure increase (30kPa/second) in order to induce pain. Pressure algometry was used in study I-III because it is a clinically valid stimulation that allows for a semi-objective quantification of the sensitivity to pressure. Tenderness to pressure is a diagnostic criterion for FM and previous studies have shown that assessments at a limited number of sites of the body give a good estimate of the overall pain sensitivity.

The method for inducing experimental pain in study I-III was pressure pain. All stimulations were applied to the thumbnail using an automated, pneumatic, computer-controlled stimulator with a plastic piston that applies pressure via a 1

cm^2 hard rubber probe. The thumb was inserted into a cylindrical opening and positioned so that the probe applied pressure to the nail bed. Each pressure lasted for 2.5 seconds with at least 30 seconds interval. Pressure pain was chosen as stimulation modality for study I-III because it elicits a deep pain sensation that is clinically valid in the group of patients investigated in these studies. Tenderness to pressure is a diagnostic criterion for FM.

In study IV heat pain was repeatedly induced to different locations of the dorsum of the hand using a 3x3 cm heat probe (Medoc TSA, Medoc, Israel). Hence, no part of the skin was affected by the heat probe more than once as to prevent increased sensitivity to the pain stimulus by means of local mechanisms. The order of the different positions on the hand was counter-balanced and in total five blocks of 30 seconds of tonic heat (48° Celsius) was administered. There was at least a 10 minutes pause between all pain stimuli.



8.3 FMRI EXPERIMENT

The subjects were placed in the scanner with the thumb pressure device attached to their right thumb. No visual or auditory presentation was given during the experiment. Two different types of stimulations were used during scans: individually calibrated painful pressure representing each subject's 50mm VAS, and a non painful pressure perceived as light touch, representing 0mm VAS. All stimulations were randomly jittered over the scanning time, preventing subjects from anticipating the onset time and event type. The time interval between consecutive events was randomized with a mean stimulus onset asynchronicity of 15 seconds (range 10-20 seconds). Four different random sequences of jitter were created; A, B, C and D. Each subject received all four sequences but the particular order of sequences was randomized for each subject. The total duration of the scans was approximately 35 minutes. Before scanning subjects were instructed to

focus on the pressures on the thumb and to not use any distraction or coping techniques. They also received information about the sequence paradigm which means they were aware that the order of pressures and type of pressure was going to be randomly presented over the scanning time.

8.4 SELF-REPORT QUESTIONAIRRES

A commonly used strategy to find out about subjective measures is the use of questionnaires. The following questionnaires measuring different aspects of psychological- and psychical health parameters were used in all studies. The FM-specific questionnaire was administered only in study II and III.

The Beck Depression Inventory (BDI) was used to quantitatively assess the depressive symptoms in patients. The BDI is a 21-item measure of the severity of current depressive symptoms, and it has been extensively validated for use with both medical and mental health populations (Beck et al., 1961). Scoring allows for the identification of mild, moderate, and severe levels of depressive symptoms. The BDI does not provide information about a possible major depressive disorder (MDD) according to the Diagnostic and Statistical Manual of psychiatric disorders (DSM-IV). Instead it gives a quantified measure of the degree of depressive symptoms.

The Spielberger Trait-State Anxiety Inventory (STAI) was used in order to assess the participants' levels of state anxiety. STAI is a self-report questionnaire with two independent 20-item scales for measuring state-related or trait-related anxiety (Spielberger, 1983).

The Coping Strategies Questionnaire (CSQ) was used in order to assess levels of catastrophizing thoughts about pain; a parameter commonly described in studies of chronic pain. The CSQ is a self-reported measure of cognitive and behavioural responses utilized to cope with chronic pain and patients are asked to rate the frequency with which they use each strategy on a 7-point scale (Rosenstiel and Keefe, 1983).

The Short Form 36 (SF-36) is a well-established questionnaire measuring eight domains of health status: physical functioning, role limitations because of physical problems, bodily pain, general health perceptions, energy/vitality,

social functioning, role limitations due to emotional problems, and mental health (Ware and Sherbourne, 1992).

Fibromyalgia Impact Questionnaire (FIQ) is a 20-question questionnaire that assesses the overall symptom severity in patients with FM (Burckhardt et al., 1991).

The Patient Global Impression of Change (PGIC) is a 7 point scale measuring the patient's subjective report of clinical improvement in relation to a given treatment. PGIC can be used as a dichotomous scale where point 5-7 means that there was a positive treatment response and 1-4 represents no response to treatment (Hurst and Bolton, 2004).

8.5 PHARMACOLOGICAL SUBSTANCES

In study III and IV there was pharmacological treatment of pain using two different drugs. In study III included treatment of patients with FM using a substance called milnacipran. In study IV there was experimental treatment with a fast acting opioid called remifentanil in healthy controls who received pain stimuli to the hand.

Milnacipran is the first of a new class of agents known as NSRI's, or Noradrenaline Serotonin Reuptake Inhibitors. This type of substance is marked by its preference for noradrenaline reuptake inhibition over serotonin at a 3:1 ratio. This typical preference distinguishes NSRI's from Selective Serotonin Reuptake Inhibitors (SSRI's) that have limited effect in FM patients.

Remifentanil is an opioid drug acting on μ-type receptors. It has a rapid onset of action and a very short duration. Remifentanil is often used in pain experiments because of its effectiveness in acute nociceptive pain.

9 SUMMARY OF STUDIES I-IV

9.1 STUDY I - IMPAIRED INHIBITION OF PAIN IN FM PATIENTS

This study compared the cerebral response during pain in 16 patients with FM and 16 matched controls. Brain activity was measured using fMRI during individually calibrated painful pressures.

Patients exhibited higher sensitivity to pain provocation than controls as they required less pressure to evoke equal pain magnitudes. Despite lower pressures applied in patients at VAS 50mm, the fMRI-analysis revealed no difference in activity in brain regions relating to attention and affect or regions with sensory projections from the stimulated body area. However, in the primary link in the descending pain regulating system, the rostral ACC (rACC), the patients failed to respond to pain provocation.

The attenuated response to pain in this brain region is the first demonstration of a specific brain region where the impairment of pain inhibition in FM patients is expressed. These results validate previous reports of dysfunctional endogenous pain inhibition in FM and advance the understanding of the central pathophysiologic mechanisms, providing a new direction for the development of successful treatments in FM.

9.2 STUDY II – PAIN PROCESSING IN FM IS UNAFFECTED BY MOOD

In this study the differential impact of depressive symptoms, anxiety and catastrophizing thouhgts on clinical pain ratings and brain processing was investigated in patients with FM.

Female FM patients (n=92), mean age 44.2 (SD=8.2), fulfilling the ACR-1990 diagnostic criteria participated. Patients rated pain intensity (VAS), severity of FM symptoms (FIQ), general health status (SF-36), depressive symptoms (BDI), anxiety (STAI) and catastrophizing (CSQ). Experimental pain was induced to the thumb using a computer-controlled stimulator. Functional magnetic resonance imaging (fMRI) was performed during individually calibrated painful stimuli representing 50 mm on a 100 mm VAS, as well as non-painful pressures. The

stimulations were randomly jittered over the scanning time preventing subjects from anticipating the onset time and event type.

A correlation analysis including results from all self-ratings showed that depressive symptoms, anxiety and catastrophizing scores were correlated, but did not correlate with ratings of clinical pain, nor with sensitivity to pressure pain. However, the subjective rating of general health was negatively correlated with ratings of depressive symptoms and anxiety. Results from imaging analyses using self-rated psychological measures as co-variates, showed that brain activity during experimental pain in FM patients was not modulated by depressive symptoms, anxiety, or catastrophizing. This data provide evidence for two segregated neurofunctional mechanisms dealing with pain and negative affect in FM patients.

9.3 STUDY III – PHYSIOLOGY OF PLACEBO AND MILNACIPRAN RESPONSE

The aim of this study was to investigate the differences underlying a positive treatment outcome for FM patients taking milnacipran or placebo. 92 female FM patients took part in a 13-week, multicenter, double-blind, placebo controlled, randomised trial assessing the effect of milnacipran 100 mg b.i.d. Positive treatment outcome was defined by the patient's subjective rating on the PGIC improvement scale. Those who rated an improvement were defined as treatment responders.

Milnacipran responders (n=21) had a shorter duration of widespread pain compared to non-responders (n=23), while no differences in ratings of depressive symptoms, anxiety or catastrophizing was seen between the groups. Following treatment, pain intensity decreased and sleep and fatigue improved in both milnacipran and placebo responders. Pressure tolerance (measured on the thumb) increased in milnacipran responders, but not in placebo responders nor in milnacipran non-responders. Also, there was a positive correlation between decreased sensitivity to stimulus-induced pain on the thumb and decreased intensity of spontaneous ongoing pain in milnacipran responders.

The results from this study suggest that early treatment of FM patients with milnacipran might increase the response rate for this particular drug. The analgesic effects of milnacipran in FM are mediated by decreased sensitivity to stimulus induced pain, either by direct antinociceptive effects and/or by the strengthening of the endogenous pain inhibitory mechanisms.

9.4 STUDY IV – GENETIC CORRELATES TO PAIN SENSITIVITY

The aim of this study was to assess the impact of a functional genetic polymorphism (COMT val^{158}met) on sensitivity to repeated pain stimuli and the injection of an opioid drug. In line with previous findings of increased pain sensitivity and lower μ-opioid activation in met/met carriers we hypothesized that these individuals would exhibit higher pain sensitization and opioid-induced hyperalgesia in response to repeated pain stimuli and the opioid drug.

Participants were 43 healthy subjects who went through an experiment where five blocks of pain were induced to the hand using a heat probe. After each stimulus subjects rated the pain on a VAS from 0mm (no pain) to 100mm (worst imaginable pain). Before the second stimulus there was an intravenous injection of a rapid and potent opioid drug.

At baseline there was no difference in pain ratings between the COMTval^{158}met genotypes. However, a repeated measures analysis for all five stimuli revealed a main effect for COMTval^{158}met genotype. Met/met individuals reported significantly more pain compared to val/val. Interestingly, a pairwise comparison between baseline and the opioid intervention demonstrated that analgesia was effectively induced in all groups without separating different genotypes. This suggests that the initial response of the descending pain system is not influenced by the COMTval^{158}met polymorphism but when the system is challenged the difference is revealed. An important clinical implication of this may be that the COMTval^{158}met related differences may be more expressed in individuals where the inhibitory system is already challenged and sensitive, e.g. chronic pain patients. This has to be proven in future studies where the impact of the COMTval^{158}met polymorphism on opioid treatment in patients is addressed.

10 GENERAL DISCUSSION

All four studies in this thesis investigate different factors that contribute to descending inhibition of pain. It has been suggested that patients with FM exhibit impaired inhibition of pain and the correlating cerebral mechanisms were investigated in Study I. Firstly, results from Study I suggest that subjectively calibrated painful stimulations in FM patients and healthy controls have equal cerebral representations in regions attributed to sensory-discriminative and emotional-motivational aspects of pain (Jensen et al. 2009b). This speaks against hypervigilance or augmented affective modulation of pain as plausible pathophysiological mechanisms in FM. This was interpreted as a validation of the response style and subjective report of pain in FM patients; demonstrating that it does not differ from controls.

Secondly, patients failed to activate the rACC during pain, confirming previous evidence for impaired inhibition of nociceptive input in FM. The rACC is well known for its involvement in descending inhibition of pain and the regions in the brainstem which are known to depend on the rACC displayed a corresponding abnormality in response to pain. The pain inhibitory network is always part of the normal pain response and constitutes an essential role for regulation of nociceptive input. Results from the present study demonstrate that the homeostatic relation between afferent input of nociceptive signals and descending inhibition is out of balance in FM. The pain inhibitory network is partly mediated by serotonergic, noradrenergic, and opioidergic pathways and it has been shown that treatment with SNRI's is effective in treatment of FM. Furthermore, it is known that the positive treatment outcome of SNRI's is not attributable to a general effect on mood but to reduction of FM related symptoms such as pain and fatigue (Arnold et al., 2005; Vitton et al., 2004). There is need for better understanding of the mechanisms involved in successful treatment with SNRI's in patients with FM. It seems likely, however, that there is impact on the noradrenergic pain inhibitory system, resulting in reduction of some aspects of FM pathophysiology and concomitant reduction of FM symptoms.

In order to explore the possible physiological mechanisms involved in response to SNRI drugs, Study III involved investigations of FM patients treated with either milnacipran or placebo. The patients rated their level of treatment outcome subjectively, using the PGIC scale (described in Chapter 8). The different outcome measurements used in the drug trial were then compared in order to distinguish milnacipran from placebo response. When comparing milnacipran responders to milnacipran non-responders there was only one significant factor that would determine treatment outcome: duration of pain symptoms. Results showed that milnacipran responders had a shorter duration of widespread pain compared to non-responders (unrelated to age). This indicates that early recognition of the FM diagnosis and initiation of treatment will increase the chances for symptom reduction with SNRI drugs and possibly even lead to a full remission in certain patients. This result also suggests that the pathology in FM could be increasingly rooted with time leading to a more pronounced central dysfunction (i.e., facilitation/central sensitisation/disinhibition) which could counteract the antinocieptive effects of milnacipran.

When comparing placebo responders to milnacipran responders we found that the average stimulus pressure corresponding to pain ratings of 50mm VAS increased selectively in milnacipran responders. This means that patients in both the placebo and the milnacipran group would report a general improvement of their health whereas only those in the milnacipran group exhibited decreased sensitivity to experimental pain. Our finding indicates that there is an antinociceptive effect of milnacipran in those who respond favourably to the treatment. The reduced pressure pain sensivitity seen in milnacipran responders is therefore not just an unspecific effect related to increased well-being. Furthermore, it is not an unspecific effect seen in all patients treated with milnacipran. It probably reflects direct antinociceptive effects and/or strengthening of the endogenous pain inhibitory mechanisms in milnacipran responders.

As expected, psychological factors such as depression, anxiety and catastrophizing at baseline did not differ between milnacipran responders and

non-responders. This is in accordance with previous studies showing that positive treatment outcomes in FM patients treated with antidepressants was unrelated to the degree of depression and anxiety at baseline as well as the treatment effect on mood. Interestingly, the placebo responders were characterized by significantly lower ratings of depression and catastrophizing compared to placebo non-responders and had a tendency to lower ratings of depression and catastrophizing also compared to milnacipran responders. This suggests that the recruitment of endogenous analgesia and concomitant symptom reduction is helped by low ratings of negative mood. It is possible that there is segregation between the nociceptive processes in FM and negative mood symptoms, reflected in the lack of effect of milnacipran on depression, anxiety and catastrophizing. However, in relation to the activation of endogenous analgesia through placebo treatment there seem to be a negative impact of depression, anxiety and catastrophizing. In conclusion this means that the positive effect of an SNRI drug on FM symptoms is not dependent on psychological factors like depression and anxiety. On the other hand, the positive treatment effect of placebo is dependent on low rating of depression and anxiety.

In Study II we addressed the differential impact of depressive symptoms, anxiety and catastrophizing on cerebral processing of pressure pain in a large number of FM patients. We found no relationship between negative mood and cerebral processing of pain in FM patients. We also found that the reports of clinical and experimentally induced pain were unaffected by levels of depressive symptoms, anxiety and catastrophizing thoughts. This validates that the aberrations in pain sensitivity in FM patients exist independently of negative mood. Our results contradict previous speculations about a generally exaggerated emotional response in FM patients, suggesting the disorder is caused by psychological vulnerability (Ehrlich, 2003; Hadler, 1996). The results of study II is in line with the increasing evidence showing that there is no hypervigilance or increased affective modulation in FM patients. However, in two previous studies investigating the impact of depression (Giesecke et al., 2005) and catastrophizing (Gracely et al., 2004) on neuronal activity in FM patients there were findings of brain regions with altered response during pain. In the depression study there were findings of increased neuronal activity in the

bilateral amygdalae and contralateral anterior insula in relation to depressive symptoms, indicating more involvement of emotional processing in response to the pain stimulation in depressed patients. In the study investigating the impact of catastrophizing on pain processing in FM there was an association between high levels of catastrophizing thoughts and increased brain activity in regions pertaining to attention and anticipation of pain. It is likely that the difference in results between the two previous studies and the results presented in the present thesis can be explained by 1) the difference in pain paradigms and 2) the difference in sample size. The paradigm used in the study by Gracely et al. and Giesecke et al. was a block design with inherent predictability of the pain stimuli (blocks of 25 seconds of pain). It is possible that the non-depressed and non-catastrophizing patients had better possibilities to cope with the situation by steeling themselves before the pain-block; therefore not activating the emotional structures as much as the depressed/catastrophizing patients. The results from previous studies could thus be due to a difference in sensitivity for pain anticipation but does not necessarily say anything about the pain processing *per se*. Therefore, we conclude that mood does not affect the perception or processing of experimentally induced nociceptive stimuli in FM patients, when anticipation is controlled for. Moreover, the number of patients in the present study was much larger than any comparable imaging study, indicating that there is still need for validation of the previous two studies investigating depression and catastrophizing in FM patients.

The understanding of the factors involved in endogenous regulation of pain is essential in understanding complex pain pathology. For example, Harris et al. used PET to investigate the opioid binding potential in patients with FM and found that there was an altered function of the endogenous opioid activity compared to healthy controls (Harris et al., 2007). Now, there is emerging data from studies investigating the genetic influence on the function of the endogenous pain inhibitory system. The noradrenalin- and opioid-dependent inhibition of pain is genetically influenced by the activity of the catecholamine breakdown enzyme COMT (Lotta et al., 1995). We wanted to investigate the influence of the COMTval^{158}met polymorphism on the response to pain after opioid treatment and repeated pain stmulations. Initially, this study was performed in healthy controls

but it would be of great importance to also investigate this in patients with FM. Study IV provided evidence for the contribution of the COMTval^{158}met polymorphism in descending pain regulation in healthy individuals (Jensen et al., 2009). Results suggest that the initial response of the descending pain inhibitory system is not influenced by the COMTval^{158}met polymorphism but when the system is challenged the difference is revealed (Jensen et al., 2009). It is possible that this difference can be explained by the degree of involvement of the frontal lobes. The COMTval^{158}met polymorphism is implied in frontal lobe function and the effect of different genotypes might be revealed first when the inhibitory system requires more frontal lobe involvement. This is crucial for clinical interventions where the biological system is already challenged (Nagel et al., 2008), e.g. neurodegeneration, chronic pain or psychiatric disorders with impaired function of the frontal lobes. In order to find out more about the role of functional polymorphisms in clinical states, it is important to design experiments that challenge the biological system. For example, it is possible that genetic stratification can help us to determine who will respond to analgesic treatment. In the case of FM, only a limited number of patients respond to NSRI drugs. Therefore the clarification of the genetic influence on the descending pain inhibitory functions can help us to find better treatment possibilities in the future.

11 FUTURE OF PAIN RESEARCH AND TREATMENT

It is clear that we are moving towards a more individualized view of pain and treatment of pain. Anecdotally it has been known that different individuals have very different pain tolerance but the reason for this has mainly been attributed to personal character. However, recent research has shown that the mental experience of the individual has a corresponding representation in the central nervous system. For pain, we know that this neural representation is largely dependent on psychological and contextual factors but also there are biological and genetic factors that will influence the final pain experience. Consequently, the contemporary understanding of inherent differences in pain perception and tolerance should increase the respect for differences in complaints between individuals; we simply are not equipped with the same abilities to handle pain. It is the responsibility of pain researchers to spread new findings and oppose any time there is presence of unscientific statements that will lead to dysfunctional beliefs and guilt for those suffering from pain. Also, novel findings of inter-individual differences in perception of pain will hopefully enhance future pain experiments. The use of subjectively calibrated pain stimulations and genetic stratification of subjects will probably be more common in a near future.

To date, schoolbooks in clinical medicine for physicians include little or no information at all about the possible genetic influence on pain perception. This is unfortunate since the difference in the ability to modulate pain is an important factor in understanding why some individuals develop chronic pain disorders. It has been shown that some individuals with a localized pain problem develop chronic widespread pain and severe disability. In fact, a localized pain problem is the most common precursor of FM. Health care providers should pay close attention to this in order to identify those with early symptoms of pain syndromes. The early recognition of pain pathology leads to better treatment possibilities and will increase the likelihood of a full remission for the patient. Today there is no simple gene-test that could tell who is at risk for developing chronic pain. Results are always valid on a group level and the complex interactions between gene-gene and gene-environment are not understood. In the future, however, there will likely be genetic tests that physicians could use in

order to identify those at risk of developing chronic pain. Those individuals should be offered extensive preventive interventions.

There are still few imaging studies investigating the pathophysiology of chronic pain syndromes. It has been shown that there is altered brain activity associated with certain pain syndromes and clinical studies just started to use neuroimaging techniques to assess the neural correlates to positive treatment effects. It is possible that there will be many more studies using neuroimaging to determine the mechanisms involved in a particular treatment effect. Not only behavioral interventions have poorly defined mechanisms of action but also pharmacological treatment needs to be closer investigated. If the mechanisms behind a positive treatment outcome can be better defined we have better opportunities of giving the right treatment to the right patient. As proposed earlier, we are moving towards a more individualized pain signature where the traditional pain matrix is not a sufficient construct. Also, there is good hope for the possibility of performing fMRI of the spinal cord in the near future (Govers et al., 2007) . If we can combine information about cerebral and spinal function in pain processing there can be increased understanding of the treatment mechanisms that are poorly defined today.

One shortcoming to our current understanding of the representation of pain in the brain is that we can not yet distinguish between the representation of pain and pain produced by peripheral nociceptive input (Raij et al., 2005). For example, the cerebral representation of watching someone else in pain overlaps with the activity evoked by pain in the first person (Singer et al., 2004). It is a challenge of the future to further disentangle afferent input and the cerebral processing leading to the final pain perception.

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