Characterization of subgroups in fibromyalgia syndrome

Charakterisierung von Subgruppen des Fibromyalgie-Syndroms

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Inhalt

1 Summary

The present cumulative dissertation summarizes three clinical studies, which examine subgroups of patients within the fibromyalgia syndrome (FMS). FMS entails chronic pain and associated symptoms, and its pathophysiology is incompletely understood (1). Previous studies show that there is a subgroup of patients with FMS with objective histological pathology of the small nerve fibers of the peripheral nervous system (PNS). Another subgroup of FMS patients does not show any signs of pathological changes of the small nerve fibers. The aim of this dissertation was to compare FMS patients with healthy controls, and these two FMS subgroups for differences in the central nervous system (CNS) in order to explore possible interactions between PNS and the CNS. Regarding the CNS, differences of FMS patients with healthy controls have already been found in studies with small sample sizes, but no subgroups have yet been identified. Another aim of this thesis was to test whether the subgroups show a different response to different classes of pain medication. The methods used in this thesis are structural and functional magnetic resonance imaging (MRI), magnetic resonance diffusion imaging and magnetic resonance spectroscopy. For the evaluation of clinical symptoms, we used standardized questionnaires. The subgroups with and without pathologies of the PNS were determined by skin biopsies of the right thigh and lower leg based on the intraepidermal nerve fiber density (IENFD) of the small nerve fibers.

1) In the first MRI study, 43 female patients with the diagnosis of FMS and 40 healthy control subjects, matched in age and body mass index, were examined with different MRI sequences. Cortical thickness was investigated by structural T1 imaging, white matter integrity by diffusion tensor imaging and functional connectivity within neuronal networks by functional resting state MRI. Compared to the controls, FMS patients had a lower cortical volume in bilateral frontotemporoparietal regions and the left insula, but a higher cortical volume in the left pericalcarine cortex. Compared to the subgroup without PNS pathology, the subgroup with PNS pathology had lower cortical volume in both pericalcarine cortices. Diffusion tensor imaging revealed an increased fractional anisotropy (FA) of FMS patients in corticospinal pathways such as the corona radiata, but also in regions of the limbic systems such as the fornix and cingulum. Subgroup comparison again revealed lower mean FA values of the posterior thalamic radiation and the posterior limb of the left internal capsule in the subgroup with PNS pathology. In the functional connectivity analysis FMS patients, compared to controls, showed

a hypoconnectivity between the right median frontal gyrus and the posterior cerebellum and the right crus cerebellum, respectively. In the subgroup comparisons, the subgroup with PNS pathology showed a hyperconnectivity between both inferior frontal gyri, the right posterior parietal cortex and the right angular gyrus. In summary, these results show that differences in brain morphology and functional connectivity exist between FMS patients with and without PNS pathology. These differences were not associated with symptom duration or severity and, in some cases, have not yet been described in the context of FMS. The differences in brain morphology and connectivity between subgroups could also lead to a differential response to treatment with centrally acting drugs. Further imaging studies with FMS patients should take into account this heterogeneity of FMS patient cohorts.

2) Following the results from the first MRI study, drug therapies of FMS patients and their treatment response were compared between PNS subgroups. As there is no licensed drug for FMS in Europe, the German S3 guideline recommends amitriptyline, duloxetine and pregabalin for temporary use. In order to examine the current drug use in FMS patients in Germany on a cross-sectional basis, 156 patients with FMS were systematically interviewed. The drugs most frequently used to treat pain in FMS were non-steroidal anti-inflammatory drugs (NSAIDs) (28.9%), metamizole (15.4%) and amitriptyline (8.8%). Pain relief assessed by patients on a numerical rating scale from 0-10 averaged 2.2 points for NSAIDs, 2.0 for metamizole and 1.5 for amitriptyline. Drugs that were discontinued for lack of efficacy and not for side effects were acetaminophen (100%), flupirtine (91.7%), selective serotonin reuptake inhibitors (81.8%), NSAIDs (83.7%) and weak opioids (74.1%). Patients were divided into subgroups with and without PNS pathology as determined by skin biopsies. We found no differences in drug use and effect between the subgroups. Taken together, these results show that many FMS patients take medication that is not in accordance with the guidelines. The reduction of symptoms was best achieved with metamizole and NSAIDs. Further longitudinal studies on medication in FMS are necessary to obtain clearer treatment recommendations.

3) Derived from previous pharmacological and imaging studies (with smaller case numbers), there is a hypothesis in the FMS literature that hyperreactivity of the insular cortex may have an impact on FMS. The hyperreactivity seems to be due to an increased concentration of the excitatory neurotransmitter glutamate in the insular cortex of FMS patients. The hypothesis is supported by magnetic resonance spectroscopy studies with small number of cases, as well as results from pharmacological studies with glutamate-inhibiting medication.

Studies from animal models have also shown that an artificially induced increase in glutamate in the insular cortex can lead to reduced skin innervation. Therefore, the aim of this study was to compare glutamate and GABA concentrations in the insular cortex of FMS patients with those of healthy controls using magnetic resonance imaging. There was no significant difference of both neurotransmitters between the groups. In addition, there was no correlation between the neurotransmitter concentrations and the severity of clinical symptoms. There were also no differences in neurotransmitter concentrations between the subgroups with and without PNS pathology. In conclusion, our study could not show any evidence of a correlation of glutamate and GABA concentrations with the symptoms of FMS or the pathogenesis of subgroups with PNS pathologies.

2 Zusammenfassung

Die vorliegende kumulative Dissertation fasst drei klinische Studien zusammen, welche Unterschiede zwischen Patientinnen mit Fibromyalgiesyndrom (FMS) und gesunden Kontrollen, sowie Subgruppen des FMS untersuchen. Das FMS wird als chronisches Schmerzsyndrom mit Begleitsymptomen wie Depressionen, gastrointestinalen Symptomen oder Erschöpfung definiert. Die Pathophysiologie ist noch nicht vollständig geklärt (1). Frühere Studien zeigen, dass es eine Subgruppe von PatientInnen mit FMS gibt, welche objektive, histologische Pathologien der kleinen Nervenfasern des peripheren Nervensystems (PNS) aufweisen. Eine andere Subgruppe von FMS-Patienten zeigt keinerlei Anzeichen für pathologische Veränderungen dieser kleinen Nervenfasern. Ziel dieser Dissertation ist es, diese beiden Subgruppen auf Unterschiede im zentralen Nervensystem (ZNS) hin zu vergleichen, um mögliche Wechselwirkungen zwischen dem PNS und ZNS zu untersuchen. Hinsichtlich des ZNS wurden bereits Unterschiede zwischen FMS-Patienten und gesunden Kontrollpersonen in Studien mit kleineren Fallzahlen festgestellt, jedoch wurden noch keine Subgruppen identifiziert. Ein weiteres Ziel dieser Arbeit war es, zu prüfen, ob die Subgruppen von FMS PatientInnen unterschiedlich auf verschiedene Arten von Schmerzmedikamenten ansprechen. Die in dieser Arbeit verwendeten Methoden sind die strukturelle und funktionelle Magnetresonanztomographie (MRT), die Magnetresonanz-Diffusionsbildgebung und die Magnetresonanzspektroskopie. Für die Bewertung der klinischen Symptome wurden standardisierte Fragebögen verwendet. Die Subgruppen mit und ohne Pathologien des peripheren Nervensystems (PNS) wurden durch Hautbiopsien des rechten Ober- und Unterschenkels anhand der intraepidermalen Nervenfaserdichte der kleinen Nervenfasern bestimmt.

1) In der ersten MRT-Studie wurden 43 Patientinnen mit der Diagnose eines FMS und 40 gesunde Kontrollpersonen, die hinsichtlich Alter und Body-Mass-Index gematcht waren, mit verschiedenen Sequenzen der Magnetresonanztomographie (MRT) untersucht. Das Volumen des Kortex wurde mittels struktureller T1-Bildgebung, die Integrität der weißen Substanz mittels Diffusionstensor-Bildgebung und die funktionelle Konnektivität innerhalb neuronaler Netzwerke mittels einer funktionellen Ruhezustands-MRT untersucht. Im Vergleich zu den Kontrollpersonen hatten FMS-Patientinnen ein geringeres Kortexvolumen der bilateralen frontotemporoparietalen Regionen und der linken Inselrinde, aber ein höheres Kortexvolumen im linken pericalcarinen Kortex. Im Vergleich zu der Untergruppe ohne PNS-Pathologien wies

die Untergruppe mit PNS-Pathologien ein geringeres Kortexvolumen in beiden pericalcarinen Kortizes auf. Die Diffusions-Tensor-Bildgebung zeigte eine erhöhte fraktionelle Anisotropie (FA) der FMS PatientInnen in kortikospinalen Bahnen wie der Corona radiata, aber auch in Regionen des limbischen Systems wie dem Fornix und dem Cingulum. Ein Subgruppenvergleich ergab wiederum niedrigere mittlere FA-Werte in der Subgruppe mit PNS-Pathologien bezüglich der hinteren Thalamusausstrahlung und des hinteren Schenkels der linken Capsula interna. In der Analyse der funktionellen Konnektivität zeigten FMS-Patienten im Vergleich zu den Kontrollen eine Hypokonnektivität zwischen dem rechten medianen frontalen Gyrus und dem hinteren Kleinhirn bzw. dem rechten Kleinhirn. In den Subgruppenvergleichen zeigte die Subgruppe mit PNS-Pathologien eine Hyperkonnektivität zwischen beiden inferioren frontalen Gyri, dem rechten posterioren parietalen Kortex und dem rechten Gyrus angularis. Zusammengefasst zeigen diese Ergebnisse, dass zwischen FMS Patienten mit und ohne PNS-Pathologie Unterschiede in der Hirnmorphologie und funktionellen Konnektivität bestehen. Diese Unterschiede waren nicht mit der Dauer oder Ausprägung der Symptome assoziiert und sind teilweise noch nicht im Zusammenhang mit dem FMS beschrieben worden. Die Unterschiede in der Hirnmorphologie und Konnektivität zwischen den Subgruppen könnte auch zu einem unterschiedlichen Ansprechen auf die Behandlung mit zentral wirksamen Medikamenten führen. Weitere bildgebende Studien mit FMS-PatientInnen sollten diese Heterogenität von FMS-Patientenkohorten berücksichtigen.

2) Den Ergebnissen der ersten MRT-Studie folgend wurden die medikamentösen Therapien von FMS-PatientInnen und ihr Ansprechen auf die Behandlung zwischen den PNS-Subgruppen verglichen. Da es in Europa kein zugelassenes Medikament für das FMS gibt, empfiehlt die deutsche S3-Leitlinie Amitriptylin, Duloxetin und Pregabalin zur vorübergehenden Anwendung. Um den aktuellen Medikamenteneinsatz bei FMS-Patienten in Deutschland im Querschnitt zu untersuchen, wurden 156 PatientInnen mit FMS systematisch befragt. Die am häufigsten verwendeten Medikamente zur Schmerzbehandlung bei FMS waren nicht-steroidale Antirheumatika (NSAIDs) (28,9 %), Metamizol (15,4 %) und Amitriptylin (8,8 %). Die von den Patienten auf einer numerischen Bewertungsskala von 0-10 bewertete Schmerzlinderung betrug im Durchschnitt 2,2 Punkte für NSAIDs, 2,0 für Metamizol und 1,5 für Amitriptylin. Medikamente, die wegen mangelnder Wirksamkeit und nicht wegen Nebenwirkungen abgesetzt wurden, waren Paracetamol (100 %), Flupirtin (91,7 %), selektive Serotonin-Wiederaufnahmehemmer (81,8 %), NSAIDs (83,7 %) und schwache Opioide (74,1 %).

Die Patienten wurden in Subgruppen mit und ohne PNS-Pathologien eingeteilt, welche, wie schon beschrieben, anhand von Hautbiopsien bestimmt wurden. Wir fanden keine Unterschiede zwischen den Subgruppen in Bezug auf die Medikamenteneinnahme und deren Wirkung. Insgesamt zeigen diese Ergebnisse, dass viele FMS-PatientInnen Medikamente einnehmen, die nicht mit den Leitlinien übereinstimmen. Die Reduzierung der Symptome wurde am besten mit Metamizol und NSAIDs erreicht. Weitere Längsschnittstudien zur Medikation bei FMS wären hilfreich, um breitere Behandlungsempfehlungen zu erhalten.

3) Abgeleitet aus den bisherigen pharmakologischen und bildgebenden Studien (mit kleineren Fallzahlen) besteht in der FMS Literatur die Hypothese, dass eine Hypersensitivität der Inselrinde einen Einfluss auf die FMS-Symptomatik haben könnte. Diese Hypersensitivität könnte durch eine erhöhte Konzentration des erregenden Neurotransmitters Glutamat in der Inselrinde von FMS Patienten bedingt sein. Diese Hypothese wird durch Magnetresonanzspektroskopie-Studien mit kleinen Fallzahlen, sowie Ergebnissen aus pharmakologischen Studien mit Glutamat-hemmender Medikation gestützt. Studien aus dem Tiermodell konnten außerdem zeigen, dass ein künstlich herbeigeführter Anstieg von Glutamat in der Inselrinde zu einer Reduktion der kleinen Nervenfasern im PNS führen kann. Ziel dieser Studie war es deshalb, mittels Magnetresonanztomographie die Glutamat- und GABA Konzentrationen der Inselrinde von FMS Patienten mit denen von gesunden Kontrollen zu vergleichen. Es zeigte sich kein signifikanter Unterschied beider Neurotransmitter zwischen den Gruppen. Es konnte ebenfalls kein Zusammenhang zwischen den Konzentrationen und der Ausprägung der klinischen Symptomatik bewiesen werden. Auch zwischen den Subgruppen mit und ohne PNS Pathologie zeigten sich keine Unterschiede in der Neurotransmitterkonzentration. Zusammenfassend konnte unsere Studie keinen Hinweis auf einen Zusammenhang der Glutamat- und GABA- Konzentrationen in der Inselrinde mit der Symptomatik des FMS oder der Entstehung von Subgruppen mit PNS Pathologien zeigen.

3 Introduction

Acute pain is a sensation that is a combination of many signals from peripheral nociceptors, due to damage or potential damage to tissue. These signals are transmitted via various classes of nerve fibers, the dorsal root ganglion and the spinal cord to the brain, where they are processed in subcortical and cortical regions before reaching consciousness. Disruption of physiological processes in any of these parts of the peripheral and CNS can cause pain. However, chronic pain can also be felt without peripheral signals. As Socrates once said: "Every pleasure or pain has a sort of rivet with which it fastens the soul to the body and pins it down and makes it corporeal, accepting as true whatever the body certifies [1]."

The various influences of the nervous systems on different pain syndromes such as fibromyalgia syndrome (FMS) have not yet been conclusively clarified in the current scientific community [2, 3]. Fibromyalgia syndrome is a chronic pain disorder and has a prevalence of about 2% in the general population [4]. Changes in various physiological body systems have been observed in patients, but so far, no clear causal link has been proven. The most commonly reported pathphysiological factor is sensitization of the CNS to sensory stimuli and altered central processing of pain [5, 6]. Since 2013, however, several studies have been published that indicate pathology of the small nerve fibers in the PNS in certain subgroups of FMS patients [7, 8]. The severity of this reduction in skin innervation has been associated with the severity of FMS symptoms [9]. It is unclear at the current state of research, whether the term FMS refers to syndromes with similar symptoms but different pathogenesis, with pathologies in the CNS in one group and in the PNS in the other. The present dissertation therefore deals in several studies with the changes in the PNS and CNS and individual drug responses in subgroups of FMS. The methods used are structural and resting state functional magnetic resonance imaging (MRI & rsfMRI), diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS) and clinical analyses of drug intake and effects. Data on the PNS, especially the IENFD, were obtained by skin biopsies of the thigh and lower leg.

To give a brief overview about the complex processing of pain, the different influencing factors and neural processing stations, from nociceptor to spinal cord to brain, or respectively from the molecular level to the level of neural processing networks, are introduced. Later on, the current scientific status of changes in these systems will be discussed more specifically in FMS and the neuroimaging methods, as well as other methods used in the studies of the

cumulative dissertation are explained. At the end of the discussion, a summary of the main research questions as well as aims and their implementation in each study will be presented.

3.1 The processing of pain

External stimuli, such as cold, heat, pressure, cuts, vibration or chemicals irritate sensory receptors, such as Merkel cells, Ruffini endings, Meissner corpuscles, Pacinian corpuscles or free nerve endings in our skin and other organs. This is a protective mechanism of our body against potential tissue damage [10]. The different sensors can perceive different stimuli [11]. The stimuli are then transmitted by the unmyelinated C fibers and the thinly myelinated Aδ fibers. The fibers differ in the type of stimuli they are responsible for transmitting, Aδ fibers transmit, for example, pinprick stimuli or pulling on hair [12], while C fibers perceive heat or cold pain. The nerve endings of C fibers extend up to our epidermis, so the measurement of IENFD by biopsies in the studies of this dissertation is a relatively specific examination of small, unmyelinated nerve fibers. If an external stimulus is strong enough, it creates a generator potential through a cation influx in the different receptors, which is large enough to create an action potential, especially meditated by activated voltage-gated Na+ channels. This is then transmitted along the nerves to the spinal cord [13]. Hypersensitivity to pain can occur not only at the central level, but also already at the peripheral level. Examples are the transient receptor potential vanilloid type 1 channel (TRVP1) or the ankyrin 1 channel (TRPA1) in C fibers, which are responsible for sensations of heat as well as cold. By released cytokines, neurotrophic factors or other immune modulators, these channels in nociceptors can be irritated and release further immune modulators, which can lead to further inflammation and pain [14].

After the Aδ and C fibers transmit the signals of the peripheral nociceptors to the spinal dorsal horn, it forwards the signals via projection neurons across the brain stem to higher CNS regions. The superficial regions of the spinal dorsal horn, such as the lamina I, are mostly involved here [15]. These regions exclusively transmit nociceptive signals, for example directly from the C fibers and from the receptors for neurokinin 1 (substance P). A targeted switch-off of these neurons and their substance P receptors could be experimentally associated with a reduction of hyperalgesia [16]. Neuroplasticity can already be observed at this level of the CNS, because intensive peripheral nociceptive stimuli can trigger a long-term increased excitability of these neurons, which is also known as Long Term Potentation (LTP) [17]. In the presence of

such a LTP, the activation of glial cells and cytokines can also excite other nociceptor synapses at the spinal level, which can lead to a generalized, wide-spread pain [18], as reported by patients with FMS. However, the sensory spinal cord does not only transmit afferent excitatory signals to the brain. About 30% of the dorsal horn consists of inhibitory neurons, which inhibit nociceptive signals via spinal GABA and glycine receptors [19]. Also the brain, especially the brain stem, has an influence on the subjective pain perception on the spinal level through serotonin and norepinephrine modulated inhibitory, descending control pathways and interneurons [20]. Peripheral inflammatory reactions or nerve damage can also lead to reduced activity of the inhibitory GABA and glycinergic receptors through activation of microglia, thus contributing to a chronicity of pain [21]. Inflammatory pain due to these processes can be alleviated if this pathway is suppressed by taking CNS-penetrating cyclooxygenase inhibitors [22].

Before the nociceptive signals in the brain reach the level of consciousness, they are influenced by cognition, emotions, memories and attention. Among other things, these influences determine whether the nociceptive signal is perceived as painful or unpleasant. Nociceptive signals, but also pain are normally important parts of all physiological signals for the perception of our body in relation to the outside world. They help to react quickly to dangers and take the appropriate countermeasures to ensure rapid healing of the tissue. If, however, the pain persists for a significantly longer period of time, even if a danger situation no longer exists, it is considered chronic and is referred to as pathological, since the affected subject feels pain in this situation and can no longer take any alleviating measures [23].

The current state of research assumes that the spinal nociceptive signals are transmitted mainly via the parabrachial nuclei in the pons to the subcortical structures in the brain [24]. Yet until the signal becomes conscious, it is influenced by many brain regions. It is assumed that the amygdala and thalamus transmit these signals mainly to the cortex regions of the primary/secondary sensory cortices and insular cortices [25]. The thalamus seems to have a modulating function [26]. In the anterior cingulate cortex, alterations of activity could be related with the transition of acute into chronic pain [27]. These patterns could even predict pain chronicity before it actually occurred in patients [28]. However, for the chronification of pain, behavior and movement patterns learned through conditioning are also important, which are significantly influenced by the amygdala and its radiations into the medial prefrontal cortex and the spinal cord [29].

3.2 The pathophysiology of fibromyalgia syndrome

FMS is a chronic pain disorder with many possible accompanying symptoms, including cognitive impairment and comorbidities such as Irritibale Bowel syndrome, psychiatric and rheumatic disorders [30]. The former diagnostic criteria from 1990, in which tender points, painful pressure points on various muscle tendons, were still decisive [31], have now been replaced by the diagnostic criteria 2010 of the American College of Rheumatology [32]. Here, the focus was placed on the severity of the symptoms, such as chronic pain, sleep disorders, exhaustion and cognitive problems. The prevalence of FMS in the general population is estimated at 2-4% and most of the patients are middle aged (40-65 years) [33]. While with the old diagnostic criteria mainly women with FMS were diagnosed, in the newer studies the gender ratio is approximately balanced [34].

Clear, causal relationships could not yet be discovered in the pathophysiology of FMS, but several influencing factors have been identified [35, 36]. There seem to be genetic influences, as direct relatives have an eight times higher risk of developing FMS symptoms [37] and several gene polymorphisms have been discovered, which are mostly associated with the development and degradation of neurotransmitters involved in nociception. These include in particular genes that encode catechol-O-methyltransferase, the dopamine type 4 receptor, the serotonin 5-hydroxytryptamine 2A receptor and the serotonin transporters [38]. However, the genetic effects are too small to substantiate the complete pathophysiology on them. In the autonomic nervous system, analyses of heart rate variability, sympathicotonic skin reaction and the reaction during a tilting table examination showed a predomination mainly in the sympathetic nervous system [39]. In some patients, the symptoms can also be induced by triggers, including prolonged infections such as those caused by the Ebstein-Barr virus or Lyme disease [40], chronic peripheral pain of a different origin such as rheumatoid arthritis or ankylosing spondyloarthritis [41], but also psychological stress such as childhood trauma or chronic stress [42].

Hypersensitivity at the level of the CNS has long been hypothesized as a potential cause of the symptomatology of FMS patients [43]. Imaging techniques have proven to be particularly useful here, although most studies on FMS to date have only a small number of cases, which lead to a low statistical power when using a method like MRI, which is based on thousands of

data points (voxels). Accordingly, the previous literature is inconsistent in structural imaging, with some studies finding increases in grey matter, while some find decreases in the same areas [44]. Most often, however, the anterior and posterior areas of the cingulate cortex (ACC and PCC) and the medial prefrontal cortex are affected, both of which are involved in the emotional and cognitive processing of pain [45]. Probably due to the stronger effect size of task-related fMRI studies, more consistent results are seen in similar case numbers. For various nociceptive stimuli, increased activity in pain-processing regions such as the sensory cortex, insular cortex and thalamus is seen in comparison to healthy control groups [46]. Increased activity in pain anticipation and cognitive processing areas, such as the dorsolateral prefrontal cortex (DLPFC), amygdala [47] and the ACC, was found in the announcement of pain [48]. Resting state fMRI studies have shown increased functional connectivity between the DMN and the insular cortex, indicating increased interoception and thus pain perception [49, 50]. Investigations of the white matter using diffusion tensor imaging (DTI), with relatively low case numbers up to now, have not shown consistent results either, but in several areas such as the prefrontal, temporal and insular cortex a correlation between the number of white fibers and pain sensitivity could be established [51]. An increased number of white fibers indicates an increased activity on these connections and has also been associated with an increased activity of immunomodulatory substances such as microglia [52]. This increased microglia activity has already been measured in FMS patients using positron emission tomography and indicates an immunological response in the CNS in patients with FMS [53].

In the last decade, however, there have been increasing findings not only in the CNS, but of objective pathology at the level of the PNS [54, 55]. Alterations in the small peripheral nerve fibers could be structurally determined with the help of IENFD by skin biopsies [54] or the corneal nerve fiber density as measured by confocal corneal microscopy [56]. Also functional limitations of these small nerve fibers have been identified in quantitative sensory testing "QST", which tests detection thresholds for several sensory qualities, such as heat or pressure [8].

Evdokimov et al. showed that there are several subgroups of FMS patients regarding small fiber pathology [9].This publication demonstrated the presence of three subgroups in the FMS population with reduced IENFD at either both biopsy sites (proximal (hip) and distal (calf)), only one of both biopsy sites, or normal IENFD at both biopsy sites. For this dissertation, patients of the subgroup with normal IENFD and of the subgroup with reduced IENFD at both

biopsy sites (proximal and distal) were recruited. In the latter nociceptive signals could come from pathologies in the the Aδ and C fibers, which have been described many times in FMS in recent years [7]. Differences of these two “PNS subgroups” on the level of the CNS have not been investigated yet. The objective of this dissertation was therefore to investigate possible differences between these subgroups with and without pathology of the small nerve fibers of the PNS in the CNS using the following methods.

3.3 Techniques for the differentiation of subgroups

3.3.1 Imaging techniques

All imaging in this dissertation was performed with a magnetic resonance tomograph, because this technique provides a high contrast for the organ brain with a high spatio-temporal resolution, without any harm to the examined subject. Thus, all imaging methods are based on the principle of nuclear magnetic resonance. Using different stimulation pulses, different sequences can be programmed, which are more specific for individual substances in the CNS. These include T1 and T2 relaxation time measurements, diffusion measurements, blood level oxygen level (BOLD) measurements and spectra of key biochemical species. To provide the physical basis for the methodological understanding of the methods in this dissertation, the following paragraphs will deal with them briefly.

3.3.1.1 Magnetic Resonance Imaging

The physical basis of nuclear magnetic resonance (NMR) is that all nuclei with an odd number of protons or neutrons have a spin property [57]. Classical magnetic resonance imaging has specialized in the spin of hydrogen nuclei, since it has the highest concentration of all spinning isotopes in the human body (88 Molar) in the form of water. This reduces the measuring time required for good image quality. A nucleus has an angular momentum due to its spinning, measured in Planck's constant, which means that the nuclei continue to rotate until prevented from doing so by an external force. However, since the nuclei are also electrically charged, the rotation around the respective axis creates a magnetic property. So when the nuclei are exposed to a very strong magnetic field, as it is in the MRI, they begin to align themselves according to the magnetic field, also called B_0. There they rotate almost parallel to the magnetic field lines. The proton’s precessional frequency, known as the Larmor frequency (ω) is

determined by the strength of the magnetic field and gyromagnetic ratio. This means that a doubling of the magnetic field strength, measured in Tesla (T), from 1.5 T to 3 T also doubles the Larmour frequency. While the magnetic moments normally disperse due to their non-directional nature, in a strong magnetic field a slightly higher number of nuclei are aligned along the magnetic field lines than in the opposite direction. The number of differences between these directions of the nuclei is sufficient to generate the NMR signal for imaging. To generate this signal the nuclei have to be pushed away from this equilibrium alignment by radio frequency (RF) pulses of another magnetic field, also called B_1, which is in the same ω frequency. Special receiving coils are tuned to signals in this frequency range and transmit the signal into a so-called K-space, where they are converted into image data by a Fourier transformation and further algorithmic processing [58]. To obtain the cleanest possible signal, the magnetic field must be very homogeneous. However, since the tissue to be examined causes spatial deviations in the magnetic field, a subsequent homogenization of the magnetic field, also called shimming, is conducted. To obtain a spatial resolution of the signals, the magnetic field is traversed by magnetic gradients in different planes. A typical gradient can have a value of 10 mT/m. The time needed for the nuclei excited by the RF pulses to reach equilibrium of the longitudinal magnetic B0 field again is called T1 time. Due to the magnetic properties of the nuclei, or protons, they also influence each other, which changes their delay on the transverse axis (-90° of the B0 field). Local magnetic field changes, for example due to the different magnetic properties of oxygenated and deoxygenated blood or components of nearby hemosiderin, also change the transverse magnetization. The time constant for decay/dephasing of transverse magnetization is called T2 time [59]. T1 times are longer the stronger the B0 field is, while T2 times are mostly independent of this, although there can be deviations due to weakly dia- and paramagnetic substances in the brain. Each tissue has specific T1 and T2 times due to its nature, which produces the image contrast in most MRI sequences. The sequences used in this dissertation also use data from T1 and T2 values.

3.3.1.2 Diffusion Imaging

Diffusion imaging is physically based on the principles of the Brown molecular movement, which depends on the temperature [60] and Albert Einstein's mathematical description of particles in liquids at rest, which states that diffusion is directly proportional to temperature, or Boltzmann constant, which is the relation of kinetic energy to temperature, and indirectly

proportional to the size of the particles or the viscosity of the medium [61]. To measure this diffusion in an MRI, a pulsed gradient spin echo sequence is used, which first creates a T2-weighted image (B0 map), and then uses two strong gradients interrupted by a 180° pulse. While solid particles are reset by the second gradient in phase accumulation, diffusing particles between the gradients change their position and thus fall out of phase and loose signal [62]. These changes are recorded in rapidly following source images, which are combined with the original B_0 map to form the Apparent Diffusion Coefficient (ADC) map [63]. To eliminate signal interference by local artifacts in the magnetic field, bidirectional data acquisition was used in this thesis. From this data, further calculations, such as Diffusion Tensor Imaging, can then be performed [64]. This is based on the assumption that water particles diffuse more directionally in neuronal axons because they are prevented from free diffusion by the structure of the axons. This is called fractional anisotropy. FA is a value between 0 and 1 and represents the diffusion asymmetry within a voxel. In neuroimaging, and also in study #1 of this dissertation, this is used as a value of the neuronal consistency of the axons, primarily of the white matter, whereby it is unclear which cellular events have an influence on an increase or decrease in FA. In clinical routine, diffusion imaging is used on the basis that the water content in the extracellular fluids increases in case of cell damage due to toxic or ischemic factors, and the resulting energy-dependent Na-K ion pumps no longer function. This leads to a signal decrease due to shortened diffusion times. The increased water content also leads to changes in the T1 and T2 values. In the case of cell-rich tumours or acute ischemia, diffusion may be restricted and the signal enhanced [65].

3.3.1.3 Functional magnetic resonance imaging

Functional MRI imaging is mostly based on blood oxygen level dependent imaging, a technique developed by Seiji Ogawa [66], which does not require contast agents. A basic distinction is made between task-related fMRI and resting state fMRI. The physical principles are the same, whereby in one type the examined subject performs tasks, such as motor or cognitive, and in the other the subject is simply measured in the resting state. Using the BOLD technique it is possible to map increased neuronal activity anatomically in the brain, by analyzing the temporal contrast of the local ratio of (paramagnetic) deoxyhemoglobin to (diamagnetic) oxyhemoglobin. The altered susceptibility of hemoglobin, caused by four exposed electrons per iron center after the release of oxygen [67], affects the T2* rate, also called the "observed" T2

value, a value that reflects the T2 value and local magnetic field inhomogeneities [68]. When brain regions are activated, the corresponding area is supplied with more oxygenated blood than it can metabolise. This excess of oxygenated blood changes the rate to deoxygenated blood and causes an increase in the BOLD signal [69]. In resting state fMRI, the subject is measured at rest and the BOLD signals are recorded throughout the brain. In a subsequent statistical processing of the data, strong correlations of synchronous fluctuations, at relatively low frequency (<0.1 Hz), between different localizations can be determined [70]. These correlations represent network connections, and show that different brain regions work together to process sensory or auditory input, for example.

3.3.2 Magnetic resonance spectroscopy

While frequency differences are used in MRI via readout gradients to decode the spatial resolution, they are used in magnetic resonance spectroscopy (MRS) to analyse the chemical composition of a tissue in vitro, but also in vivo, noninvasively. The underlying effects here are the differences in the local magnetic fields around the nucleus at the atomic level, caused by the electrons around the nucleus. These electrons generate a local magnetic field B_{loc}, which counteracts the external magnetic field B_0 and thus creates diamagnetic susceptibility effects [71]. This frequency change, also called chemical shift, is converted into spectra by a Fourier transformation, with the respective peaks representing the individual substances [72]. The area under the peak correlates approximately with the number of the respective nuclei in the examined area/voxel. The x-axis, which distinguishes the substances in peaks, represents the chemical shift, which is expressed in parts per million (ppm). Since water and fat are the most common substances in the brain and their chemical shift effect overlaps that of all other substances, which are a thousandth of the amount of water and fat and are measured in millimolar (mM), they are suppressed. In principle, MRS distinguishes between Single Voxel Spectroscopy (SVS) and 2D/3D Chemical Shift Imaging (CSI), while SVS offers a better signal to noise ratio (SNR) with lower spatial resolution [73]. Since the composition and localization of the peaks of most substances is now known, fitting algorithms can be used to measure the area under the peak and thus the relative quantity of each substance [74]. Using external reference values, such as phantom measurements, or internal reference values, such as the N-acetylaspartate (NAA) peak, even absulte quantities can be obtained, but the consistency of these measurements is highly variable and should be used with caution [75]. The GABA and

glutamate analysis required in study #3 was achieved with a special Mega Point Resolved Spectroscopy (MPRESS) sequence to detect the overlap of GABA A and GABA B peaks with other substances, especially creatine [76].

3.3.3 Histology

Patients were divided into subgroups of patients with reduced intrapidermal and normal and skin innervation. Skin punch biopsies were taken from the right thigh and lateral lower calf and were of 6-mm diameter. Specimens were fixed in fresh paraformaldehyde for 30 minutes, washed and frozen after cryoprotection. To visualize the nerve fibers, they were then immunoreacted with a fluorescent antibody to the pan-axonal marker protein-gene product 9.5 (PGP 9.5). Several sections were examined under a microscope to quantify the IENFD. The unit used is nerve fibers per mm epidermal length. Based on comparative values from previous studies with n = 120 healthy women, patients with an IENFD on the lower leg with 8.2 (+/- 2.8) fibers/mm and on the upper thigh with 11.8 (+/- 3.3) fibers/mm were included in the group with normal nerve fiber density values (noPNS). This technique has already been described and published for biopsies from patients with neuropathies and FMS [9, 77]. All patients with values below normal were included in the group with pathologies in small nerve fibers, futher subdivided into those with pathology only at one site (lower leg or thigh) or at both sites. For this thesis, the subgroup with normal skin innervation (NoPNS) and the subgroup with reduced skin innervation at both sites (PNS) were compared.

3.4 Gap in the literature

The previous paragraphs have illustrated that there is an extensive literature already available regarding the alterations of the CNS, but also of the PNS, in patients with FMS. However, it is unclear whether the nervous system changes are reciprocal or indicative of subgroups with different pathophysiology. To date, no scientific work had examined the PNS and CNS within the same FMS patients, thus investigating possible interactions. The recently published results on the subgroups of FMS patients, which differ in the severity of the pathologies of the PNS, but also in the severity of the clinical symptoms [9], also raise the question whether these subgroups also differ in the response to, for example, neuropathic pain medication. Furthermore, the evidence from an animal study in rats that insular cortex glutamate

concentrations may be related to loss of skin innervation [78] has not yet been tested in vivo in humans.

3.5 Concluding aims and hypothesis of the thesis

The objectives of this dissertation can be summarized as answering the following questions: 1) Do FMS patients and controls and the subgroups of FMS patients with and without PNS pathology differ in various structural and functional markers of the CNS? 2) Do these subgroups differ in their clinical response to different pain medications? 3) Are the concentrations of glutamate and GABA in the insular cortex associated with the expression of PNS pathology and the severity of clinical FMS symptomatology?

Hypotheses for these questions were, following the literature: 1) The FMS subgroup with PNS pathologies shows minor changes in the CNS, while the FMS subgroup without pathologies shows more pronounced CNS changes (which could explain the FMS symptomatology in this subgroup as a hypersensitization). 2) FMS patients with PNS pathology respond better than FMS patients without PNS pathologies to certain medications approved for the treatment of neuropathic pain such as gabapentoids, SNRIs or amitriptyline. 3) Increased glutamate levels or decreased GABA levels in the insular cortex of FMS patients are associated with more severe PNS pathology and clinical symptomatology.

4 Manuscript section

4.1 CNS imaging characteristics in fibromyalgia patients with and without peripheral nerve involvement - Scientific Reports (2022)

Hans-Christoph Aster, Dimitar Evdokimov, Alexandra Braun, Nurcan Üçeyler, Thomas Kampf, Mirko Pham, György A. Homola*, Claudia Sommer* (*Authors contributed equally);
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CNS imaging characteristics in fibromyalgia patients with and without peripheral nerve involvement

Hans-Christoph Aster[1,2]✉, Dimitar Evdokimov[1], Alexandra Braun[1], Nurcan Üçeyler[1], Thomas Kampf[3], Mirko Pham[3], György A. Homola[3,4] & Claudia Sommer[1,4]

We tested the hypothesis that reduced skin innervation in fibromyalgia syndrome is associated with specific CNS changes. This prospective case–control study included 43 women diagnosed with fibromyalgia syndrome and 40 healthy controls. We further compared the fibromyalgia subgroups with reduced (n = 21) and normal (n = 22) skin innervation. Brains were analysed for cortical volume, for white matter integrity, and for functional connectivity. Compared to controls, cortical thickness was decreased in regions of the frontal, temporal and parietal cortex in the fibromyalgia group as a whole, and decreased in the bilateral pericalcarine cortices in the fibromyalgia subgroup with reduced skin innervation. Diffusion tensor imaging revealed a significant increase in fractional anisotropy in the corona radiata, the corpus callosum, cingulum and fornix in patients with fibromyalgia compared to healthy controls and decreased FA in parts of the internal capsule and thalamic radiation in the subgroup with reduced skin innervation. Using resting-state fMRI, the fibromyalgia group as a whole showed functional hypoconnectivity between the right midfrontal gyrus and the posterior cerebellum and the right crus cerebellum, respectively. The subgroup with reduced skin innervation showed hyperconnectivity between the inferior frontal gyrus, the angular gyrus and the posterior parietal gyrus. Our results suggest that the subgroup of fibromyalgia patients with pronounced pathology in the peripheral nervous system shows alterations in morphology, structural and functional connectivity also at the level of the encephalon. We propose considering these subgroups when conducting clinical trials.

The fibromyalgia syndrome (FMS) is a chronic pain disorder with a prevalence of approximately 2% in the general population[1]. Abnormalities in pain processing regions in the CNS, neurotransmitter levels, the autonomic nervous system, and in small fibers of the peripheral nervous system are frequent findings associated with FMS, but their causal connection to the manifestation and course of its symptoms is still unclear. Altered pain processing at the level of the CNS is regarded as a major pathophysiological factor[2,3]. However, structural lesions and functional deficits were also observed at the level of the PNS, where specifically small fiber pathology is a robust finding in a substantial group of patients fulfilling the established diagnostic criteria of FMS[4]. These findings of structural and functional alterations in FMS at both CNS and PNS level were reproducible: CNS structural measurements, like voxel-based-morphometry or cortical reconstruction, have revealed atrophy of the grey matter in the left prefrontal cortex and the posterior cingulate cortex[5,6]. Diffusion tensor imaging (DTI) has shown changes in white matter integrity, e.g. in the corpus callosum[7], and functional magnetic resonance imaging (fMRI) has identified hyperactivity in many regions related to pain processing[8], such as the left prefrontal cortex and in the posterior cingulate cortex, the insular cortex and the cerebellum. Functional connectivity was increased in the default mode network (DMN) and pain related areas, such as the insular cortex[9–11]. In the PNS, we and other groups described a decrease in intraepidermal nerve fiber density (IENFD)[12–17], which was related to symptom severity[4].

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The relative importance of CNS and PNS abnormalities for FMS pathophysiology has been a matter of debate. A continuum between peripherally driven pain at one end and centrally driven pain at the other end has been suggested[3]. Whether CNS and PNS abnormalities coexist in the same patients, or whether CNS and PNS pathology define two non-overlapping subgroups in FMS has never been studied and presents a particular methodological challenge. For FMS, we addressed this challenge in the following manner: We established robust differences between two cohorts of FMS patients using objective and validated criteria of injury at the PNS level (FMS with markedly reduced IENFD vs. FMS with normal IENFD). We hypothesized that structural or functional remodeling of the brain would occur differentially in these two subgroups on a global or regional level. We tested this hypothesis in these two FMS subgroups versus case matched healthy controls using MRI methods to measure brain morphometry, structural and functional connectivity.

Materials and methods

Subjects. Forty-three female patients with FMS were recruited at the Department of Neurology, University Hospital Würzburg, who also had taken part in a previous study investigating small fiber pathology in FMS[4]. Forty healthy female age and sex matched controls were recruited via public announcements. All patients had been diagnosed with FMS and examined by a rheumatologist and a neurologist, fulfilled the diagnostic criteria for FMS according to the guidelines released by the American College of Rheumatology[18], and had been comprehensively examined in our hospital for possible differential diagnoses (see Evdokimov et al. 2019[4]). Specifically, patients must have had widespread pain for more than three months that could not be explained by other diseases, have a Widespread Pain Index (WPI) ≥ 7 and the Symptom Severity Score ≥ 5[18].

All patients were off their pain medication for 3 days before the examination. None of the patients and controls had been taking anticonvulsants, antihistamines, muscle relaxants or benzodiazepines within the 4 weeks before the examination. All participants in the study gave there written informed consent according to Declaration of Helsinki. The study was approved by the Ethics Committee of the University of Würzburg Medical Faculty (63/18). The exclusion criteria for patients and controls were other current autoimmune or inflammatory diseases that can cause pain, such as rheumatoid arthritis, systemic lupus erythematosus, or chronic inflammatory bowel disease, as well as neurological, cardiovascular, psychiatric diseases, such as major depression, in the past and at present, any contraindication for MRI like cardiac pacemakers, cochlear implants, vascular stents or metal splinters in the body, a history of drug abuse, a history of head trauma requiring medical attention or brains with significant structural abnormalities.

Subgrouping according to intraepidermal nerve fiber density. Patients from the previous study[4] who had either normal IENFD at the lower leg (above the lower limit of normal 5.4 fibers/mm) and at the upper thigh (above the lower limit of normal 8.5 fibers/mm) or a non-length dependent abnormal IENFD, which means the IENFD was below the lower limits at both biopsy sites, were re-recruited, i.e. were contacted by H.-C. A. and invited to a follow-up appointment for MRI imaging. The first group was termed "noPNS", the second group "PNS". These cut-off values were determined based on skin biopsies of these two regions of 120 healthy women (median age = 50 years, range = 20–84 years) in our department. The cut-off values represent the lower limit of the standard deviation of the IENFD results of all the healthy controls investigated in our laboratory.

Fibromyalgia related symptoms. Results of the questionnaire and clinical examination data of the FMS patients have already been published[4]. To evaluate pain severity, two pain scores were used (Graded Chronic Pain Scale (GCPS) and Neuropathic Pain Symptom Inventory (NPSI)). In order to assess the depressiveness of the patients, the "Allgemeine Depressionskala" (ADS) was used, which is a German version of the Center for Epidemiological Studies—Depression scale questionnaire[19]. To evaluate catastrophizing, the Pain Catastrophizing Scale (PCS)[20], which is a self-report measure, consisting of 13 items scored from 0 to 4, resulting in a total possible score of 52, was assessed. To test the anxiety level, the State-Trait Anxiety Inventory (STAI) was used[21], which is a commonly used measure of trait and state anxiety. In order to assess the influence of the disease on daily experience, the Fibromyalgia Impact Questionnaire (FIQ)[22] was used. Also, the Symptom Severity Scale (SSS) was used to query other FMS-associated symptoms[18]. It measures three key symptoms during the past week: Fatigue, unrefreshed wakening and cognitive impairment. The O'Leary-Sant Symptom and Problem Index assesses the impairment by bladder dysfunction[23] and was selected, as FMS patients frequently report abdominal pain and problems with urination. Data collected in the context of the clinical diagnostics, such as the conduction studies of the sural nerve and the blood values, for example HbA1c and vitamin D, were also analyzed.

MR imaging and analysis. *Data acquisition.* Magnetic resonance imaging was performed on a Siemens MAGNETOM Prisma fit Scanner (Siemens Healthcare GmbH, Erlangen, Germany), operating at 3 T, equipped with a 64-channel head coil at the Department of Neuroradiology, University Hospital Würzburg. For each participant we included a structural T1-weighted (T1w) sequence, diffusion weighted imaging (DWI), fieldmap data and resting-state functional MRI (rs-fMRI) series. The T1w gradient echo MPRAGE sequence (repetition time (TR) 2400 ms, echo time (TE) 3.17 ms, flip angle (FA) 8°, inversion recovery (IR) 1000 ms) contained 176 sagittal slices with an isotropic voxel size of 1 × 1 × 1 mm. The visual examination of the T1w-structural images revealed no gross morphological abnormalities for any patient or subject. DWI was obtained using multiband echo-planar imaging (EPI) with the following parameters: TR = 3100 ms, TE = 89 ms, FA = 90°, isotropic voxel size of 2 × 2 × 2 mm. Diffusion data were collected with reversed phase-encode blips, resulting in pairs of b0-images with distortions in opposite directions for further susceptibility induced distortion correction. Resting state fMRI data was acquired using a T2*-weighted multiband EPI sequence with TR = 1610 ms, TE = 30 ms, FA = 70°, isotropic voxel size of 2 × 2 × 2 mm, 69 slices. During the 9-min resting state fMRI acquisition period

with 300 volumes the subjects were told to lie still and remain awake with their eyes open. Participants' motion was minimized using tight foam pads around the head, their physiology was monitored.

Structural analysis. Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite v6.0.0 (Martinos Center for Biomedical Imaging, Boston, MA, USA) using the 3D T1w data. The technical details of these procedures are described in prior publications[24,25]. Parcellations were classified according to the Desikan-Killiany Atlas[26]. The exact listing of all ROIs used can be found under supplementary material 1a. Volume was measured in mm^3. In addition to the exploratory whole-brain approach, hypothesis-driven group comparisons were also performed with volumes of cortical regions that had been shown in a meta-analysis to be specifically affected in FMS[5] (namely, the left medial frontal cortex and the right posterior cingulate cortex). Since the factor age has been shown to be associated with differences in white and grey matter volume[27], we decided to include this factor as a covariate. We also included the pain intensity score of the GCPS as a covariate.

Structural connectivity: diffusion tensor imaging. The Oxford Centre for Functional Magnetic Resonance Imaging of the Brain software library (FSL, Oxford, UK, https://www.fmrib.ox.ac.uk)[28] was used for DTI data analysis and preprocessing. Our diffusion data, recorded in reversed phase-encode blips, were preprocessed using the FSL tools "topup"[29], "eddy (correction)", "BET"[30], and "FNIRT". FA images and eigenvalue images were created by fitting a tensor model to the preprocessed diffusion data using the FSL FDT toolbox (Functional MRI of the Brain Diffusion Toolbox, DTIFIT). For ROI specific evaluation of the FA data we created a mask with the ICBM-DTI-81 white-matter labels atlas (Laboratory of Brain Anatomical MRI, Johns Hopkins University[31]) in the same space and calculated the average FA value of all voxels in 48 ROIs. The exact listing of all tracts used as ROIs can be found under supplementary material 1b.These data were analyzed for group comparisons with ANCOVAs including post-hoc testing (Tukey) and correlated with clinical data and questionnaires using a spearman Rho correlation for non-normally-distributed z-standardized clinical data analysis (significance level of 0.01, two-tailed, confidence interval 0.95). In addition to the exploratory whole-brain approach, hypothesis-driven group comparisons were also performed with white matter tracts that had been shown to be affected in FMS (namely the thalamus[32], the corpus callosum[7], the cingulum and the white matter adjacent to the insula (anterior limb of the internal capsula[33])).

Functional connectivity: resting state BOLD fMRI. Resting state functional data were spatially preprocessed using SPM12 (Welcome Trust Centre for Neuroimaging, University College London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm/) and the CONN Toolbox v18 (https://www.nitrc.org/projects/conn, RRID:SCR_009550[34]) running in Matlab R2019a (The Mathworks Inc, USA). The reason for changing from FSL to the Conn Toolbox run in SPM was the extensive ROI to ROI analysis provided by this toolbox. Functional data were realigned, slice-time corrected, spatially normalized to the Montreal Neurological Institute (MNI) space, and spatially smoothed with a FWHM Gaussian kernel of 8 mm. We collected fieldmaps and undistorted the EPI images using the Fieldmap Toolbox (SPM). Motion parameters from realignment were evaluated, and a motion artefact threshold (translation > 3 mm, rotation > 1°) was employed for exclusion. Participant motion parameters were included as first-level covariates. No participants displayed gross movements to require total exclusion. Slices with motion parameters outside of the threshold were discarded. After denoising, quality control measurements (mean motion and max motion) were correlated and plotted with the functional connectivity values to control for influences (QC-FC correlations). To remove blood-oxygen-level-dependent (BOLD) signal from the cerebral white matter and ventricles, each participant's T1-weighted MPRAGE image was automatically segmented into grey matter, white matter, cerebrospinal fluid, normalized and transformed to MNI space using the Computational Anatomy Toolbox (CAT12; http://www.neuro.uni-jena.de/cat/) running in SPM12. BOLD data were bandpass filtered (0.008–0.09 Hz) to reduce low-frequency drift and noise effects. We then generated seed-to-seed connectivity maps for each individual using 164 seeds. These seeds are provided in the CONN software[35]. The exact classification of all seeds and the MNI coordinates of all network hubs are documented in supplementary material 1c. Individual correlation maps were generated. These results were subsequently used for second-level analysis of relative functional connectivity using an ANCOVA, implemented in the CONN toolbox, to investigate differences in seed-to-seed connectivity between groups. We applied a seed-to-seed analysis to investigate which brain areas show hyper- or hypoconnectivity between patients and controls and between subgroups. In addition to the exploratory whole-brain approach, hypothesis-driven group comparisons were also performed with seed regions that had been shown to be affected by FMS (namely the insular cortex[36], the frontoparietal network[37], the default mode network[10] and the somatosensory network[38]). Pain intensity (GCPS) and ADS (depression) scores were included as second-level covariates. The influence of the IENFD data on the FC-values was analyzed using a linear regression model. False discovery rate (FDR) correction was applied at the cluster level ($p < 0.05$).

Statistical analysis. Data were analyzed with IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Armonk, NY, USA) and JASP (JASP Team (2021) (Version 0.14.1, Windows 10). We tested the clinical data for normal distribution with a Shapiro–Wilk test and then, depending on the result, examined for group differences with a two-tailed t-test or a Mann–Whitney-U test. Data are given as mean ± SD or median/range unless otherwise specified. We used the Levene test with a significance threshold of 0.05 to check the data for equivalence of variance. The confidence interval was set at 95%. ROI group means of the structural, DTI and functional connectivity data were compared using an ANCOVA after controlling for interactions between the covariate and fixed factor and Tukey-tests for post-hoc comparisons. For the ANCOVA, effect sizes are displayed as $\acute{\omega}^2$,

	Patients all (n = 43)	Controls (n = 40)	
	Mean ± SD	Mean ± SD	p-value
Age	53.5 ± 6.5	52.6 ± 6.7	< 0.51
BMI	28.2 ± 5	26.6 ± 5	< 0.14

Table 1. Clinical data compared between patients and controls.

	PNS (n = 21)	noPNS (n = 22)	
	Mean ± SD/median (range)	Mean ± SD/median (range)	p-value
Age	53.5 ± 6.7	53.4 ± 6.5	< 0.9
BMI	30.9 ± 4.2	25.5 ± 4.2	**< 0.001**
IENFD lower leg (fibers/mm)	3.9 ± 1.5	10 ± 2.6	**< 0.001**
IENFD upper thigh (fibers/mm)	5.7 ± 1.5	11.5 ± 2.8	**< 0.001**
Time since diagnosis (years)*	5 (1–19)	5 (0–14)	< 0.51
Duration of pain due to the disease (years)	16.8 ± 10.8	18. 8 ± 12.7	< 0.71
Number of tender points*	14 (11–18)	15 (7–18)	< 0.23
WPI*	13.0 (10–19)	15 (8–18)	< 0.82
SSS*	7 (6–10)	7 (5–11)	< 0.87
HbA1c (%)	5.4 ± 0.3	5.3 ± 0.2	< 0.16
Sural nerve SNAP (μV)	22.6 ± 7.8	25.1 ± 12.5	< 0.45
Sural nerve conduction velocity (m/s)	48.3 ± 4.05	50.4 ± 3.5	< 0.09
Serum vitamin D (μg/l)	30.1 ± 14.1	30.1 ± 11.1	< 0.99
Highest education level**	3 (2–5)	3 (2–5)	< 0.13
NPSI sum score	31. 1 ± 4.2	25.5 ± 4.2	< 0.09
GCPS pain intensity	73.6 ± 10.8	64 ± 15.1	**< 0.02**
GCPS disability due to pain*	66.7 (10–83.3)	53.3 (16.6–86.6)	< 0.09
Pain catastrophizing scale	26.7 ± 10.1	20.7 ± 10.3	< 0.06
ADS	27.8 ± 11.8	21.2 ± 11.4	< 0.07
FIQ	51.9 ± 12	42.3 ± 13.2	**< 0.01**
The O'Leary-Sant symptom index and problem index*	12 (0–33)	9 (1–22	< 0.28
STAI	47.1 ± 11.6	44.3 ± 13.3	< 0.48

Table 2. Clinical and questionnaire data compared between subgroups. *ADS* Allgemeine depressionskala, *BMI* body mass index, *FIQ* Fibromyalgia Impact Questionnaire, *GCPS* Graded Chronic Pain Scale, *IENFD* intraepidermal nerve fibre density, *NPSI* Neuropathic Pain Symptom Inventory, *SNAP* sensory nerve action potential, *SSS* Symptom Severity Score, *STAI* State-Trait Anxiety Inventory, *WPI* Widespread Pain Index. *These data are not normally distributed, therefore the median and the range are shown here and a Mann–Whitney U test was applied. ** (1: Elementary school, 2: Primary school, 3: Secondary school, 4: High school, 5: University).

which is based on Cohens f^2 ($f^2/(1+f^2)$) and Cohen's d. The correlation analyses were performed with a Pearson correlation (after controlling for the distribution of the data), 1000 samples of bootstrapping and a significance level of 0.01. All post-hoc group comparisons were corrected for multiple comparisons using the false discovery rate algorithm[39].

Data availability. The raw, skull stripped, data used to analyze the following results can be obtained upon request from the corresponding author. The processing and statistical analysis of the data was done using established neuroimaging software, as described in the methods. The STROBE Statement-Checklist was used for the quality control of our case–control study.

Results

Patient population. The patient group (n = 43, mean age 53.5 ± 6.5 years, mean BMI 28.2 ± 5.0) and the healthy control group (n = 40, mean age 52.5 ± 6.7 years, mean BMI 26.6 ± 5.0) did not differ in age and BMI. The subgroups noPNS (normal IENFD) and PNS (decreased IENFD) differed in BMI, with a higher BMI in the PNS subgroup (Tables 1, 2).

Clinical data and questionnaires. We included patients with normal skin innervation and patients with reduced IENFD both at the lower leg and the upper thigh from the cohort described in[4]. In patients with reduced

	Cortex parcellation	p-adjusted	F-value	ώ-square
Patients vs controls	Left fusiform	0.04	4,2	0,09
	Left inferiorparietal	0.04	3,6	0,08
	Left inferiortemporal	0.04	4,6	0,1
	Left insula	0.04	3,4	0,08
	Left pericalcerine	0.03	3,8	0,09
	Right middletemporal	0.01	5,4	0,12
	Right parsopercularis	0.04	3,2	0,07
	Right superiorfrontal	0.03	3,5	0,08
	Right superiortemporal	0.04	3,5	0,08
	Right supramarginal	0.04	4,6	0,1
PNS vs NoPNS	Left pericalcarine	0.049	4.1	0.06
	Right pericalcarine	0.03	7.2	0.13

Table 3. Results of cortical volume analysis after FDR-correction.

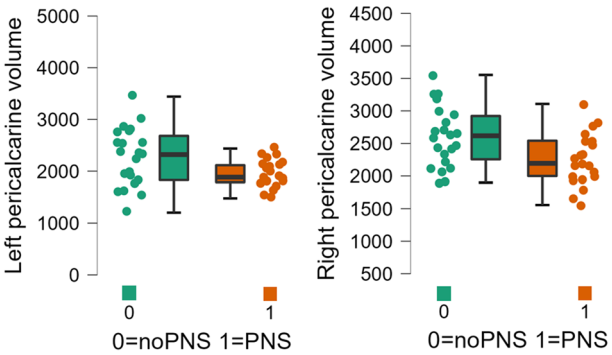

Figure 1. Group differences of cortical thickness. Plots showing a lower volume of the bilateral pericalcarine cortex in the PNS subgroup compared to the noPNS subgroup. Volume is measured in mm^3.

distal and proximal IENFD (PNS group), FMS symptoms were more severe ($p = 0.02$) and quality of life was lower compared to FMS patients with normal distal and proximal IENFD ($p = 0.01$) as reflected by the values of the GCPS pain intensity and the FIQ questionnaire (Table 2). There was no difference between the subgroups regarding parameters evaluating how widespread the pain was (WPI or tender points).

Structural analysis. With the values of the cortical volume per parcellation calculated by Freesurfer, we performed an ANCOVA with post-hoc testing including all patients ($n = 43$), PNS patients ($n = 21$) and noPNS patients ($n = 22$). Cortical volume differed between the FMS and control groups in 10 cortical regions (see Table 3). Cortical volume differed between the subgroups (PNS versus noPNS) in the left pericalcarine cortex ($F = 4.1$, p-adjusted $= 0.049$, $ώ2 = 0.06$) and the right pericalcarine cortex ($F = 7.2$, p-adjusted $= 0.03$, $ώ2 = 0.13$) (see Fig. 1). Except for the left pericalcarine cortex, all cortical regions of FMS patients showed lower volumes than those of healthy controls (see supplementary material 2).

To examine possible influences of clinical data including the severity of pain and depression on cortex volume, correlation analyses between questionnaire data for pain and depression and cortical volumes were calculated. This was a-priori restricted to the 10 ROIs, which showed significant alterations in the FMS group compared to the control group. We found no significant influence of clinical data including the severity of pain and depression on cortex volume in the correlation analysis after FDR correction.

Structural analysis of FMS "specific" regions. Following a meta-analysis that analyzed FMS data from voxel-based morphometry[5], we explicitly tested group differences in the volume of the left medial frontal cortex, as well as the right posterior cingulate cortex. Indeed, the FMS group showed a smaller cortex volume in the left frontal pole ($p = 0.03$, $\eta^2 = 0.05$), in the posterior cingulate cortex ($p = 0.04$, $\eta^2 = 0.05$), and trendwise in the left rostral midfrontal cortex ($p = 0.08$, $\eta^2 = 0.04$) compared to the control group. The subgroup comparison PNS versus noPNS showed no differences in these regions (left frontal pole ($p = 0.26$, $\eta^2 = 0.03$), posterior cingulate cortex ($p = 0.27$, $\eta^2 = 0.03$), left rostral midfrontal cortex ($p = 0.6$, $\eta^2 = 0.006$)).

Diffusion tensor imaging. In the ROI-based analysis comparing patients and controls, a significant increase in FA was found in 14 out of 48 ROIs in FMS patients (after FDR-correction). This was evident in corticospinal pathways such as the corona radiata, but also in regions of the limbic systems such as the fornix and cingulum.

	White matter tract	t-value	p-adjusted	Cohen's d	Group	N	Mean	SD	SE
Patients vs controls	Anterior corona radiata l	−3.292	0.009	−0.719	Controls	41	0.540	0.004	0.004
					Patients	43	0.556	0.003	0.003
	Body of corpus callosum	−3.705	0.004	−0.809	Controls	41	0.825	0.003	0.003
					Patients	43	0.840	0.002	0.002
	Cingulum 40	−4.384	0.001	−0.957	Controls	41	0.609	0.006	0.006
					Patients	43	0.642	0.005	0.005
	Cingulum 41	−3.456	0.009	−0.754	Controls	41	0.599	0.006	0.006
					Patients	43	0.626	0.005	0.005
	Fornix 44	−3.843	0.004	−0.839	Controls	41	0.573	0.004	0.004
					Patients	43	0.595	0.004	0.004
	Genu of corpus callosum	−2.939	0.01	−0.642	Controls	41	0.740	0.004	0.004
					Patients	43	0.757	0.004	0.004
	Pontine crossing tract	−2.992	0.02	−0.653	Controls	41	0.768	0.004	0.004
					Patients	43	0.785	0.004	0.004
	Posterior corona radiata r	−2.992	0.01	−0.642	Controls	41	0.534	0.02	0.004
					Patients	43	0.551	0.02	0.004
	Posterior limb of internal capsule l	−3.215	0.01	−0.702	Controls	41	0.650	0.004	0.004
					Patients	43	0.668	0.004	0.004
	Posterior thalamic radiation 34	−3.242	0.01	−0.708	Controls	41	0.617	0.004	0.004
					Patients	43	0.634	0.004	0.004
	Superior corona radiata l	−2.832	0.02	−0.618	Controls	41	0.551	0.005	0.005
					Patients	43	0.568	0.004	0.004
	Superior corona radiata r	−3.129	0.01	−0.683	Controls	41	0.540	0.004	0.004
					Patients	43	0.556	0.003	0.003
	Superior longitudinal fasciculus r	−2.773	0.02	−0.605	Controls	41	0.566	0.005	0.005
					Patients	43	0.584	0.004	0.004
	Uncinate fasciculus l	−0.367	0.04	−0.080	Controls	41	0.586	0.008	0.008
					Patients	43	0.590	0.007	0.007
	White matter tract	**F-value**	**p-adjusted**	**ώ-square**	**Group**	**N**	**Mean**	**SD**	**SE**
PNS vs noPNS	Posterior limb of internal capsule l	4.8	0.034	0.08	PNS	21	0.658	0.020	0.004
					NoPNS	22	0.678	0.029	0.006
	Posterior thalamic radiation 34	4.9	0.048	0.09	PNS	21	0.626	0.022	0.005
					NoPNS	22	0.642	0.025	0.005

Table 4. Between group comparisons of the FA data (ROI-wise).

A detailed list of these regions with the respective FA values can be found in Table 4. Scatter plots to check for the distribution of the data can be found in the supplementary material 3a/b. The ROI-based comparison of the two subgroups PNS and noPNS showed elevated FA levels in the left posterior limb of the internal capsule and the posterior thalamic radiation (after FDR-correction) (Fig. 2).

The Pearson correlation analysis, a-priori restricted to the 14 regions that revealed differences in the group comparison, showed a negative association with the anxiety questionnaire (STAI-S) and the FA of the fornix (Pearson's $r = -0.4$, $p = 0.006$), the posterior thalamic radiation (Pearson's $r = -0.4$, $p = 0.006$) and the right posterior corona radiata (Pearson's $r = -0.4$, $p = 0.005$). This means that higher anxiety scores were associated with lowered FA in the respective areas.

Diffusion tensor imaging of FMS "specific" regions. White matter tracts that had already shown changes in patients with FMS in the literature are the corpus callosum, the thalamus, the cingulate, and the insular cortex connecting tracts (anterior limbs of the internal capsule). Our data indicated also an increased FA in the FMS group compared to controls in the cingulum ($p < 0.001$, $\eta^2 = 0.13$), in the body of the corpus callosum ($p < 0.001$, $\eta^2 = 0.14$), in the genu of the corpus callosum ($p = 0.004$, $\eta^2 = 0.1$), and in the posterior thalamic radiation ($p = 0.002$, $\eta^2 = 0.12$). No significant differences were found in the anterior limb of the left ($p = 0.2$, $\eta^2 = 0.02$) and right ($p = 0.62$, $\eta^2 = 0.003$) internal capsule and the splenium of the corpus callosum ($p = 0.53$, $\eta^2 = 0.005$).

Subgroup comparison between PNS and noPNS showed an increased FA of the posterior thalamic radiation in the noPNS subgroup ($p = 0.03$, $\eta^2 = 0.1$). No subgroup differences were seen in the cingulum ($p = 0.9$, $\eta^2 < 0.001$), body of the corpus callosum ($p = 0.57$, $\eta^2 < 0.001$), genu of the corpus callosum ($p = 0.99$, $\eta^2 < 0.001$), splenium of the corpus callosum ($p = 0.47$, $\eta^2 = 0.01$), and the anterior limb of the left ($p = 0.7$, $\eta^2 = 0.003$) and right ($p = 0.92$, $\eta^2 < 0.001$) internal capsule.

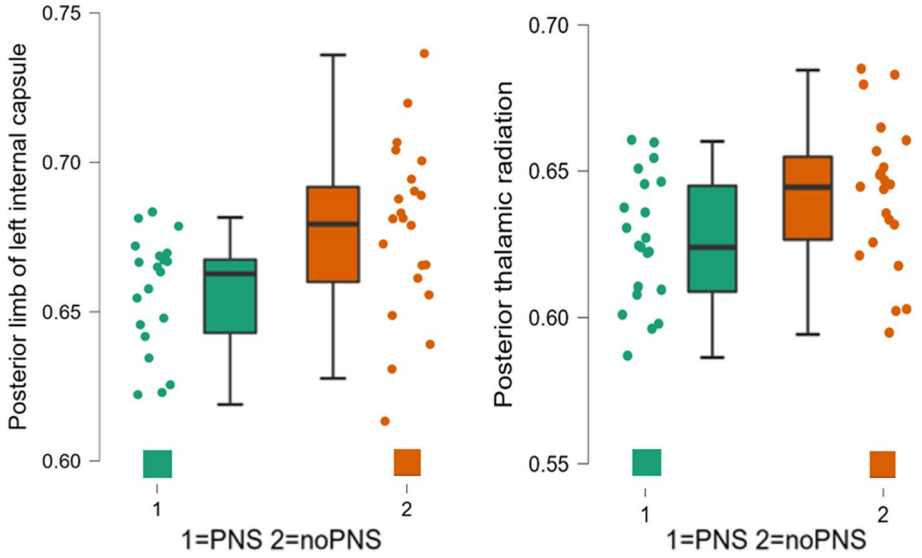

Figure 2. Group differences of white matter integrity. Plots showing decreased FA of two white matter tracts in the PNS subgroup compared to the noPNS subgroup.

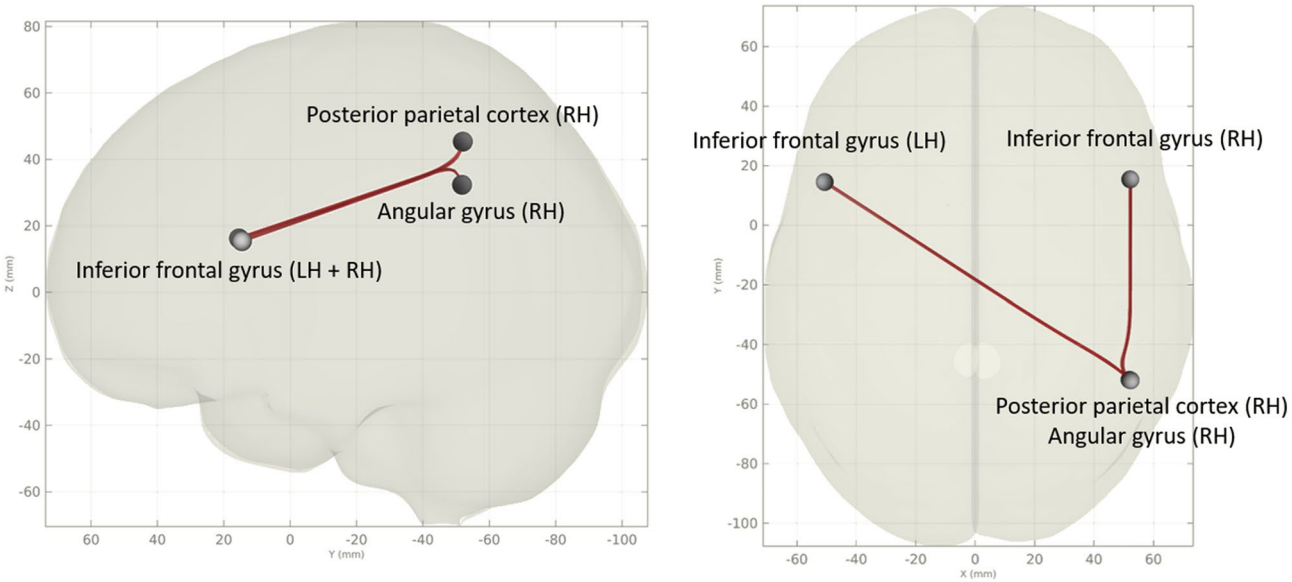

Figure 3. Group differences of functional seed-to-seed connectivity. Hyperconnectivity cluster in the PNS subgroup compared to the noPNS subgroup (LH: left hemisphere, RH: right hemisphere).

Functional resting state imaging. Seed-to-seed analysis between patients and controls showed significant hypoconnectivity of the right midfrontal gyrus to the posterior cerebellum (p-FDR = 0.048) and to the right crus cerebelli 1 (p-FDR = 0.048) in FMS patients. Seed-to-seed analysis between the subgroups noPNS and PNS showed one FDR-corrected cluster of the PNS subgroup compared to the noPNS subgroup (F = 12.8, p-adjusted = 0.049) with hyperconnectivity between the left and right inferior frontal gyrus (IFG) and the right angular gyrus (left IFG: T = 3.33; right IFG: T = 3.27) and posterior parietal cortex (left IFG: T = 2.93; right IFG: T = 3.27) respectively (Fig. 3).

The linear regression model with the IENFD values as independent variables showed no significant associations with the ROI-ROI functional connectivity after FDR-correction.

Functional resting state imaging of FMS "specific" network hubs. Network hubs that had already shown changes in FMS patients in previous publications are the default mode network, the somatosensory network, the frontoparietal network, and the insular cortex. Even after restricting the analysis to these regions of interest, we could

not find any connectivity cluster differences between the FMS and the control group or between the PNS and noPNS subgroups in our data.

Discussion

In this study, two group comparisons were conducted using structural, DWI and functional MRI data: Firstly, we compared FMS patients to healthy controls, secondly, we divided the FMS group into two subgroups with and without PNS pathology (PNS and noPNS groups) and compared these subgroups with each other. While the structural and functional differences in MRI studies of FM patients have been described in the literature, so far no study has investigated the possible interaction between the peripheral nervous system and the brain of FMS patients.

We show that in FMS (1) cortical volume is decreased in the left and right frontal/temporal cortices and the left insula, (2) FA is generally increased in corticospinal tracts and regions of the limbic system and (3) functional connectivity is reduced between the right midfrontal gyrus and the posterior cerebellum as well as the right crus cerebelli.

Comparison of the noPNS and PNS subgroups showed (1) lower volumes in the bilateral pericalcarine cortex in the PNS group, (2) lower FA in the left posterior limb of internal capsule and in the posterior thalamic radiation in the PNS group and (3) a hyperconnectivity cluster between the bilateral inferior frontal gyri, the angular gyrus and the posterior parietal cortex in the PNS group. In summary, the noPNS group showed greater deviations from healthy controls in structural MRI measures than the PNS group.

Comparison of the present findings with published data. Our results on the cortical volume are for the most part (regarding the alterations in the temporal, parietal and insular cortices) in line with the results of a meta-analysis which pooled structural and functional MRI studies comparing FMS patients to healthy controls[40]. These regions also appear to change their cortical thickness as the disease progresses[41]. Decreased gray matter in the left fusiform and prefrontal cortex was also found in FMS patients in another voxel morphometry-based meta-analysis[42]. In our hypothesis-driven analysis restricted to regions that showed lower cortex volumes in a meta-analysis of structural FMS data (left medial frontal cortex and right posterior cingulate cortex), we were able to reproduce the results of the meta-analysis[5]. However, in our subgroup comparisons, these regions showed no significant differences. The prefrontal cortex is a known site of pain modulation. Indeed, a dual role has been described including antinociceptive effects by modulating sensory afferent influx, as well as the furthering of chronic pain via corticostriatal projections. Interestingly, decline of prefrontal cortex volume in chronic pain can be reversed with successful biopsychosocial therapy, be it cognitive behavioral therapy, exercise or transcranial magnetic stimulation[43].

Our subgroup comparison of cortex volume data showed a bilateral decrease in the volume of the pericalcarine cortex in the PNS group. Interestingly, in our results, the pericalcarine cortex is the only region that shows larger volumes in the FMS patients compared to the healthy controls. Thus, the noPNS group has a greater change in pericalcarine volume compared to the healthy controls. The pericalcarine cortex is part of the visual cortex. In our literature research, this region has not yet been associated with FMS symptoms. A magnetoencephalography study showed that the visual cortex in FMS patients has decreased connectivity to other brain regions[44]. This hypoconnectivity was also demonstrated in another study using resting state fMRI[45] and was associated with decreased resiliency towards pain[46]. However, the pericalcarine cortex is also involved in other pain disorders, for example, its volume changes during acute migraine attacks and normalizes in post-ictal phases[47]. Our results do not allow us to determine whether the pericalcarine cortices decrease in volume during the course of the disease in the PNS group or whether the difference exists at the onset of the disease. Longitudinal studies are needed to explore the role of the pericalcarine cortex in pain development.

Regarding FA, a marker for the integrity of the white matter, our whole brain analysis showed an increase in FA in the corona radiata and regions of the limbic system (e.g. fornix and cingulate cortex) in the FMS group compared to controls. The previous results of diffusion imaging in FMS patients are not consistent, and the results here vary widely. Regions that frequently showed changes in FA in the literature were the corpus callosum, the cingulum, the thalamus, and the anterior limb of the internal capsule adjacent to the insular cortex[7,33]. Except for the anterior limbs of the internal capsule, we were able to reproduce these results in our hypotheses driven analyses. Regarding our subgroups analyses, two regions showed a significant decrease of FA in the PNS group compared with the noPNS group (left posterior limb of internal capsule and the posterior thalamic radiation). Increased FA of these regions has already been found in studies with FMS patients or other chronic pain disorders and was associated with pain severity[48]. It has also been shown in FMS patients that white matter pathways with increased FA after a prolonged period of increased activity[49], in this case in pain processing regions, show decreased FA again after pain chronification and show lower values than healthy controls[33]. Longitudinal study designs are needed to clarify the extent to which FA changes over the course of chronic pain disorders and the influences of a reduction or increase in FA on symptoms.

Regarding functional connectivity, even after limiting the regions of interest included in the analysis to network hubs already published in the FMS literature (default mode network, somatosensory network, frontoparietal network, insular cortex)[10,36,38,50] we could not reproduce alterations in these hubs with our data. The reason for this could be the lack of control for depression or pain intensity in other studies or different methods of analysis. The cluster found in our subgroup analysis has not been described in the FMS literature before. All involved regions (inferior frontal gyrus, angular gyrus and posterior parietal cortex) are involved in attention and evaluation of external and internal stimuli. Overactivation of the angular gyrus in fMRI has been associated with a stronger negative evaluation of pain[51], while the inferior frontal gyrus seems to be involved in the regulation of

emotions[52]. The posterior parietal gyrus with its connections to the somatosensory cortex appears to have an important role in the spatial perception of pain stimuli[53].

Are the findings specific for FMS? Most of our findings have been described in other publications about chronic pain imaging[54]. For example, it has already been suggested that a lower activity of the prefrontal cortex, a well-known pain modulation area, could lead to a failure in the elimination of subcortically driven fear behaviors, thereby resulting in pain chronification[55]. It is currently unclear whether these processes are adaptive, maladaptive or cause some of the symptoms. In order to better understand the pathophysiology of FMS, it is therefore important to first understand the role of brain neuroplasticity in chronic pain, as a brain signature of pain appears to be found across various pain syndromes[56]. Neuroimaging studies with multiple pain syndromes as comparison groups are needed here before finding brain regions specific to FMS that could potentially trigger some of the symptomatology.

Limitations of our study. Our study has some limitations. Because our study was designed as a cross-sectional study, the question of the reasons for and the effects of our detected group differences cannot be answered. By including individual pain intensity as a covariate in our group statistics, we attempted to account for a possible influence of pain intensity on our MRI results. However, because none of the MRI modalities showed a significant association with IENFD scores after FDR correction, we cannot rule out the possibility that subgroup differences were driven by other factors not captured in our clinical examinations. Furthermore, even structural MRI markers, such as cortical volume, are subject to temporal variations, depending, for example, on acute stimulus severity[57]. This emphasizes the need for longitudinal studies.

The healthy controls in our study did not receive a skin biopsy, so we cannot rule out that some persons with reduced IENFD might have been in this group. However, in our previous study[4], only 2% of normal controls had reduced IENFD at the lower and upper leg, so that it is highly unlikely that a large number of our present controls would have had this finding.

Conclusions

While structural and functional MRI changes in FMS patients have already been investigated, our study first demonstrated differences between FMS subgroups with and without peripheral nerve involvement. The study design obviously does not allow any conclusions to be drawn about the reasons for and effects of these subgroup differences. While most clinical trials on FMS therapy included only patients diagnosed according to current diagnostic criteria, one has to consider that FMS is a heterogeneous condition with potentially different underlying pathophysiological processes within subgroups. These subgroups might respond differentially to specific treatments. Psychiatric comorbidities, such as depression and anxiety, also affect the brain structure in FMS and thus influence the results in MRI imaging. We therefore advocate that future studies should take into account the different subgroups of patients both on the basis of small nerve fiber pathology, symptom severity, and psychiatric comorbidities.

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Research Article

Analgesic Medication in Fibromyalgia Patients: A Cross-Sectional Study

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There is no approved drug for fibromyalgia syndrome (FMS) in Europe. In the German S3 guideline, amitriptyline, duloxetine, and pregabalin are recommended for temporary use. The aim of this study was to cross-sectionally investigate the current practice of medication in FMS patients in Germany. We systematically interviewed 156 patients with FMS, while they were participating in a larger study. The patients had been stratified into subgroups with and without a decrease in intraepidermal nerve fiber density. The drugs most commonly used to treat FMS pain were nonsteroidal anti-inflammatory drugs (NSAIDs) (41.0% of all patients), metamizole (22.4%), and amitriptyline (12.8%). The most frequent analgesic treatment regimen was "on demand" (53.9%), during pain attacks, while 35.1% of the drugs were administered daily and the remaining in other regimens. Median pain relief as self-rated by the patients on a numerical rating scale (0–10) was 2 points for NSAIDS, 2 for metamizole, and 1 for amitriptyline. Drugs that were discontinued due to lack of efficacy rather than side effects were acetaminophen, flupirtine, and selective serotonin reuptake inhibitors. Reduction in pain severity was best achieved by NSAIDs and metamizole. Our hypothesis that a decrease in intraepidermal nerve fiber density might represent a neuropathic subtype of FMS, which would be associated with better effectiveness of drugs targeting neuropathic pain, could not be confirmed in this cohort. Many FMS patients take "on-demand" medication that is not in line with current guidelines. More randomized clinical trials are needed to assess drug effects in FMS subgroups.

1. Introduction

Fibromyalgia syndrome (FMS) is a chronic pain disorder associated with fatigue, sleep, memory, and mood disturbances, defined by a widespread pain index (WPI) and the symptom severity scale, symptom persistence over 3 months, and exclusion of all other diseases that might cause pain [1]. The etiology of FMS is still largely unknown. The majority of patients are women [2].

Systematic reviews of randomized clinical trials have shown that serotonin–norepinephrine reuptake inhibitors (SNRIs) [3], pregabalin [4], noradrenaline reuptake inhibitors (NRIs) [5], tricyclic antidepressants [6], and cyclobenzaprine [7] have a small but significant effect on FMS pain severity. Opioids or dopaminergic agents had no effect on pain and carry the risk of drug dependency [5]. In the German S3 guideline of 2017 [8] and the European Alliance of Associations for Rheumatology (EULAR) recommendations of 2016 [9], amitriptyline, duloxetine, and pregabalin are recommended as temporary drug therapies for FMS. The Canadian and Israeli guidelines advise to use SNRIs and anticonvulsants (pregabalin and gabapentin) [10, 11]. All guidelines also point out that nonpharmacological therapy such as aerobic training or cognitive-based behavioral therapy may be more efficient in the relief of pain and fatigue, with fewer side effects.

No drug is licensed specifically for FMS in Europe, while the United States Food and Drug Administration approved pregabalin, duloxetine, and milnacipran for this indication [12]. In Europe, the European Medical Agency (EMA) has approved amitriptyline for the treatment of neuropathic pain as part of multimodal treatment, tramadol for

moderate-to-severe musculoskeletal pain [13], strong opioids for cancer pain and chronic noncancer pain as a last therapeutic option, and pregabalin and gabapentin for the treatment of neuropathic pain.

We have prospectively recruited and comprehensively investigated a large cohort of patients with FMS [14]. Here, we were interested in how these patients were medically treated in the absence of specifically licensed drugs and in the context of current guidelines. We report the current pharmacological treatment of these patients, which drugs were discontinued and why, and how well the individual drugs reduced pain. Previously, we showed that FMS patients with small fiber pathology as indicated by reduced intraepidermal nerve fiber density (IENFD) at the lower and upper legs had more severe clinical symptoms [14]. Hence, we hypothesized that drug efficiency might differ in patient subgroups stratified for small fiber pathology reflecting a potential neuropathic component.

2. Materials and Methods

Patients were recruited for a larger study on FMS and small fiber pathology at the Department of Neurology, University Hospital Würzburg, Germany, between 2014 and 2019. A flowchart of the inclusion process is shown in Figure 1. The study was approved by the Ethics Committee of the University of Würzburg Medical Faculty (number 121/14), and all study participants gave written informed consent. Before study inclusion, all patients were diagnosed by a board-certified rheumatologist. All patients were then examined by a neurologist, and a structured medical history focusing on pain and current and former FMS treatment was recorded. All patients were diagnosed according to the 1990 and 2010 criteria of the American College of Rheumatology [15] after alternative diagnoses had been excluded [14]. The exclusion criteria included amongst others a diagnosis of a manifest psychiatric or neurological disease, possible somatic underlying causes of neuropathy or other pain disorders, and a history of cancer in the last 5 years. Further details on the recruitment and exclusion criteria can be found in [14, 16]. Patients were asked about their current medication, the indication, the dose, the effect, and treatment regimen. Furthermore, the medication history was taken, and the reasons why previous medication was discontinued were elicited. These data were stored electronically in standardized forms. Since many patients took several pain medications, data are given relative to the total number of patients' replies to a specific drug. Only the general frequency of medication classes used in Table 1 is given relative to the absolute number of patients (Table 1).

Having determined IENFD in skin biopsies of the lower and upper leg, we had identified patients at the two opposite ends of the spectrum, which resulted in a group with pathologic IENFD in both the distal and the proximal biopsy and a group with normal IENFD in both biopsies [14]. Here, we investigated whether drug intake and efficacy differed between these previously determined subgroups.

To evaluate pain relief by the drugs, we used a numeric rating scale (NRS, 0–10; 0 = no pain; 10 = worst possible pain). This scale was used for all analyses regarding the effectiveness of individual drugs in relieving pain. The remaining pain questionnaires were only used to obtain a more comprehensive clinical characteristic but were not related to the effectiveness of the medications. To evaluate persistent pain severity, we used the Graded Chronic Pain Scale (GCPS), which reflects two dimensions of chronic pain: pain intensity and pain-related disability [17]. To assess the presence of depressive symptoms, we used the "Allgemeine Depressionsskala" (ADS), which is a German version of the Center for Epidemiological Studies-Depression Scale questionnaire [18]. To evaluate the extent of catastrophizing, we applied the Pain Catastrophizing Scale [19], We further used the State-Trait Anxiety Inventory (STAI) [20], which is a commonly used measure of trait and state anxiety. In order to record the impact of FMS symptoms on everyday life activities, we used the Fibromyalgia Impact Questionnaire (FIQ) [21]. The O'Leary-Sant symptom and problem index assesses the impairment by bladder dysfunction [22]. Since some patients also report problems or pain during urination, we used this questionnaire to evaluate secondary symptoms and possible side effects.

We categorized diclofenac, ibuprofen, and acetylsalicylic acid as nonsteroidal anti-inflammatory drugs (NSAIDs); etoricoxib and nimesulide as cyclooxygenase-2 (COX-2) inhibitors; tilidine and tramadol as weak opioids; oxycodone, tapentadol, and fentanyl as strong opioids; tolperisone as a muscle relaxant; fluoxetine and sertraline as selective serotonin reuptake inhibitors (SSRI); and duloxetine as serotonin–norepinephrine reuptake inhibitors (SNRI). Some patients reported guaifenesin treatment explicitly against FMS symptoms; hence, we also included this mucus diluent in our analysis.

For statistical analysis, the program IBM SPSS Statistics for Windows version 25.0 (IBM Corp. Armonk, NY, USA) was used. Data were converted into the dichotomic multiple answer system of SPSS and evaluated using crosstabs. the Shapiro–Wilk test was performed to check for normal distribution of the data. For normally distributed data (all questionnaires except the GCPS and the STAI), we used a two-sided t-test for group comparisons. For nonnormally distributed data, the group comparison was performed by the Mann–Whitney U test. The crosstabs were tested for significance using the chi-square test. Correlation analysis was performed by the two-sided Spearman–Rho test. The confidence interval was 0.95, and the significance threshold was $p < 0.05$. In order to compare the effectiveness of the pain medication between the small nerve fiber groups, only medication classes that were taken by more than 15 patients were included for sufficient statistical power.

3. Results

One hundred and fifty-six patients (144 women, 12 men) were included in our analysis. The median age was 50.6 years (range 21.5–74.7). The sum scores of the patients' symptom questionnaires and the proportion of frequent FMS comorbidities are displayed in the Supplementary Table 1. There was no difference between the groups with and

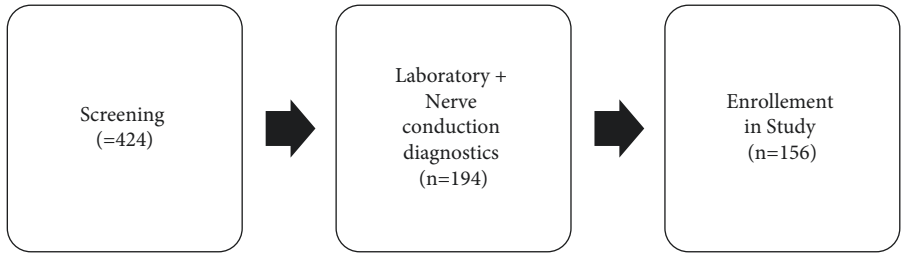

Figure 1: Flowchart of the inclusion process of patients.

Table 1: Current medication of fibromyalgia patients (total number of patients = 156) and previously discontinued medication. Some of the patients took more than one medication.

Medication	Current use Number of patients currently using the drug (% of all patients)	Past use Number of patients having used the drug in the past (% of all patients)
NSAID	64 (41.0)	53 (35.1)
Metamizole	35 (22.4)	14 (9.3)
None	25 (16.0)	26 (17.2)
Amitriptyline	20 (12.8)	57 (37.7)
SNRI	18 (11.5)	33 (21.9)
Weak opioid	9 (5.8)	27 (17.9)
COX-2 inhibitor	8 (5.1)	2 (1.3)
Pregabalin	8 (5.1)	28 (19.2)
Muscle relaxant	7 (4.5)	3 (2.0)
Acetaminophen	6 (3.8)	12 (7.9)
Cannabinoid	4 (2.6)	—
Strong opioid	3 (1.9)	5 (3.3)
Guaifenesin	3 (1.9)	—
Triptan	3 (1.9)	—
Flupirtine	3 (1.9)	13 (8.6)
SSRI	3 (1.3)	11 (7.3)
Corticosteroid	1 (0.6)	1 (0.7)
Lidocaine	1 (0.6)	1 (0.7)
Magnesium	1 (0.6)	—
Mirtazapine	1 (0.6)	—

without pathologic IENFD in the results of the questionnaires (Supplementary Table 2).

3.1. Current Medication. The most frequently taken class of drugs was NSAIDs with 41.0% of all patients, followed by metamizole with 22.4% and amitriptyline with 12.8% (Table 1). Opioids were taken by 7.7% of the patients. 16% of the patients in our study did not take any medication against FMS symptoms. The most frequent analgesic treatment regimen was "on demand" during pain exacerbations (53.9% of all prescribed drugs), while 35.1% of the drugs were administered according to a fixed regime. Antidepressants were mostly taken on a daily basis (Table 2). 57.6% of patients took one analgesic drug, 27.3% two drugs, 5.2% three drugs, and 1.2% four drugs.

Only 29.6% of the patients had drug therapy according to the German S3 guideline. However, 78.8% of the patients had already tried at least one of the drugs recommended in the guideline in the past and had discontinued it due to side effects or lack of efficacy. Amitriptyline (37.7% of all

Table 2: Treatment regimens for each category of medication.

	On demand (%)	Fixed daily regime (%)
NSAID	97.0	3.0
Metamizole	82.4	17.6
Amitriptyline	5.3	94.7
SNRI	0.0	100.0
Weak opioid	55.6	44.4
Pregabalin	0.0	100.0
Strong opioid	14.0	86.0
COX-2 inhibitor	71.4	28.6
Muscle relaxant	28.6	71.4
Acetaminophen	100.0	0.0
Cannabinoid	25.0	75.0
Guaifenesin	0.0	100.0
Triptans	100.0	0.0
Flupirtine	100.0	0.0
SSRI	0.0	100.0
Corticosteroid	100.0	0.0
Lidocaine	0.0	100.0
Magnesium	0.0	100.0
All	60.3	39.4

Table 3: Effect of the medication on pain relief.

	Percentage of patient replies indicating pain reduction by x points on the NRS with a given drug (retrospective evaluation).									N
	0	1	2	3	4	5	6	8	Pain reduction in NRS (median, range)	
NSAID	6.2	18.5	38.5	16.9	16.9	3.1	0.0	0.0	2.3 (2, 0–5)	64
Metamizole	12.1	15.2	51.5	15.2	0.0	3.0	0.0	3.0	2.0 (2, 0–8)	33
Amitriptyline	45.0	15.0	15.0	20.0	5.0	0.0	0.0	0.0	1.3 (1, 0–4)	20
SNRI	38.9	33.3	16.7	11.1	0.0	0.0	0.0	0.0	1.0 (1, 0–3)	18
Drugs taken by < 15 patients										
Weak opioid	0.0	22.2	44.4	0.0	22.4	11.0	0.0	0.0	2.6 (2, 1–5)	9
Pregabalin	12.5	0.0	50.0	37.5	0.0	0.0	0.0	0.0	2.1 (2, 0–3)	8
Strong opioid	0.0	14.3	0.0	71.4	0.0	14.3	0.0	0.0	3.0 (3, 1–5)	7
COX-2 inhibitor	0.0	14.3	28.6	42.9	14.3	0.0	0.0	0.0	2.6 (3, 1–4)	7
Muscle relaxant	33.3	16.7	33.3	0.0	16.7	0.0	0.0	0.0	1.5 (2, 0–4)	6
Acetaminophen	16.7	16.7	33.3	16.7	16.7	0.0	0.0	0.0	2.0 (2, 0–4)	6
Cannabinoid	0.0	0.0	25.0	25.0	25.0	0.0	25.0	0.0	3.7 (4, 2–6)	4
Guaifenesin	33.3	0.0	0.0	0.0	33.3	0.0	33.3	0.0	3.3 (4, 0–6)	3
Flupirtine	0.0	33.3	33.3	0.0	33.3	0.0	0.0	0.0	2.3 (2, 1–4)	3
SSRI	0.0	50.0	0.0	50.0	0.0	0.0	0.0	0.0	2.0 (2, 1–3)	2
Triptans[1]	0.0	0.0	0.0	50.0	50.0	0.0	0.0	0.0	3.5 (3.5, 3-4)	2
Corticosteroid	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	4.0 (4)	1
Lidocaine	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	4.0 (4)	1
All									2.1 (2, 0–8)	195

N, the number of patients' replies when asked about a given drug. [1]Used in migraine attacks.

Table 4: Effect of the medication categories (current treatment) on pain relief in NRS-points, in the subgroups with and without reduction of skin innervation.

	Reduced IENFD		Normal IENFD		P	All	
	N	Response (median, range)	N	Response (median, range)		N	Response (median, range)
NSAID	16	2, 0–5	21	2, 0–4	0.33	65	2, 0–5
Metamizole	9	2, 0–3	5	2, 1–3	0.36	33	2, 0–8
Amitriptyline	5	0, 0–2	6	1, 0–4	0.24	20	1, 0–4
SNRI	8	1, 0–2	3	0, 0	0.13	18	1, 0–3

N, the number of patients replies when asked about a given drug; IENFD, normal and reduced intraepidermal nerve density.

patients) was the most frequently discontinued drug in the past, followed by NSAIDs (35.1%) and pregabalin (19.2%). Current therapy was the first medical treatment attempt for only 9.1% of the patients. Most often, previous drugs had been discontinued due to lack of effect (63.7% of all prescribed drugs). The median duration of the current drug therapy up to study enrollment was 3 years (range from 1 month–30 years).

3.2. Pain Relief by Type of Medication. The patients were asked to rate the pain reduction by the individual drugs on an NRS of 0–10. We analyzed all drug classes taken by $n > 15$ patients. These were NSAIDs with a median pain reduction of 2 points (range 0–5), SNRIs with a median of 1 point (range 0–3), amitriptyline with 1 point (range 0–4), and metamizole with 2 points (range 0–8) (Table 3).

3.3. Pain Relief in Patient Subgroups. We compared the groups with prominent small fiber pathology (reduction of IENFD in distal and proximal biopsy, $n = 36$) and with entirely normal skin innervation ($n = 42$). In the overall response and also analyzing the frequently taken drugs NSAIDs or metamizole, we did not find intergroup differences in treatment response (Table 4).

3.4. Reasons for Discontinuing Previous Medication. 33.7% of patients had already used other drugs before their current therapy, 25.5% two drugs, and 29.1% three drugs. Lack of efficacy was the most frequently mentioned reason for discontinuing past treatment with opioids, NSAIDs, SSRIs, flupirtine, and acetaminophen. Intolerable side effects were the most frequently mentioned reason to discontinue SNRI, amitriptyline, and pregabalin (Table 5).

3.5. Correlations between Medication and Clinical Symptoms. We hypothesized that the choice of drug might be guided by symptom, severity, and phenotype. For example, patients with more severe pain might more often be prescribed opioids, and patients with a more "neuropathic" phenotype might more often receive antineuropathic drugs. This was not the case.

We found several correlations between the intake of distinct drugs and clinical parameters (Table 6). Intake of SNRIs ($r = -0.25$) or guaifenesin ($r = -2.0$) was negatively

Table 5: Reasons for discontinuing medication given in % of treatment episodes.

	No effect (%)	Side effects (%)	No reason given (%)	*N*
Amitriptyline	42.3	57.7	8.8	57
NSAIDs	83.7	16.3	7.5	53
SNRI	42.4	57.6	0	33
Pregabalin	48.3	51.7	0	29
Weak opioids	74.1	25.9	0	27
Metamizole	57.1	28.6	14.3	14
Flupirtine	84.6	7.7	7.7	13
Acetaminophen	100.0	0.0	0	12
SSRI	81.8	18.2	0	11
Strong opioids	60.0	40.0	0	5
COX-2 inhibitors	100.0	0.0	0	2
Cyclobenzaprine	33.3	33.3	33.3	3
Corticosteroids	100.0	0.0	0	1
Lidocaine	100.0	0.0	0	1
All	60.1	34.1	5.8	261

N, the total number of treatments with the respective drug in the past.

Table 6: Correlations between the use of certain classes of medication and clinical symptoms and the IENFD in the lower leg.

Medication	Questionnaire (CC; p value)			
No medication	STAI (0.18; 0.02)			
Weak opioid	GCPS disability due to pain (−0.16; 0.03)			
Strong opioid	O' Leary (0.23; 0.005)			
NSAID	NPSI (0.17; 0.02)	GCPS grade (0.19; 0.01)	ADS (0.2; 0.01)	
SNRI	Pain Catastrophizing Scale (0.18; 0.02)	FIQ (0.016; 0.04)	O′ Leary (−0.2; 0.01)	IENFD lower leg (−0.25; 0.001)
Muscle relaxant	GCPS disability due to pain (−0.15; 0.04)		ADS (−0.1; 0.04)	
Guaifenesin	IENFD lower leg (−0.2; 0.01)			
Flupirtine	Paresthesia (0.2; 0.01)			

correlated with the IENFD in the distal leg. Interestingly, the use of strong opioids was associated with higher scores in the "O´Leary/Sant voiding and pain indices." To validate this correlation, we conducted a direct group comparison. In this direct comparison of the questionnaire results between patients taking opioids ($n = 12$) and those not taking any ($n = 146$), we found one difference, namely, higher scores ($p = 0.02$) in the "O´Leary/Sant voiding and pain indices," which asks about urinary problems. Since these correlation analyses had an exploratory purpose to enable us to test hypotheses from them later in large cohort studies, we did not apply the Bonferroni correction. These data should therefore be regarded as pilot results and warrant replication.

3.6. Dosage of FMS Analgesic Medication. Only 29.6% of FMS patients took recommended medication according to the German FMS guideline [8]. In the group of patients taking pregabalin, 25% used the recommended dosage of 150–450 mg/day, while 75% of patients used a lower dose (median 75 mg/d, range 25–500 mg/d). For amitriptyline, recommended doses between 10 mg/d and 50 mg/d were used by 84.2% patients, in 10.5% of cases, the dose was lower, and in 5.3% of cases, the dose was higher (median 25 mg/d, range 10–75 mg/d). Two patients took an SSRI such as fluoxetine (recommended dosage 20–40 mg/d) for an accompanying depressive disorder: one of these patients was underdosed (10 mg/d) and the other overdosed (50 mg/d). We did not detect any overdoses in our cohort for the frequently used drugs: metamizole (maximum recommended dose 4000 mg/d), COX-2 inhibitors such as etoricoxib (maximum recommended dose 120 mg/d) and acetaminophen (maximum recommended dose 4000 mg/d), and NSAIDs, such as ibuprofen (maximum recommended dose 2400 mg/d).

3.7. Medication due to Comorbidities. As shown in Table 7, 22.9% of the patients had no other comorbidities requiring drug treatment. The three most frequently treated comorbidities were thyroid dysfunction (16.7%), arterial hypertension (13.2%), and depression (7.6%). Table 7 shows the respective medication that was taken for each of these conditions. The most commonly taken drugs were l-thyroxine (14.8%), proton pump inhibitors (5.5%), and vitamin *D* (5.9%); drugs are listed in Table 7. Some drugs such as SSRIs that might also be used for the treatment of FMS symptoms, in these cases, were explicitly prescribed for other indications, e.g., depression.

Table 7: Concomitant medications and their indications.

Indication	Generic	*N*	%
None	None	52	22.9
Thyroid dysfunction	L-Thyroxin	35	14.9
	Iodine	3	1.4
Hypertension	Beta-blocker	12	5.3
	Angiotensin-converting enzyme (ACE) inhibitor	6	2.6
	Angiotensin II blocker	4	1.8
	Calcium channel blocker	5	2.2
Depressive symptoms	SSRI	11	4.8
	SNRI	3	1.4
	Herbal agent	2	0.8
Sleep disturbances	Tricyclic antidepressant	7	3.0
	Zopiclone	1	0.5
	SSRI	1	0.5
	Pregabalin	1	0.5
Stomach pain	Proton pump inhibitor	16	7.0
Vitamin substitution	Vitamin D	13	5.7
	Estrogen	3	1.4
Asthma	Beta II agonist	11	4.6
	Corticosteroid	7	3.1
Other pain disorder	NSAID	2	0.9
Anxiety symptoms	SSRI	1	0.5
Osteoporosis	Vitamin D	1	0.5
Others		32	14.1

N, the number of treatment regimens; %, percentage of the whole cohort.

4. Discussion

In this cross-sectional study of 156 patients with FMS, we found that NSAIDs and metamizole on demand were the most frequently used drugs. Drugs with proven efficacy in randomized controlled trials (RCTs) and with recommendations in national and international guidelines [8] were only used by 29.6% of the patients (amitriptyline 12.8%, pregabalin 5.1%, and duloxetine (SNRI) 11.5%). Other drugs with efficacy in RCTs such as milnacipran were not encountered in our cohort. Over the course of their disease, more patients had been using either amitriptyline (37.7%) or pregabalin (19.2%); however, these drugs had been discontinued due to lack of efficacy or side effects.

Among the few studies worldwide that have investigated the current use of drugs in FMS, one explicitly deals with opioids. A study from the United States of America (USA) examined the intake of opioids by FMS patients from 2011 to 2017 [23]. In 2011, 42% of FMS patients were taking opioids as pain medication, but in 2016, the rate had dropped to 27%, probably due to higher awareness towards the side effects and addictive potential of opioids. The second study was also based on the USA and investigated multimorbidity and polypharmacy in elderly FMS patients [24]. The authors described that the most frequently taken drugs were sleeping aids with 33.3%, SSRIs with 28.7%, and SNRIs with 21.0%. In this study, opioids accounted for 22.4% of all drugs.

Two population-based studies focused on the choice of drug against FMS symptoms [25, 26]. Both studies examined cohorts in the USA, one of which showed that less than 20% of the drug therapies were retained for more than a year [26]. More than 50% of the patients in this study took opioids. At treatment initiation, the average daily dose of pregabalin was 75 mg/d, and in 52% of patients treated with pregabalin, this dose was not increased. Of these 52%, 78% discontinued pregabalin within 3 months. This shows some similarities with our data, since we also see a relatively low dosing of pregabalin. One explanation for the retention of pregabalin at higher doses may be that higher doses are more effective and thus lead to a longer duration of treatment. The second study examined the factors influencing the prescription of drugs in FMS patients with a focus on duloxetine and found that, among other factors, prior intake of pregabalin made the prescription of duloxetine more likely [25].

Our cohort is smaller compared to the previously mentioned studies, but similar in patients' characteristics. Here, as well, the average age is approximately 50 years, and on average, about 80% of the patients are women. However, the number of other pain disorders was lower in our cohort compared to others [25]. This may be due to our relatively strict exclusion criteria [14]. Our patients were extensively examined rheumatologically and neurologically for other possible causes of pain until the diagnosis of FMS was made. Furthermore, the proportion of opioids was 7.7%, which is lower than in the US-American cohorts with up to 50%. The reason may be a higher sensitivity to opioid related problems, stricter prescription rules [27, 28], and adherence to guidelines [29]. In contrast to the abovementioned studies, however, our patients were all volunteers in a prospective study, so our patient population may be less severely affected than those studied in pain clinics or population studies,

more aware of nondrug therapies, and motivated for treatment.

The reason for the low number of patients taking the drugs according to the guidelines (29.6%) remains unclear. One obvious reason may be that duloxetine and pregabalin are off-label for FMS in Germany. Other reasons may be lack of information in the group of the treating physicians or that physicians decided to discontinue an ineffective drug therapy after consulting the guidelines, which recommend initial nondrug therapy. Another reason may be a lack of adherence by patients. Often the term "antidepressants" is misunderstood and patients feel stigmatized by taking such a drug. Many patients also report side effects, such as weight gain or fatigue, which can lead to severe loss of quality of life. We show in our results that this varies greatly depending on the medication taken. This may lead to patients preferring complementary medicine to classical medicine. Since there are no drugs specifically approved for FMS in Europe, a standardized therapy is more difficult. Well-planned RCTs or register studies might lead to additional safety and possibly to the licensing of helpful drugs in Europe.

Although the German guidelines explicitly do not recommend the use of opioids in FMS [8], 7.7% of the patients were taking them. Our correlation analysis found an increased number of problems during urination in these patients. This might be explained by an opioid side effect on the detrusor muscle [30, 31]. Our hypothesis that there are differences in the intake and efficacy of the drugs between the subgroups with "neuropathic" and "nonneuropathic" pain, as evaluated by the presence or absence of small fiber pathology, could not be confirmed; however, our subgroups were too small to exclude such an effect. The question should be investigated in a prospective study with a larger number of cases with the goal to provide more personalized therapy.

Our study has a number of limitations. For certain classes of drugs, the number of patients was low, so the conclusions in these cases are limited. This is a cross-sectional study; therefore, the recall of medication effects may be biased. We did not query the compliance of the patients, which could have been influential on the results. Patients were asked to distinguish between multiple medications individually; however, overlapping effects may have occurred. In addition, the different drugs were taken over different periods of time; we could not control this parameter with our data. Previously published small RCTs do not show a superiority of NSAIDs over the placebo effect [32]; however, the fixed regime in the RCTs cannot be compared with the on-demand application by our patients, and the impact of a placebo effect in our cohort is unclear. Furthermore, the question whether the correlations between the intake of certain drugs and patient reported symptoms reflect medication side effects or insufficiently treated FMS symptoms cannot be answered by our cross-sectional study.

5. Conclusions

In conclusion, FMS patients in Germany take many different medications for their pain, which are not officially recommended for the treatment of FMS. However, these lead to moderate therapeutic success. These substances, such as NSAIDs and metamizole, should be tested in randomized controlled clinical studies in FMS. To assess possible differences in therapeutic response between the subgroups with and without small nerve fiber pathology, studies with larger cohorts are needed. Physicians treating FMS patients should also pay attention to the recommended dose ranges with regard to the tolerability of the medication. Limitations of the study were the small number of patients in the subgroups, the cross-sectional design that did not allow for conclusions about placebo effects or overlapping effects with multiple medications, and a lack of control for patients' medication adherence.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

HA, DE, and AB declare no conflicts of interest. NÜ has received honoraria for presentations from Sanofi Genzyme, Takeda, and Astellas; NÜ has received travel grants from Pfizer, Sanofi Genzyme, Takeda, Astellas; NÜ has received research support from Sanofi Genzyme, Takeda, and Idorsia. CS has received fees for consulting related to the treatment of neuropathic pain from the companies Algiax, Air Liquide, and Bayer.

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Supplementary Materials

Supplementary Table 1: clinical characteristics of the cohort and proportion of FMS comorbidities. Supplementary Table 2: clinical and questionnaire data compared between subgroups according to IENFD. (*Supplementary Materials*)

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4.3 Comparison of gamma-aminobutyric acid, glutamate, and N-acetylaspartate concentrations in the insular cortex between patients with fibromyalgia, rheumatoid arthritis, and healthy controls - a magnetic resonance spectroscopy study - MedRxiv (2022)

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Comparison of gamma-aminobutyric acid, glutamate, and N-acetylaspartate concentrations in the insular cortex between patients with fibromyalgia, rheumatoid arthritis, and healthy controls - a magnetic resonance spectroscopy study

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Abstract

Fibromyalgia syndrome (FMS) is a chronic pain disorder with hypersensitivity to painful stimuli. A subgroup of patients shows damage to small peripheral nerve fibers. Previous studies support the hypothesis that increased activation of the pain-processing insular cortex is mediated by an imbalance of insular glutamate and γ-aminobutyric acid (GABA) concentrations. Here, we aimed to test this hypothesis in a large cohort of FMS patients comparing data of patients and healthy controls. In addition, we tested the hypothesis whether a reduction in small peripheral nerve fibers relates to glutamate concentrations in the insular cortex. We recruited 102 subjects (all female, 44 FMS patients, 40 healthy age-matched controls, and 19 patients with rheumatoid arthritis (RA) as disease controls. Study participants underwent single-voxel magnetic resonance spectroscopy of the right and left insular cortex. All patients completed questionnaires on symptom severity (pain intensity, impairment due to symptoms, depression). FMS patients were further stratified into subgroups with and without reduced intraepidermal nerve fiber density (IENFD) assessed on skin punch biopsies. We found no intergroup difference of the glutamate/GABA metabolite concentrations between FMS and RA patients and healthy controls. Glutamate/GABA levels did not correlate with symptom severity. Cerebral glutamate concentrations were independent of skin innervation. We found similar insular glutamate/GABA concentrations in FMS patients and disease and healthy controls. Therefore, our data cannot support the hypothesis that a glutamate/GABA mismatch leads to a sensitization of the insular cortex of fibromyalgia patients and thereby induces the symptoms.

Introduction

Patients with fibromyalgia syndrome (FMS) suffer from widespread pain and comorbid symptoms like depression and anxiety [1]. FMS prevalence ranges from 2-4%, with more women affected [2]. The pathophysiology of FMS is not fully understood. Currently, a multifactorial origin including alterations in the peripheral [3, 4], autonomic [5], and central nervous systems (CNS) [6] is assumed. Genetic [7], hormonal [8], and psychosocial factors such as childhood trauma may facilitate the development of FMS [9]. These findings suggest that there are FMS patient subgroups with potentially distinct pathophysiological mechanisms at play, which may also be reflected by diversity in symptom manifestation [4, 10].

One of the most substantiated hypothesis on the pathophysiology of FMS is that of central sensitization. Central sensitization is defined as an amplification of neural signaling within the CNS that leads to pain hypersensitivity. However, it is unclear whether central sensitization happens primarily ("top-down") or is a secondary consequence of peripheral nociceptive input ("bottom-up") [1]. A primary central sensitization, which might lead to reduced descending pain inhibition, is supported by findings in the serotonergic-adrenergic [11] and opioid systems [12] in FMS patients. The excitatory neurotransmitter glutamate was investigated in context with central sensitization. Indicators are higher glutamate levels in cerebrospinal fluid of FMS patients compared to healthy controls or rheumatoid arthritis patients [11] and in MRI spectroscopy findings in FMS patients compared to healthy controls, especially of the insular cortex [13]. The clinical efficacy of memantine, an NMDA receptor antagonist, in alleviating pain [14], also points to a potentially important role of glutamate in the pathophysiology of FMS. Indicators of peripherally triggered sensitization in

FMS are, for example, findings from microneurography, showing spontaneous activity and sensitization in C-fibers [15].

In the peripheral nervous system, damage to small nerve fibers has been shown in a clinically more severely affected subgroup of FMS patients [4, 16]. In an experimental animal study in rats, elevated glutamate levels in the insula led to a decrease in intraepidermal nerve fiber density (IENFD) [17].

The insular cortex is involved in different dimensions of pain [18, 19]. It is assumed to play an important role in the affective, cognitive, and sensory-discriminative aspect of chronic pain [20-22]. Previous neuroimaging studies discovered stronger insular connectivity to other pain processing brain areas through fMRI studies and higher glutamate levels in chronic pain disorders through magnetic resonance spectroscopy [23, 24]. As the anterior and posterior insular cortex have different functional connectomes to cortical and subcortical regions, both insular regions are assumed to perform different tasks in the processing of pain signals [25].

The anterior insular cortex is mainly involved in affective processing of pain stimuli and interospective awareness [26, 27]. In a magnetic resonance spectroscopy (MRS) study, low GABA levels were found in the anterior insular cortex of FMS patients compared to healthy controls. Electro acupuncture treatment of FMS patients was associated with an increased concentration of these GABA levels in the anterior insular cortex, which was associated with the subjective report of patients` pain intensity [28].

The posterior insular cortex is considered a potential "gateway" to the somatosensory network. Focal epilepsies in this area may trigger pain sensations [29]. A MRS study showed

higher glutamate levels in the right posterior insular cortex of FMS patients compared to healthy controls [13]. Lower GABA levels in the posterior insular cortex correlated with lower pressure-pain thresholds [30].

Most MRS studies investigated small groups of patients or did not compare findings with healthy and/or disease controls. Since CNS alterations reported in FMS were not specific, such comparisons are crucial. This is particularly true when assessing relative levels of neurotransmitters. Hence, in order to enable a direct comparison, this study recruited a large cohort of FMS patients, RA disease controls and healthy controls. We aimed to test the hypothesis that FMS patients have higher glutamate or lower GABA concentrations in the insular cortex, respectively. We further aimed to test the hypothesis that this mismatch is related to symptom severity. In an exploratory analysis, we further tested the hypothesis that higher glutamate levels are associated with a lower skin innervation in FMS patients.

Materials and Methods

Study design

In this case-control study, we recruited three groups of study participants: 1. A patient group with a diagnosis of FMS. 2. A patient group matched for age, sex and BMI with a diagnosis of RA as a disease control group. 3. A healthy control group matched for age, sex, and BMI. All groups received a single MRS examination. The patient groups (1, 2) also filled in clinical questionnaires.

Participants

Patients were eligible if they were female, with an age range from 40 to 65 years, and a body-mass-index (BMI) between 20 and 35. Patients diagnosed with FMS met the diagnostic criteria according to the 2010 guidelines by the American College of Rheumatology [31]. MRI and clinical data of the FMS patients have already been published [4, 10]. RA patients (disease controls) were diagnosed according to the American College of Rheumatology/European League against Rheumatism (ACR/EULAR) classification criteria and had a disease duration of at least 5 years [32] with a respectively long chronic pain history. To assure ongoing disease activity, we only included RA patients with a disease activity score (DAS28) [33] $\geq$ 2.6 (exclusion of RA patients in remission) and clinically significant pain (minimum pain intensity on a numeric rating scale $\geq$ 4). No RA patient fulfilled the diagnostic criteria for FMS. The reason for including RA patients as disease controls was their persistent pain over several years, but with a well-defined inflammatory cause. The control group consisted of healthy women, also matched by age and BMI.

Patients and controls were excluded if they had any form of MRI incompatibility, other severe pain syndromes, other inflammatory diseases, any other neurological, psychiatric or

severe cardiovascular diseases, history of drug abuse, head trauma requiring medical attention, or any structural anomaly of the brain. Written informed consent was obtained from all patients and controls. The ethics committee of the University of Würzburg University provided approval of the study (105/19).

Clinical evaluation

In all patients, we evaluated pain intensity and pain-related disability through the Graded chronic pain scale (GCPS) [34] and used the German version of the center for epidemiological studies – Depression scale questionnaire ("Allgemeine Depressionsskala" (ADS)) [35] to record depressive symptoms. We further applied the "Pain Catastrophizing scale" [36]. We evaluated daily life impairment due to pain in all patients with the Fibromyalgia Impact Questionnaire (FIQ) [37]. All questionnaires are self-report measures. Disease activity of RA patients was assed using the DAS28, which includes the examination of swelling and pressure pain of 28 joints and the one hour erythrocyte sedimentation rate (ESR) [33].

Evaluation of intraepidermal small fiber density

As described and published previously [4], FMS patients were evaluated for IENFD by skin punch biopsy at two sites (lower and upper leg). Based on the results, patients were divided into subgroups [10] with normal and reduced IENFD.

MRS data acquisition

Magnetic resonance scanning was conducted on a 3T MR Scanner (Prisma, Siemens, Germany) with a 64-channel head coil at the Institute of Neuroradiology, University Hospital

of Würzburg. We used a "MPRAGE" sequence to acquire T1-weighted anatomical images for spectroscopic voxel placement (repetition time TR = 2400ms, echo time (TE) = 3.17ms, flip angle (FA) = 8° inversion recovery (IR) = 1000ms, 176 sagittal slices, isotropic voxel size = 1 x 1 x 1 mm). MR-Spectroscopy is a non-invasive *in vivo* method to measure metabolites in the brain. GABA has proven to be difficult to detect due to its spectral peaks partially overlaping with peaks of other major metabolites especially creatinine (Cr), which is present in much greater concentrations. However, the use of editing sequences like MEshcher-GArwood Point-RESolved Single-voxel Spectroscopy (MEGA-PRESS) increased the reliability and is commonly used for GABA acquisition [38-42]. Therefore, a MEGA-PRESS sequence was used to detect GABA, glutamate and N-acetyl-aspartate (NAA) in the regions of interest (ROIs), with the following scanning parameters: TR = 2500 ms, TE = 68 ms, acquisition bandwidth = 1500 Hz, editing pulses frequency applied (off) at 1,9 ppm and (off) at 7.5 ppm, editing pulse bandwidth = 52 Hz. The 30 x 15 x 20 mm voxel was placed manually successively in the right and left IC. The voxel size was chosen as compromise between localization and signal quality to compensate the low signal to noise ratio (SNR) for GABA (0.7 to 1.4 mM/cm^3) [39, 43]. To compromise between SNR (thus reliable results) and anatomical specificity, the entire insular cortex was used as voxel content rather than the subdivision into anterior and posterior. Before the measurement, the magnetic field was automatically shimmed in 3 rounds on the respective voxel. Only measurements with magnetic field inhomogeneity <30 Hz average linewidth were accepted for the defined ROI. An unsuppressed water measurement was used for frequency and phase correction and as reference to tissue water.

Spectroscopy analysis

Preprocessing and fitting of the MR spectra was performed using the Totally Automatic Robust QUantitation in Nuclear MR (TARQUIN) software. The metabolite concentrations of GABA, Glutamate and NAA were analyzed. Glutamate concentrations were obtained from the off spectra. GABA and NAA concentrations were obtained from the difference spectrum (on – off).

Statistical Analysis

Statistics were performed with JASP Team (2022). JASP (Version 0.16.2) [Computer software]. The data were tested for normal distribution with a Shapiro-Wilk test. We used the Levene test with a significance threshold of 0.05 % to check the data for equivalence of variance. To compare the groups to each other regarding the concentration of the metabolites and the clinical parameters, we calculated ANOVAs. Due to the different group sizes, a Welch correction was performed and the following post-hoc comparisons were corrected for multiple comparisons using the Games-Howell method. Depending on the condition of normal distribution, correlation analyses were calculated using Pearson's or Spearman's correlation. Due to higher standard deviations, we calculated subgroup comparisons using the Mann-Whitney-U Test. The confidence interval was 95 %, the significance level at 0.05 %.

Results

Cohort characteristics

The final sample size consisted of 102 participants (all female), who were divided into three groups: FMS group (n = 44; subgroup with reduced IENFD (PNS) n = 21; subgroup with normal IENFD (noPNS) n = 23), RA group (n = 19), and healthy controls (n = 40). FMS patients showed higher scores than RA patients in the self-report questionnaires on pain intensity, impairment in daily life, depression and pain catastrophizing. The group differences in clinical symptoms and structural and functional imaging between the FMS group and healthy controls have already been published [10]. All baseline characteristics can be found in Table 1.

Cohort characteristics (Fibromyalgia (FMS), Rheumatoid arthritis (RA), Controls (HC))						
	Gruppe	**Mean/Median***	**Standard deviation/Range***	**p-value**	**Effect size (ω^2)**	**Post hoc Tests (Games-Howell p-value)**
Age	FMS	51*	26*	<0.05	0.005	FMS-RA: 0.05
	RA	58*	23*			FMS-HC: 0.7
	HC	52*	23*			RA-HC: <0.05
BMI	FMS	28.2	5.0	<0.05	0.058	FMS-RA: 0.5
	RA	26.8	4.6			FMS-HC: 0.3
	HC	26.6	5.0			RA-HC: 1

Pain characteristics (Fibromyalgia (FMS), Rheumatoid arthritis (RA))					
	Group	**Mean/Median**	**Standard deviation/Range**	**p-value**	**Effect size (Cohens d)**
PCS	FMS	25*	39*	**<0.05**	0.412
	RA	11*	52*		
ADS	FMS	23*	42*	**<0.01**	0.438
	RA	11*	52*		
FIQ	FMS	46.6	13.5	**<0.01**	0.786
	RA	34.7	18.5		
GCPS Disability	FMS	4*	6*	**<0.001**	1.027
	RA	2*	6*	**<0.001**	0.541
GCPS pain intensity	FMS	71.7*	63.3*	**<0.001**	0.536
	RA	53.3*	50.0	**<0.001**	0.536

Table 1: Group characteristics. BMI: Body Mass Index, FMS: Fibromyalgia-syndrome, RA: Rheumatoid arthritis, PCS: Pain catastrophizing scale (sum score), ADS: Allgemeine Depressionsskala, FIQ: Fibromyalgia Impact Questionnaire, GCPS: Graded chronic pain scale. *This variable was not normally distributed. The data are therefore characterized as median/range.

Group differences in glutamate, GABA, and NAA

Glutamate and GABA concentrations did not differ in the insular cortex between the FMS and the control groups. Only the concentration of NAA in the insular cortex of RA patients was lower compared to the FMS group (left hemisphere p-value: 0.08, right hemisphere p-value: 0.003) and the control group (left hemisphere p-value: 0.02, right hemisphere p-value: 0.02) (Figure 1, Table 2).

Group comparisons of metabolite concentrations						
Metabolite	**Group**	**Mean/Median**	**Standard deviation/Range**	**p-value Levene test**	**p-value (Welch corrected)**	**Effect size ω^2**
Right GABA	FMS	6.3	1.2	0.229	0.353	0.000
	RA	5.8	1.2			
	Controls	6.1	1.6			
Right NAA	FMS	15.5	1.5	0.681	**0.003**	0.100
	RA	14.0	1.5			
	Controls	15.1	1.4			
Right Glutamate	FMS	3.4	0.9	0.592	0.107	0.021
	RA	3.1	0.8			
	Controls	3.5	0.7			
Left GABA	FMS	5.8	1.3	0.019	0.729	0.000
	RA	5.6	1.4			
	Controls	5.4	2.4			
Left NAA	FMS	13.5*	12.9*	0.478	**0.025**	0.056
	RA	12.2*	6.4*			
	Controls	13.6*	8.4*			
Left Glutamate	FMS	2.7	1.2	0.128	0.645	0.000
	RA	2.8	1.0			
	Controls	2.6	0.9			

Post-hoc comparisons

Metabolite	Group comparison	Mean Difference	Standard error	t-value	p-value (Games Howell corrected)
Right NAA	Controls - FMS	-0.32	0.32	-1.01	0.57
	FMS - RA	1.46	0.4	3.61	**0.003**
	Controls - RA	1.14	0.4	2.81	**0.02**
Left NAA	Controls - FMS	0.26	0.41	0.63	0.79
	FMS - RA	1.24	0.55	2.23	0.07
	Controls - RA	1.5	0.53	2.83	**0.02**

Table 2: Group comparisons of metabolite concentrations in the left and right insular cortex. NAA: N-acetylasparate, GABA (gamma-amino-butyric acid), FMS: Fibromyalgia syndrome, RA: Rheumatoid arthritis. *This variable was not normally distributed. The data are therefore characterized as median/range.

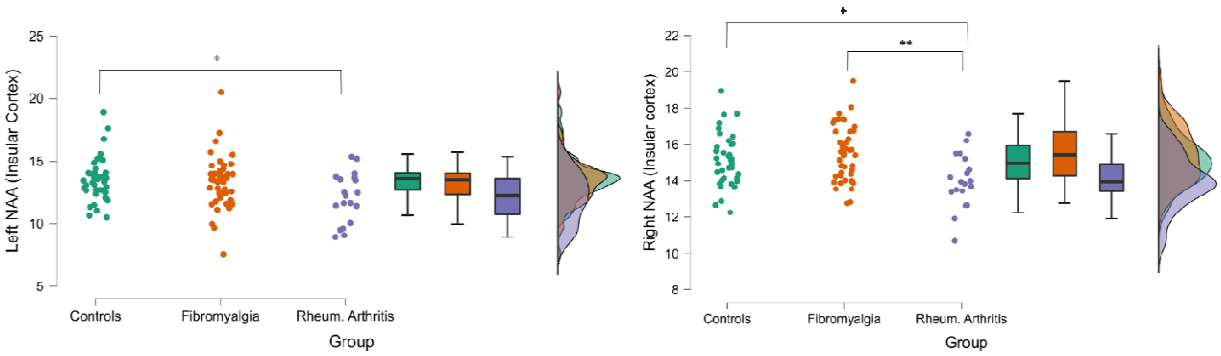

Figure 1: Lower NAA concentration of the insular cortex of RA patients compared with controls (left and right hemisphere) and with fibromyalgia patients (right hemisphere).

Subgroup comparisons

Subgroups with normal IENFD (noPNS; n=23) and reduced IENFD (PNS; n=21) did not differ in metabolite concentrations (Table 3).

Subgroup metabolite concentrations					
Metabolite	**Subgroup**	**Median**	**Range**	**p-value**	**Cohen's d**
Right GABA	noPNS	8.6	3.2	0.38	0.16
	PNS	6.0	4.5		
Right NAA	noPNS	15.6	5.3	0.9	0.01
	PNS	15.1	5.7		
Right Glutamate	noPNS	3.6	4.2	0.78	0.05
	PNS	3.6	3.2		
Left GABA	noPNS	5.7	4.0	0.59	0.09
	PNS	5.7	4.9		
Left NAA	noPNS	13.6	12.9	0.85	-0.03
	PNS	13.3	6.0		
Left Glutamate	noPNS	2.4	4.9	0.59	-0.09
	PNS	3.0	4.7		

Table 3: Comparison of metabolite concentrations in the subgroups PNS (reduced IENFD) and noPNS (normal IENFD).

Associations of clinical symptom severity and cerebral levels of glutamate and GABA

We found no evidence that an increased insular glutamate concentration or decreased GABA concentration in FMS patients are related to more severe clinical symptoms. We found no

correlations in the FMS group nor in the patient group (RA + FMS patients combined). More detailed statistics on "metabolite ~ clinical parameter" associations in FMS patients can be found in Table 4.

Clinical parameter		Right Glutamate	Right GABA	Left Glutamate	Left GABA
PCS	Spearman's rho	-0.089	0.054	-0.114	-0.003
	p-value	0.572	0.732	0.465	0.984
ADS	Spearman's rho	0.148	-0.125	0.164	-0.110
	p-value	0.338	0.418	0.288	0.476
FIQ	Spearman's rho	0.040	-0.178	0.224	0.002
	p-value	0.795	0.247	0.144	0.992
GCPS Pain intensity	Spearman's rho	-0.262	0.077	-0.078	0.040
	p-value	0.086	0.621	0.614	0.795
GCPS Disability due to pain	Spearman's rho	-0.049	-0.065	-0.074	0.053
	p-value	0.754	0.674	0.635	0.731

Table 4: Associations with metabolite concentrations and the severity of clinical parameters of FMS Patients. PCS: Pain catastrophizing scale (sum score), ADS: Allgemeine Depressionsskala, FIQ: Fibromyalgia Impact Questionnaire, GCPS: Graded chronic pain scale.

Discussion

In this study including 102 participants, we found no evidence for higher glutamate or lower GABA levels in the insular cortex of FMS patients compared with healthy and disease controls. We also found no evidence that higher levels of glutamate correlated with lower IENFD. The levels of glutamate and GABA in the insular cortex did not correlate with the severity of classic symptoms in FMS patients in our analysis.

The utility of non-invasive MRS as a method to better explore the pathophysiology of chronic pain disorders has increased [44, 45]. In addition to measuring glutamate, there are also some studies that have found decreased levels of NAA in various brain regions of FMS patients, such as the dorsolateral prefrontal cortex [46] and the hippocampus [47]. Higher concentrations of Glx (glutamine + glutamate) were also found in other regions such as the posterior gyrus of 10 FMS patients compared to 10 healthy controls [48]. Regarding the target region of the present study, the insular cortex, the body of evidence for a static change in glutamate/GABA changes is relatively small. An MRS study of 19 FMS patients and 14 controls found an increase in glutamate in the right posterior insular cortex in FMS, which correlated with decreased pain pressure thresholds [13]. A few years later, in a study of 16 FMS patients and 17 controls, the same research group published decreased GABA concentrations in the anterior right insular cortex but not in the posterior. However, in the posterior insular cortex of FMS patients, GABA levels still correlated with the pressure pain threshold [30]. A PET study suggested that the increased $GABA_a$ receptor density in different brain regions in FMS patients might be a compensation for the decreased GABA concentrations [49]. In recent years, the focus has shifted from static differences in

glutamate/GABA concentrations to dynamic concentrations after interventions. A combined MRS and fMRI study with 67 FMS patients but no controls showed that electrical acupuncture could elicit pain relief in FMS patients. This pain relief correlated with increased connectivity of the anterior insular cortex and a part of the somatosensory cortex. The connectivity strength in turn correlated with an increase in GABA in the anterior insular cortex [28]. A similar study with 17 FMS patients, also without controls, showed that the administration of pregabalin alleviated FMS pain. This pain relief correlated with a reduced connectivity of the anterior insular cortex with the default mode network. This reduced connectivity, in turn, correlated with a decreased Glx concentration in the posterior insular cortex [50]. Thus, there is some evidence that suggests a mismatch of glutamate/GABA in the insular cortex of FMS patients. The glutamate/GABA mismatch seems to correlate with the severity of clinical symptoms, however, the sample size was small.

Our finding that RA patients show decreased levels of NAA, a marker of neuronal integrity [51], has already been supported in the literature regarding RA and other rheumatic diseases [52]. The most consistent conjecture on the background of decreased NAA concentrations in the magnetic resonance spectroscopy literature on pain is that of neuronal damage. Decreased NAA levels also appear to be related to increased inflammatory markers. [53]. Therefore, the normal NAA concentrations in our study in FMS patients may indicate intact neuronal integrity of the insular cortex. Only in one brain region, the hippocampus, a meta-analysis found decreased NAA levels FMS patients. [47].

There are several potential reasons why our study did not report group differences, which is not in line with previous findings on a glutamate/GABA mismatch of the insular cortex in FMS patients: Most of the previous studies had subdivided the insular cortex into the anterior and posterior insular cortex as the regions of interest (ROI) [13, 30, 50]. Splitting the

insular cortex in two subregions is reasonable, as there is evidence from fMRI studies that the anterior and posterior insular cortex perform different functions in pain processing [22]. However, in planning the study design, we had opted to measure the entire insular cortex for several reasons. One reason was that previous literature has been incongruent as to whether the glutamate/GABA mismatch is present in the anterior or posterior insular cortex [13, 30]. Another reason for measuring the entire insular cortex was that MEGA-PRESS sequences have a particularly unfavorable signal to noise ratios [43].

In a recent MRS study increased GABA concentrations were found in FMS patients in the anterior insula after electronic acupuncture [28]. Administration of pregabalin decreased glutamate concentrations in the posterior insula of 17 FMS patients [50]. Decreased connectivity with the default mode network then correlated with decreased expression of FMS symptoms. However, because our study used a static setting to measure metabolite concentrations and the aforementioned dynamic studies did not include a comparison with healthy controls in their study design, no comparisons can be made here.

Finally, MEGA-PRESS sequence are susceptible to confounding and therefore do not only depend on physical prerequisites, such as the correct shimming for homogenization of the magnetic field lines and the voxel size, but also on the analysis method [43]. It has been shown that the standard softwares in MRS analysis differ in their technique of fitting the spectra, resulting in different quantification results of the MEGA-PRESS metabolite concentrations [54].

Our study has several limitations. Because of the measurement of the entire insular cortex, a subdivision into anterior and posterior insular cortex and a direct comparability with previous studies is only possible to a limited extent. Furthermore, due to the overlapping

spectra of glutamine and glutamate, it is currently technically impossible to completely differentiate these two metabolites in MRS. Different analysis software may do the fitting of these two metabolite spectra to different extents. Another issue that may generate possible variations in metabolite concentrations is the subjective ROI placement on the insular cortex directly at the scanner. Because the anatomy of the insular cortex is individual, the ROI was placed directly at the scanner to allow the highest possible proportion of gray matter in the ROI. In addition, many cell types are also present in the gray matter besides neurons. Since glutamate is also a product of metabolism in, for example, glial cells, and not only a neurotransmitter of the synapses, this study describes metabolites rather than neurotransmitters concentrations. A final limitation is that measurements were performed at only one time point. A longitudinal study design could account for possible individual variations in metabolite concentrations in the future.

Conclusion

Our study found no differences of glutamate/GABA concentrations between FMS and RA patients, nor healthy controls. Our data therefore does not substantiate the glutamate/GABA hypothesis of the insular cortex in the pathophysiology of FMS, which has been previously described in the literature. For the future, it would be beneficial to find uniform measurement methods for the quantification of the metabolites glutamate/GABA in order to compare them more directly. In addition, it would be important to conduct further longitudinal studies that could detect a potential causal effect on the glutamate/GABA metabolite concentrations with the help of an intervention.

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Conflicts of interest

HCA, VH, GH, TK, NÜ, MP, MS and CS declare no conflict of interests.

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5 Summarising discussion

5.1 Objective of the PhD thesis

In the studies summarized in this dissertation, an attempt was made to examine subgroups of the FMS, which were defined on the basis of their IENFD in skin biopsies, for differences using a wide variety of techniques. Several MRI techniques such as structural and functional imaging, diffusion imaging and magnetic resonance spectroscopy were used as imaging techniques. The data were analyzed using the standard analysis tools. Furthermore, data on drug intake and efficacy in FMS patients were collected in a clinical study. The aims of the studies were to determine whether differences in PNS morphology are relate to changes in CNS function and structure. Furthermore, we aimed to determine whether subgroups with different PNS morphology respond differently to different pain medications for the treatment of FMS in terms of decreasing their symptoms. All presented studies are cross-sectional. Therefore, the aim of the studies was not to causally investigate which nervous system might originally trigger the FMS symptoms and which part of the nervous system might possibly react with functional and structural changes as a consequence of the symptoms.

Our initial hypotheses at the beginning of the dissertation period were as follows:

1) The FMS subgroup without pathology in the PNS shows more pronounced functional and structural changes in the CNS, since the FMS symptomatology in this subgroup is not induced by damage to peripheral nerve pathways, but by central hypersensitivity.

2) Patients with FMS show increased glutamate and decreased GABA concentrations in the insular cortices compared to healthy controls and RA patients. These altered concentrations correlate with the severity of FMS symptoms. Higher glutamate levels also correlate with lower IENFD values (measure of PNS pathology).

3) FMS patients in the subgroup with pathology in the PNS, compared to patients in the subgroup without pathology in the PNS, show a greater reduction in pain symptoms with drugs that are approved for the treatment of neuropathic pain.

5.2 Summary of results

Regarding structural and functional MRI differences in the central nervous system, FMS patients in general showed decreased cortex volumes in 10 centrotemporoparietal regions

compared to healthy controls. Structural subgroup comparison showed a decrease in volume in the subgroup with PNS pathology in the bilateral pericalcarine cortices, a part of the primary visual cortex. Diffusion tensor imaging showed an increase in fractional anisotropy in 14 of 48 ROIs representing parts of the corticospinal pathways (for example the corona radiata, pons, thalamus and capsula interna) or parts of known pain processing regions (cerebellum and cingulate cortex) in FMS patients compared with healthy controls. Subgroup comparison showed a decreased fractional anisotropy of parts of the capsula interna, as well as the thalamic radiation, in the subgroup with pathology in the PNS. Functional connectivity imaging showed hypoconnectivity of FMS patients compared to healthy controls between the right midfrontal gyrus and parts of the cerebellum. In subgroup comparison, the group with PNS pathology showed a hyperconnectivity cluster between the left and right inferior frontal gyrus, the right angular gyrus and the posterior parietal cortex. Our hypothesis that FMS patients without PNS pathology show more pronounced functional and structural changes in the CNS could not be substantiated in publication #1. On the contrary, multimodal MRI analyses showed more significant deviations from the control group in the subgroup with PNS pathology. This was evident in the cortical morphology and functional connectivity.

In publication #2, we systematically surveyed the medication regimens of a larger cohort of FMS patients, as well as their discontinuation reasons and side effects. We were unable to confirm our original hypothesis that FMS patients with pathology of the PNS would respond better to medications directed at neuropathic pain. There were no differences in medication intake, as well as efficacy, between the two subgroups. With respect to the overall cohort, it was found that most patients were treated with non-steroidal anti-inflammatory drugs or metamizole as an on-demand medication. These two medications also produced the most effective pain relief according to self-report. None of these drugs is approved for the treatment of FMS or recommended for treatment according to the German guideline. Our analysis also showed that some patients with FMS are treated with opiates, which is contraindicated according to German guidelines.

The aim of publication #3 was to test the long-standing hypothesis that FMS symptoms are associated with increased glutmatate or decreased GABA levels in the insular cortex in a larger sample. We also wanted to test whether an association between peripheral nerve fiber pathology and insular cortex glutamate levels published in an animal study with rats could be reproduced in humans. Both hypotheses could not be confirmed in a cohort consisting of a

total of 102 subjects (FMS, controls, rheumatoid arthritis). Concerning glutamate and GABA concentrations, we found no group differences and no correlations with symptom expression or peripheral nerve fiber density.

5.3 Interpretation of the data and comparison with existing literature

An important difference of our publication #1 from other neuroimaging studies in FMS was our exploratory "whole-brain approach." Whereas most studies using functional or structural imaging have used already known pain regions as pre-specified regions (in order to achieve more statistical power), we decided to use an exploratory approach without pre-specifying regions. This was due to the fact that a subgroup analysis of FMS patients with and without PNS pathologies has never been conducted before. Although this fact reduced our statistical power, it allowed us to discover involved regions that had not previously been part of the discussion. The lower cortex volumes of FMS patients in the temporoparietal cortex and insular cortex have been previously described in meta-analyses [79]. As the disease progresses, cortex thickness also appears to decrease in these regions, suggesting a secondary effect of the disease [80]. Our subgroup analysis showed a significant and bilateral decrease in the bilateral pericalcarine cortices in the subgroup with PNS pathology. This region has not been frequently described in the context of FMS, and when it has, it has been described in functional studies. In these, MEG and fMRI studies showed hypoconnectivity of the pericalcarine cortex with other brain regions, which was also partially associated with weaker resilience to pain.

The FMS literature is not congruent regarding FA values, which are supposed to measure the state of the neurons by the ability of the water to diffuse [81]. Other diffusion imaging studies with smaller case numbers have already published some of our results, for example in the right and left anterior cingulate cortex (ACC), the dorsal lateral prefrontal cortex (DLPFC) [82], the anterior thalamic radiation, and the anterior limb of the internal capsula up to the putamen [83]. However, there are also studies that postulate a decrease in FA in FMS, especially in the corpus callosum [84]. The study situation is ambiguous in this respect, although our study is the largest diffusion imaging study in FMS to date, which also has an impact on the statistical power.

To conjecture why in our study FA levels are so consistently higher in FMS patients compared to healthy controls, it is worthwhile to look into the physiological background. There is first evidence which physiological processes have an influence on FA. It is assumed that the

reduced diffusion in the axons, i.e. the increased FA, is caused by an increase in oligodendrocytes [81]. These are, among other cells, responsible for the production of myelin and microglia [85]. Oligodendrocytes also play a role in pain processing mechanisms by producing and influencing chemokines and cytokines and by that interacting with microglia cells. A study focusing on microglial cells in the CNS of FMS patients could show that human-induced microglia-like cells isolated from the blood of FMS patients produced more TNF alpha compared to healthy controls. The up-regulation of TNF alpha also correlated with pain intensity [86]. It is also assumed that not only TNF alpha, but also other pro-inflammatory cytokines such as Interleukin-6 or Substance P are excreted more in the CNS of FMS patients [87]. In order to measure microglia activity in FMS patients directly in the brain with spatial resolution, a special PET marker has been developed in recent years. PET measurements have shown that the activity of microglia in FMS patients is increased in the temporal lobe and in the medial and lateral areas of the frontal lobe [53]. This is consistent with the areas of our study where FA is elevated. Our results could therefore point to an inflammatory process in the brain of FMS patients.

Since in most other chronic pain disorders or psychiatric diseases a decrease in FA is often reported after a certain period of illness, a possible hypothesis for the reduced FA of the 2 ROIs found in the PNS group compared to the noPNS group can be deduced from this. A decrease in FA of such white matter structures such as internal capsula and the anterior corona radiata, has been described in other pain disorders [88] or psychiatric diseases [89] and may even serve as predictive markers for the transition between acute and chronic pain [28, 90]. It has also been shown specifically in FMS patients that white matter pathways, whose FA increases after a period of increased activity [91], in this case in pain processing regions, decrease again after pain chronification and show lower values than healthy controls [83]. The PNS group showed an overall stronger symptomatology, such as increased pain intensity or disability due to pain. Therefore one could speculate that the 2 ROIs in the PNS group, such as the capsula interna and the thalamic radiation, have decreased in FA only in the course of the disease due to the stronger symptoms of this subgroup. Since the brain is known to change as we age, we tested our data for the age factor by correlating the FA data with the age of the patients. Only the splenium of the corpus callosum correlated with the FA values, a fact that has already been shown in studies with larger case numbers [92]. Thus, we do not assume that age had an influence on our results in our cohort with the age range of 40-65 years. In order to

verify the above mentioned hypothesis of increasing and decreasing FA in FMS, and to understand the brain development of FMS patients in general during their lifetime, longitudinal studies using diffusion imaging, PET imaging and preferably CSF examinations are needed.

The previous literature on functional network analyses in FMS is diverse. In summary, most studies point to hyperconnectivity of the insular cortex [50, 93, 94] or default mode network [95]. These findings support the "central hypersensitivity hypothesis," which posits that brain hypersensitivity triggers FMS symptoms. In our main analysis, we could not reproduce any of the mentioned connectivity differences between FMS patients and controls. While our main analysis was an exploratory "whole brain approach", which included all brain regions as ROI, we also analyzed possible connectivity differences between previously published regions with connectivity differences in FMS (default mode network, somatosensory network, frontoparietal network, insular cortex) at the request of a reviewer. This analysis also showed no relevant group differences between these regions. Although the ventromedial cortex is part of the default-mode network, its hypoconnectivity to the cerebellum found in our data has not been described in other FMS-specific studies. However, this hypoconnectivity has been found in studies with psychiatric patients [96, 97]. The hyperconnectivities found in our subgroup with PNS pathology between the left and right inferior frontal gyrus, the right angular gyrus and posterior parietal cortex are also not previously described in the FMS literature, but are known hubs in the general pain literature. While a more negative evaluation of pain symptoms has been associated with a stronger fMRI activation in the angular gyrus [98], the posterior parietal gyrus, with its strong connections to the somatosensory cortex, appears to be responsible for the spatial perception of pain stimuli [99]. The inferior frontal gyri, which showed bilateral hyperconnectivity with the above regions in our subgroup analysis, appear to be responsible for emotion regulation [100]. The connectivity differences found in the group comparisons FMS/controls and between subgroups cannot fully explain a clear causal cause of FMS symptoms and may rather indicate predispositions in the central networks that may favor a stronger development of FMS symptoms. This would be supported by the more pronounced connectivity differences in the subgroup with peripheral pathologies, which is also significantly more severely affected in terms of FMS symptoms. Resting state data has already been used in initial studies as a predictor of individual pain sensitivity [101]. This subgroup might be able to develop a stronger attention to peripheral stimuli due to hyperconnectivities in attention-

regulating brain regions. The fact that these regions also show abnormalities in patients with other chronic pain diseases also suggests that these connectivity changes are a consequence of the chronic pain diseases. However, the subgroups in our study did not differ in the duration of the disease, so this hypothesis is not substantiated by our data. Why the previously published connectivity differences in FMS sufferers did not emerge in our analyses is a matter of conjecture. While there are some whole-brain studies in FMS, most studies focus on hypothesis-driven ROI-based analyses, which generate higher statistical power. Here, for example, one could perform seed-to-voxel analyses that are not limited to ROI regions but analyze connectivities to voxel clusters throughout the brain. However, this analysis method was not compatible with the exploratory approach of our study. Another conjecture (supported by our unpublished analysis attempts) is that our control variables "depression" and "pain intensity" filtered out connectivities in the models that have been published as significant in other studies. However, we included these control variables in the model, as it is known that chronic pain and depressive symptom can affect brain connectivities [102-104].

Regarding study #2, there is no other study to date that investigated the potential influence of FMS subgroups with and without PNS pathology on the intake patterns and subjective pain relief of various medications. This may be due to the invasive method used to determine IENFD, which has not yet provided clinical benefit to patients. Our data does not indicate a clear clinical benefit of subgroup ascertainment either. While some meta-analyses and reviews have been published on randomized controlled trials regarding specific medications for the treatment of FMS [105-107], our study (in addition to subgroup analysis), as a cross-sectional study, aimed to address the question of how the guideline recommendations are actually implemented in clinical practice. Most guidelines recommend, depending on the severity of symptoms, such as sleep disturbances, severe pain or restrictions in the daily routine, exercise, cognitive-behavioral therapy, multimodal pain therapy, serotonin noadrenaline reuptake inhibitors (SNRIs), amitriptyline and anticonvulsants, such as pregabalin or gabapentin [108]. However, most guidelines recommend drug therapy only for a limited period of time, for example 6 months, to motivate patients to try non-pharmacological therapies. A literature search identified several studies regarding the pharmacological treatment practices in various countries. Most of them specialised on one certain drug class. One study, which focused on pregabalin, was conducted in Japan. An important result of our study was that pregabalin is often prescribed at insufficient doses. Pregabalin also appeared to

be frequently underdosed in Japan, with 47.7% of patients with neuropathic pain taking a dose below the 150 mg/d recommended for FMS patients [109]. While the recommended maximum daily dose for FMS is 450 mg, the recommended daily dose for patients with general neuropathic pain is up to 600 mg in most countries. Although pregabalin may also have rare serious side effects such as angioedema or heart failure, it may be useful for patients with neuropathic pain not only because of its analgesic but also because of its neuroprotective effect [110]. This is also pointed out by an American population-based study, which shows that at the start of treatment of FMS with pregabalin the average dose is 75 mg/d and 52% of patients do not increase this dose as recommended in the treatment guidelines. Of these 52% of patients, 78% discontinued therapy with pregabalin, in other words before a dose was reached which was described in the guidelines as the minimum dose for effective therapy [111]. This observation is consistent with our study. The fact that patients with higher doses of pregabalin took the therapy over a longer period of time could therefore indicate that it was more effective in alleviating FMS symptoms. With regard to pregabalin, another American population-based study, which investigated the influence of taking certain drugs on the likelihood of taking other drugs, showed that a prior intake of pregabalin made taking duloxetine, one of the three drugs recommended for the treatment of FMS, more likely [112]. A study analyzing the opioid intake of FMS patients in America between 2011 and 2017 showed that the rate of opioid intake decreased from 42% in 2011 to 26% in 2016. The authors believe the reasons for this are the higher public awareness of the risks of opioid dependence and side effects in recent years [113]. This higher sensitivity to opioid-related problems is also reflected in stricter prescribing criteria and greater adherence to guidelines. Nevertheless, this rate is still significantly higher than in our cohort (7.0%), however, this difference between European and American population studies is already well evidenced, although in Europe the rates tend to rise [114]. The higher sensitivity to opioid-related problems in Europe is also reflected in the stricter prescription criteria [115, 116] and greater adherence to the guidelines [117]. An American study, which examined the intake of substances of all drug classes in older FMS patients for the correlation between morbidity and polypharmacy, showed different rates compared to our cohort. Here, the most frequent rates were sleeping aids with 33.3%, SSRIs with 28.7% and SNRIs with 21.0% [118]. Opioids were taken by 22.4% of patients, similar to the study by Sarmento et al. However, a comparison of these study results with those of our cohort is difficult due to the different age averages and national differences. Apart from that, our cohort is comparable to the studies

mentioned above. Since most of the studies were able to access data from national registries, the case numbers were substantially higher, but we were able to pre-select our patients much better during the recruitment phase and to perform detailed inclusion and exclusion diagnostics prior to recruitment. This is also shown by the comparison of our cohort with regard to the number of other pain disorders in the patients. Since patients were included with other chronic pain that could be clearly distinguished from FMS pain, such as a disc herniation, the rate of patients in our cohort taking pain medication for this was 3.5%. This low rate made it possible to determine the effectiveness and routines for taking pain medication more specifically for FMS. However, a major difference to subjects in other cross-sectional studies is that our participants were voluntary participants in a clinical study, for which most of them had to travel to our clinic and take part in a full day of examinations. Our patients may therefore have a higher resilience than other typical FMS patients or be more severely affected by the disease than these. Nevertheless, we consider our results to be relevant, since corresponding data from Europe are missing so far and our patients were carefully examined from neurological and rheumatological side before the diagnosis of FMS was made, which excludes other reasons for the pain, and thus other influencing factors, to a large extent. For the clinical interpretation of this study the question why such a small number (20.2%) of FMS patients in Germany are not treated according to the guidelines is another important point. On the one hand, this may be due to the guideline, as it can only refer to the already published and high-quality drug studies in FMS. For drugs that are frequently taken by FMS patients, such as NSAIDs, the guideline can only refer to 2 small clinical studies or, in the case of metamizole, to no relevant studies and therefore cannot make a positive or negative statement [108, 119]. However, since many of these drugs can be sold over the counter, it is important to check the effect of these drugs in randomized controlled trials (RCTs). Another reason could be a lack of awareness or information of the treating physicians, as FMS has been treated as a psychosomatic illness among physicians for decades. The latest scientific findings in recent years have often not yet been applied in practice. It is therefore important that specialised pain therapists make their colleagues in general practice or orthopaedics aware of the current guidelines. Sometimes, however, in practice one also experiences a lack of compliance on the part of patients who discontinue or take on as needed the therapies that have been set up, for example in the case of SNRIs, which depend on continuity. In this case, it is important that the treating physicians are informed in detail, because often the term "antidepressants" is misunderstood or

stigmatized and patients think that they are not taking it seriously and labeled as a psychiatric patient. Patients then often consult so-called "alternative doctors" who offer private medical treatments, such as heavy metal binding, without any scientific basis. It is also possible that patients evaluate the effectiveness of antidepressants before the known delayed onset of action. Still, the relatively high percentage of patients who have already taken a guideline-compliant therapy but had already stopped it before our study shows that especially with these drugs, such as SNRIs, amitriptyline or pregabalin, side effects are the most frequent reason for discontinuation. Patients often report fatigue, weight gain or lack of concentration, which are known side effects of these drugs and lead to a loss of life quality. In our study, we see that the reason for discontinuation of drugs that do not comply with the guidelines is rather the lack of effect. Especially opioids should be taken into account, as their effect on microglia cells [120] could be counterproductive in FMS, as these cells are suspected to be involved in the pathophysiology [53]. Since no drug is approved in Europe for the treatment of fibromyalgia and the German guideline can only refer to so-called off-label use drugs, a standardized therapy is still difficult. High-quality RCTs or registered studies could better investigate the safety and effectiveness and thus lead to the approval of effective drugs in Europe.

Regarding study #3, the hypothesis that a dysbalance between glutamate and GABA concentrations in the insular cortex leads to hyperexcitability of the latter, which in turn provides hypersensitivity to peripheral stimuli, has existed in the FMS literature for several decades [121, 122]. It fits well into the inferral of pathophysiology based on a central cause [123, 124]. This thesis of "hypersensitation" of the central nervous system in FMS has already been introduced above in the introduction, and is quite conclusive, since GABA is known to be an inhibitory and glutamate as an excitatory neurotransmitter [125]. The role of the insular cortex in the processing of pain stimuli is a commonly accepted concept in the literature [126, 127]. It is assumend that the anterior and posterior insular cortex contribute differently to the processing of pain [128]. Since there have been no previous studies examining the peripheral and central nervous systems in the same patients, this was the gap in the literature that the study was designed to fill. It has been hypothesized in the literature that there may be subgroups of FMS patients in whom either the aforementioned "hypersensitization" or peripheral nerve damage causes the similar symptomatology [35]. Other studies suggested a causal relationship between central neurotransmitters and the objective damage of the peripheral nerves [78]. Previous findings in the literature on altered glutamate and GABA using

altered glutamate and GABA using MR spectroscopy could show that lower GABA concentrations in FMS patients in the posterior insular cortex (which is called the "gateway" to the somatosensory network [129]) were related to increased pain sensitivity [130]. In the anterior insular cortex (which is thought to be responsible for affective processing of pain stimuli and for introspection [131]), FMS patients showed lower GABA concentrations compared to healthy controls [130]. These GABA concentrations were increased by electroacupuncture in FMS patients in a study without healthy controls [132]. Regarding glutamate concentrations, there is one study that showed an increased glutamate concentration in FMS patients compared to healthy controls in the right posterior insular cortex [122]. Other regions of the insular cortex showed no differences in neurotransmitter concentrations.

Our study differs from all of these studies not only in its results, but also in the way the ROIs were placed. We chose the entire insular cortex as the ROI because this was the only way to ensure reliable signal quality according to the recommendations for MEGA-PRESS spectroscopy sequences [133]. The previously mentioned studies separated the insular cortex into an anterior and posterior ROI. This makes sense functionally and anatomically, but provides poor signal to noise ration and poor data quality [76]. In addition, the previous studies were either small in their group size (usually less than 20 subjects), or did not control for their effects with healthy controls. In addition, the data were all analyzed with a toolbox that does not disclose its code, so the analysis cannot be understood precisely [134]. In summary, our spectroscopy study was the first study to investigate the glutamate/GABA hypothesis in FMS patients including a healthy control group and a disease control group in a sufficient number of cases (n=102). We did not find any evidence supporting the hypothesis or links between the glutmatate concentrations and the pathology in the PNS.

5.4 Limitations of the studies

In the research on the pathophysiology of FMS, two questions are currently in the spotlight: 1. Are there subgroups with possibly different etiologies and similar symptoms? 2. Are the findings in the CNS and PNS the cause or consequence of the symptoms? While this dissertation may contribute in part to answering the first question, the cross-sectional study design reveals the first limitation of this dissertation. Due to the lack of longitudinal data, it is not clear whether the changes in the central nervous system found by neuroimaging causally cause the

development of the symptoms, whether they might merely exacerbate the symptoms, or whether they are long-term consequences of the chronic disease. The subgroup differences found in our MRI study (#1) did not correlate significantly with nerve fiber density (except for differences in pericalcarine cortices volume). Therefore, based on the data available to us, we cannot exclude that the subgroup differences were influenced by, for example, clinical parameters that we did not include in our data collection. Because of the exploratory approach of our MRI study (#1), we barely limited the ROIs for the correlation analyses with clinical parameters, so correction for multiple comparisons would have been necessary to draw statistically definite conclusions. To preserve the exploratory nature of the correlation analyses, we limited ourselves to a p-value threshold lowering to $p<0.01$. Group and subgroup comparisons were all corrected for multiple comparisons. Another limitation of our study is that our control subjects (healthy and rheumatoid arthritis) did not receive assessment of the intraepidermal nerve fibers. Thus, we cannot exclude the possibility that peripheral nerve fiber pathologies also existed in these groups. However, a preliminary study from our lab showed that the proportion of individuals with pathology of the small peripheral nerve fibers in the normal population is approximately 2% [9]. In our medication study (#2), we had chosen the naturalistic cross-sectional study design in order to obtain a realistic assessment of the current medication regimen of FMS patients in Germany and their subjective assessment regarding their medication. Because in this study we also queried reasons for discontinuation of former medications and an assessment of current medication efficacy since initiation of dosing, the results may be biased by recall bias. In addition, we could not discount the possibility that patients on multiple concurrent medications attributed the effects to a particular medication when, in fact, another medication caused the subjective symptom relief. Therefore, study #2 can only be an impetus to initiate targeted controlled and randomized trials of specific drug classes in FMS treatment. In study #3, the most obvious limitation in terms of comparability with other studies is already addressed in the discussion. Because the previous studies separated the insular cortex into anterior and posterior ROIs, direct comparison of the data is limited. In addition, the single-voxel spectra have not been adapted to the proportion of gray matter in the voxel, which more modern analysis methods are starting to establish.

5.5 Impact of the studies

Several conclusions can be drawn from the results of this dissertation for further research. From the data of study #1, it appears that the reason for the formation of subgroups with and without peripheral nerve pathologies probably has no underlying central cause. Only two MRI findings appear to be specific and have not been clearly associated with FMS or chronic pain outcomes in general. These are the increased FA (of multiple ROIs) in the FMS group compared with healthy controls in our data, and the cortex volume differences of the bilateral pericalcarine cortices between the subgroups. All other regions and connectivity differences in the groups and subgroup comparisons show changes that have already been demonstrated in other chronic pain disorders. Thus, the specificity of these findings is limited and rather suggests that they are a consequence of the patients' chronic pain. This in turn indicates that FMS do actually experience pain as a symptom, which is nowadays also widely acknowledged in clinical care. For further research on neuroimaging in FMS, the following suggestions can be summarized: 1. Due to the lack of information on whether the alterations in the brain morphology and connectivity of FMS patients and their subgroups are a consequence or cause of the symptoms, longitudinal neuroimaging studies should be performed, preferably directly at the onset of the disease symptoms. If the alterations would be a consequence of chronic pain, they should be very mild at the onset of symptoms and then become more apparent as the disease progresses. 2. Our Data indicated subgroup differences of the pericalcarine cortex bilaterally. The role of the pericalcarine cortex is considered mainly as being a part of the visual cortex. Subsequent studies should address the question of why this region shows subgroup differences. The increased FA in several pain processing regions in FMS patients could indicate an inflammatory process with increased glial cell activity. The fields of neuroimmunology and neuroimaging have also shown interdisciplinary evidence of altered glial cell activity in FMS patients in recent years [53, 86, 87, 135]. Possible neuroimmunological processes in the peripheral and central nervous system of FMS patients will probably be in scientific focus in the next years. 4. Our functional network analyses indicate an increased capacity of introspection and altered emotional evaluation to peripheral stimuli in FMS patients, especially in patients with PNS pathologies. Psychotherapeutic approaches using mindfulness-based approaches, as already practiced in specialized pain centers, could also be an effective therapeutic method in FMS. An interesting question in the future could be whether this form of therapy can ameliorate the alterations in

the functional networks and whether this amelioration is associated with an improvement in clinical symptoms.

From study #2, the following implications can be summarized: 1. Metamizole and NSAIDs, as widely used pain medications with relatively low side effect profiles, showed subjective effectiveness on reducing pain symptoms in FMS patients. Since the available evidence for the pharmaceutical treatment of FMS is still scarce, these medications are not recommended in the German guideline. A better evidence base through more RCTs would be useful to improve the care of patients. 2. The medications amitriptyline, duloxetine, and pregabalin, which are recommended for temporary use in the German guideline for the treatment of FMS, are, according to our data, predominantly not dosed in the recommended therapeutic range, but often below or above it. This causes unnecessary side effects in patients and thus possibly high discontinuation rates of the recommended medications. The same is true for underdosing, which could potentially lead to early discontinuation of recommended medications due to a lack of efficacy. 3. We found no evidence in our study for differences in medication adherence and subjective effectiveness of medication between the subgroups with and without PNS pathologies. However, we think that the numbers of cases in the subgroup analysis were insufficient to draw firm conclusions. Therefore, it would be good to re-address this question with a larger study population. 4. Although several publications and the German guideline for the treatment of FMS clearly advise against the prescription of opioids for treatment of FMS, we see these prescriptions, albeit in relatively smaller numbers compared to the US, in our data. Better education of pain societies on this topic could potentially further reduce prescribing rates.

The data from Study #3 contradict the hypothesis that the symptoms of fibromyalgia, as well as the development of peripheral pathologies, are associated with increased glutamate or lower GABA concentrations in the insular cortex of FMS patients. The difficulties of comparability of our data with the previous studies, which have already been discussed in the discussion, indicate that uniform guidelines should be agreed upon not only for the analysis procedures of MEGA-PRESS spectroscopy data but also for the data acquisition at the scanner in order to minimize the possible influencing factors (ROI placement, duration of the sequences, blood glucose level, time of day) and thus the scatter of the data.

Concluding remarks

There is no clear evidence so far that all previous findings in the CNS in FMS patients are the cause of the symptoms. They could also be the result of neuroplastic changes, due to chronic symptoms or peripheral nociceptive signals. There are already first indications that structural changes in the CNS in chronic pain patients are reversible as soon as their symptoms are treated efficiently [136, 137]. Therefore, it is also necessary in FMS to plan longitudinal studies in order to be able to differentiate exactly between causes and consequences or more precisely between the influences of the peripheral and central nervous system. The aim of this dissertation was to find out whether subgroups of FMS patients with and without pathology in the small nerve fibers of the PNS show structural and functional differences in the central nervous system and in the type of therapy, or therapy effectiveness. Therefore several MRI examinations and a clinical study were conducted. It was shown that the PNS subgroup is more severely affected in the CNS in most investigations, such as structural and functional imaging. Only diffusion imaging showed an increased density of white matter in both subgroups, which may indicate an increased activity of the immune system, for example of microglia cells. It is still unclear whether the changes in the CNS are the cause or consequence of the FMS symptoms. To further find out whether different pathophysiologies are responsible for the subgroups, longitudinal studies with multimodal examinations such as MRI, PET or CSF examinations will be needed in the future. A small, but not significant, difference in the effectiveness of drug therapy was found between the subgroups. However, the group size in this study was relatively small. To find more targeted therapies for the subgroups, controlled, randomized and longitudinal studies with larger case numbers are therefore needed. However, the results of this dissertation show that this could be a worthwhile further step.

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9 Affidavit

I hereby confirm that my thesis “Characterization of subgroups of fibromyalgia syndrome” is the result of my own work. I did not receive any help or support from commercial consultants. All sources applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Würzburg, January 09, 2023

10 Eidesstattliche Erklärung

Hiermit bestätige ich, dass meine Arbeit "Charakterisierung von Subgruppen des Fibromyalgie-Syndroms" das Ergebnis meiner eigenen Arbeit ist. Ich habe keine Hilfe oder Unterstützung von kommerziellen Beratern erhalten. Alle verwendeten Quellen sind in der Arbeit aufgeführt und benannt.

Weiterhin versichere ich, dass diese Arbeit bisher weder in gleicher noch in ähnlicher Form im Rahmen eines anderen Prüfungsverfahrens eingereicht worden ist.

Würzburg, den 09.01.2023

11 Appendix

11.1 List of scientific publications

1. **Aster HC.**, Evdokimov D., Braun A., Üçeyler N., Sommer C., (2022), Analgesic Medication in Fibromyalgia Patients: A Cross-Sectional Study, Pain Research and Management

2. **Aster H.C.**, Hahn V., 2, Schmalzing M., Homola G. A., Kampf T., Pham M., Üçeyler N., Sommer C. (2022), Comparison of gamma-aminobutyric acid, glutamate, and N-acetylaspartate concentrations in the insular cortex between patients with fibromyalgia, rheumatoid arthritis, and healthy controls - a magnetic resonance spectroscopy study. MedRxiv

3. **Aster HC.**, Evdokimov D., Braun A., Üçeyler N., Kampf T., Pham M., Homola G.A., Sommer C., (2022) CNS imaging characteristics in fibromyalgia patients with and without peripheral nerve involvement. Scientific Reports

4. **Aster H.C**., Romanos M., Walitza S., Gerlach M., Mühlberger A., Hasenauer N., Hartrampf P., Nerlich K., Reiners C., Lorenz R., Buck A., Deserno L., (2022) Responsivity of the striatal dopamine system to methylphenidate – a within-subject I-123-ß-CIT-SPECT study in children and adolescents with Attention-Deficit/Hyperactivity Disorder. Frontiers in Psychiatry

5. Schwartz C., **Aster H.C.**, Al-Schameri R., Müller-Thies-Broussalis E., Griessenauer C.J., & Killer-Oberpfalzer M. (2018) Microsurgical clipping and endovascular treatment of middle cerebral artery aneurysms in an interdisciplinary treatment concept: Comparison of long-term results. Intervent. Neuroradiology.

6. **Aster H.C.**, Bohrer T. (2019) Big Data Algorithms in Medicine – Medical Competence and Critical Judgment Required? International Medicine Review

7. **Aster HC.**, Sommer C. (2019) Chronic Pain and Society / Chronischer Schmerz und Gesellschaft. Der Schmerz

8. Daub J., **Aster HC.**, Gauger H., Gallasch T., Schmidt M., Koenigshausen J., Bohrer T. (2019) The Philosophicum – Model Project of Philosophy of Medicine in Medical Education and Practice in Germany. American Journal of Internal Medicine

11.2 Statement of individual author contributions and of legal second publication rights

Manuscript 1: CNS imaging characteristics in fibromyalgia patients with and without peripheral nerve involvement

Hans-Christoph Aster, Dimitar Evdokimov, Alexandra Braun, Nurcan Üçeyler, Thomas Kampf, Mirko Pham, György Homola, Claudia Sommer

Scientific Reports (2022)

Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design Methods Development	CS GH	HCA HCA	MP		
Data Collection	HCA	GH	DE	AB	NÜ
Data Analysis and Interpretation	HCA	GH	CS	TK	
Manuscript Writing Writing of Introduction Writing of Materials & Methods Writing of Discussion Writing of First Draft	HCA	CS	GH	NÜ	

Manuscript 2: Analgesic Medication in fibromyalgia patients – a cross sectional study

Hans-Christoph Aster, Dimitar Evdokimov, Alexandra Braun, Nurcan Üçeyler, Claudia Sommer

Pain Research and Management (2022)

Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design Methods Development	CS CS	NÜ NÜ	HCA HCA		
Data Collection	DE	HCA	AB	NÜ	
Data Analysis and Interpretation	HCA	CS			
Manuscript Writing Writing of Introduction Writing of Materials & Methods Writing of Discussion Writing of First Draft	HCA	CS	NÜ		

Manuscript 3: Comparison of gamma-aminobutyric acid, glutamate, and N-acetylaspartate concentrations in the insular cortex between patients with fibromyalgia, rheumatoid arthritis, and healthy controls - a magnetic resonance spectroscopy study

Hans-Christoph Aster, Viola Hahn, Marc Schmalzing, Thomas Kampf, Mirko Pham, György Homola, Nurcan Üçeyler, Claudia Sommer

medRxiv (2022)

medRxiv (2022)					
Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design Methods Development	HCA GH	GH TK	CS HCA	TK	MP
Data Collection	HCA	VH	TK	NÜ	MS
Data Analysis and Interpretation	HCA	VH	GH	TK	
Manuscript Writing Writing of Introduction Writing of Materials & Methods Writing of Discussion Writing of First Draft	HCA	VH	CS	GH	

The doctoral researcher confirms that she/he has obtained permission from both the publishers and the co-authors for legal second publication.

The doctoral researcher and the primary supervisor confirm the correctness of the above mentioned assessment.

Hans-Christoph Aster

Doctoral Researcher's Name Date Place Signature

Claudia Sommer

Primary Supervisor's Name Date Place Signature

11.3 Statement of individual author contributions to figures/tables of manuscripts included in the dissertation

Manuscript 1: CNS imaging characteristics in fibromyalgia patients with and without peripheral nerve involvement

Hans-Christoph Aster, Dimitar Evdokimov, Alexandra Braun, Nurcan Üçeyler, Thomas Kampf, Mirko Pham, György Homola, Claudia Sommer

Scientific Reports (2022)

Figure	**Author Initials,** Responsibility decreasing from left to right				
1	HCA	GH			
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Table	**Author Initials,** Responsibility decreasing from left to right				
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4	HCA	CS			

Manuscript 2: Analgesic Medication in fibromyalgia patients – a cross sectional study

Hans-Christoph Aster, Dimitar Evdokimov, Alexandra Braun, Nurcan Üçeyler, Claudia Sommer

Pain Research and Management (2022)

Figure	**Author Initials,** Responsibility decreasing from left to right				
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Table	**Author Initials,** Responsibility decreasing from left to right				
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6	HCA	CS			
7	HCA	CS			

Manuscript 3: Comparison of gamma-aminobutyric acid, glutamate, and N-acetylaspartate concentrations in the insular cortex between patients with fibromyalgia, rheumatoid arthritis, and healthy controls - a magnetic resonance spectroscopy study

Hans-Christoph Aster, Viola Hahn, Marc Schmalzing, Thomas Kampf, Mirko Pham, György Homola, Nurcan Üçeyler, Claudia Sommer

medRxiv (2022)

Figure	**Author Initials,** Responsibility decreasing from left to right				
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Table	**Author Initials,** Responsibility decreasing from left to right				
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3	HCA	CS			
4	HCA	CS			

I also confirm my primary supervisor's acceptance.

Hans-Christoph Aster

Doctoral Researcher's Name Date Place Signature

11.4 Abbreviations

ACC: Anterior cingulate cortex

ADC: Apparent Diffusion Coefficient

BOLD: Blood-oxygenation-level-dependent

CNS: Central nervous system

CSI: Chemical shift imaging

DLPFC: Dorsolateral prefrontal cortex

DMN: Default Mode Network

DTI: Diffusion tensor imaging

FA: Fractional anisotropy

GABA : γ-aminobutyric-acid

IENFD: Intraepidermal nerve fiber density

LTP: Long-term potentiation

mM: Millimolar

MPRESS: Mega Point Resolved Spectroscopy

MPRESS: MEGA-point-resolved spectroscopy

MRI: Magnetic resonance imaging

MRS: Magnetic resonance spectroscopy

NAA: N-acetylaspartate

NMR: Nuclear magnetic resonance

NSAIDs: Non-steroidal anti-inflammatory drugs

PCC: Posterior cingulate cortex

PGP: Protein-gene product

PNS: Peripheral nervous system

PPM: Parts per million

QST: Quantitative sensory testing

RCT: Randomized controlled trial

RF: Radio frequency

ROI: Region of interest

rsfMRI: Resting state functional magnetic resonance imaging

SNR: Signal to noise ratio

SNRI: Serotonin noadrenaline reuptake inhibitor

SVS: Single voxel spectroscopy

T: Tesla

TRPA1: Ankyrin 1 channel

TRVP1: Transient receptor potential vanilloid type 1