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MULTIMODAL NEUROIMAGING OF PAIN AND INFLAMMATION IN THE CENTRAL NERVOUS SYSTEM IN CHRONIC PAIN PATIENTS WITH FIBROMYALGIA AND RHEUMATOID ARTHRITIS

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Multimodal Neuroimaging of Pain and Inflammation in the Central Nervous System in Chronic Pain Patients with Fibromyalgia and Rheumatoid Arthritis

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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POPULAR SCIENCE SUMMARY OF THE THESIS

Under normal circumstances, pain is an adaptive physiological protection system that can be likened to a fire alarm designed to warn the body of potentially harmful situations. In the central nervous system (including the brain, brainstem and spinal cord), pain signals are regulated and filtered out. This filtering process works normally in healthy people, but patients with long-term pain exhibit clear changes in the way the brain handles pain signals. One of today's challenges is to understand why some patients with rheumatoid arthritis (RA) continue to experience pain even though they have received satisfactory treatment for the inflammation in their joints. One hypothesis is that these patients with RA develop a functional reorganization in the central nervous system, so-called nociplastic pain, which characterizes patients with fibromyalgia (FM). There is also a surprisingly high incidence of concomitant fibromyalgia in patients with rheumatoid arthritis.

The purpose of this thesis was to use two brain imaging methods to identify and fill contemporary knowledge gaps that relate to how the brain process and filter out pain signals in patients with well-characterized rheumatoid arthritis (so-called nociceptive pain) who do not show symptoms of fibromyalgia, and patients with well characterized fibromyalgia (so-called nociplastic pain) who do not show symptoms of rheumatoid arthritis.

In **study I**, positron emission tomography (PET) was used to study the brain's immunocompetent cells, glia. These showed signs of activation in the brains of patients with FM, indicating the presence of neuroinflammation.

Study II used functional magnetic resonance imaging (fMRI) which showed that RA patients processed pain normally when receiving painful pressure over an unaffected area, but showed aberrant brain activation in the frontal lobe when stimulated over the most inflamed finger joint.

In **studies III and IV**, fMRI was used to study the role that expectations play in how fibromyalgia patients experience painful pressure and how their brain processes painful pressure. **Study III** revealed that individuals with FM showed a higher pain-related brain activation when the pain stimulation was higher than expected, while healthy individuals showed higher pain-related brain activation in relation to painful stimulation that was lower than expected. Specifically, a moderate pressure increased brain activation in a region associated with reluctance or aversion (insula) in individuals with FM when the pressure followed a low pain signal compared to if the same pressure followed a high pain signal. Increased activity in the insula was observed in healthy individuals under opposite conditions. In **study IV**, brain activation was examined during a period when individuals expected high or low pain stimulation, i.e. during the signal period until the painful stimulation. In this case, individuals with FM had a reduced response in the frontal lobe compared to healthy individuals during the signal for high pain (when it was followed by a lower moderate pressure), which may explain why individuals with FM continued to rate high pain intensity after the signal for high pain, when they actually received moderate pressure.

In **Study V** we used fMRI brain imaging to compare how the brain process pain in patients with RA and FM. The results showed a less pronounced involvement of the frontal lobe during pain stimulation in individuals with RA compared to FM. In contrast, individuals with FM showed reduced engagement of medial structures such as the rostral anterior cingulum, a brain region involved in the descending pain inhibitory system. Higher ratings of clinical pain in individuals with FM, but not RA, correlated with more pronounced disturbances in brain regions involved in pain inhibition, which indicates that disturbed pain regulation is important for the manifestation of nociplastic pain.

In summary, in individuals with FM, we were able to demonstrate a glial cell activation indicating neuroinflammation and document deviations in how expectations affected the experience of pain and pain-related brain activation. Furthermore, pronounced differences in pain-related brain activation were seen between individuals with nociceptive (RA) and nociplastic (FM) pain. The intensity of the spontaneous fibromyalgia pain was related to a reduced activation of descending pain-inhibiting systems in connection with pain stimulation, which shows that disturbed pain regulation has clinical relevance in nociplastic pain.

POPULÄRVETENSKAPLIG SAMMANFATTNING

I normala förhållanden är smärta ett adaptivt fysiologiskt skyddssystem som kan liknas med ett brandlarm som är utformat för att varna kroppen för potentiellt skadliga situationer. I centrala nervsystemet (inkl. hjärnan, hjärnstammen och ryggmärgen) sker reglering och bortfiltrering av smärtsignaler. Hos friska personer fungerar denna bortfiltrering, men patienter med långvarig smärta uppvisar tydliga förändringar i hjärnans sätt att hantera smärtsignaler. En av dagens utmaningar är att försöka förstå varför vissa patienter med ledgångsreumatism (RA) fortsätter att uppleva smärta trots att de fått tillfredsställande behandling av inflammationen i deras leder. En hypotes är att patienter med RA utvecklar en funktionell omorganisation i det centrala nervsystemet, sk. nociplastisk smärta, vilket karaktäriserar patienter med fibromyalgi (FM). Det finns även en förbryllande hög förekomst av samtidig fibromyalgi hos patienter med ledgångsreumatism.

Syftet med denna avhandling var använda sig av två hjärnavbildningsmetoder för att identifiera och fylla samtida kunskapsluckor som relaterar till hur hjärnan bearbetar och filtrerar bort smärtsignaler hos patienter med väl karakteriserad ledgångsreumatism (sk. nociceptiv smärta) som inte uppvisar symptom av fibromyalgi, samt patienter med väl karakteriserad fibromyalgi (sk. nociplastisk smärta) som inte uppvisar symptom av ledgångsreumatism.

I **studie I** användes positronemissionstomografi (PET) för att studera hjärnans immunokompetenta celler, glia. Dessa visade tecken på aktivering i hjärnorna hos patienter med FM, vilket tyder på förekoms av neuroinflammation.

I **studie II** användes funktionell magnetresonanshjärnavbildning (fMRI) som visade att RA patienter bearbetade smärta normalt när fick smärtsamt tryck över icke-drabbat område, men uppvisade en avvikande hjärnaktivering i frontalloben när de stimulerades över den mest inflammerade fingerleden.

I **studie III och IV** studerade vi vilken roll förväntningar spelar för hur fibromyalgipatienter upplever och hur deras hjärna bearbetar ett smärtsamt tryck. I **studie III** dokumenterades att individer med FM uppvisade en högre smärtrelaterad hjärnaktivering när smärtstimuleringen var högre än förväntat, medan friska individer uppvisade högre smärtrelaterad hjärnaktivering i samband med smärtstimulering som var lägre än förväntat. Specifikt, ett medelstarkt tryck ökade hjärnaktivering i en region associerad med motvilja eller aversion (insula) hos individer med FM när trycket följde en signal för låg smärta jämfört med om samma tryck följde en signal för hög smärta. Ökad aktivitet i insula observerades hos friska individer under motsatta förhållanden. I **studie IV** undersöktes hjärnaktivering under en period då individerna förväntade sig hög eller låg smärtstimulering, det vill säga under signal perioden fram till smärtstimuleringen. Därvid hade individer med FM en minskad respons i frontalloben jämfört med friska individer under signalen för hög smärta (när det sedan följdes av ett lägre medelstarkt tryck), vilket kan förklara varför individer med FM fortsatte att skatta hög smärtintensitet efter signalen för hög smärta trots att de erhöll återkommande stimulering med medelstarkt tryck.

I **studie V** jämfördes hjärnans smärtbearbetning hos patienter med RA och FM. Resultaten visade ett mindre uttalat engagemang av frontalloben under smärtstimulering hos individer med RA jämför med FM. Däremot uppvisade individer med FM ett minskat engagemang av mediala strukturer såsom rostrala anteriora cingulum, en hjärnregion som är involverad i

nedåtstigande smärthämmande system. Högre grad av klinisk smärta hos individer med FM, men inte RA, korrelerade med uppvisade störningar i nedåtgående smärthämmande system, vilket indikerar att störd smärtreglering är viktig för uppkomsten av nociplastisk smärta.

Sammanfattningsvis kunde vi hos individer med FM påvisa en gliacellsaktivering talande för neuroinflammation och dokumentera avvikelser i hur förväntningar påverkade upplevelsen av smärta och smärtrelaterad hjärnaktivering. Vidare sågs uttalade skillnader i smärtrelaterad hjärnaktivering mellan individer med nociceptiv (RA) och nociplastisk (FM) smärta. Intensiteten av den spontana pågående fibromyalgismärtan var relaterad till en minskad aktivering av nedåtstigande smärthämmande system i samband med smärtstimulering, vilket visar att störd smärtreglering har klinisk relevans vid nociplastisk smärta.

ABSTRACT

The prevalence of concomitant fibromyalgia (FM) is puzzlingly high among rheumatoid arthritis (RA) patients and a contemporary challenge is to resolve why some RA patients continue to report pain despite adequate treatment of their peripheral inflammation. While recent literature has concentrated on the link between cerebral and inflammatory mechanisms in RA patients with concomitant FM, little attention has been directed towards commonalities and divergences among these two patient groups when they are well-characterized. The overarching aim of the current thesis was to identify and filling contemporary gaps of knowledge related to cerebral pain processing and associated mechanisms (i.e. contextual influences and neuroinflammation) in patients with well-characterized rheumatoid arthritis (nociceptive pain) and well-characterized fibromyalgia (nociplastic pain) condition.

In **study I,** multi-ligand positron-emission tomography (PET) was used to investigate brain glial activation (i.e. neural inflammation) in FM patients compared to healthy controls (HC). The results supported a role for glial activation in FM pathophysiology, as FM vs. HC exhibited wide-spread cortical elevations of translocator protein (TSPO) binding, a sign of activated glia. Increased subjective ratings of fatigue in FM correlated with increased TSPO binding in the midcingulate cortex.

In **study II**, functional magnetic resonance imaging (fMRI) was used to Investigate cerebral pain processing in RA patients at disease-affected (most inflamed finger joint) and non-affected (thumb nail) sites. Corresponding sites were used in HC. The results indicated normal pain sensitivity and cerebral pain processing in RA for non-affected sites, while disease-relevant pain processing was marked by a failed initiation of cortical top-down regulation.

In **study III**, combined behavioral and fMRI data suggested that FM subjects display a predisposition to form new pain-related associations while simultaneously maintaining high-pain associations that are no longer relevant. **Study IV** extended these findings, and revealed that FM vs. HC exhibited reduced prefrontal activation during repeatedly violated high pain associations. These results may help explain why ratings of high pain persist in FM subjects despite that the subsequent pressure stimulation had been lowered (i.e. high pain replaced by a lower mid-intensity painful pressure).

In **study V**, fMRI was used to directly compare cerebral pain processing in well-characterized RA and FM patients without comorbidities. The results suggested that cerebral pain processing in RA was associated with dysfunction in the early initiation of the pain modulatory system, i.e. reduced activation of the dorsolateral prefrontal cortex. Whereas, cerebral pain processing in FM was associated with reduced engagement of more medial structures such as medial prefrontal cortex and rostral anterior cingulate cortex. In FM patients only, disruptions in pain-related cerebral activation correlated with higher degrees of clinical pain, which indicate more pronounced disruptions in patients suffering from nociplastic pain.

In conclusion, the results from the above-mentioned studies in the current thesis noted distinct aberrations in cerebral pain modulation between well-characterized FM and well-characterized RA. Specifically, while cerebral pain modulatory aberrations were restricted to affected sites (i.e. most inflamed finger joint) in RA, cerebral pain processing in FM was found to be marked by notably complex cognitive processes and associated with overall clinical pain. These results may indicate more prominent pain-related cerebral disruptions in patients suffering from nociplastic pain. However, it remains elusive to which extent contextual factors and pain catastrophizing interact with cerebral pain modulation (independent of mood) in RA.

LIST OF SCIENTIFIC PAPERS

I. Daniel S. Albrecht#, Anton Forsberg#, **Angelica Sandström**, Courtney Bergan, Diana Kadetoff, Ekaterina Protsenko, Jon Lampa, Yvonne C. Lee, Caroline Olgart Höglund, Ciprian Catana, Simon Cervenka, Oluwaseun Akeju, Mats Lekander, George Cohen, Christer Halldin, Norman Taylor, Minhae Kim, Jacob M. Hooker, Robert R. Edwards, Vitaly Napadow, Eva Kosek*, Marco L. Loggia* (2019). Brain glial activation in fibromyalgia – A multi-site positron emission tomography investigation. *Brain, Behavior and Immunity, 75,* 72-83. #co-first authors, *co-senior authors.

II. **Angelica Sandström**, Isabel Ellerbrock, Karin Jensen, Sofia Martinsen, Reem Altawil, Philip Hakeberg, Peter Fransson, Jon Lampa, Eva Kosek (2019). Altered cerebral pain processing of noxious stimuli from inflamed joints in rheumatoid arthritis: An event-related fMRI study. *Brain, Behavior and Immunity, 81,2* 72-279.

III. **Angelica Sandström**, Isabel Ellerbrock, Jeanette Tour, Diana Kadetoff, Karin Jensen, Eva Kosek (2020). Neural correlates of conditioned pain responses in fibromyalgia subjects indicate preferential formation of new pain associations rather than extinction of irrelevant ones. *PAIN, 161,* 2079-2088.

IV. **Angelica Sandström**, Isabel Ellerbrock, Jeanette Tour, Diana Kadetoff, Karin Jensen, Eva Kosek. Dysfunctional activation of the dorsolateral prefrontal cortex during pain anticipation is associated with altered subsequent pain experience in fibromyalgia subjects. *Submitted.*

V. **Angelica Sandström**, Isabel Ellerbrock, Monica Löfgren, Reem Altawil, Indre Bileviciute-Ljungar, Jon Lampa, Eva Kosek. Distinct Aberrations in Cerebral Pain Processing Differentiating Fibromyalgia from Rheumatoid Arthritis Patients. *Submitted.*

ADDITIONAL PUBLICATIONS

Additional publications by the author from the Department of Clinical Neuroscience which are not included in the thesis:

I. Isabel Ellerbrock, **Angelica Sandström**, Jeanette Tour, Diana Kadetoff, Martin Schalling, Karin Jensen, Eva Kosek (2020). Polymorphisms of the μ-opioid receptor gene influence cerebral pain processing in fibromyalgia. *Eur J Pain. 2020 Oct 16. doi: 10.1002/ejp.1680.*

CONTENTS

LIST OF ABBREVIATIONS

A1	Classically activated, pro-inflammatory astrocytic (glial) cell
A2	Alternatively activated, neuroprotective astrocytic (glial) cell
ACC	Anterior cingulate cortex
ACR	American College of Rheumatology
BOLD	Blood-oxygen-level-dependent
CNS	Central nervous system
CPM	Conditioned pain modulation
CR	Conditioned response
CRP	C-reactive protein
CS	Conditioned stimulus
DAS28	Disease activity score in 28 joints
dlPFC	Dorsolateral prefrontal cortex
DMN	Default mode network
EIH	Exercise induced hypoalgesia
ESR	Erythrocyte sedimentation rate
FM	Fibromyalgia
fMRI	Functional magnetic resonance imaging
FMSs	Fibromyalgia subjects
HC	Healthy controls
HRF	Haemodynamic response function
IASP	International Association for the Study of Pain
IL	Interleukin
IPL	Inferior parietal lobe
M1	Primary motor cortex

M1	Classically activated, pro-inflammatory microglial cell
M2	Alternatively activated, neuroprotective microglial cell
MCC	Midcingulate cortex
mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
P10	Painful pressure corresponding to 10mm/100mm VAS
P30	Painful pressure corresponding to 30mm/100mm VAS
P30green	P30 stimulus following green cue
P30red	P30 stimulus following red cue
P50	Painful pressure corresponding to 50mm/100mm VAS
PAG	Periaqueductal gray
PCS	Pain catastrophizing scale
PET	Positron emission tomography
PIPS	Proximal interphalangeal joint
PPI	Psychophysiological interaction (connectivity analysis)
RA	Rheumatoid Arthritis
rACC	Rostral anterior cingulate cortex
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
TNF	Tumour necrosis factor
TSPO	Translocator Protein
US	Unconditioned stimulus
VAS	Visual analogue scale

1 INTRODUCTION

At present, the pain research field has yet to answer the question of why certain individuals develop nociplastic pain, which denotes pain that arises as a result of functional changes in the central nervous system[1], while others with seemingly the same tissue damage do not. Rheumatoid arthritis (RA) is a nociceptive chronic peripheral inflammatory pain disorder characterized by peripheral synovial inflammation that causes swelling, stiffness, and destruction of the affected joints[2,3]. Fibromyalgia (FM) is a nociplastic[1], chronic wide-spread pain disorder, characterized by a change in function of nociceptive pathways, leading to generalized hypersensitivity to sensory stimuli, often in combination with disturbed sleep, fatigue, memory difficulties and psychological distress[4,5].

Despite excellent control of peripheral inflammation in RA joints, some patients continue to report pain[6–8], and the prevalence of concomitant FM is especially high among RA patients. Among patients with RA, an estimated 15-23% have comorbid FM[8,9], compared to a world-wide incidence of FM in the general population of approximately only 2-3%[4,8]. It has been suggested that, in certain RA patients, the central nervous system (CNS) may become sensitized via peripheral inflammatory processes acting on pronociceptive pathways[10] which may drive the CNS towards a nociplastic state[11], a state that characterizes FM[1]. Moreover, accumulating evidence supports a neural inflammatory reflex[12], as direct stimulation to the vagus nerve inhibit cytokine production and attenuates disease severity in RA[13]. Moreover, the possibility that altered brain function (activity and connectivity) drives peripheral inflammation via endocrine pathways has also been discussed[14].

Recent literature has focused on the link between cerebral and inflammatory mechanisms in RA patients with concomitant FM[14–16]. Yet, research on well-characterized RA (without FM co-morbidity) and well-characterized FM patients (without RA) indicates fundamental differences when compared to healthy controls (HCs) in descending pain inhibition, through for example, conditioned pain modulation[17–19] and exercise induced hypoalgesia[17,20–24].

The overarching aim of this thesis is to contribute to filling an important knowledge gap through investigating commonalities as well as divergences, in cerebral pain modulatory mechanisms between patients with *nociceptive* and *nociplastic* chronic pain, and to study neuroinflammation and contextual influences on pain perception in nociplastic pain.

Increased knowledge of these mechanisms is of vital importance for understanding the manifestation of nociplastic pain and, ultimately (in the long run), improving treatment outcomes for affected patients.

2 LITERATURE REVIEW

2.1 DEFINITIONS OF PAIN

The international association for the study of pain (IASP) defines pain as: *"An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"*[25]. Acute pain is an adaptive physiological protective system, which can be seen analogous to a fire alarm, designed to warn biological systems against physical damage or injury. However, if the pain becomes chronic (> 3 months), it has lost its early warning signalling value and can in many instances be considered maladaptive (Woolf, 2010; Dydyk 2020). Previously, chronic pain was described as either nociceptive or neuropathic, with the former being the most common human experience of pain [1], and the latter created to differentiate from or as a contrast to the former [26].

Nociceptive pain is defined as: *"Pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors."*[26]

Neuropathic pain is defined as: *"Pain caused by a lesion or disease of the somatosensory nervous system."*[27]

Note that, while the former term (nociceptive) is used to describe pain occurring with a normally functioning somatosensory nervous system, it is designed to contrast with the abnormal function seen in the latter (neuropathic)[26]. However, this dichotomy excludes many patients with chronic persistent pain that does not result from an obvious activation of nociceptors nor from a proven lesion or disease of the somatosensory nervous system. Such as, for example, the case with fibromyalgia. Hence, a third mechanistic descriptor was proposed by Kosek et al. 2016[1] and adapted by IASP in 2017:

Nociplastic pain is defined as: *"pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain."*[28]

Important to note, is that patients can have a combination of nociceptive and nociplastic pain[1,28], which may be the case of persistent pain in patients with well-controlled rheumatoid arthritis.

2.2 CLASSIFICATIONS

2.2.1 Fibromyalgia (FM): Nociplastic Pain Disorder

Fibromyalgia (FM) is a nociplastic pain disorder characterized by altered function of the somatosensory system[1]. FM patients exhibit widespread pain, generalized hypersensitivity to sensory stimuli, often in combination with fatigue, disturbed sleep, memory difficulties and psychological distress[4,5]. A specialist in rehabilitation medicine and pain relief examined all FM subjects to ensure they met the ACR-1990[29(p1)] diagnostic criteria for the classification fibromyalgia (**Study I, III, IV, V**) as well as the ACR-2011, i.e., the modified ACR 2010 criteria[30] (**Study I, III, IV**). Given the controversy regarding the diagnosis of FM, we chose to use two sets of criteria (ACR 1990 and 2011) in all of our studies, except from study V. In which, the data collection preceeded the publication of the ACR 2010/2011 criteria and thus only the ACR were available. More detailed information on the FM classification criteria used in this thesis can be found in **table 1a and 1b**. The ACR 2010/2011 criteria were further revised in 2016[31] but this revision had not been published when the inclusion of subjects began and was therefore not applied.

2.2.2 Rheumatoid Arthritis (RA): Nociceptive Pain Disorder

Rheumatoid arthritis (RA) is a nociceptive chronic pain disorder characterized by peripheral joint inflammation that causes pain, swelling, stiffness, and destruction of the affected joints[2,3]. Pain is the most common and utmost challenging symptom in RA[7] and the primary priority for patients when seeking medical healthcare. In **study II and V**, RA patients were screened by a medical doctor to ensure that the patients met the American College of Rheumatology (ACR) 1987 criteria for the classification of rheumatoid arthritis[32]. Further information on RA classification criteria can be found in **table 2**.

Table 1a.

The 1990 American College of Rheumatology diagnostic criteria for the classification fibromyalgia (Wolfe et al., 1990).

1. ***History of widespread pain.***

Definition. Pain is considered widespread when all of the following are present: pain in the left side of the body, pain in the right side of the body, pain above the waist and pain below the waist. In addition, axial skeletal pain (cervical spine or anterior chest or thoracic spine or low back) must be present. In this definition, shoulder and buttock pain is considered as pain for each involved side. "Low back" pain is considered lower segment pain.

2. ***Pain in 11 of 18 tender point sites on digital palpation.***

Definition. Pain, on digital palpation, must be present in at least 11 of the following 18 tender point sites:
Occiput: bilateral, at the suboccipital muscle insertions.
Low cervical: bilateral, at the anterior aspects of the intertransverse spaces at C5-C7.
Trapezius: bilateral, at the midpoint of the upper border.
Supraspinatus: bilateral, at origins, above the scapula spine near the medial border.
Second rib: bilateral, at the second costochondral junctions, just lateral to the junctions on upper surfaces.
Lateral epicondyle: bilateral, 2 cm distal to the epicondyles.
Gluteal: bilateral, in upper outer quadrants of buttocks in anterior fold of muscle.
Greater trochanter: bilateral. posterior to the trochanteric prominence.
Knee: bilateral, at the medial fat pad proximal to the joint line.

Digital palpation should be performed with an approximate force of 4 kg.
For a tender point to be considered "positive" the subject must state that the palpation was painful. "Tender" is not to be considered "painful."

* For classification purposes, patients will be said to have fibromyalgia if both criteria are satisfied. Widespread pain must have been present for at least 3 months. The presence of a second clinical disorder does not exclude the diagnosis of fibromyalgia.

Table 1b.

The modified 2010 American College of Rheumatology diagnostic criteria for the classification fibromyalgia (Wolfe et al., 2011), referred to as the ACR-2011 criteria.

Criteria

A patient satisfies modified ACR 2010 fibromyalgia diagnostic criteria if the following 3 conditions are met:
(1) Widespread pain index ≥ 7 and symptom severity score ≥ 5 OR WPI of 3–6 and symptom severity score ≥ 9.
(2) Symptoms have been present at a similar level for at least 3 months.
(3) The patient does not have a disorder that would otherwise sufficiently explain the pain.

Ascertainment

1) Widespread Pain Index (WPI): Note the number of areas in which the patient has had pain over the last week. In how many areas has the patient had pain? Score will be between 0 and 19.

Shoulder girdle, left	Hip (buttock, trochanter), left	Jaw, left	Upper back
Shoulder girdle, right	Hip (buttock, trochanter), right	Jaw, right	Lower back
Upper arm, left	Upper leg, left	Chest	Neck
Upper arm, right	Upper leg, right	Abdomen	
Lower arm, left	Lower leg, left		
Lower arm, right	Lower leg, right		

2) Symptom Severity Score: Fatigue; Waking unrefreshed; Cognitive symptoms

For each of the 3 symptoms, indicate the level of severity over the past week using the following scale:

0 = No problem
1 = Slight or mild symptoms; generally mild or intermittent
2 = Moderate; considerable problems; often present and/or at a moderate level
3 = Severe: pervasive, continuous, life-disturbing problems

The Symptom Severity Score is the sum of the severity of the 3 symptoms (fatigue, waking unrefreshed, cognitive symptoms) plus the sum of the number of the following symptoms occurring during the previous 6 months: headaches, pain or cramps in lower abdomen, and depression (0-3). The final score is between 0 and 12.

Table 2.

The 1987 revised criteria for the classification of rheumatoid arthritis (Arnett, 1987).

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.
3. Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint.
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, or MTPs is acceptable without absolute symmetry).
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician.
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects.
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).

*For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is *not* to be made. PIPs = proximal interphalangeal joints; MCPs = metacarpophalangeal joints; MTPs = metatarsophalangeal joints.

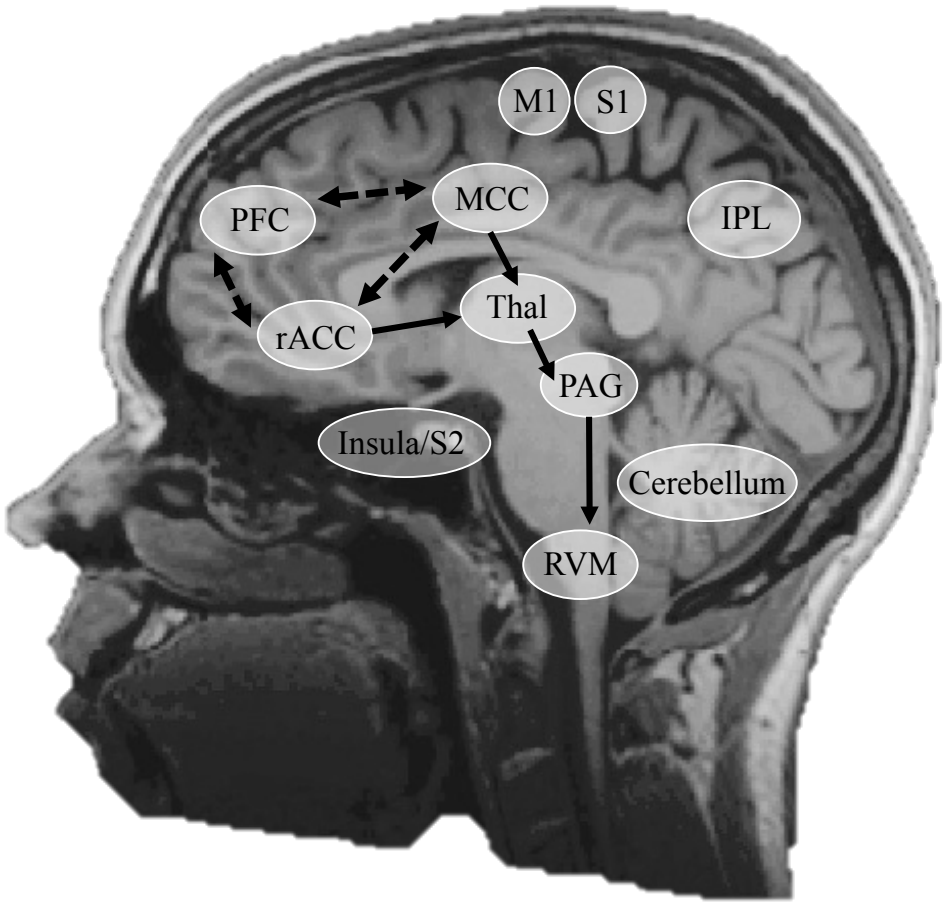

Figure 1. ***Illustrates the Brain Regions Frequently Mentioned Throughout the Thesis.***

Depicts an MRI image of the midsagittal section of the human brain. Light grey circles mark the approximate locations of the brain regions that are frequently mentioned throughout the current thesis. The marked brain regions are commonly associated with human pain processing and/or pain modulation. Unidirectional arrows exemplify descending pain modulatory pathways. Bidirectional dashed arrows indicate that the directionality of communication between these brain regions during human descending pain modulation is less certain.

M1 = primary motor cortex; S1 = primary somatosensory cortex; S2 = secondary somatosensory cortex; PFC = prefrontal cortex; MCC = midcingulate cortex; IPL = inferior parietal lobe; rACC = rostral anterior cingulate cortex; Thal = thalamus; PAG = periaqueductal gray; RVM = rostroventromedial medulla. The subject on the image has agreed to participate in print.

2.3 CEREBRAL PAIN PROCESSING AND PAIN MODULATION

In normal conditions, pain is an adaptive physiological alarm system that warns the body of a potentially harmful situation. However, in some circumstances, such as the case with persistent chronic pain, pain may become maladaptive and loose its function of an early warning signal[4]. Brain regions involved in human descending pain inhibition involve the frontal cortex (including dorsolateral prefrontal cortex, dlPFC), anterior- and mid-cingulate cortex (ACC and MCC), insula, thalamus, brainstem periacqueductal gray (PAG) and rostral ventromedial medulla (RVM)[33] (Figure 1). Specifically, the dlPFC is a key brain region involved in descending pain inhibition through is implication in cognitive-, affective- and sensory processing[34]. The dlPFC can activate the ACC/MCC through opioid-dependent signalling, which in turn, can engage lower parts of the descending pain regulatory system such as thalamus and PAG[34,35]. The insula has been suggested to serve a fundamental role in pain[36] as it is engaged in multisensory integration including intensity and location processing (the posterior part) and interoceptive representation or subjective experience of pain (the anterior part)[11,36]. Meta-analysis on pain-related functional neuroimaging studies, suggest that healthy subjects compared to various patients with various forms of chronic pain, commonly exhibit aberrant brain activation in MCC, ACC, thalamus, insula, inferior parietal lobe (IPL), middle frontal gyrus (dlPFC), claustrum, cerebellum, inferior frontal gyrus[37].

2.3.1 Pain Processing in FM

FM is a nociplastic pain disorder characterized by nociceptive plasticity, i.e. a change in function of nociceptive pathways[1], leading to generalized and wide-spread hypersensitivity to sensory stimuli, in combination with a number of other symptoms related to aberrant functioning of the central nervous system (CNS) such as memory difficulties, sleep disturbances, fatigue, and mood disorders[5,38]. The pathogenesis of FM is not fully understood, but contemporary hypotheses suggest that genetic predisposition, stressful life events, peripheral (inflammatory) and central (cognitive-emotional) factors contribute to functionally rearranging the CNS and generating a dysperception of pain[4].

Neuroimaging studies have repeatedly reported aberration in the CNS of FM patients. Observed abnormalities include dysfunctional descending pain modulation[39,40], gray matter decrements in pain-inhibitory brain regions[41,42] and altered brain connectivity at rest[43–46]. Moreover, microscopic alterations have been detected in FM, such as altered brain chemistry[47] including altered endogenous opioids[48] and wide-spread upregulation of inhibitory $GABA_A$ receptors[49]. A meta-analysis of functional brain aberrations in FM patients compared to healthy subjects, suggest that primary somatosensory cortex (S1), rostral anterior cingulate cortex (rACC) and amygdala are the brain regions most likely to be found hypoactivated, whereas insula, left secondary somatosensory cortex (S2) and lingual gyrus are the brain regions most likely to be found hyperactivated during evoked pain[39]. Although the neural processes of applied experimental pain has been widely investigated in FM, it remains unclear to what extent contextual factors such as emotional distress and pain expectations are related to chronic pain disorders such as FM, and to what extent these factors may alter cerebral pain processing[50].

2.3.1.1 Contextual Influences on Pain Perception

The research on FM has advanced in the understanding of relevant mechanisms implicated in cerebral pain processing and is increasingly moving on towards studying the importance of contextual factors. One hypothesis is that certain states of chronic pain are a result of an interaction between socio-environmental stressors (e.g. fear, trauma, distress and pain catastrophizing) and neurophysiological factors, in which, psychological stress is hypothesized to induce receptor sensitization and neuroplasticity[4,51].

Although depression and anxiety are not directly related to the severity of clinical symptoms or pain sensitivity, nor to cerebral pain processing in FM *per se*[52,53], accumulating research suggest that pain in FM is influenced by notably complex cognitive processes particularly related to an increased response to pain-related threat[54–58]. These processes can be studied through conditioning, which is an integral part of several models of chronic pain[58,59]. Within the framework of pain, conditioning takes place when a cue (conditioned stimulus, CS), is repeatedly associated with a painful stimulation or experience (unconditioned stimulus, US), until the cue becomes pain-predictive and evokes a pain-related response by itself (conditioned response, CR), such as for example, fear of pain.

Behavioral studies have demonstrated impaired contingency learning, in which FM patients display a bias towards a higher expectancy of aversive events following high- and low pain cues alike[54,55], and once pain-related fear has been established in FM subjects, it is difficult to extinguish[56]. However, the neurological mechanisms underlying these processes in FM remain elusive.

As such, **study III and IV** in the current thesis, involved creating expectations of high- and low pain through an instructed pain conditioning paradigm. Specifically, the aim of **study III** was to investigate the neural correlates of conditioned pain responses and its relationship to emotional distress (particularly pain catastrophizing) in FM patients and HC. The aim of **study IV** was to investigate the neural correlates of pain anticipation (i.e. prior to painful stimulus onset) for pain in FM patients and HC during congruent (correctly cued pain associations) and incongruent (incorrectly cued pain associations) tasks.

2.3.2 Pain Processing in RA

While the research on cerebral pain processing in FM is well advanced[39], far fewer attempts has been made to investigate cerebral pain modulation in patients with RA. While peripheral inflammation is the major contributor to RA pain, central mechanisms may also play a role, as some patients continue to report persistent pain, despite therapeutic improvements in peripheral joint inflammation[6–8]. This notion is supported by, for example, a longitudinal study demonstrating that RA patients today do not experience less pain and disability compared to RA patients in the 1990's, despite treatment advances[60]. This suggests that more active dampening of peripheral inflammation is not directly related to a reduction in RA pain symptomatology. Accumulating research suggest that RA is accompanied with structural changes in brain organization[16,61], functional connectivity[16,62], and altered central pain processing in brain regions such as prefrontal cortex[63,64], ACC and MCC[63] compared to healthy subjects. Moreover, cerebral pain processing in RA may be influenced by concomitant depression[65].

Study II in the current thesis, was the first (to the extent of our knowledge), to use fMRI to investigate cerebral pain processing in RA patients compared to healthy subjects when painfully stimulated at disease-relevant (most inflamed joints) vs. non-affected (thumbnail) sites. Corresponding sites were used in healthy subjects. **Study V** in the current thesis was the first to use fMRI to directly compare cerebral pain processing in well-characterized

patient cohorts of fibromyalgia (without RA comorbidity) and rheumatoid arthritis (without FM comorbidity).

2.4 THE ROLE OF INFLAMMATION IN CHRONIC PAIN

Historically, chronic pain was attributed a pure neuronal response to injured tissue involving central nervous mechanisms such as central sensitization, including facilitation and disinhibition[66]. As of today, it is well-known that the immune system and nervous system are intimately intertwined, and can involve bidirectional neuron to non-neuron (immunocompetent cells) nociceptive signalling through, for example, cytokines[10,66,67]. Cytokines are pleiotropic proteins and polypeptides that play key roles in inflammatory responses in both the periphery and central nervous system (i.e. neuroinflammation) and are known to influence and maintain different types of pain. One straight-forward example is sensory nerve damage in neuropathic pain, where the pain has been found to be related to the concentration of various cytokines in the blood as well as the cerebrospinal fluid[68].

In inflammatory nociceptive pain, such as RA, elevated cytokine levels have been reported in the inflamed joints[69]. Pro-inflammatory cytokines can sensitize nociceptive nerve endings thus decreasing excitation thresholds at the primary nociceptive afferents. Previously inactive or silent pain fibres can become activated by the increased surrounding inflammation and begin to respond to mechanical, thermal, or chemical stimuli[2,3,10]. Biological treatments that aim to inhibit pro-inflammatory cytokines such as tumour necrosis factor- α (TNF-α), Interleukin (IL)-1β, and IL-6 are associated with successfully decreased peripheral joint inflammation in RA[2,3,69]. Yet the exact mechanisms behind the experienced pain in RA remain unresolved, as some patients continue to report pain despite well-controlled peripheral joint inflammation[6–8,70].

These clinical data are in concordance with results from animal studies[71], which have revealed that injection of anticitrullinated protein antibodies (ACPA) from RA patients into mice induce long-lasting pronounced pain-like behavior in the absence of joint inflammation. The effect was mediated by osteoclasts releasing the chemokine CXCL1/IL-8, which in turn activates peripheral nociceptive afferents[71]. Further, in analogy with clinical findings, pain-like behaviors are seen in animal models of RA both during and following visual signs of arthritis. In these models, anti-inflammatory treatments are efficient only during the arthritic phase whereas gabapentin, a drug used to treat

neuropathic and nociplastic pain conditions, including FM, is effective during the post-inflammatory period[72,73].

2.4.1 Inflammatory Substances in the Cerebrospinal Fluid

RA and FM have demonstrated different inflammatory profiles. RA patients, compared to FM, have demonstrated increased cerebrospinal fluid (CSF) levels of pro-inflammatory IL-1β, and reduced anti-inflammatory levels of IL-1Ra, IL-4, and IL-10[74]. Increased IL-1β levels in RA were found to be inversely correlated with parasympatetic activity, and RA revealed a reduced parasympathic tone compared to healthy controls, which confirms a decreased vagus activity in the patients. Conversely, FM patients compared to RA, exhibited higher CSF levels of pro-inflammatory IL-8 and anti-inflammatory IL-4 and IL-10. No correlation was found between sympathetic tone and CSF IL-8, although FM patients show increased sympathetic activity compared to HC[74]. Further, FM patients compared to healthy subjects, exhibited increased CSF levels of pro-inflammatory chemokine fractalkine (CX3CL1), which among other cytokines and chemokines, has been shown to be implicated in neuron-to-glia communication[75]. Taken together, these results suggest different CSF cytokine profiles in RA and FM. Whereas RA show evidence of pro-inflammatory cytokine profile, FM show a diverge profile of both pro- (e.g. IL-8, fractalkine) and anti-inflammatory (e.g. IL-4, IL-10) cytokines[74,75].

2.4.2 Glial Cell Activation

Increased levels of multifunctional cytokines in the CSF may indicate ongoing neural inflammation through activated glial cells such as microglia and/or astrocytes. When glial cells become active, they release pro-inflammatory cytokines to communicate with neurons, which has the capacity to amplify nociceptive transmission[66]. Increased release of TNF-α, IL-1β, IL-6, IL-8, and fractalkine are potential mechanisms contributing to CNS aberrations, central sensitization, and pain amplification in chronic pain disorders such as FM and RA[66,74,75]. In the human brain, glial activation can be studied *in vivo* using positron emission tomography (PET) and radioligands that bind to the translocator protein (TSPO), such as [^{11}C]PBR28. TSPO expression is low in healthy CNS tissue, but is intensely upregulated in activated microglia (and astrocytes to some extent) under inflammatory conditions[76,77]. TSPO PET has been used to image various chronic pain disorders such as

chronic low back pain[78], rheumatoid arthritis[79] and migraine[80]. **Study I** in the current thesis is the first to use multi-ligand PET ([^{11}C]PBR28 and [^{11}C]-$_{L}$-Deprenyl-$_{D2}$) to investigate brain glial inflammation in FM.

2.5 BRAIN IMAGING TECHNIQUES

2.5.1 Functional Magnetic Resonance Imaging (fMRI)

Magnetic resonance imaging (MRI) is a non-invasive imaging technique that can generate images of internal bodily organs *in vivo*. Instead of using radiation, MRI utilize a strong magnetic field, magnetic field gradients, and radio frequency pulses[81]. In brief, MRI is based on the magnetic qualities of hydrogen protons (properly known as *spin* or *spin angular momentum*). Within the MRI scanner, the spins align with the strong magnetic field. A radio frequency pulse can be added to disrupt the alignment of the spins. The misalignment is an energy demanding state, and followed by a relaxation back to equilibrium (i.e. the spins relax back to the strong magnetic field of the scanner). The energy that is released from the relaxation is measured by the (head) coil in the scanner, and depending on the tissue type (e.g. gray matter, white matter or fluid), the time constant of the relaxation differ. By varying the use of pulse sequences with different timing and excitation characteristics, different tissue types can be imaged with high spatial resolution[82].

Functional MRI (fMRI) is a non-invasive imaging technique developed in the early 1990s to measure brain activity associated with blood flow[81]. Specifically, fMRI utilize the blood oxygen level-dependent (BOLD) contrast to indirectly measure neuronal activity, as observations confirm that neuronal activation and cerebral blood flow are coupled. The underlying assumption is that, local neuronal activity requires increased flow of oxygenated blood, and the increase in the oxy-/deoxy-hemoglobin ratio induce local magnetic changes that can be detected though fMRI[83,84]. Typically, fMRI software model the BOLD signal through fitting it to a standard model of the hemodynamic response function (canonical HRF), which is characterized by (an initial dip followed by) a gradual rise, peaking ~5-6 seconds after stimuli onset, return to baseline at ~12 seconds, followed by a small undershoot before stabilizing again at ~25-30 seconds after stimuli onset[81].

Statistical analysis of fMRI data[85] is a complex process that is dominated by computational models and require disciplined interpretation of the experimental results[86–88]. For example, analysis of the same fMRI dataset and research questions, can yield extensive variability in

results and data interpretation[89]. Nevertheless, analytical flexibility is a challenge in scientific domains other than neuroimaging[90] and thorough meta-analytical approaches can aid in reaching consensus in (many fields, including) the field of neuroimaging[89]. For detailed information on fMRI data preprocessing steps and analysis, see included articles (**study II-V**).

2.5.1.1 Event-related Task-based fMRI

In event-related fMRI, participants are placed within the scanner and asked to perform a task. In this way, neuronal responses to various forms of stimuli can be investigated. Typical experimental designs include comparing brain activation during a task with brain activation during baseline or a "rest" condition[81]. For example, in **study II and IV** in the current thesis, painful stimuli were used as the task condition, and sensory stimulation was the comparison condition. In **study III and IV**, the event-related task designs included multiple events such as: anticipation for high pain (red cue), anticipation for low pain (green cue), high painful stimulus, mid-intensity painful stimulus, low painful stimulus, time to rate perceived pain intensity, and time to rest (fixation cross) in between events.

2.5.1.2 Psychophysiological Interaction Analysis

Psychophysiological interaction (PPI) is a task-based functional connectivity analysis that can be used to investigate the interaction between an experimental condition (psychological) and a source region (physiological) that is based on a selected volume of interest. The analysis provides information about the contribution of one region to another (i.e., functional connectivity between brain regions) in relation to an experimental context[91]. PPI analysis is used in **study II-V**, and in these studies, the experimental context either refers to the pain anticipation prior to the painful stimulation (**study IV**), or the application of painful stimulation itself (**study II, III, IV**).

2.5.2 Positron Emission Tomography (PET)

Positron emission tomography (PET) is a nuclear imaging technique developed in the 1970s for clinical use and is based on the injection of radiolabelled substances, known as radiotracers. Unique features of PET include visualizing and measuring physiological activity at nanomolar concentration levels (or less) such as metabolic pathways, receptor

density or molecular targets[92]. The sensitivity of PET relies on (1) the radiochemists' ability to produce labelled compounds with very high specificity (i.e. radiolabelling a high percentage of the compound to be injected) and (2) the ability to detect and localize the positron-emitting nuclei by using coincidence counting to capture the paired annihilation photons emitted following positron annihilation with an electron[93]. PET imaging technique was used in **study I** in combination with the radio ligands detailed below.

2.5.2.1 [^{11}C]PBR28 Ligand

In the human CNS, glial activation can be studied *in vivo* using positron emission tomography (PET) and radioligands that bind to the 18-kDa translocator protein (TSPO), such as [^{11}C]PBR28. TSPO is mainly located on the outer mitochondrial membrane and, in general, sparsely expressed in the brain under normal conditions, but widely upregulated under inflammatory conditions in glial cells such as microglia and astrocytes[76,77]. While TSPO upregulation in neuroinflammatory responses consistently co-localizes with microglia, an accompanying astrocytic component has been observed in some[77,94,95], but not all cases[96,97].

2.5.2.2 [^{11}C]-$_L$-Deprenyl-D_2 Ligand

In order to better differentiate between microglia and astrocytic glial cells, a subsample of FM patients received a second PET scan with ligand [^{11}C]-L-deprenyl-D_2, which binds to monoamine oxidase B (MAO-B). In the brain, MAO-B is thought to be mainly, if not exclusively, expressed within astrocytes[98].

3 RESEARCH AIMS

The overarching aim of the current thesis was to investigate similarities and differences in cerebral pain processing of individuals with a nociceptive pain condition (rheumatoid arthritis) and nociplastic pain (fibromyalgia). The aim of **study I** was to assess if glia cell activation is present in FM. Study **II-IV** aimed to illuminate and characterize mechanisms implicated in CNS pain processing in FM and RA. **Study V** aimed to make a direct comparison of cerebral pain processing between these two patient groups. Specifically, five research papers were included in the current thesis, with four defined aims:

- **Study I**: Examine the potential role of glia activation in the brain of FM patients.
- **Study II**: Investigate disease-relevant cerebral pain processing in patients with inflammatory nociceptive pain (RA).
- **Study III and IV**: Characterize how contextual factors may influence and disrupt cerebral pain processing in nociplastic pain (FM).
- **Study V**: Investigate divergencies (and commonalities) in cerebral pain processing in patients with well-characterized nociceptive inflammatory pain (RA) compared to well-characterized nociplastic pain (FM).

4 MATERIALS AND METHODS

The work in the current thesis utilizes two brain imaging techniques (PET and fMRI) to assess inflammatory and pain-related mechanisms in the central nervous system (CNS). Participant cohorts originate from 4 independent data collections. Shared among all works is that all patients were screened by- and had received their respective diagnosis from a medical doctor to ensure well-characterized fibromyalgia (FM) and well-characterized rheumatoid arthritis (RA) patient cohorts.

In brief, **Study I** was a collaborative project that combined FM patient data and healthy subject data from two independent research centres: Karolinska institutet (KI) in Stockholm, Sweden and Massachusetts General Hospital (MGH) in Boston, MA, United States. In **study II**, RA patients were recruited to participate in a randomized, placebo-controlled trial investigating the effects of a tumour necrosis factor (TNF-alpha) inhibitor on inflammation and pain through the rheumatology clinic at the Karolinska Hospital in Stockholm, Sweden. Important to note is that **study II** only included baseline-data, i.e. comparing cerebral pain modulation prior to anti-inflammatory treatment onset in RA patients with healthy controls. **Study III and IV**, included FM subjects and healthy participants recruited through advertisement in the daily press to participate in a fairly large study (FM n >80; HC n >40) investigating multiple aspects of pain sensation and cerebral pain processing (e.g. fMRI, pain modulation, cognitive and affective aspects, peripheral and central inflammation and pain-related genes). **Study V** share RA patient cohort with **study II**, but the FM patients were initially recruited to participate in a multi-centre longitudinal intervention study investigating the cerebral effects of 15-week physical exercise or relaxation therapy. Important to note, is that **study V** only included baseline-data prior to treatment onset in both RA and FM cohorts. Namely, the identical procedures, including the same fMRI scanner and facilities, same data collection period, same paradigm and the same pain stimulator probe.

4.1 PARTICIPANTS

All patients were screened and diagnosed by a medical doctor to ensure proper diagnosis and that RA patients did not have concomitant FM, or that FM patients did not have

concomitant RA, and that no patients exhibited signs of any other pain condition than their primary diagnosis. In **study I**, a total of 31 FM patients and 27 healthy controls received a [^{11}C]-PBR28 PET brain scan, and 11 FM patients and 11 controls received a [^{11}C]-L-deprenyl-D_2 brain scan. In **study II**, fMRI scans of 31 RA patients and 23 HC were analysed. In **study III**, fMRI data were analysed from 67 FMSs and 34 HCs. In **study IV**, fMRI data were analysed from 65 FMSs and 33 HCs (same subjects as in study III). In **study V**, fMRI data were analysed from 31 RA patients (same subjects as in study II) and 26 FM subjects.

4.1.1 Fibromyalgia Patients

All fibromyalgia subjects (**study I, III, IV and V**) underwent a physical examination by a specialist in rehabilitation medicine and pain relief on a separate occasion to ensure that they fulfilled the inclusion criteria. Inclusion criteria for all FMSs (**study I-V**) were female sex, right handed, and meet the ACR-1990 FM criteria[29]. Additionally, in **study I, III and IV**, FM subjects were also screened to meet the modified ACR-2010 criteria (referred to as ACR- 2011)[30]. Exclusion criteria for all studies (i.e. **study I-V**) were other dominant pain conditions than fibromyalgia, rheumatic or autoimmune diseases, other severe somatic diseases (neurological, cardiovascular, cancer, etc.), psychiatric disorders, ongoing treatment for depression or anxiety, substance abuse, pregnancy, magnetic implants, previous brain or heart surgery, hypertension (>160/90 mm Hg), obesity (body mass index >35), smoking (>5 cigarettes/day), medication with antidepressants or anticonvulsants, inability to speak or understand Swedish, self-reported claustrophobia, not being able to refrain from non-steroidal anti-inflammatory drugs, analgesics, or hypnotics for at least 48 hours before study participation (48 hours before the first visit, and 72 hours before the second visit, i.e., the scanning session). In **study I, III, IV** included FM subjects were of working age (20-60 years). In **study V**, FM patients could be of age up to 65 years and excluded if reporting high consumption of alcohol (Audit >6), participation in any other rehabilitation program within the past year, contemporary regular resistance exercise training or relaxation exercise training ≥ 2/week.

4.1.2 Rheumatoid Arthritis Patients

All rheumatoid arthritis patients (**study II and V**) were screened by a medical doctor to ensure that patients were fulfilling the ACR 1987 classification criteria for RA[32], working

age (≥18 years), clinical indication for use of TNF-blockers, and approved for MR examination. Exclusion criteria were left handedness, fibromyalgia comorbidity, neurological disease, comorbid depression, ongoing treatment with antidepressants, severe cardiovascular disease, latent tuberculosis, claustrophobia, pregnancy, previous treatment with biologics or other motives based on the judgment of the responsible physician. A specialist in rheumatology identified the most inflamed finger joint in RA patients.

4.1.3 Healthy Subjects

Healthy controls were recruited in parallel to their respective patient cohorts (**study I-IV**), with the attempt to balance sex and age between the groups. Exclusion criteria for the HC were identical to the patients, with the additional exclusion criteria of recurrent pain problems, including RA and FM.

4.2 BEHAVIOURAL AND CLINICAL ASSESSMENTS

American college of Rheumatology 2011[30] self-report survey for the assessment of FM was used in **study I**. Subscales include symptom severity, wide-spread pain index, trouble thinking, fatigue and waking up tired. See **table 1b** for additional information.

Beck's Depression Inventory[99] is a 21-item multiple-choice questionnaire assessing the severity of depression. Scoring allows for the identification of the degree of depressive symptoms, ranging from mild, moderate, to high. Higher BDI scores indicate more severe depressive symptoms. BDI was used in **study I, III, IV**.

Disease Activity Score based on 28 joint count (DAS 28)[100] is a composite measurement to assess disease activity in RA patients. A rheumatologist or specialist nurse collect information regarding (1) number of swollen joints, (2) number of tender joints, (3) blood sample to measure erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP), (4) asking the patient to indicate "global assessment" on a 100mm visual analogue (VAS)-scale anchored by 0 "very bad" to 100 "very good". The assessment, including ESR, was used in **study II and V**.

Disease Duration (in months) was collected in **all studies (I-V)**.

Euro Quality of Life 5-dimension scale (EQ-5D)[101] is a self-assessment of current health. The scale consists of 5 items (surveying mobility, self-care, usual activities, pain/discomfort and anxiety/depression) and one 100mm VAS-scale anchored by 0 "worst imaginable health state" to 100 "best imaginable health state." The assessment was used in **study II**.

Fibromyalgia Impact Questionnaire[102,103] is a 20-item self-report assessment developed for clinical and research settings to assess the current health status in individuals with fibromyalgia. Measures pain, stiffness, fatigue, morning tiredness, work status (missed days of work and difficulty), depression, anxiety and well-being over the past week. The assessment was used in **study I, III, IV and V**.

Health Assessment Questionnaire[104] is an instrument developed to assess level of difficulty patients with rheumatoid arthritis have experienced the past week when dressing and grooming, arising, eating, walking, reaching, gripping, and during hygiene routines and common daily activities. The instrument contains 8 sections with 2 or 3 items in each section. Scoring within each section range from 0 to 3 (0= without any difficulty; 1 = with some difficulty; 2 = with much difficulty; 3 = unable to do). The assessment was used in **study II and V**.

Hospital Anxiety and Depression scale[105] is a 14-item self-assessment divided into two subscales: anxiety (HAD-a) and depression (HAD-d) consisting of 7 items each. Subjects rate on a 4-likert scale (ranging from 0-3). A total score of <7 points of either subscale is regarded as of no clinical relevance, 8-10 points indicate intermediate levels, and >11 points indicate clinically relevant levels of depression or anxiety. The assessment was used in **study II and V**.

Multidimensional Fatigue Inventory[106] is a 20-item self-assessment instrument designed to measure five dimensions of fatigue. In the current study, we used the subscale "general fatigue" which is suggested to be used if only a short instrument of fatigue is required [106], and it has been found to be highly correlated with 100mm VAS fatigue assessment in clinical populations [107]. The assessment was used in **study V**.

Pain Catastrophizing Scale [108] is a self-assessment 13-item scale, with a 5-point scale ranging from 0 "not at all" to 4 "all of the time". The PCS is divided into 3 subscales: rumination, magnification, and helplessness. Example of items corresponding to the 3 subscales in order: "I keep thinking about how much it hurts," "I become afraid that the pain may get worse" and "there is nothing I can do to reduce the intensity of the pain."

Higher PCS scores indicate more intense pain catastrophizing. PCS was used in **study I, III and IV**.

The State-Trait Anxiety Inventory—State[109] is a self-assessment used for measuring state-related anxiety, i.e., current feelings of anxiety. The questionnaire consists of 20-items, with a 4-point scale ranging from "almost never" to "almost always". Items include "I worry too much over something" and "I feel like a failure." Scores range from 20 to 80, with higher scores indicating higher levels of anxiety (clinically significant cut-off point for STAI-S scale is 39-40). The assessment was used in **study III and IV**.

Visual Analogue Scale (VAS) is a one-dimensional self-assessment instrument to assess subjective experience of pain. The scale consists of a 100mm scale anchored by 0 "no pain" to 100 "worst imaginable pain". The VAS can be used to assess subjective experienced pain at a particular location or general/overall in body, either in the present moment or retrospectively such as in the past week. The VAS instrument was used to assess participants' current subjective experience of pain in **all studies (i.e. I-V)**. Further, VAS can be used to measure levels of fatigue[110] by asking subjects to indicate their level of fatigue on a 0-100mm VAS (ranging from 0 "no fatigue" to 100 "severe fatigue"). The assessment was used in **study V**.

4.3 PRESSURE PAIN ASSESSMENT

Pressure pain assessment was used in **study II, III, IV and V**. An automated, pneumatic, computer-controlled plastic piston was used to apply pressure in **study II and V**. A rapid cuff inflation system was used to apply pressure in **study III and IV**. In all these studies (II-V), all participants underwent a subjective calibration procedure one day prior to brain scanning, in order to assess which pressures to be delivered in the fMRI scanner the next day.

4.3.1 Study II and V: Pressure Probe

In **study II and V**, subjective calibration of pressure pain sensitivity (to be used in the fMRI scanner) was performed using an automated, pneumatic, computer-controlled stimulator that applies pressure via a plastic piston with a 1 cm^2 hard rubber probe. In RA

patients, the probe was placed on the clinically most affected proximal interphalangeal joint of the patient's left hand and at the non-affected left thumbnail. In FM patients, the probe was placed on the left thumbnail. Following each pressure, subjects were instructed to rate the pain intensity on a visual analogue scale (VAS, anchored by 0 = "no pain" and 100 = "worst imaginable pain"). All pressures were applied for 2.5 sec with 30 sec intervals.

The aim of the subjective calibration procedure was to ultimately determine which individual pressure (kPa) that corresponded to a subjective rating of 50mm VAS (designated as P50). First, subjects received one ascending series, with increasing steps of 50 kPa, to determine the pressure pain threshold (first VAS >0 mm) and stimulation maximum (first VAS > 60 mm). Next, subjects received three randomized series of five different pressure intensities within the range of each patient's pressure pain threshold and stimulation maximum. Last, the final 15 ratings from the randomized series of pressure pain were fitted using a polynomial regression in order to ultimately determine each subject's representation of P50.

4.3.2 Study III and IV: Rapid Inflatable Pressure Cuff

In **study III and IV**, subjective calibration of pressure pain sensitivity was performed using a rapid cuff inflation system (CPA, Hokanson E20/AG101). The aim of the calibration procedure was to ultimately determine each participant's subjective rating corresponding to 10/100mm (P10) and 50/100mm (P50) VAS. These pressures were used in the fMRI scanner the next day. The cuff was placed on subjects' left calf. Subjects received one ascending series of 5-second stimuli with increasing steps of 25 mmHg to determine the pressure pain threshold (first VAS > 0 mm) and the stimulation maximum (first VAS > 60 mm) to determine the endpoints of pressure stimuli. Next, subjects received two randomized series of 5 second pressure stimuli and were asked to rate their perceived pain following each stimulus on a 100mm handheld visual analogue scale (VAS, anchored by 0 = "no pain" to 100 = "worst imaginable pain"). The randomized series to determine P10 used the pressure pain threshold as a starting point with increments of +/- 10 mmHg or 25 mmHg, and the randomized series to determine P50 used the stimulation maximum as a starting point with decreasing increments of 25 mmHg.

4.3.2.1 Pain Conditioning: Contextual Influences on Pain Perception

Following the individual calibration procedure, the subjects in **study III and IV** completed an acquisition phase of a conditioning paradigm outside the scanner. In which, subjects trained in front of a computer monitor to associate a green circle with their individually calibrated P10 stimulation and a red circle with their individually calibrated P50 stimulation. The stimuli were presented in a pseudo-randomized order of 10xP10 and 10xP50. Following each stimulus, subjects rated their perceived pain on a computerized 100mm VAS.

In total, all subjects underwent three phases of the conditioning experiment, i.e., (1) an acquisition phase (CS is repeatedly associated with US) outside the scanner on day 1, (2) an acquisition phase (CS is repeatedly associated with US) inside the scanner on day 2, followed by (3) an experimental test phase (testing the strength of the CR) inside the scanner also on day 2.

4.4 BRAIN IMAGING

4.4.1 Study I: PET Brain Glial Inflammation

Study I used multi-tracer positron emission tomography (PET) to investigate the presence of neuroinflammatory responses, i.e activated neuroimmune brain glial cells, in the fibromyalgia brain compared to healthy subjects. The PET ligand [^{11}C]PBR28 was used to assess glial cell activation (including microglia and astrocytes). The PET ligand [^{11}C]-L-deprenyl-D_2, which primarily binds to astrocytes, was used in order to help differentiate which glial cell that would be driving the [^{11}C]PBR28 signal. Theoretically, if brain regions demonstrate increased [^{11}C]-L-deprenyl-D_2 signal within brain regions also showing [^{11}C]PBR28 signal, the result would suggest an astrocytic contribution to the signal. Whereas, the absence of [^{11}C]-L-deprenyl-D_2 in brain regions showing elevated [^{11}C]PBR28, would suggest that the signal is dominated by microglial cells.

4.4.2 Study II and V: fMRI Cerebral Pain Modulation

The day after subjective pain calibration, all subjects in **study II and V** entered the fMRI scanner and underwent stimulation with a pressure probe using their previously individually calibrated painful pressure (P50) and a non-painful pressure stimulus (50 kPa). Prior to scanning, each subject was instructed to focus on the pressure delivered to the left thumbnail or joint and not to use any coping or distraction techniques. In **study II**, RA patients were exposed to two runs of stimuli applied to the affected joint and two runs of stimuli applied to the thumbnail. The corresponding sites were used for healthy controls. In **study V**, only the two runs of disease-relevant joint stimulation were analysed for RA and compared with two runs of thumbnail stimulation in FM. Each run consisted of 30 pressure stimuli, 15 painful and 15 non-painful stimuli, which were presented in a pseudo-randomized manner. All stimuli inside the MR scanner were delivered for 2.5 seconds and jittered over time with a mean interval between onsets of stimuli of 15 seconds (range 10-20s). Total duration of each run was 8 minutes and 15 seconds.

4.4.3 Study III and IV: fMRI Contextual Influences on Pain Perception

The day after subjective pain calibration and contingency acquisition, all subjects in **study III and IV** entered the fMRI scanner to complete two paradigms investigating contextual influences on pain perception (Figure 2). The first fMRI paradigm was a congruent paradigm, identical to the paradigm the subjects had trained on outside the scanner the day before (*see* 4.3.2.1). In this paradigm, subjects were presented with red and green cues and received correctly cued congruent painful stimulation, i.e., high (P50) and low (P10) pain, respectively. After a short break, subjects were asked to repeat the same paradigm. However, this time, only the initial first four stimulations were identical to the preceding run and served as a reminder boost (pseudo-randomized 2 x P10green and 2 x P50red) and then the experimental test phase began. The purpose of the experimental test phase was to test the acquired cue-pain associations. This phase consisted of an incorrectly cued incongruent paradigm where subjects did not receive the painful stimulation that matched their previously acquired cue-pain association. Specifically, both green and red cues were followed by an identical and novel medium intensity painful pressure (P30) that the subjects had not previously been exposed to. The medium intensity pressure corresponded to each subject's calculated average between P10 and P50 (P30=(P10+P50)/2)), and was presented in a pseudo-randomized order of 10 x P30green trials and 10 x P30red trials.

Painful stimuli were delivered for 5 seconds and jittered over time with a mean interval between onsets of stimuli of 20 seconds, including 8 sec rating time. Both sessions lasted for approx. 11 minutes in total. **Study III** investigated neural mechanisms in response to the painful stimulus in the latter paradigm only. That is, whether the cerebral response to mid-intensity pressure pain may vary depending on whether it was preceded by a high or low pain signalling cue. **Study IV** investigated cerebral activation during pain anticipation (prior to pain stimuli onset) across both congruent and incongruent paradigms.

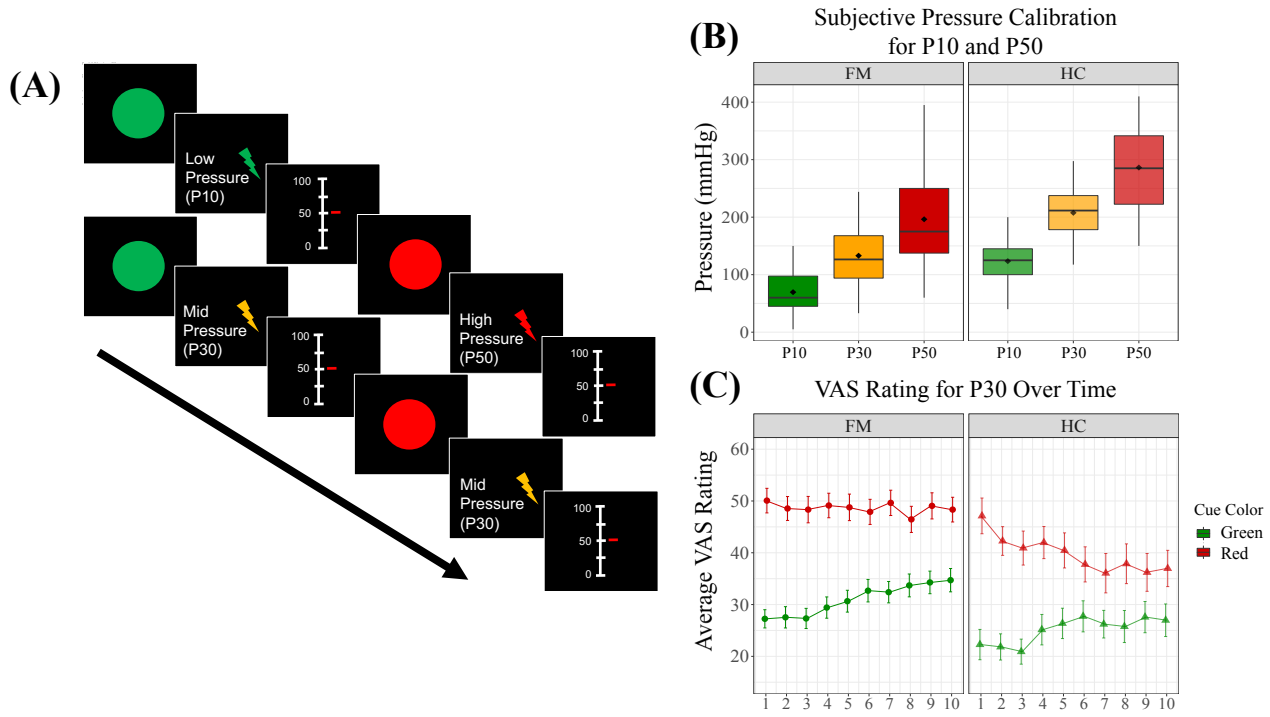

Figure 2. ***Illustrates the experimental paradigm in study III and IV.***

(A) Top row: exemplifies the conditioning paradigm in which green and red cues were followed by low and high painful pressure, respectively. Bottom row: exemplifies the experimental paradigm investigating conditioned pain responses in FM and HC. Here, both green and red cues were followed by an identical mid-intensity painful pressure. **(B)** Boxplots illustrate average pressure (mmHg) corresponding to subjectively calibrated pain ratings of 10mm VAS (P10) and 50mm VAS (P50). P30 corresponds to each subjects' calculated average between P10 and P50. Fibromyalgia values are displayed in the plot to the left, and HC values are displayed in the plot to the right. Horizontal lines within boxes represent median values. Black dots represent mean values. Box top and bottom frames represent 25th and 75th percentile. Whiskers represent minimum and maximum values. **(C)** Illustrates changes in pain ratings over time in FM (left) and HC (right) in response to mid-intensity painful pressure P30. Pain ratings varied significantly depending on whether the P30 pressure was following a red (top row, red color) or a green (bottom row, green color) visual cue (p <0.001). Dots (FM) and triangles (HC) represent mean rating values, and error bars represent standard deviations. FM = fibromyalgia; HC = healthy control; VAS = visual analogue scale.

5 RESULTS

5.1 STUDY I: PET IMAGING IN FM

The results in **study I** revealed upregulated [^{11}C]PBR28 binding in FM patients compared to healthy controls (HC) in many cortical regions such as primary somatosensory cortex (S1), dorsolateral prefrontal cortex (dlPFC), precuneus, and anterior midcingulate cortex (MCC). Subjective ratings of fatigue, which is one of the most common symptoms reported by FM patients [30,111], was correlated with increased TSPO binding in anterior and posterior MCC in FM patients. No group differences were found in [^{11}C]-$_L$-deprenyl-D_2 signal, which suggests that the increased [^{11}C]PBR28 signal in FM patients vs. HC reflect increased microglial- and not astrocytic activation.

5.2 STUDY II: FMRI CEREBRAL PAIN PROCESSING IN RA

The results indicated normal pain sensitivity and cerebral pain processing in RA (without fibromyalgia co-morbidity) for non-affected sites, while increased pain sensitivity (i.e. lower P50) at inflamed joints indicate peripheral/spinal sensitization.

Brain imaging data revealed significantly less pain- and somatosensory processing (S1, M1, insula, S2, MCC) in RA patients compared to HC when painfully stimulated over the disease-affected joint, whereas no group differences were found when painfully stimulated over the non-affected thumbnail. Similarly, RA patients revealed significantly less brain activation during pain processing at disease-affected joint vs. non-affected thumbnail in bilateral S1, S2 and insula.

Specifically, when RA patients were stimulated over the most inflamed finger joint, RA patients exhibited a significant decrease in the activation of the right dlPFC. PPI seeding from the right dlPFC, revealed significantly positive coupling between right dlPFC and left dlPFC during painful stimulation at the inflamed finger joints, indicating that activity in both right and left dlPFC changes conjointly (i.e. in this case decreasing) during the evoked pain.

5.3 STUDY III: FMRI CONDITIONED PAIN RESPONSES

The results in **study III** demonstrated increased P30green ratings over time in FM subjects (FMSs), while P30red ratings remained elevated. HC adapted all pain ratings to resemble moderate pain. FMSs exhibited increased activation for [P30green>P30red] in M1/anterior insula, whereas HC showed increased S2/mid insula response to [P30red>P30green].

Contextual influences on cerebral pain processing were more pronounced among high pain catastrophizing FMSs. Specifically, high pain catastrophizing scores (PCS) in FMSs co-varied with heightened brain activation for mid-intensity pressure pain following green cues (i.e. contrast [P30green]*PCS) in dlPFC and medial prefrontal cortex (mPFC)/orbitofrontal cortex (OFC); and with mid-intensity pressure following green cues compared to mid-intensity pressure following red cues (i.e. contrast [P30green>P30red]*PCS) in dorsal anterior cingulate cortex (dACC)/mid cingulate cortex (MCC), superior temporal pole extending to anterior insula, bilateral thalamus, and posterior insula. In other words, high pain catastrophizing scores interfered with cerebral pain processing of the identical painful pressures, depending on whether they followed a green or red cue. Psycho-physiological interaction analysis for contrast FMS[P30green>P30red]*PCS revealed a dissociation in functional connectivity between thalamus and bilateral inferior parietal lobe.

5.4 STUDY IV: FMRI PAIN ANTICIPATION

The results in **study IV** demonstrated that repeatedly violated *high pain* associations were associated with reduced left dlPFC activation in FMSs vs. HCs together with maintained elevated pain ratings in FMSs despite that the painful stimulus had been significantly lowered (i.e. high pain replaced by a lower mid-intensity painful pressure).

Repeatedly violated *low pain* associations (i.e. low pain replaced by a higher mid-intensity painful pressure), was associated with reduced right dlPFC activation in FMSs compared to HCs. Yet, both groups simultaneously increased their behavioral pain ratings to accurately resemble mid-intensity pain. PCS interfered with anticipatory processing in FMSs during violated low pain associations (i.e. FM[GreenCue-P30*PCS]-). Specifically, increased PCS scores were associated with reduced brain activation during repeatedly violated low pain associations in medial frontal gyrus and rostral anterior cingulate cortex (rACC).

Moreover, a group x green cue congruency interaction was detected in the right dlPFC. Specifically, HC vs. FMSs revealed significantly more de-activation of right dlPFC activation during correctly cued congruent low pain anticipation. Whereas, during violated low pain anticipation, HC vs. FMSs revealed significantly increased right dlPFC activation. Psychophysiological interaction connectivity analysis, seeding from the interaction cluster in the right dlPFC, revealed increased functional connectivity during correctly cued low pain anticipatory trials, in HC compared to FMSs between right dlPFC and supplementary motor area encapsulating MCC, and right S1.

5.5 STUDY V: COMPARING CEREBRAL PAIN MODULATION IN RA AND FM

The results from **study V** revealed disease distinct differences in the activation of the brain's descending pain modulatory system in RA and FM patients, which could neither be explained by individual pressure intensity, gender, depression, anxiety nor fatigue. Specifically, whereas RA vs. FM patients had a reduction in the initiation of early cortical top-down regulation (i.e. dorsolateral prefrontal cortex, dlPFC) during evoked pain, FM vs. RA revealed reduced pain-related functional connectivity between dlPFC and deeper structures within the cortex (i.e. medial prefrontal cortex/rostral anterior cingulate cortex, mPFC/rACC). Pain-related disruptions in modulatory and attentional brain regions were related to ongoing clinical pain intensity in FM only, which indicate more prominent pain-related cerebral disruptions in patients suffering from nociplastic pain.

6 DISCUSSION

The prevalence of concomitant FM is inexplicably high among RA patients[8,9] and a contemporary challenge is to resolve why some RA patients continue to report pain despite adequate treatment of their peripheral inflammation[6–8]. Therefore, many attempts have been made to study the link between cerebral and inflammatory mechanisms in RA patients with concomitant FM. Yet, on the behavioural level, previous studies indicated fundamental differences in descending pain modulation between these two patient groups when they are well-characterized. The overarching aim of the current thesis was to identify and fill contemporary gaps of knowledge related to cerebral pain processing and associated mechanisms (i.e. contextual influences and neuroinflammation) in patients with a well-characterized rheumatoid arthritis (nociceptive pain) and well-characterized fibromyalgia (nociplastic pain) condition (**Study I-IV**). The ultimate goal was to directly compare disease-related cerebral pain processing mechanisms in these two patient groups (**Study V**). Increased knowledge of these mechanisms is of vital importance for understanding the manifestation of nociplastic pain and, in the long run, improving treatment outcomes for affected patients.

6.1 CEREBRAL PAIN MODULATION IN NOCIPLASTIC AND NOCICEPTIVE CHRONIC PAIN

While aberrant cerebral pain processing has been widely demonstrated in FM[39], **study II** was the first, to the extent of our knowledge, to investigate disease-specific (disease-affected vs. non-affected sites) cerebral pain modulation in RA patients compared to healthy controls (HC), and **study V** was the first to directly compare disease-relevant cerebral pain processing in RA (without FM co-morbidity) and FM (without RA co-morbidity) patients. Together, the results from **study II and V** in the current thesis are the first to propose distinct disruptions in pain modulatory and attentional brain networks and regions between RA (primarily dlPFC) and FM (primarily mPFC/rACC). Moreover, in FM patients, disruptions in pain-related cerebral activation was correlated with higher degrees of clinical pain, which indicate more pronounced disruptions in patients suffering from nociplastic pain (FM).

The dlPFC and rACC play prominent roles in the descending pain modulatory system, communicating primarily via opioid-dependent signalling, and can engage lower parts of the descending pain regulatory system such as thalamus and brainstem[34,35]. The observed group-differences in **study V** were not confounded by individual pressure intensity, gender, depression, anxiety or fatigue, and the brain regions implicated in **study II and V** are well in alignment with previous literature[37,39,40,42,63,64]. Regarding RA patients, the results of reduced dlPFC activation in RA vs. FM during painful stimulation are in alignment with previous reports (using other imaging modalities) of aberrant pain-related dlPFC activation in RA patients compared to HC[63,64]. Regarding FM patients, a meta-analysis suggest that the ACC is among the top brain regions likely to find reduced activation in FM during pain modulation[39] and is the most likely brain region to observe estimates of gray matter loss in FM[42]. Moreover, previous studies have demonstrated that FM compared to HC display reduced functional connectivity between rACC and other descending pain modulatory brain regions[112]. Worthy of note from **study V**, was that in FM patients only, increased self-assessed overall clinical pain correlated with diminished pain-related brain activation throughout the entire cingulate cortex (ACC, MCC, PCC), as well as other brain regions implicated in descending pain modulation such as dlPFC, hippocampus/anterior insula, cerebellum, which may indicate more prominent disruptions in patients with nociplastic pain. It has been suggested that the (commonly observed) decreased pain-evoked neural activity in FM subjects is associated with decreased mu-opioid receptor availability in FMS, which suggests that the opioid system is a central contributor to FMS related pain[48].

Taken together, the results from **study II and V** show normal cerebral pain processing from non-affected sites in RA, while cerebral processing of painful joint stimulation is associated with prefrontal dysfunction (i.e. dlPFC), which is involved in early initiation of the pain modulatory system. In FM, cerebral pain processing was associated with overall clinical pain and reduced engagement of more medial structures such as mPFC and rACC. The results are consistent with previous investigations where RA or FM patients were compared to healthy subjects, and suggest that previous observations hold for group comparisons between nociceptive and nociplastic pain patients even when controlling for pain sensitivity and anxiety.

6.2 CONTEXTUAL FACTORS AND CEREBRAL PAIN MODULATION IN NOCIPLASTIC PAIN

The experience of pain in chronic pain patients is multifaceted and influenced by numerous cognitive and psychological aspects, which in many cases, can be more disabling than the sensory aspect of pain itself[113]. **Study III** was the first study to combine behavioral and neuroimaging data suggesting that FMSs display a predisposition to forming new pain-related associations while simultaneously maintaining high-pain associations that are no longer relevant. **Study IV** extended these findings, and revealed that FMSs vs. HC exhibited reduced dlPFC activation during repeatedly violated high pain associations, which may help explain why ratings of high pain persist in FMSs despite that the subsequent pressure stimulation has been lowered, i.e. high pain replaced by a lower mid-intensity painful pressure. Taken together, **study III** and **study IV** suggest that dysfunctional update of high pain-associations in FMSs result in a continuous accumulation of new painful associations, together with an undermined extinction process, until all (i.e. both high and low) pain-signaling cues are associated with a subsequent high pain response.

Specifically, during correctly cued low pain anticipatory trials (i.e. low pain predictive cue followed by a low painful stimulus), FMSs vs. HCs exhibited lower functional connectivity between brain regions implicated in cognitive modulation of pain (dlPFC), and nociceptive processing (S1 and SMA/MCC). These results may help elucidate FM behavioral reports of impaired safety processing and reports of expecting to receive pain in all situations, even if they are non-painful[54,55]. Likewise, when low pain associations were repeatedly violated (i.e. low pain replaced by a higher mid-intensity painful pressure), FMSs exhibited non-activation of the right dlPFC, which diverged from the significantly increased dlPFC activation seen in HC. Following the aberrant processing of violated low pain associations, FMS exhibited increased insular response to the identical mid-intensity pressure pain when it followed a conditioned low- vs. high pain signaling cue. The insular cortex is involved in multisensory integration, interoceptive experience of pain[11,36], and modulates aversion responses in uncertain situations[114,115]. In FMS, the degree of insular activation in response to aversive stimuli, has been found to correlate with FM patients' self-reported clinical pain and can be directly diminished following administration of pregabalin (but not placebo)[116]. The behavioral ratings in **study III** demonstrated that FMSs detected that the pressure following the violated low pain predictive cues had been increased (from low to mid-intensity), but they did not detect that the pressure following the violated high pain

predictive cue has been significantly lowered (from high to mid-intensity). Taken together, the combined results from **study III and IV** during low pain associative trials, showing that FMS formed new (perhaps aversive) associations linking higher pain to the green cue, are in alignment with literature suggesting that FMSs and other chronic pain patient groups are more focused on detecting pain threats[58], more efficient in forming new pain-related associations[55], and that low pain associations are more easily extinguished and more sensitive to sensory experience than high pain associations[117–120]. Moreover, the observations of identical right dlPFC non-activation in FMSs during congruent and incongruent low pain anticipation trials, may be related to behavioral reports of aberrant safety processing in FMS[54,55] and that patients expect to receive pain in all sorts of situations even if they are non-harmful[55]. It is tempting to speculate that, increased protective responding (e.g. failure to extinguish) may be an adaptive process in the short term[58], in alignment with a "better-safe-than-sorry" approach to pain. However, in the long run, an exaggerated protective response may worsen pain disability and possibly contribute to chronic pain maintenance[58,59] as disproportionate responses to non-harmful events may lead to increased anxiety as more cues in the environment have the potential to signal harm[58,59,121].

6.2.1 The Influence of Pain Catastrophizing

High pain catastrophizing scores (PCS) in FMSs were associated with altered brain activation throughout pain anticipation and processing of painful stimuli exclusively during violated low pain associations. Specifically, high PCS in FM subjects was associated with reduced mPFC/rACC brain activation in the repeatedly violated low pain anticipatory phase (**study IV**), which was followed by an increased wide-spread cortical brain activation (inclusive mPFC) during the mid-intensity pain painful pressure stimulation itself (**study III**). These results are in alignment with previous observations in FMSs, suggesting that high pain catastrophizing interferes with pain-anticipatory brain activation[122] and cognitive modulation of pain[123]. The relationship between catastrophizing and heightened pain experience is hypothesized to be mediated through attentional processes[124]. The mPFC is a region eminently implicated in evaluating behavioral and emotional salience[125]. In this way, the increased mPFC activation (**study IV**) in high pain catastrophizing FMS may reflect an increased salience directed toward the forthcoming mid-intensity pressure following the low-pain predictive cue. Indeed, the effect of PCS was even more pronounced in **study III**, when comparing FMS brain activation in response to the identical mid-painful pressure

depending on whether it was following a low vs. high pain predictive cue. This contrast engaged multiple brain regions consistently overlapping with neuroimaging meta-analysis of pain catastrophizing[126] and instructed fear conditioning[127]. Namely, in dACC/MCC, insula, bilateral thalamus, mPFC, putamen, superior-, and middle parts of the temporal lobe. Taken together, **study III and IV** suggest that contextual factors disrupt pain anticipation and the subsequent pain modulation in nociplastic pain. Higher pain catastrophizing ratings in FMS interfere with neural processing of anticipation, as well as the perception of pain, during violated low pain associations exclusively. Specifically, FM subjects exhibited reduced prefrontal (mPFC/rACC) activation during pain anticipation, followed by wide-spread cortical hyperactivation during painful stimulus application itself.

Pain catastrophizing is a feature present among RA patients as well, and has been associated with increased pain sensitivity, increased perceived severity of pain, impaired physical functioning, an elevated risk of developing persistent long-term pain and predict more severe depressive symptoms[128]. Higher levels of depressive symptoms have, in turn, been found to increase mPFC neural responses to painful stimulation over the joints in RA patients[65]. These results may, arguably, be similar to the results observed in **study III and IV**, where PCS co-varied with increased mPFC response during violated low pain anticipation (**study IV**) as well as during the identical mid-intensity painful pressure (**study III**) following a low vs. high pain predictive cue. Taken together, pain catastrophizing modulates the experience of pain in both FM and RA, and has specifically been found to interact with FM neurobiology as it influences cerebral pain processing of the identical mid-intensity painful pressure (**study III)**. However, the extent to which contextual factors and PCS interact with cerebral pain modulation independent of mood, remain elusive in RA.

Moreover, in **study III**, psychophysiological interaction (PPI) task-based functional connectivity analysis revealed a functional dissociation between thalamus and bilateral inferior parietal lobe (IPL), that co-varied with increased PCS, during identical mid-intensity pressure stimulation depending on whether the pressure was following a high or low pain predictive cue. Specifically, higher PCS among FM subjects, was associated with increased thalamic brain activation, but reduced connectivity to bilateral IPL, during identical mid-intensity pressure stimulation following low vs. high pain predictive cue, and vice versa (i.e. reduced thalamic activation, but increased pain-related connectivity to bilateral IPL, following high vs. low pain predictive cue). The parietal cortex is involved in pain perception through its functional engagement in sensorimotor integration and

supporting body awareness[129,130]. Atrophy to the parietal cortex is associated with interoceptive impairments[131] and a meta-analysis on neuroimaging studies suggests that IPL and thalamus are among the most likely clusters of activation when investigating interoception[132]. In FM, low interoceptive accuracy is associated with increased symptom severity [133], and higher disruption of external signals[134]. Further, it has been proposed that associative fear learning may impair interoception and the ability to discriminate between different bodily sensations, ultimately contributing to more intense and frequent pain experiences[135]. Speculatively, the observed disrupted thalamic-IPL functional connectivity may reflect a sensory disintegration that is more pronounced among high pain catastrophizing FMS, which may lead to a tendency to over-estimate incoming sensory signals and/or excessively shut off incoming sensory signals.

Aberrant functional connectivity to the IPL has also been observed among RA patients with concomitant FM. Specifically, higher levels of peripheral inflammation (in RA patients with FM), was associated with increased functional connectivity between the IPL and multiple brain regions such as mPFC, ACC, mid/posterior insula and medial frontal gyrus (i.e. dlPFC)[14]. In RA patients without FM, higher levels of peripheral inflammation were associated with lower functional connectivity between IPL and insula, but higher functional connectivity between IPL and superior temporal gyrus[14]. The parietal lobes together with the mPFC, comprises the default mode network (DMN), which is a network commonly activated during introspection, or when healthy subjects are at rest. In FM, hyperconnectivity between the DMN and insula has been associated with amplified ongoing pain[45,46,136]. This hyperconnectivity has also been confirmed among RA patients with increased FM symptomatology[137]. Taken together, these results may suggest a central role for connectivity to the parietal lobe in RA patients with high FM symptomatology, as the results suggest established neurobiological patterns of pronociceptive connectivity that co-vary with FM symptomatology as well as level of peripheral inflammation.

6.3 THE ROLE OF CENTRAL INFLAMMATORY MECHANISMS IN FM AND RA

The aim of **study I** was to examine the potential role of glia activation in the brain of FM patients. The results showed upregulated [^{11}C]PBR28 binding in FM patients compared to HC in S1/M1, dlPFC, MCC and precuneus. In particular, increased [^{11}C]PBR28 binding in

the MCC co-varied with increased levels of fatigue in FM subjects. The lack of [^{11}C]-$_{L}$-deprenyl-D_2 signal suggested that the [^{11}C]PBR28 signal reflected microglial (not astrocytic) activation. This study suggest that central inflammation may play a role in FM pathophysiology, but there is a need for further investigation.

One particular problem with the TSPO [^{11}C]PBR28 radioligand is that it is currently uncertain which phenotype of activated glia increase their TSPO expression, which challenges the interpretation of the PET findings. In short, microglia and astrocytes can be differenctially activated and thus exhibit various phenotypes, including the classically activated, pro-inflammatory (microglia M1, astrocyte A1) and the alternatively activated, neuroprotective (microglia M2; astrocyte A2) states[138,139]. Recent data suggest that pro-inflammatory M1 microglia and macrophages may show lower [^{11}C]PBR28 binding in humans[140–142], and increased thalamic [^{11}C]PBR28 binding in chronic low back pain patients was found to negatively correlate with pro-inflammatory serum IL-6 levels[122]. Further, [^{11}C]PBR28 binding has been investigated in the brains of RA patients compared to HC[79]. Although no statistical significant group differences were detected, the results suggested numerically lower binding values in RA vs. HC. Further, lower cerebral [^{11}C]PBR28 binding was associated with higher disease activity (i.e. DAS28), which may suggest that TSPO relate to a disease modifying mechanism in RA[79]. As such, the inverse correlation of increased DAS28 disease activity and tendency of reduced [^{11}C]PBR28 binding in RA patients, could speculatively suggest that the observed decreased [^{11}C]PBR28 binding in RA is related to an increased pro-inflammatory M1 signal. This interpretation is in alignment with the high pro-inflammatory (IL-1) and significantly reduced anti-inflammatory cytokine profile (IL-4, IL-10) found in RA CSF and the more varied CSF cytokine profile of both anti- and pro-inflammatory cytokines observed in FM[74]. Further, [^{11}C]PBR28 binding in peripheral joints of 3 RA patients compared to 3 HC[143] revealed significant [^{11}C]PBR28 binding in in RA joints compared to HC, and the highest TSPO expression and [^{11}C]PBR28 binding was found on activated synovial fibroblastlike synoviocytes and M2 macrophages[143]. These results support the hypothesis that [^{11}C]PBR28 binding reflect increased M2 signal, which may suggest that M1 activation is left undetected. Other interpretations could be that the lower binding values observed in RA patients reflect lower density or an altered function of neuroimmune glial cells[142].

Together with the results from **study I**, these results may suggest that higher levels of [^{11}C]PBR28 may reflect neuro- and pain-protective effects in chronic pain patients, such as FM. Further indirect support comes from a study of patients suffering from painful

osteoarthritis demonstrating elevated CSF concentrations of fractalkine and IL-8, chemokines previously found to be elevated in the CSF of FM patients[75,144], and a negative association between the CSF levels of these chemokines and symptom severity[145]. Further, in **study I** the upregulated [^{11}C]PBR28 binding was correlated with fatigue but neither symptom severity nor disease duration. Speculatively, the increased [^{11}C]PBR28 binding in FM in **study I** could reflect an increased M2 signal, and form part of a neuroprotective response.

7 CONCLUSIONS

The results from **study I** suggested that central inflammation (microglia) may play a role in FM pathophysiology. Together with contemporary literature, these results may speculatively reflect increased anti-inflammatory signal in FM patients and increased pro-inflammatory signal in RA patients. The results from **study II and V** in the current thesis noted distinct aberrations in cerebral pain modulation between well-characterized FM and well-characterized RA. Specifically, RA patients revealed normal cerebral pain processing from non-affected sites, but dysfunctional early initiation of the pain modulatory system (i.e. dlPFC) when painfully stimulated over the most inflamed joint. On the other hand, FM vs. RA patients, exhibited reduced engagement of more medial structures such as mPFC and rACC. In FM patients only, aberrant cerebral pain modulation was associated with overall clinical pain, which indicate more prominent pain-related cerebral disruptions in patients suffering from nociplastic pain. Moreover, **study III and IV** conclude that cerebral pain processing in FM is marked by notably complex cognitive processes, including influences of contextual factors and pain catastrophizing (independent of mood). Previous literature suggests that pain catastrophizing and mood (i.e. depression) may also alter pain perception in RA, and the latter has been associated with altered cerebral pain modulation in RA. However, the extent to which contextual factors and pain catastrophizing interact with cerebral pain modulation independent of mood in RA, remain elusive.

8 POINTS OF PERSPECTIVE

It has specifically been suggested that chronic pain needs to be reframed and viewed as a disease in itself, rather than a symptom[51]. In the classical biomedical approach, nociceptive processing and the symptom of pain itself has been in focus. However, each individual's unique response to pain is influenced by a combination of genetics, neurobiology, and their interactions with vulnerability factors such as mood or sleep disturbances, as well as protective factors such as social relationships and active coping. In other words, it is clear that the field of chronic pain is moving towards an individualized view of pain and individually tailored pain treatments, focusing on including the whole patient and not only the pain symptom.

Nociplastic pain symptomatology is influences by notably complex cognition. For example, **study III and IV** demonstrated that FM subjects accumulated new pain-related associations more efficiently than extinguishing no longer relevant associations. Chronic stress and chronic pain share the common behavioral model of "failure to extinguish negative memories"[146], which suggest that both stress and chronic pain affect the frontal lobe and limbic structures that are important in learning, thus reinterpretation of pain-related associations. This sort of relationship requires further attention in the pain literature field. Moreover, the role of contextual influences on RA pain perception remain largely unexplored. It would be particularly interesting to investigate the contextual role of pain in RA patients whose pain remain persistent despite control of inflammation (i.e. RA patient with FM symptomatology). Cross-sectional studies can be designed to investigate how emotional learning, uncertainty, and pain catastrophizing may interact with current pain perception in RA patients with varying degrees of FM symptomatology. Longitudinal studies can be designed to track changes in how biopsychological factors may be connected with descending pain modulation and, ultimately, whether they are involved in functional rearrangement of the central nervous system. It should be noted that biopsychosocial aspects are relevant for chronic pain in general (i.e. neuropathic, nociceptive and nociplastic) and not restricted to FM pathology[51]. Moreover, little attention has been directed to whether pain catastrophizing can affect descending pain modulation in RA patients independent of mood, and whether this effect is more pronounced among RA patients with high FM symptomatology.

Many RA patients display comorbid FM. **Study II and V** noted that there are disease-

relevant neural aberrancies in well-characterized RA, and these aberrations hold for group comparison with FM patients. Hence, there is a great clinical value in identifying factors that can predict which among RA patients will respond vs. not respond to immunosuppressive treatment and investigate whether non-responders may benefit from other treatments commonly distributed to FM patients. However, it should be noted that decades of clinical practice and clinical trials have not yielded satisfactory outcomes when it comes to centrally acting compounds such as serotonin-norepinephrine reuptake inhibitors, tricyclic compounds, and $\alpha_2\delta$ ligands[147]. In non-responding RA patients, it would be interesting to investigate whether biopsychosocial factors such as uncertainty, pain catastrophizing, stress, poor sleep quality and other factors may contribute to persistent pain following treatment. In particular, uncertainty is a largely unexplored approach in the pain literature, although it has been demonstrated to mediate acute stress responses in humans. Specifically, the mere uncertainty of not knowing you are going to get a painful shock, is much worse than knowing for sure you will or won't receive a painful shock[148]. More surprisingly, *not* getting a shock when you expected one, is as stressful as actually getting a shock when you expected one[148]. It remains elusive whether uncertainty (especially regarding treatment outcomes) may contribute to chronic pain maintenance or perhaps to the development of nociplastic pain. It should be noted that uncertainty encompass more components than mere unpredictability[149]. For example, in some patients' unpredictability may originate in inability to predict what will elicit pain, but in another patient unpredictability may originate in a learned association that one particular movement will trigger pain and thus lead to worry about the future (e.g. maintaining a job or being able to continue with a hobby). A completely unexplored field in contemporary pain literature is the role of meta-uncertainty e.g. "How sure are you about your uncertainty?"[149]. These sorts of questions may help patients to identify erroneous ruminations about uncertainty. It would further be interesting to investigate measures of uncertainty affect brain connectivity at rest.

Finally, **Study I** emphasize the need for future study to investigate the complex psycho-neuro-immunological interactions in FM pathophysiology, and highlight the role of studying fatigue in FM. It has been shown that even a single night of total sleep deprivation can increase anxiety levels and induce generalized hyperalgesia[150]. Future studies should focus on investigating the underlying biological mechanisms of TSPO and their relevance for treatment. For example, how TSPO expression and intensity may vary in various stages of ongoing inflammatory response (acute, subacute, chronic) as well as potential triggering factors. Further, peripheral [^{11}C]PBR28 binding has been detected in the inflamed joints of RA patients[143], which suggest that future studies may advantage from performing a full

body scan in patients with ongoing peripheral inflammation in order to make sure the [^{11}C]PBR28 ligand does not excessively bind to peripheral receptors before entering the brain.

Taken together, the current thesis emphasizes the complexity of the pain experience in chronic pain and that well-characterized chronic pain patient groups can exhibit distinct aberrations in their descending pain modulatory system. Future direction of treatment requires individualized and multimodal treatment plans, including pain neuroscience education, how to cope with pain catastrophizing, manage stress and sleep.

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