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Characterisation of the Cortical and Subcortical Functional Connectivity in Adult and Paediatric CRPS patients: fMRI-based Cross-sectional and Longitudinal Studies

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List of Abbreviations

English

ACC	Anterior Cingulate Cortex
BOLD	Blood Oxygen Level Dependent
CRPS	Complex Regional Pain Syndrome
DMN	Default Mode Network
fMRI	Functional Magneto Resonance Imaging
FSL	FMRIB Software Library
FWE	Family Wise Error
GMD	Grey Matter Density
ICA	Independent Component Analysis
IFJ	Inferior Frontal Junction
IPL	Inferior Parietal Lobe
ITG	Inferior Temporal Gyrus
M1	Primary Motor Cortex
MCC	Middle Cingulate Cortex
MPFC	Medial Prefrontal Cortex
MTL	Medial Temporal Lobe
OFC	Orbitofrontal Cortex
PAG	Periaqueductal Grey
PCC	Posterior Cingulate Cortex
ROI	Region of Interest
rsFC	Resting-state Functional Connectivity
rs-fMRI	Resting-state Functional Magneto Resonance Imaging
S1	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex

SMA	Supplementary Motor Cortex
SPM	Statistical Parametric Mapping
TPJ	Temporo-parietal Junction
TRN	Thalamic Reticular Nucleus
VPL	Ventral Posterolateral Nucleus
STN	Subthalamic Nucleus
GPe	External Globus Pallidus
GPi	Internal Globus Pallidus

German

ACC	Anteriorer Zingulärer Kortex
BG	Basalganglien
CRPS	Komplexes Regionales Schmerzsyndrom
DGS	Dichte der Grauen Substanz
fMRT	funktionelle Magnetresonanztomographie
ITG	Inferiorer Temporaler Gyrus
MCC	Mittlerer Zingulärer Kortex
OFC	Orbitofrontaler Kortex
rs-fMRT	funktionelle Resting-state Magnetresonanztomographie
S1	Primärer Somatosensorischer Kortex
TRN	Thalamischer Retikulärer Nukleus
VPL	Ventraler posterolateraler Nukleus

Related Publications

Journals

Azqueta-Gavaldon, **M.**, Youssef, A. M., Storz, C., Lemme, J., Schulte-Göcking, H., Becerra, L., Azad, S. C., Reiners, A., Ertl-Wagner, B., Borsook, D., Upadhyay, J., & Kraft, E. (2020). Implications of the putamen in pain and motor deficits in complex regional pain syndrome. *Pain*, *161*(3), 595–608. doi:10.1097/j.pain.0000000000001745[1]

Schulte-Goecking, H., **Azqueta-Gavaldon, M.**, Storz, C., Woiczinski, M., Fraenkel, P., Leukert, J., Azad, S. C., & Kraft, E. (2020). Psychological, social and biological correlates of body perception disturbance in complex regional pain syndrome. *Current Psychology*, 1–11. doi:10.1007/s12144-020-00635-1

Storz, C., Schulte-Göcking, H., Woiczinski, M., **Azqueta-Gavaldon, M.**, Azad, S. C., & Kraft, E. (2020). Exergames für Patienten mit komplexem regionalem Schmerzsyndrom. *Der Schmerz*. doi:10.1007/s00482-019-00436-x

Youssef, A. M., **Azqueta-Gavaldon, M.**, Silva, K. E., Barakat, N., Lopez, N., Mahmud, F., Lebel, A., Sethna, N. F., Zurakowski, D., Simons, L. E., Kraft, E., & Borsook, D. (2019). Shifting brain circuits in pain chronicity. *Human Brain Mapping*, *40*(15), 4381–4396. doi:10.1002/hbm.24709[2]

Azqueta-Gavaldon, M., Schulte-Göcking, H., Storz, C., Azad, S., Reiners, A., Borsook, D., Becerra, L., & Kraft, E. (2017). Basal Ganglia Dysfunction in Complex Regional Pain Syndrome - A valid Hypothesis? *European Journal of Pain*, *21*(3), 415–424. doi:10.1002/ejp.975

Storz, C., Schulte-Göcking, H., **Azqueta-Gavaldon, M.**, Wania, C., Neugebauer, M., Reiners, A., Azad, S., & Kraft, E. (2017). Cognitive-perceptive approaches in the treatment of chronic pain. *Schmerz*, *31*(5), 448–455. doi:10.1007/s00482-017-0229-7

[1] Publication I, first author, contribution: concept (10%), design (50%), data acquisition (100%), data analysis (90%), data interpretation (60%), manuscript (80%)

[2] Publication II, co-author, contribution: concept (30%), design (10%), data acquisition (40%), data analysis (10%), data interpretation (10%), manuscript (10%)

Conference Presentations

Azqueta-Gavaldon, M., Youssef, A., Storz, C., Schulte-Goecking, H., Borsook, D., Kraft, E. Structural and Functional Connectivity Anomalies within the Basal Ganglia in Adult CRPS Patients. 24th Annual Meeting of the Organization for Human Brain Mapping, Singapore, July 2018

1 Introduction

As it is only too well-known chronic pain is a very debilitating condition which negatively affects the quality of life of those who suffer from it. Managing and treatment of chronic pain is a very challenging task, and complete elimination of the pain is rarely obtainable. Broader knowledge about what neurological pathologies may be present in chronic pain may help developing better and more customised therapies. The research presented here seeks to improve the understanding of the neurological signatures underlying a specific chronic pain condition: complex regional pain syndrome (CRPS). Two publications describing the cross-sectional studies done at the Department of Orthopaedic Surgery, Physical Medicine and Rehabilitation in collaboration with the Center for Pain and the Brain at the Boston Children´s Hospital are presented in this thesis.

This thesis is structured as follows. In section 1.1, a brief description of the disease CPRS is given, with a focus on the neuroimaging findings up to date as well as on differences observed between adult and paediatric populations. This pretends to show the reader the rationale behind the execution of both studies. A detailed explanation of how the neurological data dealt with in the studies can be analysed, and has been analysed, is given in section 1.2. Sections 1.3 depicts in detail which neurological networks are of relevance for the study of CRPS, in particular those involved in pain and motor control. In section 1.4, descriptions of the basal ganglia structures are presented. After having presented the reader with the rationale for the studies carried out, as well as the relevant information in terms of anatomy and methodology, the aim of the thesis is thoroughly explained at the end of the introduction.

The two research articles presented in this thesis are summarised in English and German in sections 2 and 3, respectively. Moreover, a full version of such papers is presented following those summaries.

Please note that certain parts of the thesis may almost replicate sentences of my own published work.[3] This is because, on the one hand, I considered necessary to reiterate this information in the introduction, specifically sections 1.1 and 1.4. and, on the other hand, the

[3] The published work include Azqueta et al 2017, Azqueta et al 2020 and Youssef et al 2019.

methods and results are described with very specific technical terms in the summaries presented here.

1.1 Complex Regional Pain Syndrome

Complex Regional Pain Syndrome (CRPS) is a chronic pain condition that succeeds an injury to an extremity such as fracture or sprain (Bailey et al., 2013). Depending on whether the injury presents with a definable nerve lesion or without one, CRPS has been differentiated in two subtypes, CRPS Type II and CRPS Type I, respectively. The CRPS Type I subtype is much more common than the former subtype (Harden et al., 2010). CRPS appears with a prevalence rate of 1–5% (Bruehl, 2010; Searle & Salibi, 2014), and it is three to four times higher in women than in men (de Mos et al., 2007).

The symptoms that CRPS patients commonly present with are well characterised and vary as the disease progresses. Most patients with CRPS partially recover within 6–13 months, but a substantial number of patients experience lasting symptoms, chronic pain, and disability (Bean, Johnson, & Kydd, 2014; Birklein et al., 2018). During the acute stages of CRPS (1-3 months), patients experience spontaneous or movement-induced pain sensation disproportionate to the initiating injury, together with concomitant soft-tissue oedema, disturbed sympathetic function, and movement limitation (Birklein & Dimova, 2017). In chronic stages, motor deficits may become more distinct (van Hilten, 2010), including reduced range of motion, joint stiffness, muscle weakness, tremor, dystonia, and irregular myoclonus jerks (Munts et al., 2008, 2011; van Rijn et al., 2011; Verdugo & Ochoa, 2000). Moreover, CRPS patients present with space and visual attentional impairments to the affected limb (Bultitude, Walker, & Spence, 2017), neglect-like symptoms (Lewis et al., 2007), and body perception disturbances (Lewis & Schweinhardt, 2012).

Several longitudinal studies have attempted to find characteristics that may be predictive of CRPS onset or treatment response. Demographic characteristics, as well as the nature of the trauma, show very inconsistent results in terms of CRPS development prediction. Nevertheless, a high level of pain during the weeks after the trauma seems to be the most robust risk factor for CRPS development (Birklein et al., 2018). CRPS patients often express psychological distress. However, the few prospective studies focusing on these

characteristics conclude that psychological factors are not a risk factor (Beerthuizen et al., 2011).

The pathophysiological mechanisms that either hinder a normal healing of the original injury or unfold in a CRPS phenotype are still poorly understood. Peripheral causes such as neurogenic inflammation might trigger trophic changes (Birklein & Schmelz, 2008), and small-fibre degeneration might also contribute to promote pain and autonomic dysfunction (Huge et al., 2008). Persistent pain may be explained partially by central sensitization, a process in which the membrane excitability and synaptic efficacy of the nociceptive pathways of the central nervous system (CNS) are increased in response to persistent nociceptive input. This sensitization is a manifestation of the remarkable plasticity of the somatosensory nervous system in response to activity, inflammation, and neural injury (Del Valle, Schwartzman, & Alexander, 2009; Latremoliere & Woolf, 2009). However, the extensive range and complexity of symptoms accompanying pain, including neurological symptoms, indicate that not only central sensitization, but also a broader disorder of the CNS afflicting multiple brain systems may be present in CRPS (Jänig & Baron, 2003; Maihofner et al., 2007).

1.1.1 Imaging of Central Nervous System Involvement in CRPS

Previous evidence from CRPS studies using functional magnetic resonance imaging (fMRI) to study CNS contributions, has shown morphological and functional alterations localized in different regions of the sensorimotor network, such as the primary somatosensory cortex (Lenz et al., 2011; Pleger et al., 2004), and the primary motor cortex (Gieteling et al., 2008; Maihofner et al., 2007). Past research has mainly elucidated the cortical and thalamic contributions to pain and sensorimotor deficits in CRPS (Drummond, 2010). However, little attention has been paid to the role of the basal ganglia (BG) in the CRPS pathophysiology. This is despite the well-known function of the striatum in pain and motor control as well as in related processes such as reward, aversion, and goal-directed behaviours (Borsook et al., 2010). Moreover, the observation of enhanced microglial activity within the striatum suggests that these subcortical structures may be implicated in the CRPS pathophysiology (Jeon et al., 2017). In light of previous findings, the role of the basal ganglia towards facilitating persistent pain and movement-related dysfunction in CRPS has been postulated (Azqueta-Gavaldon et al., 2017). The first publication presented here is,

presumably, the first study that investigates structural and functional abnormalities in the basal ganglia structures in relation to sensorimotor dysfunction in CRPS (Azqueta-Gavaldon et al., 2020).

1.1.2 Neurological Changes across the Lifespan in CRPS

Neuroimaging studies have shown the involvement of different brain areas across diverse age populations in CRPS. Compared with healthy controls, paediatric CRPS patients present with brain structural atrophy in motor, affective, motivational, emotional, cognitive, memory, and fear-related regions (Erpelding et al., 2016). In contrast, structural atrophy in adults appears to be more confined to affective, motivational, and cognitive areas (Barad et al., 2014; Geha et al., 2008). Additionally, resting-state functional connectivity (rsFC) within brain networks and between different a priori specified brain regions is different in paediatric and adult CRPS patients. Indeed, paediatric investigations report hyper-connectivity within the default mode network (Becerra et al., 2014) and amygdala-based connectivity (Simons et al., 2014). Adult patients, on the other hand, are associated with widespread hypoconnectivity patterns in the default mode network (Bolwerk, Seifert, & Maihöfner, 2013) and insula-centred connectivity (Kim et al., 2017). Previous studies explicitly comparing the neural differences in the CRPS brain across age populations are lacking. The second publication presented in this dissertation investigates structural and functional neurological differences between paediatric and adult CRPS patients (Youssef et al., 2019).

1.2 Resting-State fMRI: Measure of Functionality Connectivity of Brain Networks

Functional magnetic resonance imaging uses blood-oxygen-level-dependent signal (BOLD) as a proxy to measure neural activity. The firing of an active neuron relies on energy intake in the form of oxygen. Through a process called the hemodynamic response, blood releases oxygen to the firing neurons at a greater rate than to inactive neurons. The subsequent changes in the levels of oxyhaemoglobin and deoxyhaemoglobin (oxygenated or deoxygenated blood) induced by increased local blood flow can be detected using an MRI scanner (Awojoyogbe & Dada, 2011; Ogawa et al., 1990). As opposed to task-based functional MRI, resting-state functional MRI (rs-fMRI) is acquired in the absence of a stimulus or a task. With rs-fMRI, the spontaneous fluctuations of the BOLD signal at rest are measured (Lv et al., 2018).

Regions of the brain that present with synchronised activity time-series during a specific cognitive function are said to form functional networks. Interestingly, resting-state networks appear to be consistent across different individuals and across different disease states. Moreover, they are consistent and comparable with functional circuits imaged with task-based fMRI (Niazy et al., 2011).

The rs-fMRI paradigm offers several advantages over task-based fMRI, and therefore it has become a widely used tool in research over the last decades. Studies with patients with neurological, neurosurgical or psychiatric diseases who may have difficulty understanding, or executing task instructions can benefit from the rs-fMRI study design. Moreover, with rs-fMRI, several brain networks can be studied at the same time from the same data, without having to set up a different experiment for each system to be explored. The signal to noise ratio in resting-state studies is better than task-based approaches since the task-related fluctuations account only for 20% of the total BOLD activity (Fox & Greicius, 2010). Thus, approximately 80% of the signal is discarded as noise in task-based studies, whereas in rs-fMRI, most of the signal is analysed.

Nevertheless, rs-fMRI suffers from some shortcomings as well. For example, the differences in brain activity across different mental states such as sleep or wake and tired or excited are still unclear. Controlling for these mental states is challenging (Reza Daliri, 2014).

1.2.1 Analysis of Resting-State fMRI data

There are several approaches to process rs-fMRI data. The current methods examine the existence as well as the strength and spatial organisation of functional connections between brain regions. Techniques to analyse rs-fMRI data can be differentiated between model-dependent and model-free methods.

In model-dependent methods, the functional connectivity of a priori defined region of interest (ROI) is analysed. The ROI is typically termed as seed, and therefore this type of analysis is coined seed-based functional connectivity analysis. Selecting seed regions should be based on hypothesis and can be done either by a separate functional localization scan not used for the connectivity analyses, by a priori anatomical regions of interest, or by identifying task-positive regions (Kriegeskorte et al., 2009; Linnman et al., 2013). The most straightforward way to examine the functional connections of the seed regions is by correlating its resting-state time-series against the time-series of all other voxels in the brain (van den Heuvel & Hulshoff Pol, 2010a, 2010b).

In model-free or data-driven methods, there is no need for selecting any area of interest to be analysed. The independent component analysis (ICA) is the most popular data-driven method to assess resting-state functional connectivity. This method uses multivariate decomposition to separate the BOLD signal into a set of independent functional networks. These networks comprise different brain nodes that are temporally correlated (Kiviniemi et al., 2003). Furthermore, ICA analysis allows for the identifications of changes in the resting-state functional connectivity within the identified networks (Fox & Raichle, 2007).

Despite the differences in the analysis of the two approaches, very similar results have been achieved when applied to a group of healthy subjects (Rosazza et al., 2012).

1.3 Resting-State Networks Relevant for the Study of Pain and Motor Function

The resting-state functional networks that are of most relevance for the study of pain are the salience network, the default mode network (DMN), the antinociceptive network, and the motor/somatosensory network.

The dynamic pain connectome framework describes how the interactions of the three former brain systems, namely salience, DMN, and antinociceptive, shape the way an individual experiences pain (Kucyi & Davis, 2015). The dynamic interactions of these three networks underlie spontaneous fluctuations in attention to or away from pain. The salience Network (Figure 1.A) comprises the anterior insula, anterior and middle cingulate cortex (ACC, MCC), temporoparietal junction (TPJ), and dorsolateral prefrontal cortex (DLPFC) (Seeley et al., 2007). This network shows stronger activation when subjects are focusing their attention on a painful stimulus compared to when they are focusing their attention on something else during the presentation of a noxious stimulus. The default mode network (Figure 1.B) consists of the posterior cingulate cortex/precuneus, medial prefrontal cortex, lateral parietal lobe, and areas within the medial temporal lobe (Buckner, Andrews-Hanna, & Schacter, 2008). This network functions in an anticorrelated manner to the salience network. The DMN is active at rest and when attention drifts away from pain (during mind wandering). On the contrary, this network is inhibited when the mind is focused on pain. The antinociceptive system (Figure 1.C) is associated with pain intensity modulation. It includes a hub region in the periaqueductal grey (PAG) of the brainstem, which has a high density of opioid receptors (Millan, 2002). This system exhibits increased functional connectivity between the medial prefrontal cortex and PAG when attention is away from the painful sensation compared to when the mind focuses on pain. There is widespread evidence that the aforementioned networks relevant to spontaneous attentional fluctuations to pain are disrupted in chronic pain (Alshelh et al., 2018; Baliki et al., 2014; Cauda et al., 2009; Fallon et al., 2016).

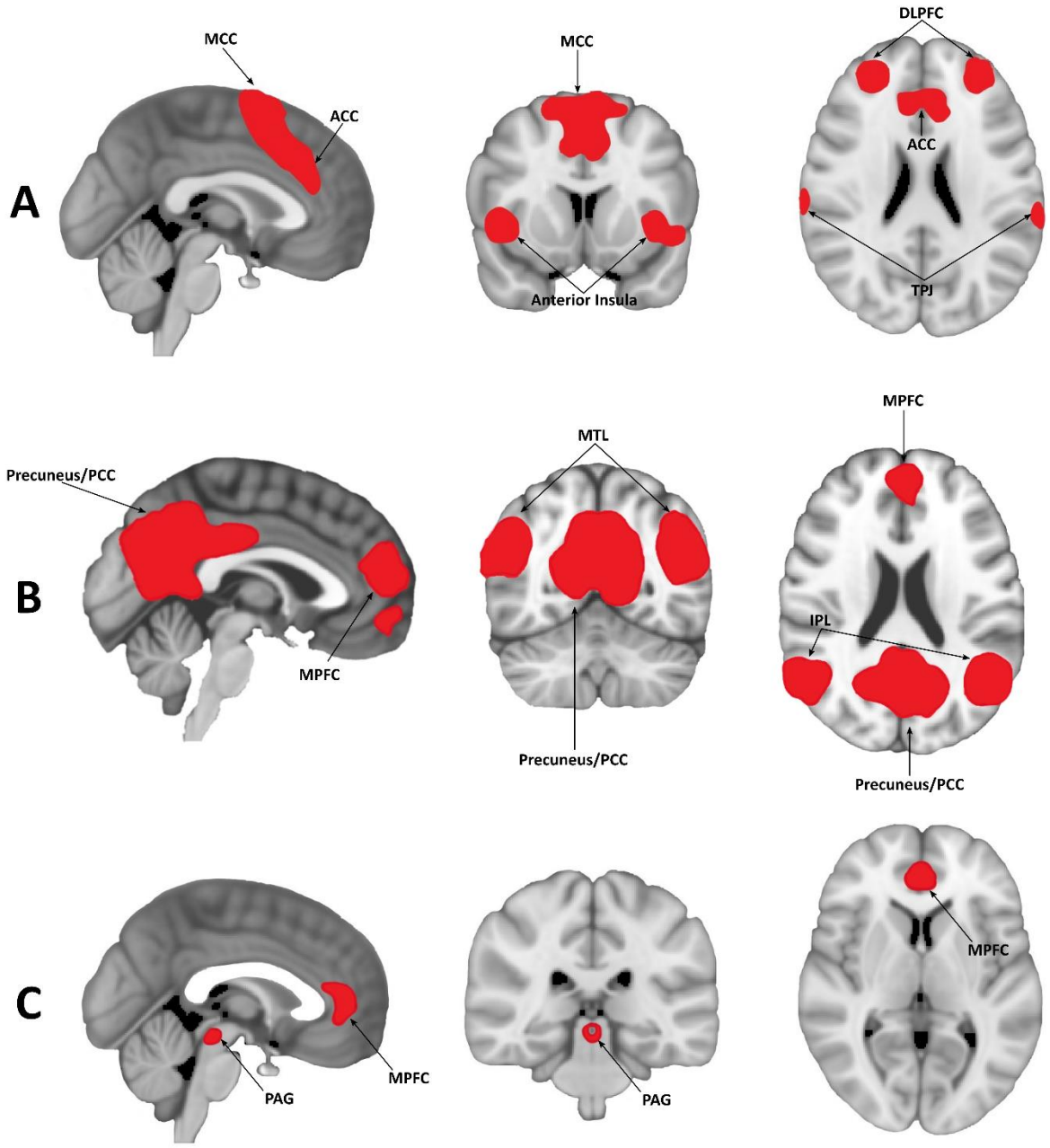

Figure 1. The dynamic relationships between three functional networks, namely the salience network, the default mode network (DMN) and the antinociceptive network shape how individuals experience pain. This framework is called the dynamic pain connectome (Kucyi & Davis, 2015). A) The salience network comprises of the anterior cingulate cortex (ACC), middle cingulate cortex (MCC), anterior insula and temporo-parietal junction (TPJ). B) The DMN comprises of the precuneus and posterior cingulate cortex (PCC), medial prefrontal cortex (MPFC), the medial temporal lobe (MTL) and the inferior parietal lobule (IPL). C) The antinociceptive system consists of the periaqueductal grey (PAG) and the medial prefrontal cortex (MPFC).

The somatotopic and motor networks represent the sensorimotor aspects of pain and play an essential role in the encoding of pain. These networks have been shown to be altered in chronic pain. Moreover, these networks are of particular interest in CRPS, since these patients

show significant sensory and motoric disabilities that may not always be present in other chronic pain conditions. The somatosensory network is in charge of the encoding of the intensity and spatial acuity of sensory perception, as well as proprioception. It comprises of several cortical and subcortical structures forming different pathways involved in different functions. The primary somatosensory cortex (S1) receives dense afferent input from the thalamus. Interactions between these structures result in the sensation of position, size and texture (Case et al., 2016; Geyer, Schleicher, & Zilles, 1999). The secondary somatosensory cortex (S2) is involved in specific touch perception and is thus integrally linked with the amygdala and hippocampus to encode and reinforce memories (Bauer, Barrios, & Díaz, 2014; Eickhoff et al., 2006). The insular cortex plays a role in the sense of bodily-ownership, bodily self-awareness, and perception (Tsakiris et al., 2007). Nodes within the parietal cortex such as the precuneus and the superior parietal lobe (SPL) are involved in locating where objects are in relation to parts of the body (Figure 2.A). The motor network is implicated in motor control and there is an established relationship between pain and motor function (Mercier & Léonard, 2011). The brain nodes forming the motor network are the primary motor cortex, the supplementary motor area, the lateral premotor cortex, the inferior frontal junction, the putamen, the thalamus and cerebellum (Figure 2.B). Both networks work in synchrony forming a larger network, namely, the sensorimotor network.

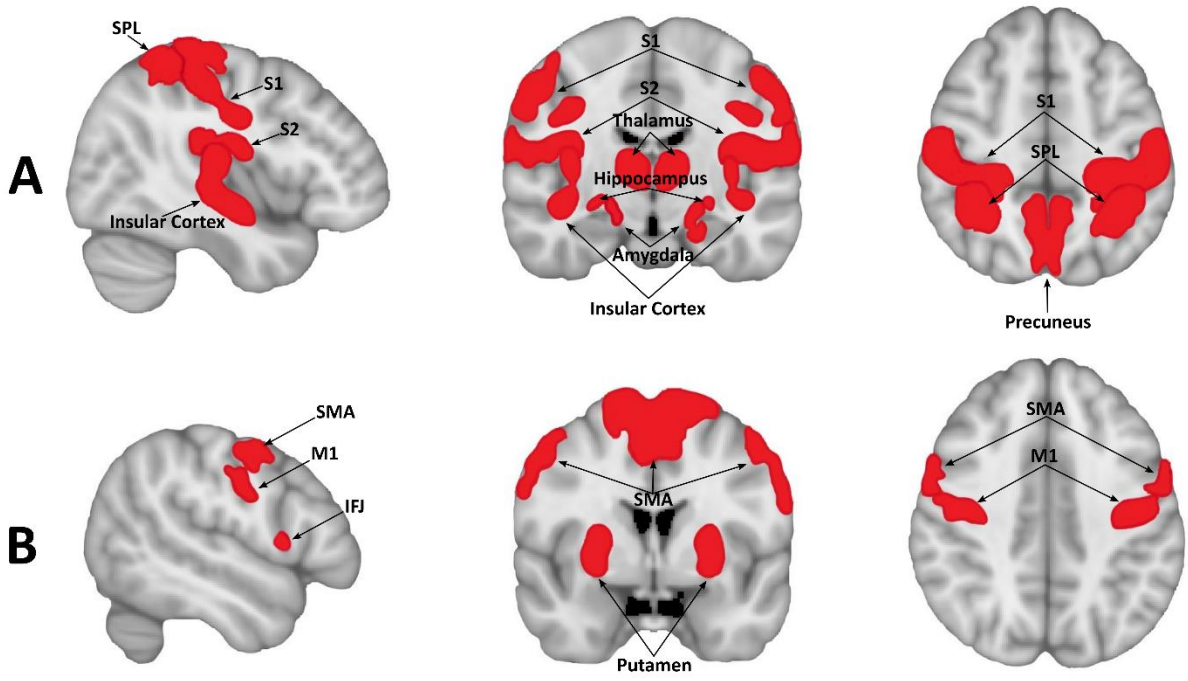

Figure 2. A) The somatosensory network plays a substantial role in perception, the sensory-discriminative aspects of pain and sensory integration. It comprises of the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), superior parietal lobule (SPL), insular cortex, precuneus, thalamus, hippocampus and amygdala. B) The motor network is implicated in motor control and there is a stablished relationship between pain and motor function. The motor network comprises the primary motor cortex (M1), the supplementary motor cortex (SMA), the inferior frontal junction (IFJ) and the putamen.

1.4 Role of the Basal Ganglia and Thalamus in Pain and Motor Function

Human neuroimaging studies have provided an improved characterisation of the anatomical structure and the functionality of the subcortical structures such as the basal ganglia and the thalamus, as well as the interaction within each other and other cortical regions (Alexander, 1986). Widespread nodes of the cerebral cortex are coupled to the above-mentioned subcortical structures forming the cortico-basal ganglia-thalamo-cortical loops (Figure 3). The loops involve connections between the cortex, the basal ganglia, the thalamus, and back to the cortex. These circuits are of particular relevance in movement, cognition, reward, and emotional processing (Di Martino et al., 2008), which are functional domains relevant for pain as well.

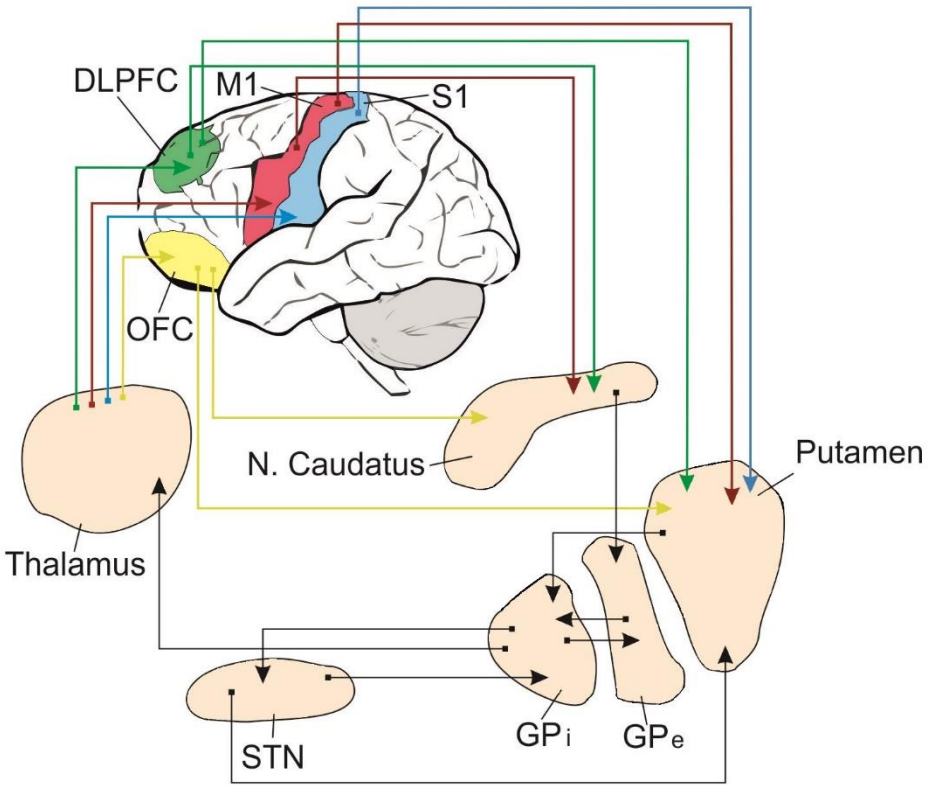

Figure 3. General, non-function-specific overview of cortical-BG-thalamic loops. Spatially dispersed cortical information from the dorsolateral prefrontal cortex (DLPFC), primary motor (M1), primary somatosensory cortex (S1) and the orbitofrontal cortex converges into different regions of the caudate nucleus and the putamen. Through other subcortical regions such as the internal and external globus pallidus (GPi, GPe), the subthalamic nucleus (STN) and the thalamus, the information diverges back to the cortex.

The basal ganglia comprise of the putamen, the caudate nucleus, the nucleus accumbens, the globus pallidus, and the subthalamic nucleus (Figure 4). Each structure has a very characteristic activation response to diverse noxious stimuli in both acute and chronic states (Borsook et al., 2010).

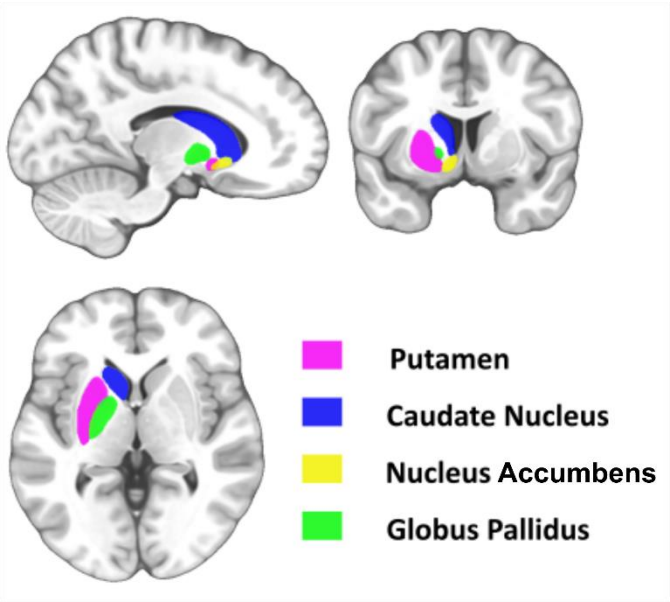

Figure 4. Main structures comprising the basal ganglia.

1.4.1 Putamen

The putamen is an important structure in analgesics given its high density of μ-opioid receptors (Upadhyay et al., 2012). It is implicated in distinct circuits of pain processing such as sensorimotor/sensory-discriminative and reward/reinforcement networks (Borsook et al., 2010) and presents with decreased activation after treatment/symptomatic reduction in CRPS (Becerra et al., 2015). The putamen plays a crucial role in motor control and sensory integration (Hening, Harrington, & Poizner, 2009).

1.4.2 Caudate Nucleus

The caudate nucleus is part of the pain modulatory system (Freund et al., 2009). It presents with higher activation with nociceptive stimulation (Freund et al., 2010) and reduction after treatment in CRPS (Becerra et al., 2015). Moreover, the caudate nucleus is involved in the smooth orchestration of motor actions (Shroff, 2011).

1.4.3 Nucleus Accumbens

The nucleus accumbens is a key component in the emotional and affective aspects of pain (Mansour et al., 2014). Grey matter volume of this structure, as well as its structural connectivity with the insula are both reduced in CRPS (Geha et al., 2008). It is involved in the cognitive processing of motor function related to reward and reinforcement (Sesack & Grace, 2010). The nucleus accumbens is heavily connected with key areas in emotional processing such as amygdala and prefrontal cortex. Moreover, this structure has been implicated in the placebo response (Scott et al., 2008).

1.4.4 Globus Pallidus

The globus pallidus has been demonstrated to play a role in normal motor behaviour and presents with somatotopic characteristics (Baker et al., 2010). Deep brain stimulation of this area has been reported to mitigate pain (Loher et al., 2002).

1.4.5 Thalamus

The thalamus is involved in the sensory discriminative and affective motivational components of pain (Ab Aziz & Ahmad, 2006). In paediatric CRPS, the thalamic nuclei exhibit volumetric differences in comparison with healthy controls. These changes normalise after treatment (Erpelding et al., 2016). The thalamus is implicated in movement control and motor learning since it is an important input and output node between motor areas of the cerebral cortex and motor-related subcortical structures (Bosch-Bouju, Hyland, & Parr-Brownlie, 2013). The ventral posterolateral nucleus of the thalamus has also shown to have a somatotopic organisation (Hong, Kwon, & Jang, 2011).

1.5 Aim of the Studies

The aim of this thesis is three-fold. First, given the motor dysfunction widely present in CRPS patients, we aim to elucidate the connection of such motor dysfunction with morphological and resting-state functional properties of the brain areas of the sensorimotor network with distinct attention to the striatum. With this purpose in mind, we evaluate a cohort of adult chronic CRPS patients with pathology unilaterally localized to the right hand or to the entire arm. Here, we hypothesize that striatal alterations may not only affect pain processing, but they may also be implicated in movement disorders in CRPS.

Secondly, we wish to determine the effects of the pain progression (acute vs chronic) in structural and resting-state functional properties in paediatric CRPS patients with pathology localized to the lower limb. Here, we hypothesize that the chronic group may display greater grey matter and functional alterations which correlate with the duration of the disease.

In addition, we wanted to determine the regional somatotopy in areas of the sensory system, namely the thalamus and sensory cortex. To do so, we evaluate resting-state functional properties in paediatric chronic CRPS patients affected in the leg in comparison to adult chronic CRPS patients with pathology in the arm. Here, we our hypothesis is that there might be a distinct somatotopic organisation of the upper or lower limb in the ventral posterolateral thalamic nucleus or the primary sensory cortex. This somatotopic organisation shows differences in CRPS patients when compared with healthy controls in both populations.

It should finally be mentioned that in all our studies we used resting-state functional connectivity, as well as grey matter volumetric analysis (VBM) to elucidate our hypotheses.

1.6 Own Contribution to the Studies

Several steps were carried out to produce two publications. These two papers were the result of a close collaboration between our team at the University Hospital of Munich (LMU) and the Center for Pain and the Brain at the Boston Children's Hospital. My tasks during these investigations are described in the following sections.

1.6.1 Publication I: Implications of the Putamen in Pain and Motor Deficits in CRPS

This first paper was mainly my responsibility: I developed it under the supervision of Dr E.K.

Study design: The concept of the study was already drafted by Dr E.K. I, however, had first to specify the eligibility criteria for the selection of the participants in the study, both CRPS patients and healthy volunteers. The right choice of participants was crucial in that we wanted to be sure that no exogenous variable would contaminate the accuracy of the statistical exercise. In agreement with my supervisor Dr E.K and Prof. B. E-W, a neuroradiologist at the University Hospital of Munich (LMU), we decided to set the eligibility criteria so that we would analyse a very homogenous group. We chose participants to be comparable in terms of handiness and place of the injury, age, and hormonal stage (the menopausal stage in case of female participants) and any other neurological pathologies. This way, we could ensure that any differences observed between patients and healthy participants, would be most likely due to the disease and not to any other factors uncontrolled for. Second, and in agreement with the technical personnel from the Department of Radiology at the University Hospital of Munich (LMU), I selected the MRI and fMRI acquisition protocols best suited to appreciate resting-state activity within the basal ganglia structures.

Data acquisition: Several preparatory steps were required for the successful completion of the data acquisition process. Firstly, we needed to recruit patients. This meant, preparing various media to advertise our study: poster and writing in self-help forums on the web, always following what is established and allowed by the ethic commission. Second, once the potentially eligible patients had been identified from the Pain Clinic database, contacting, and interviewing them to assess their eligibility. Third, other logistic steps such as sending the documents (questionnaires and study information) to the patients, keeping track of the status

of our interactions with them in a digital form, scheduling the appointments, etc. Lastly, matching the 20 healthy controls to the 20 CRPS patients according to their age and sex. During the data acquisition process itself, I was responsible for supervising every MRI scanned and assisting my colleague C.S., occupational therapist, when examining the motor function of each patient and healthy volunteer.

Data analysis: I performed both the analysis of the motor function assessment as well as most of the analysis of the neurological functional connectivity. The motor function test was a very comprehensive assessment that is widely used in the clinical practice and allows the physician to have a semi-quantitative but mostly qualitative evaluation of the patient. I simplified each test into simpler categorical scores to make them able to be analysed using inference statistics to compare both participants populations. Moreover, I carried out the statistical analysis (descriptive and inference) of the clinical and motor function data. I used the appropriate statistical methods according to the nature of the data (Fisher Test for the categorical data and Mann-Whitney Test for the continuous non-normally distributed data).

Furthermore, I performed the pre-processing and data scrubbing of the functional fMRI. I relied on the Independent Component Analysis methodology to detect physiological noise and detected and removed high spikes in the time-series to eliminate motion artefacts. Finally, I performed the seed-based functional connectivity analysis of the single-subject data, as well as the group comparison and correlations, and potential interactions with the clinical data. For this purpose, I used an ANOVA analysis. A more detailed explanation of such analysis is described in the summary of the published paper, section 2.1.

Data interpretation: Together with Dr E.K and Dr J.U of the Center for Pain and the Brain at the Boston Children's Hospital, I Interpreted the data. Along the ensuing process, quite an amount of information was discarded because it was considered irrelevant, and we were able to better calibrate our initial hypothesis with the remaining data.

Manuscript: I drafted the entire paper as well as the abstract. Moreover, I designed and implemented all the figures and tables, except Supplementary Figure 2.

As the corresponding author, I submitted the manuscript to the journal and answered to all the reviewers' comments.

1.6.2 Publication II: Shifting Brains in Pain Chronicity

Dr A.Y, under the closed supervision of Dr D.B (corresponding author), was the prime responsible for this publication. I, as the second author, had the following tasks:

Study design: Realising the lack of neuroimaging studies that compare paediatric with adult CRPS patients, I thought it would shed some light in understanding CRPS pathology, carrying out such comparative study. Children's brain is more plastic than adults' brain. I thought that having such difference in brain plasticity, we would also see how CRPS affects or it is processed differently in children's brain than in adults. During my stay in Boston, I shared this enthusiasm with Dr A.Y and Dr D.B. In the brainstorming that followed, we determined the specific hypothesis we wanted to test and the ways to do it. Here, as in the previous publication mentioned above (section 1.6.1), the selection of the study participants was of crucial importance to avoid false causalities in our findings. In the case of the adult data, which I provided, that was my responsibility.

Data acquisition: The colleagues at the Center for Pain and the Brain at the Boston Children's Hospital provided the data regarding the paediatric population, whereas I did so for the case of the adult population. For this purpose, I relied, on the data acquisition process explained in the previous article. Due to the different scope of the research project, I both refined the sample and the variables to be analysed to make it more suited for our purpose. In this second exercise, we only used fMRI data, and we discarded most of the motor function test data.

Data analysis: To prepare the data for the statistical analysis, I performed the pre-processing and motion scrubbing of the adult fMRI data. I used Independent Component Analysis methodology to detect physiological noise and detected and removed high spikes in the time-series to eliminate motion artefacts. Regarding the data analysis, Dr A.Y and Dr D.Z carried out the analysis of fMRI data as well as the statistical analysis. I was aware of their progress along the process and gave my comments when I considered it necessary.

Data interpretation: It was rewarding to see that our main hypothesis was not rejected. After close examination at the statistical exercise, I found it to be robust, and I provided such feedback in the discussions held with my colleagues.

Manuscript: apart from having read the paper and pointing out some minor corrections, I wrote the description of the adult sample data, both in terms of its composition and the fMRI protocol used. Moreover, I also provided table II of the manuscript, which describes the clinical picture of the adult patients of the study.

2 Summaries

2.1 Implications of the Putamen in Pain and Motor Deficits in CRPS

Complex Regional Pain Syndrome (CRPS) is a still poorly understood and high disabling pain disorder. In addition to pain, a substantial proportion of those affected also exhibit motor-related co-morbidities such as dystonia, rigidity, and reduced range of motion. These motor symptoms limit a patient's functionality and overall quality-of-life. Prior neuroimaging investigations have highlighted several cortical regions with divergent functional and structural properties in CRPS. Considering the role of the basal ganglia (BG) in pain mediation and movement, together with the presence of pain and motor deficits in CRPS, we hypothesized that abnormalities in the BG might underlie clinical symptoms in CRPS.

To test the above hypothesis, we selected 20 right-handed chronic (disease duration longer than 6 months) patients possessing unilateral upper limb CRPS pathology (n = 20, 15 female; age 58 ± 9 years). Moreover, 20 well-matched healthy controls were also evaluated (n = 20, 15 female; age 58 ± 9 years). We implemented a comprehensive clinical phenotyping of each study participant's pain, motor function, and medical history. In addition, all subjects underwent a cross-sectional examination with structural magnetic resonance imaging (MRI) and resting-state functional MRI (rs-fMRI). A voxel-based morphometry (VBM) analysis carried out using SPM12 (Statistical Parametric Mapping) was employed to detect group differences in grey matter density (GMD) of the BG structures. After pre-processing and data scrubbing, we performed a seed-based connectivity analysis with rs-fMRI data using the FSL toolbox (Functional Magnetic Resonance Imaging of the Brain Software Library). In our analyses, all results were corrected using cluster-correction with a z-value > 2.3 and P-value < 0.05, and where necessary, corrected for multiple comparisons.

A battery of functional tests revealed substantial motor deficits in the affected hand of CRPS patients relative to healthy controls. These bilaterally executed motor assessments demonstrated that CRPS patients harboured significant abnormalities in hand coordination, dexterity, and strength.

From a neuroimaging perspective, we detected significantly decreased GMD in the putamen in CRPS patients in comparison to healthy controls. Given the changes in putaminal volume observed in CRPS patients, we subsequently sought to determine the presence of

concomitant functional adaptations. To do so, we used resting-state functional connectivity (rsFC) analyses involving the right and left side of the putamen. Here, CRPS patients, relative to healthy controls, showed greater ipsilateral rsFC between the right putamen and pre-/postcentral gyri. Moreover, decreased rsFC occurred between the right putamen and cerebellar regions. These differences observed between CRPS and healthy volunteers may be driven by greater use of the non-affected hand in CRPS patients. Similar functional interactions between the putamen and pre-/postcentral gyri were further observed in analyses where pain and motor impairments were utilized as regressors of interests. For example, in CRPS patients, higher spontaneous pain, as well as higher motor impairment (determined by the 9-Hold-Peg test), were correlated with rsFC strengths between the putamen and the contralateral pre-/postcentral gyri, where sensory and motor processing is localized.

In summary, this investigation reveals that pain and motor-related abnormalities of the affected hand of CRPS patients correlates with structural alterations of the putamen as well as with its functional interactions with cortical sensorimotor network structures. Based on these results, we propose a framework in which functional changes involving the putamen reflect an adaptive response to maintain adequate motor functionality. Furthermore, the structural changes of the putamen could explain a loss of motor function.

2.2 Shifting Brain Circuits in Pain Chronicity

It is well-established that patients with complex regional pain syndrome (CRPS) have altered brain structure and functional circuitry. However, while reduced grey matter density (GMD) appears to be consistently reported across brain sites, resting-state functional connectivity (rsFC) reveals different patterns between paediatric and adult populations. These differences may reflect age-related effects, or disease duration, or both. Indeed, there is evidence of a shift from sensory to emotional networks with disease duration. Given these facts, we aimed to compare GMD and rsFC metrics between (1) paediatric acute patients and chronic (disease duration longer than 6 months) CRPS patients with pain in the lower extremity; (2) paediatric chronic CRPS patients and healthy controls; (3) adult chronic CRPS patients with pain in the upper extremity and healthy controls. Moreover, we intended to explore potential somatotopically-related alterations in regions such as the thalamus.

We recruited 52 patients and 52 well-matched healthy controls for the study. The patient group consisted of a paediatric group suffering from an acute ankle sprain injury (n = 16, 10 females; age: 15.8 ± 0.6 years); a paediatric group with chronic CRPS of the lower extremity (n = 16,10 females; age: 14 ± 0.6 years) and an adult group with chronic CRPS of the upper extremity (n = 20, 15 female; age: 57.9 ± 9 years). All subjects lay supine on the 3T MRI scanner with eyes closed. A T1-weighted anatomical image to determine GMD alterations and T2*-weighted BOLD contrast images to investigate rsFC changes were collected. We used SMP12 (Statistical Parametric Mapping) to analyse both structural and rs-fMRI data. GMD maps were created with the help of the Computational Anatomy Toolbox. Subsequently, image pre-processing and seed-based functional connectivity maps were created with the Functional Connectivity (CONN) Toolbox. In our analyses, significant differences were determined using an a priori primary threshold of $p < 0.001$, followed by applying a FEW (family wise error) cluster-level extent threshold to correct for multiple comparisons.

Compared with controls, chronic paediatric patients had significantly reduced GMD within the inferior temporal gyrus (ITG), the orbitofrontal cortex (OFC), the anterior cingulate cortex (ACC), the mid-cingulate cortex (MCC), and thalamic reticular nucleus (TRN). Furthermore, an increase was observed within the ventral posterolateral nucleus (VPL). On the other hand, acute pain patients presented with increased GMD within the ITG and OFC. Analogous differences were observed when the chronic group was compared with the acute

group. Compared to controls, chronic adult patients had significantly decreased GMD within the VPL, and increased rsFC within the ACC.

From a neuroimaging perspective, paediatric chronic patients presented with reduced rsFC strengths between the VPL and the postcentral gyrus or somatosensory cortex (S1). The paediatric acute patients showed increased rsFC strengths between the OFC and the hippocampus in comparison to controls. Adult chronic patients showed decreased rsFC between the VPL and the posterior cingulate cortex and increased rsFC strengths between the ACC and S1. Moreover, we reported structural alterations within VPL and functional alterations within the S1. These findings are consistent with upper and lower limb somatotopy (i.e., upper-to-lower limb: VPL, medial-to-lateral; S1, anterolateral to posteromedial).

These data show a shift in primarily concurrent grey matter atrophy and differential patterns of brain functional connectivity in paediatric patients relative to adult population. Furthermore, our data reveal a shift from sensory alterations in paediatric populations to sensory-emotional alterations in adult populations that are consistent with well-established somatotopic organisation.

3 Zusammenfassungen

3.1 Auswirkungen des Putamens auf Schmerz und motorische Defizite beim CRPS

Das komplexe regionale Schmerzsyndrom (eng. *Complex Regional Pain Syndrome, CRPS*) ist eine noch immer unzureichend verstandene und stark behindernde Schmerzkrankheit. Ein substanzieller Anteil der Betroffenen weist neben Schmerzen auch motorische Störungen wie Bradykinese, Dystonie, Rigidität und eingeschränkte Beweglichkeit auf. Diese motorischen Symptome schränken die Funktionalität und die allgemeine Lebensqualität der Patienten ein. Bildgebende Forschungsstudien haben mehrere kortikale Regionen mit unterschiedlichen funktionellen und strukturellen Eigenschaften beim CRPS beleuchtet. Unter Berücksichtigung der Rolle der Basalganglien (BG) bei der Schmerzvermittlung und Bewegung, sowie des Bestehens von Schmerzen und motorischen Defiziten beim CRPS, stellten wir die Hypothese auf, dass Dysfunktion der BG an den klinischen Symptomen beim CRPS beteiligt sind (z. B. Schmerzen, eingeschränkter Bewegungsumfang und beeinträchtigte motorische Koordination).

Um diese Hypothese zu überprüfen, wurden 20 rechtshändige chronische (Krankheitsdauer länger als 6 Monate) CRPS-Patienten (N = 20, 15 weiblich; Alter 58 ± 9 Jahre) mit einseitigem CRPS der oberen Extremität ausgewählt. Die Erkrankungsdauer betrug im Durchschnitt 4,9 Jahre. Darüber hinaus wurden 20 gesunde Kontrollen untersucht (N = 20, 15 weiblich; Alter 58± 9 Jahre). Wir führten eine umfassende klinische Phänotypisierung der Schmerzen, motorischen Funktion und Anamnese jedes Studienteilnehmers durch. Darüber hinaus wurden alle Probanden einer Querschnittsuntersuchung mit struktureller Magnetresonanztomographie (MRT) und funktioneller *resting-state*-MRT (rs-fMRT) unterzogen. Mit Hilfe der SPM12-Toolbox (*Statistical Parametric Mapping*) wurde eine Voxel-basierte Morphometrie Analyse (eng. *Voxel Based Morphometry*, VBM) durchgeführt, um Gruppenunterschiede in der Dichte der Grauen Substanz (DGS) in den BG zu erkennen. Mit Hilfe der FSL-Toolbox (*Functional Magnetic Resonance Imaging of the Brain Software Library*) eine *Seed*-basierte Konnektivitätsanalyse mit rs-fMRT-Daten durch. In unseren Analysen wurden alle Ergebnisse mittels Cluster-Korrektur mit einem z-Wert > 2,3 und einem

P-Wert < 0,05 korrigiert und, wenn nötig, wurde das Signifikanzniveau für die multiplen Vergleiche korrigiert.

Die Funktionstestungen zeigten erhebliche motorische Defizite in der betroffenen Hand von CRPS-Patienten im Vergleich zu gesunden Kontrollen. Die motorischen Untersuchungen demonstrierten, dass CRPS-Betroffene signifikante Störungen in der Handkoordination, der Feinmotorik und der Kraft aufwiesen.

Bei der Auswertung der MRT Daten stellten wir bei CRPS -Patienten im Vergleich zu gesunden Kontrollen eine signifikant verringerte DGS im Putamen fest. Angesichts dieser Veränderungen des Putamenvolumens untersuchten wir das Bestehen von begleitenden funktionellen Veränderungen. Dazu untersuchten wir bei den rs-fMRT Aufnahmen die funktionelle Konnektivität (eng. *resting-state functional connectivity*, rsFC) des rechten und linken Putamens. Hier zeigten CRPS-Betroffene im Vergleich zu gesunden Kontrollen eine stärkere ipsilaterale rsFC zwischen dem rechten Putamen und dem prä- und postzentralen Gyri. Darüber hinaus stellte sich eine reduzierte rsFC zwischen dem rechten Putamen und dem Zerebellum dar. Diese Unterschiede zwischen Betroffenen und gesunden Kontrollen können durch den verstärkten Einsatz der nicht-betroffenen Hand bei CRPS-Patienten erklärt werden. Ähnliche funktionelle Interaktionen zwischen dem Putamen und dem prä- und postzentralen Gyri wurden auch in Analysen beobachtet, bei denen Schmerzen und motorische Beeinträchtigungen als erklärende Variablen verwendet wurden. Beispielsweise korrelierten höhere spontane Schmerzen und eine höhere motorische Beeinträchtigung (bestimmt durch den 9-Hold-Peg-Test) mit dem Ausmaß der rsFC-Erhöhung zwischen dem Putamen und dem kontralateralen prä- und postzentralen Gyri. In den letztgenannten Strukturen sind Areale der sensorischen und motorischen Verarbeitung lokalisiert.

Insgesamt zeigte diese Untersuchung, dass Schmerzen und motorische Auffälligkeiten der betroffenen Hand von CRPS Patienten mit strukturellen sowie mit funktionellen Veränderungen des Putamens korrelieren. Gleichzeitig fanden wir Korrelationen zwischen den funktionellen Interaktionen des Putamens mit kortikalen sensomotorischen Netzwerkstrukturen. Auf Grund dieser Ergebnisse lässt sich vermuten, dass funktionelle Veränderungen des Putamens eine adaptive Anpassung zur Aufrechterhaltung einer adäquaten motorischen Funktionalität darstellt. Darüber hinaus könnten die strukturelle Veränderungen des Putamens einen Verlust der motorischen Funktion erläutern.

3.2 Neuordnung der Gehirnnetzwerken im Verlauf von chronischen Schmerzen

Studien haben gezeigt, dass sich Struktur und funktionelle Netzwerke des Gehirns bei Personen mit komplexem regionalem Schmerzsyndrom (eng. *Complex Regional Pain Syndrome*, CRPS) verändern. Eine reduzierte Dichte der Grauen Substanz (DGS) in verschiedenen Gehirnregionen konnte in Erwachsenen und Kindern demonstriert werden. Jedoch scheint sich die funktionelle Konnektivität im Ruhezustand (eng. *resting-state functional connectivity*, rsFC) bei pädiatrischen und erwachsenen Personen zu unterscheiden. Diese Unterschiede könnten aufgrund des Alters, der Krankheitsdauer oder beiden Faktoren auftreten. Es gibt Hinweise darauf, dass sich mit zunehmender Krankheitsdauer die sensorischen Netzwerke zugunsten der emotionalen Netzwerke verschieben. Angesichts dieser Fakten untersuchten wir die DGS- und rsFC-Messwerte zwischen (1) pädiatrischen akut und chronischen (Krankheitsdauer länger als 6 Monate) CRPS Patienten mit Schmerzen der unteren Extremität; (2) pädiatrischen chronischen CRPS Patienten und gesunden Kontrollen; (3) erwachsenen chronischen CRPS Patienten mit Schmerzen in der oberen Extremität und gesunden Kontrollen. Zusätzlich lag der Fokus auf möglichen somatotopisch bedingten Veränderungen in Regionen wie des Thalamus.

Wir rekrutierten 52 Patienten und 52 gesunde Kontrollen für die Studie. Die Patientengruppe bestand aus einer pädiatrischen Gruppe mit einer akuten Sprunggelenkverstauchung (N = 16, 10 weiblich; Alter: 15,8 ± 0,6 Jahre); einer pädiatrischen Gruppe mit chronischem CRPS der unteren Extremität (N = 16, 10 weiblich; Alter: 14, ± 0,6 Jahre) und einer erwachsenen Gruppe mit chronischem CRPS der oberen Extremität (N = 20, 15 weiblich; Alter: 57,9 ±9 Jahre). Ein T1-gewichtetes anatomisches Bild zur Bestimmung der GMD-Veränderungen sowie T2*-gewichtete Aufnahmen zur BOLD-Kontrastbilder zur Untersuchung der rsFC-Veränderungen wurden aufgenommen. Wir verwendeten SPM12 (*Statistical Parametric Mapping*), um sowohl strukturelle als auch rs-fMRT-Daten zu analysieren. DGS-Karten wurden mit Hilfe der *Computational Anatomy Toolbox* erstellt. Anschließend wurden mit der Functional *Connectivity (CONN) Toolbox* Bildvorverarbeitungs- und *Seed*-basierte funktionale Konnektivitätskarten erstellt. Unsere Analysen demonstrierten signifikante Unterschiede mit einer a priori Schwelle von P-Wert < 0,001, gefolgt von der

Anwendung einer FWE (eng. *Family Wise Error*) Cluster-level Schwelle zur Korrektur für multiple Vergleiche.

Im Vergleich zu den Kontrollen hatten pädiatrische Patienten mit chronischem CRPS eine signifikante Reduktion der DGS innerhalb des inferioren temporalen Gyrus (ITG), des orbitofrontalen Kortex (OFC), des anterioren zingulären Kortex (ACC), des mittleren zingulären Kortex (MCC) und des thalamischen retikulären Nukleus (TRN). Darüber hinaus wurde eine Zunahme innerhalb des ventralen posterolateralen Nukleus (VPL) im Thalamus beobachtet. Bei den akuten pädiatrischen Patienten wurde jedoch eine erhöhte DGS innerhalb der ITG und OFC gefunden. Ähnliche Unterschiede wurden beim Vergleich der pädiatrischen chronischen mit der akuten Gruppe beobachtet. Im Vergleich zu den Kontrollen hatten die chronischen erwachsenen Patienten innerhalb der VPL eine signifikant verringerte GMD und eine erhöhte rsFC innerhalb der ACC.

Die pädiatrischen chronischen Patienten zeigten eine reduzierte rsFC zwischen der VPL und dem postzentralen Gyrus oder somatosensorischen Kortex (S1). Die pädiatrischen Akutpatienten hatten im Vergleich zu den Kontrollen erhöhte rsFC-Werte zwischen dem OFC und dem Hippocampus. Erwachsene chronische Patienten wiesen eine verringerte rsFC zwischen der VPL und dem hinteren zingulären Kortex und erhöhte rsFC-Stärken zwischen dem ACC und S1 auf. Darüber hinaus sahen wir strukturelle Veränderungen innerhalb der VPL und funktionelle Veränderungen innerhalb der S1. Diese Befunde stimmen mit der Somatotopie der oberen und unteren Extremitäten überein (d. h. von der oberen zur unteren Extremität): VPL, medial-lateral; S1, anterolateral bis posteromedial).

Unsere Daten demonstrieren eine Verschiebung der Veränderungen der grauen Substanz und der differentiellen Muster der Konnektivität bei pädiatrischen Patienten im Vergleich zu erwachsenen Populationen. Darüber hinaus beobachteten wir Verschiebungen von sensorischen Veränderungen in pädiatrischen Populationen hin zu sensorisch-emotionalen Veränderungen in erwachsenen Populationen, die mit der gut etablierten somatotopischen Organisation übereinstimmen.

4 Publications

4.1 Publication I

Implications of the Putamen in Pain and Motor Deficits in CRPS

Azqueta-Gavaldon, **M.**, Youssef, A. M., Storz, C., Lemme, J., Schulte-Göcking, H., Becerra, L., Azad, S. C., Reiners, A., Ertl-Wagner, B., Borsook, D., Upadhyay, J., & Kraft, E.

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Implications of the putamen in pain and motor deficits in complex regional pain syndrome

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Abstract

Complex regional pain syndrome (CRPS) develops after limb injury, with persistent pain and deficits in movement frequently co-occurring. The striatum is critical for mediating multiple mechanisms that are often aberrant in CRPS, which includes sensory and pain processing, motor function and goal-directed behaviors associated with movement. Yet much remains unknown with regards to the morphological and functional properties of the striatum and its sub-regions in this disease. Thus, we investigated 20, patients (15 female, age 58 ± 9 years, right-handed) diagnosed with chronic (6+ months of pain duration) CRPS in the right hand and 20 matched, healthy controls with anatomical and resting-state, functional magnetic resonance imaging (fMRI). In addition, a comprehensive clinical and behavioral evaluation was performed, where each participant's pain, motor function and medical history were assessed. CRPS patients harbored significant abnormalities in hand coordination, dexterity and strength. These clinical pain and movement-related findings in CRPS patients were concomitant with bilateral decreases in gray matter density in the putamen as well as functional connectivity increases and decreases amongst the putamen and pre-/postcentral gyri and cerebellum, respectively. Importantly, higher levels of clinical pain and motor impairment were associated with increased putamen-pre-/postcentral gyri functional connectivity strengths. Collectively, these findings suggest that putaminal alterations, specifically the functional interactions with sensorimotor structures, may underpin clinical pain and motor impairment in chronic CRPS patients.

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Keywords

Complex Regional Pain Syndrome; motor dysfunction; chronic pain; putamen; sensorimotor network

1 Introduction

Complex Regional Pain Syndrome (CRPS) is a pain disease that ensues following injury to an extremity [8]. The pathophysiological mechanisms that hinder a normal resolution of limb injury or unfold in CRPS phenotype are poorly understood. However, the symptoms that patients present are well-characterized, including spontaneous and movement-induced pain, sensory deficits, trophic abnormalities, and autonomic dysregulation [18]. During acute stages, soft-tissue edema, disturbed sympathetic function and movement limitation are observed. In chronic stages, motor deficits may become more defined [43], including reduced range of motion, joint stiffness, muscle weakness, tremor, dystonia, and irregular myoclonus jerks [66,67,79,91]. These movement disorders together with the observation that motor tasks that cannot be performed actively, can be executed when the affected limb is passively moved, point to a central component underlying movement-related symptomatology in CRPS [56].

Neuroimaging techniques have been used to study brain alterations in chronic pain [4,28,62]. Due to methodological and clinical divergence, the findings are difficult to summarize into consistent results. However, with respect to resting-state functional magnetic resonance imaging (rsfMRI) there is converging evidence of the disruption of the Default Mode Network (DMN) across chronic pain diseases such as low back pain [2,10], fibromyalgia [68], neuropathic diabetic pain [24], knee osteoarthritis [10] and CRPS [10,19]. Components within the DMN that exhibited changes included the medial prefrontal cortex, precuneus and lateral parietal regions. Areas outside this network such as anterior cingulate cortex, anterior insula and supramarginal gyrus also presented with alterations. Overall, changes within the DMN could be interpreted as alterations of brain function related to chronic pain, but not specific to CRPS.

Previous fMRI studies addressing pain and movement disorders in CRPS showed morphological and functional alterations localized to the primary somatosensory cortex [53,74], as well as sensorimotor network regions such as pre-and postcentral gyrus [37,56]. Past research has also focused on the cortical and thalamic contributions to pain and sensorimotor deficits in CRPS [30]. However, there has been little focus on striatal structures. This is despite the known function of the striatum in pain and motor control as well as in related processes such as reward, aversion and goal-directed behaviors [21]. Neuroanatomical evidence shows that the basal ganglia (BG), cerebellum and cerebral cortex form an integrated and topographically organized network [23]. The motor, cognitive and affective territories of each node in this network are interconnected. These observations stress the importance of subcortical contributions to neuroplasticity in chronic pain.

The role of the striatum towards facilitating persistent pain and movement-related dysfunction in CRPS was recently postulated [6]. To date, structural and functional

abnormalities in BG structures have not been studied in relation to motor dysfunction in CRPS. Therefore, in this MRI-based investigation, we aimed to elucidate the morphological and functional properties of areas within the sensorimotor network with distinct attention to the striatum in a cohort of adult chronic CRPS patients with pathology unilaterally localized to the right upper limb. We hypothesize that striatal alterations do not only affect pain processing but are also implicated in movement disorders in CRPS.

2 Material and Methods

This study was approved by the Ethics Committee of the Ludwig Maximilian University of Munich (Germany) and met the Helsinki criteria for the study of pain in humans. All subjects read and signed a written informed consent prior to study participation. Patients were recruited from the Interdisciplinary Pain Unit at the University Hospital LMU and from the Department of Rehabilitation Medicine of the City Hospital Bogenhausen in Munich (Germany). Clinical screening and MRI procedures were carried out on the same day and took place at the Department of Orthopedics, Physical Medicine and Rehabilitation and at the Institute for Clinical Radiology of the University Hospital LMU Munich, respectively. Patient recruitment and screening was carried out from April 2016 to October 2017.

2.1 Study Participants

A total of 32 potentially eligible individuals possessing CRPS were assessed for eligibility and 22 patients gave consent to participate in the study. Imaging data from two patients were discarded due to observed brain lesions. Therefore, 20 (15 females; 57.9 ± 9 years old), right-handed, chronic (6+ month of pain duration) CRPS patients unilaterally affected on the right hand or arm and right-handed, gender and age-matched healthy control subjects (N=20; 15 females; 58.5 ± 10 years old) participated in this study. The enrolled patient cohort met the Budapest clinical diagnostic criteria for CRPS [41] for chronic stage and were examined by a neurologist (E.K.). Exclusion criteria included suffering from another chronic pain condition, metabolic disorders (i.e., diabetes or hypertension), severe psychiatric comorbidities, addiction, or having any contraindications for undergoing MRI procedures.

2.2 Psychophysical Evaluation

All clinical tests were performed by an occupational therapist (C.S.) under minimal distraction in a silent room, with an ambient temperature of 25–26°C. Subjects were seated on a comfortable chair and allowed to adapt to the test environment for at least 10 minutes.

2.2.1 Motor Function Evaluation—A battery of motor behavioral tests was implemented to evaluate bilateral motor function in all chronic CRPS patients and healthy controls.

9-Hole Peg Test: Finger and hand coordination was assessed with the 9-Hole Peg Test [63], where after a trial round, participants were instructed to complete the test as fast as possible and the time needed for completion was recorded.

Forearm Range of Motion (RoM): The angular velocity (ω) in degrees/s of the forearm RoM was measured with a 3D motion tracker system based on an ultrasound probe CMS-10 (Zebris Medical GmbH, Isny Germany). During this test, patients were instructed to perform a forearm rotation at their maximum range of movement and velocity during which pronation and supination movements were recorded across a 30-second period. To account for intra-subject variability, a symmetry index (RoM_{SI}) of the angular velocity that determines side performance dominance was computed ($\omega_{right}/\omega_{left}$), where $RoM_{SI} > 1$ indicates better performance of the dominant/affected hand; $RoM_{SI} < 1$ corresponds to better performance of the left hand; and $RoM_{SI} = 1$ indicates total symmetry or equal performance between both hands.

Rigidity: The rigidity of the upper limbs was evaluated following the Unified Parkinson's Disease Rating System (UPDRS-III) measurement for rigidity [38]. Scores for each individual limb ranged from 0 – 3. The total rigidity score accounting for both limbs was calculated by the sum of the two individual scores (range 0 – 6).

Grip Strength: A vigorimeter was utilized to measure grip strength.

Right-Left Laterality Discrimination: The computer-based tool Recognize® (Neuro Orthopedic Institute, Australia) was used to assess the ability to discriminate right from left hand images and was administered on a tablet. A symmetry index was computed using the same method as described for RoM_{SI}.

Dystonia: Arm Dystonia Disability Scale (ADDS) [32] questionnaire was used to assess reported dystonia.

2.2.2 Somatosensory Evaluation—*Monofilament Test:* Bilateral hand and finger threshold sensations were evaluated using the Semmes-Wenstein Monofilaments Examination (SWME). The monofilaments are applied to different regions of patients' hands (palmar surface of index finger and thumb, little finger and hypothenar eminence, and dorsum of the hand) perpendicularly until they bend for approximately one second. Depending on their diameter, the monofilaments can exert a pressure of 0.07, 0.4, 2, 4 or 300 g/mm^2. Having the eyes closed, patients were instructed to respond when the stimulus was felt by saying "yes". Starting with the finest monofilament, if the patients failed to feel the stimulus, the next monofilament in diameter was used for the tests. The test performance was given a clinical score of normal (Minimum force perceived = 0.07 g/mm^2), diminished light touch (Minimum force perceived = 0.4 g/mm^2), diminished protective sensation (Minimum force perceived = 2 g/mm^2), loss of protective sensation (Minimum force perceived = 4 g/mm^2), deep pressure sensation only (Minimum force perceived = 300 g/mm^2).

Two-Point Discrimination: The static two-point discrimination test was used to gauge the ability of the patients to identify two close stimulation points on a small area of the skin. The examiner randomly alternated one-point and two-point stimulation on the finger pads, while the study participants were asked to say "one" or "two" if they felt one or two points respectively. The test performance was given the clinical score of normal sensation

(Identified minimum distance = 6mm), moderately affected sensation (Identified minimum distance = 10 mm), bad sensation (Identified minimum distance = 16 mm), protective sensation (Only one point sensed).

2.2.3 Autonomic Function—The degree of autonomic dysfunction was assessed from six different perspectives. These comprised of abnormal or asymmetrical skin temperature and coloration, volume, sweat segregation, hair and nail growth. Using infrared thermometry, the skin temperature was measured three times on the bare skin, between the third and fourth metacarpal bone while avoiding the veins and hair on the dorsum of the hand, followed by three measurements on the palm of the hand. The arithmetic mean was used for further data processing and was considered to be abnormal when the temperature difference to the healthy hand was higher than 0.5°C [27]. Edema in the affected limb was assessed by measuring volume differences with the use of a hand-volumeter [64] and was classified as abnormal when the difference to the healthy limb was higher than 5% [27]. The therapist assessed sweat segregation, hair and nail growth, as well as the skin coloration through visual or/and tactile inspection.

2.2.4 Assessment of Pain, Quality of Life and Mental Health—Multiple validated self-administered questionnaires described below were completed by each participant.

Pain: Patients reported the pain intensity felt right before entering the MRI scan on a 0 – 10 visual analog scale (VAS).

Health-related quality-of-life: Health-related quality of life and disease burden was assessed with the Veterans RAND 12-item Health Survey [47]. The twelve items are summarized into two scores, a physical health (Physical Component Score, PCS) and a mental health summary measure (Mental Component Score, MCS). The PCS score ranges from 21.05 – 55.74 points, and the MCS score ranges from 12.66 – 62.88 points. For both subscales, higher scores denote better quality of life.

Depression and anxiety: Depression and anxiety status were evaluated with the German version of the Hospital Anxiety and Depression Scale (HADS-D). This 14-item self-report questionnaire comprises of two subscales (depression and anxiety) with seven items each, rated with a Likert-scale (0 – 3). The total subscale scores range from 0 – 21. The clinical cut-off score to meet the screening criteria for depression and anxiety is 8 points [17].

Kinesiophobia: Kinesiophobia or pain-related fear of movement was assessed with the 11-items Tampa Scale for Kinesiophobia (TKS-11) [92]. Items on the TSK-11 are scored from 1 (strongly disagree) to 4 (strongly agree). The total score ranges from 11 – 44 points, with higher scores indicating greater fear of pain, movement and injury. Patients with a score greater than the cut-off score of 24 are considered to have high levels of fear of movement.

2.3 Statistical Analysis (Non-imaging data)

Fulfilment of conditions of normality and homogeneity of variance of metric for psychophysical data was tested using Shapiro Wilk Test. After rejection of such fulfilment,

the Mann–Whitney U test was used to study group differences. In the case of nominal data, Fisher's exact test was used. The significant level was set to $\alpha = 0.05$.

2.4 Functional and Structural MRI Data Acquisition

All MRI data acquisition was performed on a 3T Siemens Magnetom Skyra MRI scanner using a 32-channel head coil (Erlangen, Germany). Anatomical MRI images were acquired using a magnetization prepared rapid gradient echo (MPRAGE) sequence. Images were obtained in a sagittal plane with a field of view of 256 mm^2 [160 1 mm-thick slices with an in-plane resolution of 1 mm (256 × 248 voxels)]. Resting-state fMRI images were acquired utilizing an echo-planar imaging (EPI) gradient echo sequence with isotropic voxels of 3.5 mm^3. Thirty-nine slices (64 × 64 in-plane resolution) were acquired per volume with TR/TE/Flip Angle = 2.5 secs/30 msecs/90° with 250 volumes.

2.5 Rationale for ROI selection

To elucidate the possible neurological pathologies underlying pain and motor deficits in CRPS, we chose to restrict our analysis to a priori selected anatomical regions of interest (ROI). We chose these regions following the rationale provided by prior publications from imaging studies in pain, CRPS and sensorimotor behavior.

Putamen is engaged in sensory-discriminative aspects of pain [21] and presents with decreased activation after treatment/symptomatic reduction in CRPS [14]. The putamen plays a crucial role in motor control and sensory integration [42].

Caudate Nucleus is part of the modulatory system of pain [33]. It presents with higher activation with nociceptive stimulation [34] and reduction after treatment [14] in CRPS. It is involved in the smooth orchestration of motor actions [82].

Nucleus Accumbens is a key component in the reward-aversion aspects of pain [60]. It is also involved in the cognitive processing of motor function related to reward and reinforcement [81].

Globus Pallidus has been demonstrated to play a role in normal motor behavior and presents with somatotopic characteristics [9]. Deep brain stimulation of this area has been reported to improve pain [55].

Thalamus is involved in the sensory discriminative and affective-motivational components of pain [1]. In pediatric CRPS, the thalamic nuclei exhibit volumetric differences in comparison with healthy controls that improve after treatment [31]. The thalamus is implicated in movement control and motor learning because it is an essential input and output node between motor areas of the cerebral cortex and motor-related subcortical structures [22].

Postcentral Gyrus has been proposed to play a significant role in the localization and discrimination of pain [26,50]. Consistent CRPS findings are alleging to reduced anatomical representation of the affected hand in this region [73] as disease progresses [58].

Insular Cortex is part of the affective circuits in pain processing [60] and, in CRPS, has been linked to higher activation during nociceptive stimulation in contrast to unaffected limb [57], as well as in comparison to healthy controls [34]. Moreover it plays a crucial role in sensorimotor integration [36].

2.6 Subcortical Gray Matter (GM) Volumetric Analysis

Using SPM12 (Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm/), image pre-processing and gray matter volume probability maps were created with the Computational Anatomy Toolbox (CAT12, dbm.neuro.uni-jena.de/cat). All T1-weighted images were denoised combining Markov Random Field (MRF) method with Spatially Adaptive Non-Local Means (SALNM) filter [59,78]. The denoised images were then segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) probability maps using the Adaptive Maximum A-posterior (AMAP) technique [78], which accounts for intensity inhomogeneities, followed by the Partial Volume Estimation (PVE) [88], allowing for more precise segmentation. Linear affine transformation followed by a non-linear deformation calculated with the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) tool was used [5] to register the segmented images into the Montreal Institute Neurological (MNI-152) space. Expansion (or contraction) occurring during the spatial transformation, was corrected or "modulated" by multiplying each voxel by the Jacobian determinant derived from the spatial normalization procedure. The normalized, modulated GM images were then smoothed using an isotropic 5 mm full-width-half-maximum (FWHM) Gaussian kernel. We subsequently obtained the segmentation maps of dorsal and ventral striatum regions of interest, namely caudate nucleus, putamen, nucleus accumbens and the globus pallidus. These isolated maps were used for subsequent analysis. Both intracranial volume and age were modelled as nuisance variables in the analysis. Following an a priori primary threshold of $p < 0.001$, we applied a family-wise error rate (FWE) cluster-level extent threshold to correct for multiple comparisons. Comparisons between study group (chronic stage CRPS and matched, healthy controls) and sides (left and right) for striatal volume were conducted using group x side repeated measures ANOVA. Significant linear relationships between striatal, GM densities and psychophysical measures were determined using Pearson's correlation analysis ($p < 0.05$).

2.7 Resting-state Functional Connectivity Analysis

2.7.1 Putaminal seed-based connectivity analysis—Seed-based rsfMRI analyses of the putamen were done using FSL Software version 5.0 (FMRIB's Software Library, www.fmrib.oc.ac.uk/fsl). All T-1 weighted images underwent brain extraction using Brain Extraction tool (BET) [84]. WM, GM, and CSF spatial maps were segmented using the FMRIB's Automated Segmentation Tool (FAST) [94].

rsfMRI data underwent removal of the first four volumes; motion correction using the FMRIB's Linear Motion Correction Tool (MCFLIRT) [45]; high pass filtered (0.01 Hz) to reduce low-frequency drift and noise effects.; spatial smoothing using a Gaussian kernel size of FWHM 5 mm, appropriate for striatal structures [25,61,86]; and grand-mean intensity normalization of the entire 4D dataset. Registration to anatomical MRI was carried out using

FMRIB's Linear Image Registration Tool (FLIRT) [46], and subsequently, registration to MNI-152 standard space was performed with FMRIB's Nonlinear Image Registration Tool (FNIRT) [3]. For further removal of motion-related artifacts, an Independent Component Analysis based strategy for Automatic Removal of Motion Artifacts (ICA-AROMA) was applied [77].

Using the normalized and smoothed images, regions where significant GM volume differences had been observed, namely left and right putamen, were used as seeds in the subsequent rsfMRI functional connectivity (rsFC) analyses. The seeds were transformed into the functional space of each subject using FLIRT. The single-subject time-series of the seeds were extracted and standardized (mean = 0; standard deviation = 1) to be used as explanatory variables (EV) in GLM analyses with the FMRIB's Improved Linear Model tool (FILM). Several confounding factors were included in the model to control for physiological noise and motion, since it is well documented that motion affects functional connectivity results [76]. These factors included WM and CSF time series, six motion parameters generated by motion correction during pre-processing (three rotation and three translation parameters) and a set of vectors identifying the time points when large motion displacements or spikes in the signal occurred. Comparisons of the connectivity maps between patients and controls were performed using Bayesian estimation for samples larger than 10 and robust outlier detection (FMRIB's Local Analysis of Mixed Effects, FLAME-1). Gender and age were included as nuisance factors in the model. Resulting group-level statistical maps were thresholded at z-value > 2.3 and a cluster significance threshold of $p = 0.05$. In statistical maps corrected for multiple comparisons, we looked for positive and negative differences.

A further general linear model was carried out to investigate possible modulatory effects of disease duration, pain levels, motor function, dystonia scores and kinesiophobia scores on putamen rsFC. The different scores of these variables were demeaned and introduced as regressors of interest in group-level analyses. All results were corrected for multiple comparisons using false discovery rate (FDR) and a cluster-size correction using a z-value > 2.3 and p-value < 0.05 statistical threshold.

2.7.2 ROI-to-ROI connectivity analysis—Given previous literature on neurological changes in chronic pain and motor deficits [2,7,11,48,72], we also explored functional connectivity properties in CRPS patients using a wider selection of seed regions involved in sensory perception and motor control. Namely: pre- and postcentral gyrus, insula, and thalamus. A ROI-to-ROI (region of interest) connectivity analysis was performed with the Functional Connectivity Toolbox (CONN-fMRI) v18.b [75] in conjunction with SPM 12. Here, preprocessed rsfMRI data underwent a component-based noise correction method [15] to reduce physiological and extraneous noise, providing interpretative information on positively and negatively correlated functional brain networks. Blood-oxygen-level-dependent (BOLD) signals from the cerebral WM and ventricles were removed using principal component analysis (PCA) of the multivariate BOLD signal within each of these masks obtained from the segmented T1-weighted MPRAGE scans. BOLD data were high pass filtered (0.01 Hz)

Seed ROIs were 10-mm-diameter spheres available from the CONN toolbox. They reproducibly represent core topological nodes within resting-state networks. Individual correlation maps were generated by extracting the mean BOLD time series from each seed ROI. Correlation coefficients with the BOLD time course of each target ROI throughout the whole brain were calculated. The resulting coefficients were converted to normally distributed scores using Fisher's transformation to obtain maps of voxel-wise functional connectivity for each seed ROI for each subject. The value of each voxel throughout the whole brain represents the relative degree of functional connectivity with each seed. These maps were subsequently used for second-level analysis of relative functional connectivity using a two-sided independent t-test to investigate differences in ROI-to-ROI connectivity between groups with a significance threshold of p=0.01.

3 Results

3.1 Pain levels and spectrum of movement-related abnormalities observed in CRPS patients.

Individual subject characteristics, disease duration, pain intensity and pain medication use are reported in Table 1. The enrolled patient population suffered from CRPS for approximately four years and possessed an average pain intensity of 5.15 ± 0.48 (mean ± standard error). Pain was distributed primarily on the distal hand and forearm. In comparison to healthy controls, CRPS patients showed significant motor impairment in the affected hand (Table 2 and Figure 1). Specifically, patients required a longer time to complete the 9-Hole Peg Test with the right hand than with the left, with 45% of the cohort at more than two standard deviations slower than the normal population. Forearm RoM results indicated that on average, patients moved their dominant forearm at a slower speed than the contralateral side. Interestingly, CRPS patients were characterized with an overall higher degree of rigidity in both the affected and unaffected hand. A little over half of the patients (55%) reported signs of dystonia. Although a number of movement-related deficits were identified in the CRPS cohort, only 30% reported high levels of kinesiophobia.

Patients did not perform worse than healthy controls in the right/left laterality discrimination task with Recognize® tool (Figure 1). However, somatosensory evaluation during monofilament stimulation revealed a significantly decreased sensitivity in the right hand of CRPS patients when compared to healthy controls (Table 2). Hyperhidrosis (95%), edema (60%) and vasomotor (70%) changes were present in most of the patients. CRPS diagnosis was also associated with symptoms resulting from trophic changes such as nail growth atrophy and less than half of the individuals showed skin temperature differences or abnormal hair growth patterns. CRPS patients reported significantly lower quality-of-life scores than healthy controls, in both physical and mental status. Results of the depression and anxiety scales indicated that the percentage of CRPS patients meeting the criteria for depression and anxiety was higher than in healthy controls (depression, CRPS: 45%, controls: 0%; anxiety, CRPS: 35%, healthy controls: 10%). The enrolled patients reported low levels of fear of movement, and only 30% reported high levels of kinesiophobia.

3.2 Decreases in putaminal GM volume observed in CRPS.

In comparison to healthy controls, a significant, bilateral decrease in putaminal GM volume in chronic CRPS patients was measured (Figure 2, Table 3). A negative association (Pearsońs correlation) was quantified between GM densities of the left putamen (contralateral to the affected hand in CRPS patients) and forearm mobility measured with the RoM test ($r = -0.46$, $p = 0.043$). Additionally, greater left compared to right hemisphere volumes were detected for caudate nucleus and globus pallidus across the two study cohorts.

3.3 Putamen-based functional connectivity strength associated with motor impairment in CRPS.

Following the observed decreased GM density of the putamen in CRPS patients, single-subject time series were extracted from this striatal sub-region (separately for left and right hemisphere) for subsequent seed-region, functional connectivity analyses. Head motion for all study participants during rsfMRI was first analyzed. Subjects (N=40) did not display an absolute head motion greater than 2 mm, and therefore, no imaging dataset was omitted in the analysis. Moreover, a t-test comparing mean displacement between groups showed no group-level differences (mean ± standard error, CRPS: 0.36 ± 0.037 mm; controls: 0.29 ± 0.049 mm, $p = 0.25$). The CRPS cohort compared to matched controls demonstrated significantly greater functional connectivity strength amongst the right (ipsilateral to the affected limb) putamen and sensorimotor (pre-/postcentral gyri) and superior parietal cortices, while decreased connectivity was quantified within crus I region of the cerebellum; a cerebellar node implicated in pain processing (Figure 3, Table 4) [65].

Interaction analyses involving parameters informing on motor impairment, revealed a significant profound effect between the putamen and areas involved in information processing and motor fine-tuning. Specifically, patients with poorer finger and hand coordination performance, as determined by the 9-Hole Peg Test, showed decreased functional connectivity between the left putamen and both the cerebellum (crus I and II) and precuneus, the latter of which connects to supplementary motor, premotor cortex and somatosensory cortices (Figure 4, Table 4). Patients with diminished forearm RoM presented with decreased connectivity between the left putamen and primary sensorimotor areas (Table 4, Supplemental Figure 1A), while connectivity strengths of the left putamen to the supramarginal gyrus were positively correlated with a higher degree of dystonia (Table 4, Supplemental Figure 1B). Patient specific, pain intensity ratings acquired just prior to the scan session were used in secondary regression analysis. Here, higher clinical pain levels showed greater putamen (left hemisphere)-based connectivity along the length of the left pre-/postcentral gyrus (Supplemental Figure 1C). This cortical region topologically corresponded to the hand and arm representation within the primary sensorimotor cortex. However, when further correcting these results for multiple comparisons with false discovery rate (FDR), only the correlations with the 9-Hole Peg Test were significant.

3.4 Decreased functional connectivity in motor networks

Results of the exploratory ROI-to-ROI connectivity analysis are depicted in supplementary material (Supplemental Table 1, Supplemental Figure 2). The CRPS cohort compared to matched, healthy controls demonstrated decreased functional connectivity strength amongst

the left postcentral gyrus and the precuneus and the ipsilateral hippocampus. On the other hand, the left postcentral gyrus showed increased connectivity with the contralateral frontal operculum. Decreased functional connectivity was quantified between the right and left insular cortex and vermis IX and crus II of the cerebellum, respectively. Left thalamus also exhibited decreased functional connectivity with the cerebellar vermis VII and VIII.

4 Discussion

4.1 Sensorimotor Changes in CRPS

This investigation characterized the clinical profile along with morphological and functional properties of sensorimotor network nodes with particular emphasis on the putamen in a cohort of chronic CRPS patients. In addition to a moderate level of pain, CRPS patients, relative to healthy controls, displayed multiple motor, somatosensory and autonomic abnormalities localized to the affected limb. The most prominent motor impairments observed, namely weakness, reduced distal arm mobility and limited coordination are in agreement to what has been previously reported [43]. Dystonia, which was reported by over half of the CRPS cohort, has been related to neuropathological defects in the basal ganglia [69], and is a common symptom for CRPS [66]. The symptomatic presentation of the enrolled patient population is comparable to that which is typically observed [16]. In contrast to previous studies with rsfMRI methodology on CRPS, our patients were carefully selected for unilateral distribution of ongoing pain and existing motor impairment of the dominant hand.

4.2 Putaminal and Sensorimotor Cortical Changes in CRPS

Neuroimaging data revealed bilateral decreases in putaminal volume in CRPS patients, which is in accord with recent meta-analysis findings of different chronic pain cohorts [83], and with findings specific to CRPS [11]. Decreased volume in the putamen might have a negative impact in motor function, as it is the case in Parkinson's Disease [70]. Volumetric decreased in nucleus accumbens in CRPS previously reported [35], was not replicated in our structural MRI results. Nevertheless, a parallel trend in ventral striatum across the two investigations of nearly equal sample sizes (N~20) was observed.

Putamen-based, functional connectivity differences between the two cohorts were unilaterally observed in the right cerebral hemisphere (ipsilateral to the affected hand), implicating the ipsilateral somatosensory and association cortices, as well as cerebellar cortices. Though ipsilateral hemispheric changes might seem counterintuitive, such reorganization of components of the cortical pain connectome has been observed in multiple chronic pain studies [39,71]. These observations might be the result of extensive use of the non-dominant but healthy limb. However, the specific relevance of this ipsilateral functional connectivity change of the putamen to pre-/postcentral gyrus needs to be further elucidated. Previous studies using task-dependent fMRI for investigating motor behavior in CRPS have not frequently reported changed activity in the putamen or other parts of the striatum. However, a recent study noted reduced neural responses within the putamen and nucleus accumbens in CRPS patients performing a mental rotation task, which incorporates motor and goal-directed processes to CRPS neuropathology [51]. Given the specific motor

disorders displayed in CRPS patients including hypokinesia and dystonia, it is conceivable that a more in-depth comparison between a motor and a sensory task may clarify whether altered functional connectivity of the putamen is explained by the motor-related abnormalities or pain-related processing.

From regression analyses of neuroimaging data, the relationships between properties of the putamen (contralateral to the affected limb) with clinical pain and measures of motor function were realized and showed similar involvement of structures, namely pre-/postcentral gyrus. Changes involving the cortical sensorimotor network are in line with earlier works and seem to be related to motor impairment or a maintained fear of pain and movement avoidance in children [13]. It is also worth discussing the conflicting results of decreased putaminal volume in conjunction with altered functional connectivity with sensorimotor regions. This phenomenon is also observed in motor disorders related to neurodegeneration of the nigrostriatal pathways [44]. This result could represent a (relative) overactivity of residual neurons in the putamen yielding either increases or decreases in functional connectivity with cortical regions.

The most robust finding when titrated down was finger coordination associated with decreased connectivity between the contralateral putamen and precuneus as well as parietal association areas in CRPS. The precuneus is involved in integration of information and perception of the environment. It is an important hub of the DMN with a role in self-consciousness. Alterations in the precuneus and greater DMN have been shown to contribute significantly to pain miss-processing in migraine [20], motor dysfunction in Parkinson's disease [87], as well as in CRPS [10].

Interestingly, in healthy volunteers undergoing sustained pressure pain, connectivity between the putamen and pre-/postcentral gyrus was negatively correlated with pain intensity [49], which is in contrast to our observations. This disparity potentially suggests differences in processing of pain in healthy volunteers and CRPS patients, but perhaps it may also a distinction between experimental versus clinical pain. Moreover, hypersensitivity to cold and brush stimuli applied to the affected CRPS limb have been reported to implicate the caudate as well as the putamen [52,54]. Yet, recent data solely involving healthy volunteers suggests that this may not be specific to CRPS, but rather a feature that extends to the general population, as well as to transiently evoked pain or hyperalgesic states [40].

Our findings localized to the putamen likely suggest an involvement of the reward-aversion system in CRPS, which is expected considering that persistent pain is a robust, aversive stimuli [93]. Moreover, the putamen is an integral component of both the dopamine and opioid receptor systems, which play a role in reward processes such as analgesia [21,90]. Thus, functional and structural modulation of the putamen may not only underpin enhanced aversion-related processes, but the same alterations may pose barriers to experiencing reward or analgesia.

A more widespread functional connectivity analysis (ROI-to-ROI) involving critical areas for motor control and pain showed a generalized decreased connectivity to the sensorimotor cerebellum (Crus I, II and vermis VIII) and to the cognitive network of the cerebellum

(vermis IX) [85]. The cerebellum plays an essential role in pain modulation and cognitive control [29]. Changes in connectivity involving the cerebellum with the insula and putamen may further point to deficits in integrating sensorimotor information with affective processing in CRPS. Alterations within motor regions in relation to changes within the cerebellum have also been reported in chronic pain conditions and may add to the known function of the cerebellum and how it coordinates with altered sensory-motor and emotional processing in the presence of chronic pain [62]. Moreover, decreased connectivity between postcentral gyrus and precuneus was revealed. These two regions are densely interconnected [89], and the observed connectivity changes may be related to alterations within the DMN or salience networks in response to chronic pain in CRPS patients. This is in agreement with findings in other chronic pain conditions. The functional relevance of the connectivity between postcentral gyrus and hippocampus has been related to perceptual cues [80]. Therefore, the observed decreased connectivity between these two regions may suggest impaired perceptual processing of sensory information in CRPS patients.

Altered activity within the primary somatosensory cortex and posterior insular cortex has been observed across several chronic pain diseases in presence of noxious stimuli, which suggests aberrant intensity processing of pain in chronic pain states [62]. Thus, our results involving these cortical areas might be related to chronic pain in general and not specific to CRPS. Alterations within the basal ganglia, particularly in the putamen, have been suggested to pertain to altered motor and general connectivity of the brain in chronic pain [12]. In our study, we were able to show that changes in the putamen correlated with diminished motor control, corroborating this suggestion.

4.3 Caveats

There are several caveats to consider: *Study Design*: Given the nature of this cross-sectional study, it remains difficult to confirm if the current putamen based functional changes are reflective of pathophysiological changes or adaptive processes in CRPS. To disentangle these two intertwined phenomena, future studies may involve a task-based paradigm as well as longitudinal study designs. Such approaches may help to differentiate CNS findings that are pathologic versus those that are perhaps compensatory mechanisms by which 'healthy' CNS pathways are recruited to maintain some level of normal function. *Medication Effects:* Efforts in future investigations in acute or chronic CRPS patients should better control the amount or type of medication consumed. A washout period prior to a clinical imaging examination may be valuable to implement. *Putaminal Changes in other pain conditions*: A limitation of the present study is that the findings might not be specific to CRPS [21]. Therefore, comparison with other chronic pain population, preferably such with chronic hand pain of other origin such as carpal tunnel syndrome might be more appropriate than healthy controls.

4.4 Conclusions

Undisputable, the central nervous system (CNS) contributes to pain and motor impairments in CRPS. To date, past investigations have predominately alluded to and focused upon a significant cortical network involvement in the pain processing aspects of CRPS [34,48]. Our most robust findings implicate changes in the putamen, suggesting that the striatum

might be also an essential component in motor network differences previously shown [19,56] and is a crucial component in the chronic CRPS phenotype. This is especially evident when viewing both the structural and functional neuroimaging results in the context of well-known clinical and motor function profiles of this chronic pain condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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6 References

[1]. Ab Aziz CB, Ahmad AH. The role of the thalamus in modulating pain. Malaysian J Med Sci 2006;13:11–18.

[2]. Alshelh Z, Marciszewski KK, Akhter R, Di Pietro F, Mills EP, Vickers ER, Peck CC, Murray GM, Henderson LA. Disruption of default mode network dynamics in acute and chronic pain states. NeuroImage Clin 2018;17:222–231. doi:10.1016/j.nicl.2017.10.019. [PubMed: 29159039]

[3]. Andersson JLR, Jenkinson M, Smith S, Andersson J. Non-linear registration aka Spatial normalisation FMRIB Technial Report TR07JA2. 2007 p. Available: http://www.fmrib.ox.ac.uk/datasets/techrep/tr07ja2/tr07ja2.pdf Accessed 14 Nov 2017.

[4]. Apkarian AV, Bushnell MC, Treede R-D, Zubieta J-K. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 2005;9:463–84. doi:10.1016/j.ejpain.2004.11.001. [PubMed: 15979027]

[5]. Ashburner J. A fast diffeomorphic image registration algorithm. Neuroimage 2007;38:95–113. doi:10.1016/J.NEUROIMAGE.2007.07.007. [PubMed: 17761438]

[6]. Azqueta-Gavaldon M, Schulte-Göcking H, Storz C, Azad S, Reiners A, Borsook D, Becerra L, Kraft E. Basal ganglia dysfunction in complex regional pain syndrome - A valid hypothesis? Eur J Pain 2017;21:415–424. doi:10.1002/ejp.975. [PubMed: 27805769]

[7]. Bailey J, Nelson S, Lewis J, McCabe CS. Imaging and clinical evidence of sensorimotor problems in CRPS: utilizing novel treatment approaches. J Neuroimmune Pharmacol 2013;8:564–75. doi:10.1007/s11481-012-9405-9. [PubMed: 23054370]

[8]. Bailey J, Nelson S, Lewis J, McCabe CS. Imaging and Clinical Evidence of Sensorimotor Problems in CRPS: Utilizing Novel Treatment Approaches. J Neuroimmune Pharmacol 2013;8:564–575. doi:10.1007/s11481-012-9405-9. [PubMed: 23054370]

[9]. Baker KB, Lee JYK, Mavinkurve G, Russo GS, Walter B, DeLong MR, Bakay RAE, Vitek JL. Somatotopic organization in the internal segment of the globus pallidus in Parkinson's disease. Exp Neurol 2010;222:219–225. [PubMed: 20059997]

[10]. Baliki MN, Mansour AR, Baria AT, Apkarian AV. Functional Reorganization of the Default Mode Network across Chronic Pain Conditions. PLoS One 2014;9:e106133. doi:10.1371/journal.pone.0106133.

[11]. Barad MJ, Ueno T, Younger J, Chatterjee N, Mackey S. Complex Regional Pain Syndrome Is Associated With Structural Abnormalities in Pain-Related Regions of the Human Brain. J Pain 2014;15:197–203. doi:10.1016/j.jpain.2013.10.011. [PubMed: 24212070]

[12]. Becerra L, Morris S, Bazes S, Gostic R, Sherman S, Gostic J, Pendse G, Moulton E, Scrivani S, Keith D, Chizh B, Borsook D. Trigeminal neuropathic pain alters responses in CNS circuits to mechanical (brush) and thermal (cold and heat) stimuli. J Neurosci 2006;26:10646–10657. [PubMed: 17050704]

[13]. Becerra L, Sava S, Simons LE, Drosos AM, Sethna N, Berde C, Lebel AA, Borsook D. Intrinsic brain networks normalize with treatment in pediatric complex regional pain syndrome. NeuroImage Clin 2014;6:347–69. doi:10.1016/j.nicl.2014.07.012. [PubMed: 25379449]

[14]. Becerra L, Schwartzman RJ, Kiefer RT, Rohr P, Moulton EA, Wallin D, Pendse G, Morris S, Borsook D. CNS Measures of Pain Responses Pre- and Post-Anesthetic Ketamine in a Patient with Complex Regional Pain Syndrome. 1526. doi:10.1111/j.1526-4637.2009.00559.x.

[15]. Behzadi Y, Restom K, Liau J, Liu TT. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. Neuroimage 2007;37:90–101. doi:10.1016/j.neuroimage.2007.04.042. [PubMed: 17560126]

[16]. Birklein F, Dimova V. Complex regional pain syndrome–up-to-date. PAIN Reports 2017:1. doi:10.1097/PR9.0000000000000624.

[17]. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res 2002;52:69–77. Available: http://www.ncbi.nlm.nih.gov/pubmed/11832252 Accessed 28 Sep 2018. [PubMed: 11832252]

[18]. Boerl RDH, Marinusl J, Hiltenl JJ, Huygenl FJ, Eijsl F, Kleefl M, Bauerl MCR, Gestell M, Zuurmondl WWA, Perezl RSGM. Distribution of signs and symptoms of Complex Regional Pain Syndrome type I in patients meeting the diagnostic criteria of the International Association for the Study of Pain. Eur J Pain 2011;15:830e1–830.e8. doi:10.1016/j.ejpain.2011.01.012. [PubMed: 21334934]

[19]. Bolwerk A, Seifert F, Maihöfner C. Altered Resting-State Functional Connectivity in Complex Regional Pain Syndrome. J Pain 2013;14:1107–1115.e8. doi:10.1016/j.jpain.2013.04.007. [PubMed: 23791136]

[20]. Borsook D, Maleki N, Burstein R. Migraine. Neurobiol Brain Disord 2015:693–708. doi:10.1016/B978-0-12-398270-4.00042-2.

[21]. Borsook D, Upadhyay J, Chudler EH, Becerra L. A Key Role of the Basal Ganglia in Pain and Analgesia - Insights Gained through Human Functional Imaging. Mol Pain 2010;6:1744–8069-6–27. doi:10.1186/1744-8069-6-27.

[22]. Bosch-Bouju C, Hyland BI, Parr-Brownlie LC. Motor thalamus integration of cortical, cerebellar and basal ganglia information: Implications for normal and parkinsonian conditions. Front Comput Neurosci 2013.

[23]. Bostan AC, Strick PL. The basal ganglia and the cerebellum: nodes in an integrated network. Nat Rev Neurosci 2018;19:338–350. doi:10.1038/s41583-018-0002-7. [PubMed: 29643480]

[24]. Cauda F, Sacco K, Duca S, Cocito D, D'Agata F, Geminiani GC, Canavero S. Altered Resting State in Diabetic Neuropathic Pain. PLoS One 2009;4:e4542. doi:10.1371/journal.pone.0004542.

[25]. Choi EY, Yeo BTT, Buckner RL. The organization of the human striatum estimated by intrinsic functional connectivity. J Neurophysiol 2012;108:2242–2263. doi:10.1152/jn.00270.2012. [PubMed: 22832566]

[26]. Craig ADB. Pain mechanisms: labeled lines versus convergence in central processing. Annu Rev Neurosci 2003;26:1–30. doi:10.1146/annurev.neuro.26.041002.131022. [PubMed: 12651967]

[27]. Davidoff G, Morey K, Amann M, Stamps J. Pain measurement in reflex sympathetic dystrophy syndrome 21. Pain 1988;32:27–34. Available: https://pdfs.semanticscholar.org/bd5b/7db87eeeb3e28b4228e76e0f4da9a4c5a253.pdf Accessed 14 Nov 2017. [PubMed: 3340421]

[28]. Davis KD. Introduction to a Special Issue on Innovations and Controversies in Brain Imaging of Pain. PAIN Reports 2019;4:e771.

[29]. Diano M, D'agata F, Cauda F, Costa T, Geda E, Sacco K, Duca S, Torta DM, Geminiani GC. Cerebellar Clustering and Functional Connectivity During Pain Processing. n.d. doi:10.1007/s12311-015-0706-4.

[30]. Drummond PD. Sensory Disturbances in Complex Regional Pain Syndrome: Clinical Observations, Autonomic Interactions, and Possible Mechanisms. Pain Med 2010;11:1257–1266. doi:10.1111/j.1526-4637.2010.00912.x. [PubMed: 20704674]

[31]. Erpelding N, Simons L, Lebel A, Serrano P, Pielech M, Prabhu S, Becerra L, Borsook D. Rapid treatment-induced brain changes in pediatric CRPS. Brain Struct Funct 2016;221:1095–111. doi:10.1007/s00429-014-0957-8. [PubMed: 25515312]

[32]. Fahn S. Assessment of the primary dystonias In: Munsat TL, editor. Quantification of Neurologic Deficit. Oxford: Butterworths, 1989 pp. 241–270.

[33]. Freund W, Klug R, Weber F, Stuber G, Schmitz B, Wunderlich AP. Perception and suppression of thermally induced pain: a fMRI study. Somatosens Mot Res 2009;26:1–10. doi:10.1080/08990220902738243. [PubMed: 19283551]

[34]. Freund W, Wunderlich AP, Stuber G, Mayer F, Steffen P, Mentzel M, Weber F, Schmitz B. Different Activation of Opercular and Posterior Cingulate Cortex (PCC) in Patients With Complex Regional Pain Syndrome (CRPS I) Compared With Healthy Controls During Perception of Electrically Induced Pain: A Functional MRI Study. Clin J Pain 2010;26:339–347. doi:10.1097/AJP.0b013e3181cb4055. [PubMed: 20393270]

[35]. Geha PY, Baliki MN, Harden RN, Bauer WR, Parrish TB, Apkarian AV. The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions. Neuron 2008;60:570–81. doi:10.1016/j.neuron.2008.08.022. [PubMed: 19038215]

[36]. Ghaziri J, Tucholka A, Girard G, Boucher O, Houde JC, Descoteaux M, Obaid S, Gilbert G, Rouleau I, Nguyen DK. Subcortical structural connectivity of insular subregions. Sci Rep 2018;8.

[37]. Gieteling EW, van Rijn MA, de Jong BM, Hoogduin JM, Renken R, van Hilten JJ, Leenders KL. Cerebral activation during motor imagery in complex regional pain syndrome type 1 with dystonia. Pain 2008;134:302–309. doi:10.1016/j.pain.2007.04.029. [PubMed: 17561345]

[38]. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, Poewe W, Sampaio C, Stern MB, Dodel R, Dubois B, Holloway R, Jankovic J, Kulisevsky J, Lang AE, Lees A, Leurgans S, LeWitt PA, Nyenhuis D, Olanow CW, Rascol O, Schrag A, Teresi JA, van Hilten JJ, LaPelle N. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. Mov Disord 2008;23:2129–2170. doi:10.1002/mds.22340. [PubMed: 19025984]

[39]. Gustin SM, Peck CC, Cheney LB, Macey PM, Murray GM, Henderson LA. Pain and Plasticity: Is Chronic Pain Always Associated with Somatosensory Cortex Activity and Reorganization? J Neurosci 2012;32:14874–14884. doi:10.1523/JNEUROSCI.1733-12.2012. [PubMed: 23100410]

[40]. Hansen MS, Asghar MS, Wetterslev J, Pipper CB, Mårtensson J, Becerra L, Christensen A, Nybing JD, Havsteen I, Boesen M, Dahl JB. The association between areas of secondary hyperalgesia and volumes of the caudate nuclei and other pain relevant brain structures-A 3-tesla MRI study of healthy men. PLoS One 2018;13:e0201642. doi:10.1371/journal.pone.0201642.

[41]. Harden RN, Bruehl S, Perez RSGM, Birklein F, Marinus J, Maihofner C, Lubenow T, Buvanendran A, Mackey S, Graciosa J, Mogilevski M, Ramsden C, Chont M, Vatine J-J. Validation of proposed diagnostic criteria (the "Budapest Criteria") for Complex Regional Pain Syndrome. Pain 2010;150:268–74. doi:10.1016/j.pain.2010.04.030. [PubMed: 20493633]

[42]. Hening W, Harrington DL, Poizner H. Basal Ganglia: Motor Functions of Encyclopedia of Neuroscience. Berlin, Heidelberg: Springer Berlin Heidelberg, n.d. pp. 346–350. doi:10.1007/978-3-540-29678-2_561.

[43]. Van Hilten JJ. Movement Disorders in Complex Regional Pain Syndrome. Pain Med 2010;11:1274–1277. doi:10.1111/j.1526-4637.2010.00916.x. [PubMed: 20704676]

[44]. Introduction to Parkinson's and Huntington's Disease. CNS Regen 1999:295–298. doi:10.1016/B978-012705070-6/50011-1.

[45]. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 2002;17:825–41. Available: http://www.ncbi.nlm.nih.gov/pubmed/12377157 Accessed 24 May 2018. [PubMed: 12377157]

[46]. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 2002;17:825–41. Available: http://www.ncbi.nlm.nih.gov/pubmed/12377157 Accessed 14 Nov 2017. [PubMed: 12377157]

[47]. Kazis LE, Miller DR, Skinner KM, Lee A, Ren XS, Clark JA, Rogers WH, Sprio A, Selim A, Linzer M, Payne SMC, Mansell D, Fincke BG. Applications of methodologies of the Veterans Health Study in the VA healthcare system: conclusions and summary. J Ambul Care Manage 2006;29:182–8. Available: http://www.ncbi.nlm.nih.gov/pubmed/16552327 Accessed 26 Aug 2018. [PubMed: 16552327]

[48]. Kim J-H, Choi S-H, Jang JH, Lee D-H, Lee K-J, Lee WJ, Moon JY, Kim YC, Kang D-H. Impaired insula functional connectivity associated with persistent pain perception in patients with complex regional pain syndrome. PLoS One 2017;12:e0180479. doi:10.1371/journal.pone.0180479.

[49]. Kim J, Loggia ML, Edwards RR, Wasan AD, Gollub RL, Napadow V. Sustained deep-tissue pain alters functional brain connectivity. Pain 2013;154:1343–51. doi:10.1016/j.pain.2013.04.016. [PubMed: 23718988]

[50]. Kim W, Kim SK, Nabekura J. Functional and structural plasticity in the primary somatosensory cortex associated with chronic pain. J Neurochem 2017;141:499–506. [PubMed: 28278355]

[51]. Kohler M, Strauss S, Horn U, Langner I, Usichenko T, Neumann N, Lotze M. Differences in Neuronal Representation of Mental Rotation in Patients With Complex Regional Pain Syndrome and Healthy Controls. J Pain 2019;20:898–907. doi:10.1016/j.jpain.2019.01.330. [PubMed: 30710707]

[52]. Lebel A, Becerra L, Wallin D, Moulton EA, Morris S, Pendse G, Jasciewicz J, Stein M, Aiello-Lammens M, Grant E, Berde C, Borsook D. fMRI reveals distinct CNS processing during symptomatic and recovered complex regional pain syndrome in children. Brain 2008;131:1854–1879. doi:10.1093/brain/awn123. [PubMed: 18567621]

[53]. Lenz M, Hoffken O, Stude P, Lissek S, Schwenkreis P, Reinersmann A, Frettloh J, Richter H, Tegenthoff M, Maier C. Bilateral somatosensory cortex disinhibition in complex regional pain syndrome type I. Neurology 2011;77:1096–1101. doi:10.1212/WNL.0b013e31822e1436. [PubMed: 21880999]

[54]. Linnman C, Becerra L, Lebel A, Berde C, Grant PE, Borsook D. Transient and Persistent Pain Induced Connectivity Alterations in Pediatric Complex Regional Pain Syndrome. PLoS One 2013;8:e57205. doi:10.1371/journal.pone.0057205.

[55]. Loher TJ, Burgunder J-M, Weber S, Sommerhalder R, Krauss JK. Effect of chronic pallidal deep brain stimulation on off period dystonia and sensory symptoms in advanced Parkinson's disease. J Neurol Neurosurg Psychiatry 2002;73:395–9. doi:10.1136/jnnp.73.4.395. [PubMed: 12235307]

[56]. Maihofner C, Baron R, DeCol R, Binder A, Birklein F, Deuschl G, Handwerker HO, Schattschneider J. The motor system shows adaptive changes in complex regional pain syndrome. Brain 2007;130:2671–2687. doi:10.1093/brain/awm131. [PubMed: 17575278]

[57]. Maihöfner C, Forster C, Birklein F, Neundörfer B, Handwerker HO. Brain processing during mechanical hyperalgesia in complex regional pain syndrome: a functional MRI study. Pain 2005;114:93–103. doi:10.1016/j.pain.2004.12.001. [PubMed: 15733635]

[58]. Mancini F, Wang AP, Shira MM, Isherwood ZJ, McAuley JH, Iannetti G, Sereno MI, Moseley L, Rae CD. Preserved cortical maps of the body in Complex Regional Pain Syndrome. bioRxiv 2018:409094.

[59]. Manjón J V, Coupé P, Martí-Bonmatí L, Collins DL, Robles M. Adaptive non-local means denoising of MR images with spatially varying noise levels. J Magn Reson Imaging 2010;31:192–203. doi:10.1002/jmri.22003. [PubMed: 20027588]

[60]. Mansour AR, Farmer MA, Baliki MN, Apkarian AV. Chronic pain: The role of learning and brain plasticity. Restor Neurol Neurosci 2014;32:129–139. [PubMed: 23603439]

[61]. Di Martino A, Scheres A, Margulies DS, Kelly AMC, Uddin LQ, Shehzad Z, Biswal B, Walters JR, Castellanos FX, Milham MP. Functional Connectivity of Human Striatum: A Resting State fMRI Study. Cereb Cortex 2008;18:2735–2747. doi:10.1093/cercor/bhn041. [PubMed: 18400794]

[62]. Martucci KT, Ng P, MacKey S. Neuroimaging chronic pain: What have we learned and where are we going? Future Neurol 2014;9:615–626. [PubMed: 28163658]

[63]. Mathiowetz V, Weber K, Kashman N, Volland G. Adult norms for the Nine Hole Peg Test of finger dexterity. Occup Ther J Res 1985;5:24–38. Available: https://experts.umn.edu/en/publications/adult-norms-for-the-nine-hole-peg-test-of-finger-dexterity Accessed 14 Nov 2017.

[64]. Miller LK, Jerosch-Herold C, Shepstone L. Clinical assessment of hand oedema: A systematic review. Hand Ther 2017;22:153–164. doi:10.1177/1758998317724405.

[65]. Moulton EA, Schmahmann JD, Becerra L, Borsook D. The cerebellum and pain: passive integrator or active participator? Brain Res Rev 2010;65:14–27. doi:10.1016/j.brainresrev.2010.05.005. [PubMed: 20553761]

[66]. Munts AG, Mugge W, Meurs TS, Schouten AC, Marinus J, Moseley GL, van der Helm FCT, van Hilten JJ. Fixed dystonia in complex regional pain syndrome: a descriptive and computational modeling approach. BMC Neurol 2011;11:53. doi:10.1186/1471-2377-11-53. [PubMed: 21609429]

[67]. Munts AG, Van Rootselaar A-F, Van Der Meer JN, Koelman JHTM, Van Hilten JJ, Tijssen MAJ. Clinical and neurophysiological characterization of myoclonus in complex regional pain syndrome. Mov Disord 2008;23:581–587. doi:10.1002/mds.21910. [PubMed: 18163455]

[68]. Napadow V, LaCount L, Park K, As-Sanie S, Clauw DJ, Harris RE. Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. Arthritis Rheum 2010;62:2545–2555. doi:10.1002/art.27497. [PubMed: 20506181]

[69]. Neychev VK, Gross RE, Lehéricy S, Hess EJ, Jinnah HA. The functional neuroanatomy of dystonia. Neurobiol Dis 2011;42:185–201. doi:10.1016/j.nbd.2011.01.026. [PubMed: 21303695]

[70]. Owens-Walton C, Jakabek D, Li X, Wilkes FA, Walterfang M, Velakoulis D, van Westen D, Looi JCL, Hansson O. Striatal changes in Parkinson disease: An investigation of morphology, functional connectivity and their relationship to clinical symptoms. Psychiatry Res Neuroimaging 2018;275:5–13. doi:10.1016/j.pscychresns.2018.03.004. [PubMed: 29555381]

[71]. Peyron R, Schneider F, Faillenot I, Convers P, Barral F-G, Garcia-Larrea L, Laurent B. An fMRI study of cortical representation of mechanical allodynia in patients with neuropathic pain. Neurology 2004;63:1838–46. doi:10.1212/01.wnl.0000144177.61125.85. [PubMed: 15557499]

[72]. Di Pietro F, McAuley JH, Parkitny L, Lotze M, Wand BM, Moseley GL, Stanton TR. Primary Motor Cortex Function in Complex Regional Pain Syndrome: A Systematic Review and Meta-Analysis. J Pain 2013;14:1270–1288. doi:10.1016/J.JPAIN.2013.07.004. [PubMed: 24035350]

[73]. Di Pietro F, McAuley JH, Parkitny L, Lotze M, Wand BM, Moseley GL, Stanton TR. Primary Somatosensory Cortex Function in Complex Regional Pain Syndrome: A Systematic Review and Meta-Analysis. J Pain 2013;14:1001–1018. doi:10.1016/j.jpain.2013.04.001. [PubMed: 23726046]

[74]. Pleger B, Tegenthoff M, Schwenkreis P, Janssen F, Ragert P, Dinse HR, Völker B, Zenz M, Maier C. Mean sustained pain levels are linked to hemispherical side-to-side differences of primary somatosensory cortex in the complex regional pain syndrome I. Exp Brain Res 2004;155:115–119. doi:10.1007/s00221-003-1738-4. [PubMed: 15064892]

[75]. Porcu M, Craboledda D, Garofalo P, Barberini L, Sanfilippo R, Zaccagna F, Wintermark M, Montisci R, Saba L. Reorganization of brain networks following carotid endarterectomy: an exploratory study using resting state functional connectivity with a focus on the changes in Default Mode Network connectivity. Eur J Radiol 2019;110:233–241. doi:10.1016/j.ejrad.2018.12.007. [PubMed: 30599866]

[76]. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. Neuroimage 2014;84:320–41. doi:10.1016/j.neuroimage.2013.08.048. [PubMed: 23994314]

[77]. Pruim RHR, Mennes M, van Rooij D, Llera A, Buitelaar JK, Beckmann CF. ICA-AROMA: A robust ICA-based strategy for removing motion artifacts from fMRI data. Neuroimage 2015;112:267–277. doi:10.1016/j.neuroimage.2015.02.064. [PubMed: 25770991]

[78]. Rajapakse JC, Giedd JN, Rapoport JL. Statistical approach to segmentation of single-channel cerebral MR images. IEEE Transactions on Medical Imaging.1997, Vol. 16 pp. 176–186. doi:10.1109/42.563663. [PubMed: 9101327]

[79]. van Rijn MA, Marinus J, Putter H, Bosselaar SRJ, Moseley GL, van Hilten JJ. Spreading of complex regional pain syndrome: not a random process. J Neural Transm 2011;118:1301–9. doi:10.1007/s00702-011-0601-1. [PubMed: 21331457]

[80]. Robinson JL, Barron DS, Kirby LAJ, Bottenhorn KL, Hill AC, Murphy JE, Katz JS, Salibi N, Eickhoff SB, Fox PT. Neurofunctional topography of the human hippocampus. Hum Brain Mapp 2015;36:5018–37. doi:10.1002/hbm.22987. [PubMed: 26350954]

[81]. Sesack SR, Grace AA. Cortico-basal ganglia reward network: Microcircuitry. Neuropsychopharmacology 2010;35:27–47. [PubMed: 19675534]

[82]. Shroff S. Caudate Nucleus Encyclopedia of Clinical Neuropsychology. New York, NY: Springer New York, 2011 pp. 504–506. doi:10.1007/978-0-387-79948-3_302.

[83]. Smallwood RF, Laird AR, Ramage AE, Parkinson AL, Lewis J, Clauw DJ, Williams DA, Schmidt-Wilcke T, Farrell MJ, Eickhoff SB, Robin DA. Structural brain anomalies and chronic pain: a quantitative meta-analysis of gray matter volume. J Pain 2013;14:663–75. doi:10.1016/j.jpain.2013.03.001. [PubMed: 23685185]

[84]. Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002;17:143–155. doi:10.1002/hbm.10062. [PubMed: 12391568]

[85]. Stoodley CJ, Schmahmann JD. Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. Cortex 2010;46:831–44. doi:10.1016/j.cortex.2009.11.008. [PubMed: 20152963]

[86]. Tanaka S, Kirino E. Functional Connectivity of the Dorsal Striatum in Female Musicians. Front Hum Neurosci 2016;10:178. doi:10.3389/fnhum.2016.00178. [PubMed: 27148025]

[87]. Thibes RB, Novaes NP, Lucato LT, Campanholo KR, Melo LM, Leite CC, Amaro E, Barbosa ER, Bor-Seng-Shu E, Cardoso EF, Sato JR. Altered Functional Connectivity Between Precuneus and Motor Systems in Parkinson's Disease Patients. Brain Connect 2017;7:643–647. doi:10.1089/brain.2017.0534. [PubMed: 29065697]

[88]. Tohka J, Zijdenbos A, Evans A. Fast and robust parameter estimation for statistical partial volume models in brain MRI. Neuroimage 2004;23:84–97. doi:10.1016/J.NEUROIMAGE.2004.05.007. [PubMed: 15325355]

[89]. Tomasi D, Volkow ND. Association between functional connectivity hubs and brain networks. Cereb Cortex 2011;21:2003–13. doi:10.1093/cercor/bhq268. [PubMed: 21282318]

[90]. Upadhyay J, Anderson J, Baumgartner R, Coimbra A, Schwarz AJ, Pendse G, Wallin D, Nutile L, Bishop J, George E, Elman I, Sunkaraneni S, Maier G, Iyengar S, Evelhoch JL, Bleakman D, Hargreaves R, Becerra L, Borsook D. Modulation of CNS pain circuitry by intravenous and sublingual doses of buprenorphine. Neuroimage 2012;59:3762–3773. [PubMed: 22119647]

[91]. Verdugo RJ, Ochoa JL. Abnormal movements in complex regional pain syndrome: assessment of their nature. Muscle Nerve 2000;23:198–205. Available: http://www.ncbi.nlm.nih.gov/pubmed/10639611 Accessed 10 Sep 2018. [PubMed: 10639611]

[92]. Woby SR, Roach NK, Urmston M, Watson PJ. Psychometric properties of the TSK-11: A shortened version of the Tampa Scale for Kinesiophobia. Pain 2005;117:137–144. doi:10.1016/j.pain.2005.05.029. [PubMed: 16055269]

[93]. Yager LM, Garcia AF, Wunsch AM, Ferguson SM. The ins and outs of the striatum: Role in drug addiction. Neuroscience 2015;301:529–541. [PubMed: 26116518]

[94]. Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 2001;20:45–57. doi:10.1109/42.906424. [PubMed: 11293691]

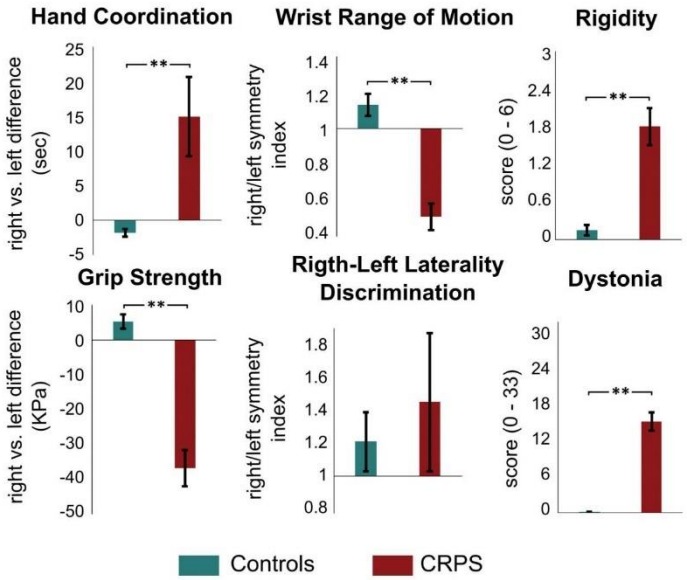

Figure 1. Functional motor tests performance.

CRPS patients presented with significantly (**$p < 0.01$) substantial motor deficits in the affected hand relative to healthy controls. The functional motor metrics reflect the performance of the affected (dominant, in case of healthy controls) hand in relation to the healthy (non-dominant) hand to account for intragroup variability.

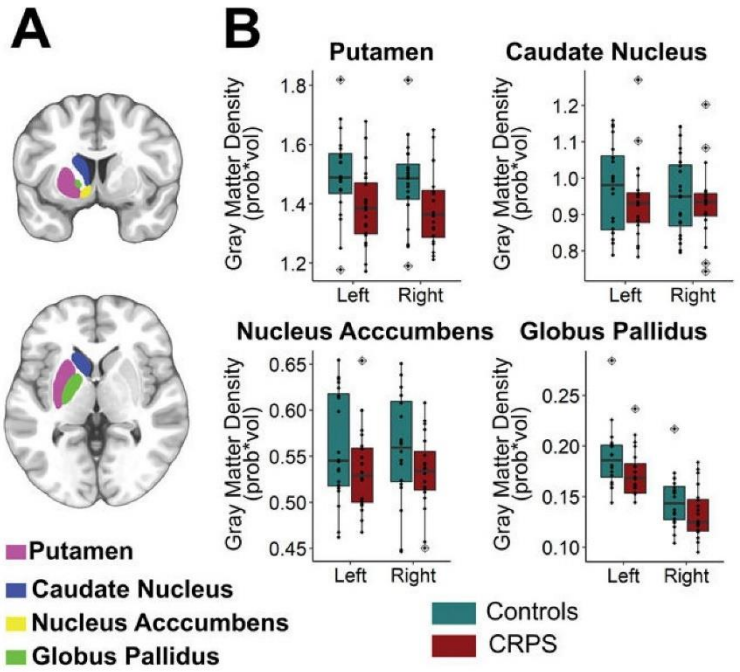

Figure 2. Box plots to show group differences in gray matter density.

Out of all of the striatal ROIs analyzed (A), only the putamen showed a significant bilateral reduction in GMD in chronic CRPS patients when compared to healthy controls. Black dots indicate singe-subjects data. Outlier observations (e.g: outside 1.5 times the interquartile range above the upper quartile and bellow the lower quartile) are denoted with a rhombis symbol. Statistical results stemming from group x side repeated measures ANOVA are given in Table 3.

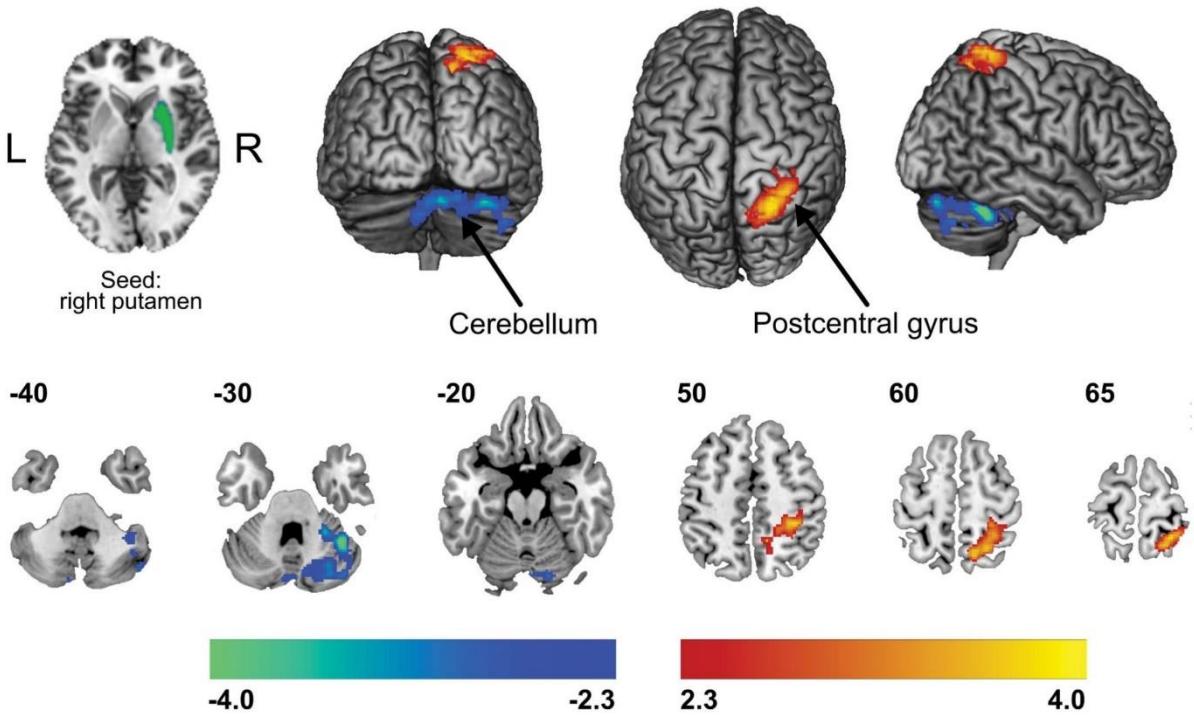

Figure 3. Group differences in resting state functional connectivity of the putamen.

Compared to matched controls, CRPS patients demonstrated greater functional connectivity strength (warm colors) amongst the right (ipsilateral to the affected limb) putamen and sensorimotor and superior parietal cortices, while decreased connectivity (cold colors) was quantified with the crus I region of the cerebellum. Statistical maps were thresholded at p-value < 0.05, corrected for multiple comparisons. Color bars show z-values.

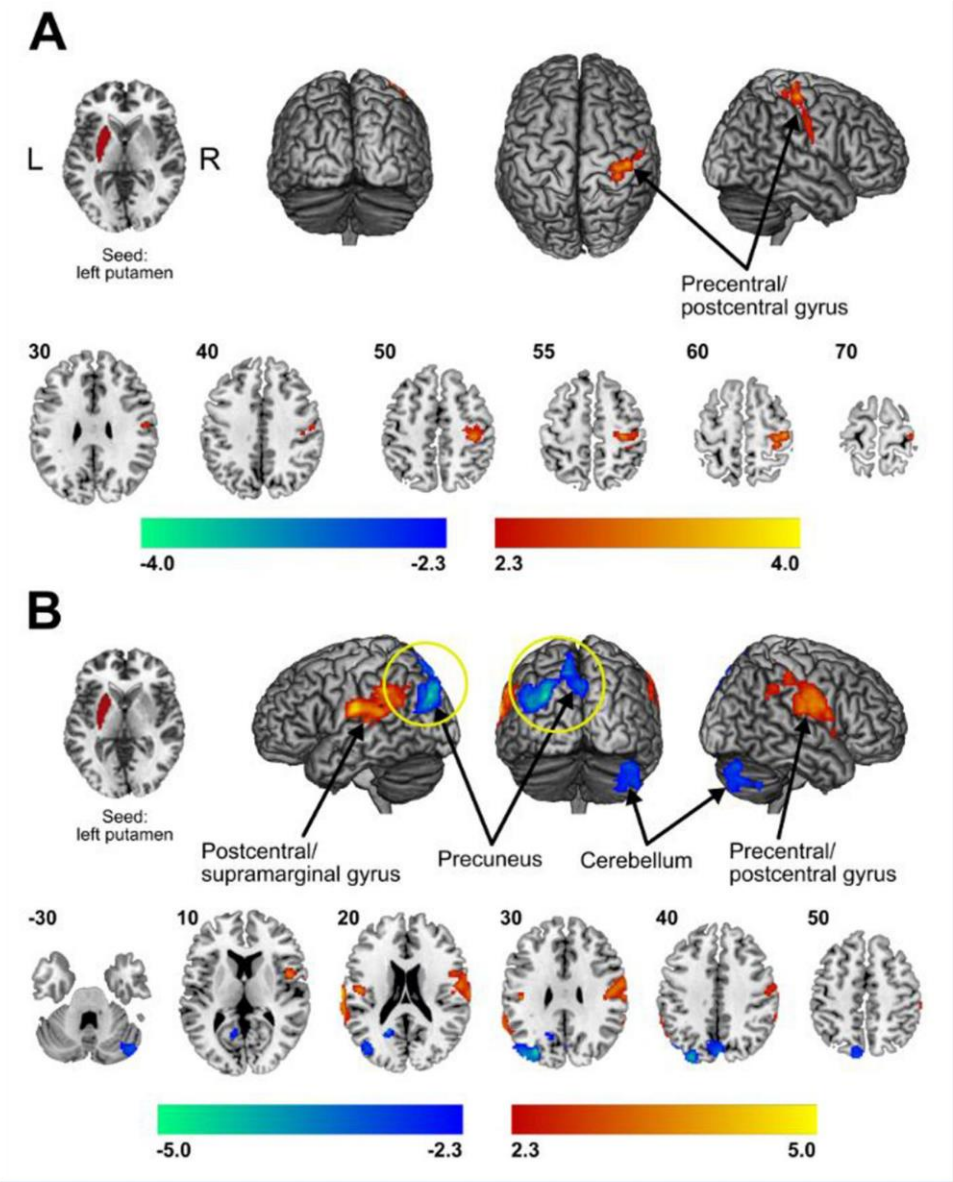

Figure 4. Correlation of resting state functional connectivity of the putamen with behavioral measures.

Patients reporting higher spontaneous pain intensity on the day of the scan showed greater functional connectivity of the left putamen with spread motor and sensory discriminative areas. The pain intensity was reported on a visual scale of 0 = "no pain" to 10 = "worse imaginable pain" (A). Interaction analysis (diseaseXmotor dysfunction) showed that patients with poorer hand and finger coordination, as evaluated with the 9-Hole Peg Test, presented increased functional connectivity strengths (warm colors) amongst the left putamen and motor and discriminative/association areas, as well as decreased functional connectivity

(cold colors) between the left putamen and both the cerebellum (crus I and II) and precuneus (B). Statistical maps were thresholded at p-value < 0.05. Color bars show z-values. Further Correction for multiple comparison using false discovery rate (FDR) showed that only decreases of functional connectivity of putamen with precuneus in the context of poor hand and finger coordination were significant – highlighted with a yellow circle in the figure.

Table 1.

Patient demographics, clinical history and medication information

Patient	Age (y)	Gender	Pain intensity (0–10)	Pain duration (m)	Pain location	Inciting event	Pain medication (doses, frequency)		Over motor deficits
							Analgesic	Co-analgesic	
1	37	female	3	103	right hand	scaphoid fracture, surgery	ibuprofen 600mg, PRN metamizole, PRN	—	weakness limited RoM poor coordination dystonia
2	46	female	2	80	right hand	carpal tunnel syn., surgery	metamizole, 500mg, PRN	—	weakness limited RoM
3	53	female	9	39	right hand	CMC osteoarthritis, surgery	—	—	weakness limited RoM poor coordination dystonia
4	54	female	1	11	right hand	finger cut with blood poisoning	metamizole 500mg, QD tramadol 500mg, QD	gabapentin 300mg, BID	weakness poor coordination dystonia
5	55	female	6	109	right arm	elbow surgery	—	—	weakness limited RoM poor coordination dystonia
6	57	female	4	50	right hand	carpal tunnel syn., surgery	ibuprofen 800mg, QD metamizole, QD tapentadol 100mg, BID	duloxetine 30mg, QD	weakness poor coordination rigidity dystonia
7	57	female	3	8	right hand	radius fracture, cast	—	—	rigidity
8	58	female	5	15	right hand, forearm	epicondylitis with partial tear	—	—	rigidity
9	62	female	8	7	right arm	radius fracture	metamizole, BID	tramadol, BID	weakness limited RoM rigidity dystonia
10	64	female	1	19	right hand	radius fracture, surgery	—	—	rigidity
11	66	female	4	6	right hand	radius fracture	metamizole 500mg, TID tilidine 50/4, BID	amitriptyline, QD	weakness limited RoM dystonia
12	67	female	5	140	right hand, forearm	radius fracture, cast	—	—	weakness limited RoM poor coordination
13	67	female	2	105	right hand	dislocated shoulder, surgery	ibuprofen 800mg, PRN	pregabalin 150mg, BID	—

Patient	Age (y)	Gender	Pain intensity (0–10)	Pain duration (m)	Pain location	Inciting event	Pain medication (doses, frequency)		Over motor deficits
							Analgesic	Co-analgesic	
14	69	female	6	144	right hand	radius fracture, surgery	ibuprofen 600mg, PRN aspirin 100mg, PRN	—	weakness
15	71	female	4	126	right hand	radius fracture	aspirin 500mg, PRN metamizole 500mg, PRN tramadol 100mg, PRN	—	limited RoM
16	47	male	8	57	right arm	radius head fracture	oxycodone 5mg, PRN	—	weakness limited RoM poor coordination rigidity dystonia
17	51	male	7	13	right hand, forearm	radius fracture, surgery	metamizole 500mg, PRN tilidine 50/4, BID	gabapentin 200mg, BID duloxetine, PRN	weakness limited RoM poor coordination dystonia
18	53	male	6	18	right arm	ulna fracture, surgery	ibuprofen 600mg, BID metamizolde 500mg, QD	—	weakness limited RoM poor coordination dystonia
19	56	male	4	64	right arm	TMC osteoarthritis, surgery	metamizole, BID	pregabalin 75mg, BID	weakness poor coordination dystonia
20	67	male	4	77	right hand	radius fracture, surgery	—	—	weakness

CMC: Carpometacarpal; TMC: trapeziometacarpal; QD: once a day; BID: twice a day; PRN: as needed; RoM: forearm range of motion

Table 2.

Group comparison of somatosensory evaluation, autonomic function and psychometrics.

	CRPS Patients		Healthy Controls		p-value
Number of patients, N	20		20		-
Somatosensory evaluation					
Monofilament test right, N (%)	7	(35%)	1	(5%)	**0.010** [a]
Monofilament test left, N (%)	4	(20%)	1	(5%)	0.171 [a]
2-point discrimination test right, N (%)	3	(15%)	1	(5%)	0.302 [a]
2-point discrimination test left, N (%)	0	(0%)	0	(0%)	
Autonomic function					
Abnormal sweating pattern, N (%)	19	(95%)	0	(0%)	**< 0.001** [a]
Abnormal nail growth pattern, N (%)	12	(60%)	0	(0%)	**< 0.001** [a]
Abnormal hair growth pattern, N (%)	4	(20%)	0	(0%)	0.053 [a]
Abnormal skin color, N (%)	14	(70%)	0	(0%)	**< 0.001** [a]
Abnormal hand volume, N (%)	12	(60%)	4	(25%)	**0.038** [a]
Abnormal hand temperature, N (%)	8	(40%)	3	(15%)	0.078 [a]
Quality of life					
Veterans RAND 12 PCS, Mean (SE)	34.52	(1.80)	53.47	(0.98)	**< 0.001** [b]
Veterans RAND 12 MCS, Mean (SE)	45.80	(3.39)	55.35	(1.76)	**0.025** [b]
Mental health					
HADS depression, Median (IQR) | N(%)	6 (6)	| 9 (45%)	2 (4)	| 0 (0%)	**0.001** [a]
HADS anxiety, Median (IQR) | N(%)	5 (5)	| 7 (35%)	4 (3)	| 2 (10%)	0.058 [a]
Kinesiophobia					
TSK-11, Median (IQR) | N (%)	22 (11)	| 6 (30%)	13(4)	| 1 (5%)	**< 0.001** [a]

N: Number of participants presenting with abnormal scores

% : Percentage of participants presenting with abnormal scores

SE: Standard Error; IQR: Inter quartile range

PCS: Physical Composite Score; MCS: Mental Composite Score

HADS : Hospital Anxiety and Depression Scale, range: 0–21

TSK-11 Tampa Scale Kinesiophobia, range: 11–44

[a] One tail Fisher Test

[b] Mann Whitney Test

Table 3.

Group (CRPS and healthy controls) x side (left and right hemisphere) comparison of striatal volumes with mixed-design analysis of variance (ANOVA).

Effect	*df*	*SS*	*MS*	*F-value*	*p-value*
Putamen					
Group	1	0.176	0.176	4.717	**0.036**
Error (Group)	38	1.417	0.037		
Side	1	0.00415	0.004153	3.842	0.057
Side x Group	1	0.00275	0.00275	2.544	0.119
Error (Side x Group)	38	0.04107	0.001081		
Caudate Nucleus					
Group	1	0.0147	0.01465	0.615	0.438
Error (Group)	38	0.9055	0.02383		
Side	1	0.004476	0.004476	6.332	**0.0162**
Side x Group	1	0.001145	0.001145	1.619	0.211
Error (Side x Group)	38	0.02686	0.000707		
Nucleus Accumbens					
Group	1	0.01353	0.013528	2.669	0.111
Error (Group)	38	0.19262	0.005069		
Side	1	0.000094	9.40E-05	0.314	0.579
Side x Group	1	0.000015	1.45E-05	0.049	0.827
Error (Side x Group)	38	0.011378	2.99E-04		
Globus Pallidus					
Group	1	0.00364	0.003638	2.758	0.105
Error (Group)	38	0.05013	0.001319		
Side	1	0.03359	3.36E-02	369.969	**<2e-16**
Side x Group	1	0.00003	3.00E-05	0.335	0.566
Error (Side x Group)	38	0.00345	9.00E-05		

df: degrees of freedom; SS: Sum of Squares; MS: Mean Square

Table 4.

Regions in which seed-based resting functional connectivity strengths were significantly different between CRPS patients and healthy controls. Note that "seed" clusters were derived from the previous gray matter volume analyses. Locations are in Montreal Neurological Institute space. Significant clusters were formed with cluster-extent thresholding (family-wise error rate) to correct for multiple comparisons.

	X	Y	Z	z-statistic	cluster	cluster size	p-value
crps > controls							
seed: right putamen							
right primary somatosensory cortex	20	−54	64	3.94	1	1545	0.00061
right superior parietal lobe	12	−50	50	3.86			
crps < controls							
seed: right putamen							
right cerebellum crus1	4.48	40	−52	−32	1	1448	0.00099
pain intensity (positive) correlation							
seed: left putamen							
right primary somatosensory	34	−32	60	3.15	1	735	0.02720
right primary motor cortex	32	−24	54	3.49			
dystonia (positive) correlation							
seed: left putamen							
left supramarginal gyrus	−52	−46	24	3.40	1	892	0.01010
Hand coordination, crps > controls							
seed: left putamen							
left postcentral gyrus	−67	−15	20	4.45	1	992	0.00339
left supramarginal gyrus	−64	−40	17	3.83			
right premotor cortex	48	2	14	4.11			
right precentral gyrus	58	−8	34	3.64	2	1482	0.00069
right postcentral gyrus	66	−18	20	3.60			
Hand coordination, crps < controls							
seed: left putamen							
left precuneus	−10	−84	52	4.27	1	1893	0.00000
left lateral occipital cortex	−30	−80	32	5.39			
right cerebellum crus I	42	−74	−28	3.39	2	784	0.03320
right cerebellum crus II	48	−70	−42	3.24			
Forearm range of motion, crps < controls							
seed: right putamen							

	X	Y	Z	z-statistic	cluster	cluster size	p-value
left precentral gyrus	−60	−4	14	3.80	1	907	0.01620
left postcentral gyrus	−50	−16	18	4.34			

4.2 Publication II

Shifting Brain in Pain Chronicity

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RESEARCH ARTICLE

Shifting brain circuits in pain chronicity

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Abstract

The evaluation of brain changes to a specific pain condition in pediatric and adult patients allows for insights into potential mechanisms of pain chronicity and possibly long-term brain changes. Here we focused on the primary somatosensory system (SS) involved in pain processing, namely the ventroposterolateral thalamus (VPL) and the primary somatosensory cortex (SI). We evaluated, using MRI, three specific processes: (a) somatotopy of changes in the SS for different pain origins (viz., foot vs. arm); (b) differences in acute (ankle sprain versus complex regional pain syndrome-CRPS); and (c) differences of the effects of CRPS on SS in pediatric versus adult patients. In all cases, age- and sex-matched individuals were used as controls. Our results suggest a shift in concurrent gray matter density (GMD) and resting functional connectivity strengths (rFC) across pediatric and adult CRPS with (a) differential patterns of GMD (VPL) and rFC (SI) on SS in pediatric vs. adult patterns that are consistent with upper and lower limb somatotopical organization; and (b) widespread GMD alterations in pediatric CRPS from sensory, emotional and descending modulatory processes to more confined sensory-emotional changes in adult CRPS and rFC patterns from sensory-sensory alterations in pediatric populations to a sensory-emotional change in adult populations. These results support the idea that pediatric and adult CRPS are differentially represented and may reflect underlying differences in pain chronification across age groups that may contribute to the well-known differences between child and adult pain vulnerability and resilience.

KEYWORDS
adult, arm, brain, complex regional pain, CRPS, leg, nerve, pediatric, S1, thalamus

1 | INTRODUCTION

Evaluation of chronic pain across pediatric and adult populations, even for the same disease, is difficult to study. Complex regional pain syndrome (CRPS) is a chronic pain condition that is observed in both age groups. CRPS is a disease that usually begins with a peripheral injury and results in multiple brain changes. Spread of pain (van Rijn et al., 2011), inattention (Bultitude, Walker, & Spence, 2017), movement disorders (Verdugo & Ochoa, 2000), neglect-like symptoms (Maihofner & Birklein, 2007) make this a notable pain disease that afflicts multiple brain systems. While this condition may extend into adulthood, studies comparing the differences in how the brain responds to a peripheral nerve injury (most common cause [Low, Ward, & Wines, 2007]) have not been well described.

Compared with pain-free controls, pediatric CRPS patients are associated with wide-spread brain gray matter atrophy in motor, affective, motivational, emotional, cognitive, memory, and fear-related regions (Erpelding et al., 2016), whereas atrophy in adults appears to be more confined to affective, motivational and cognitive regions (Barad, Ueno, Younger, Chatterjee, & Mackey, 2014; Geha et al., 2008). Additionally, more apparent, are the mixed reports in child and adult populations across resting functional connectivity metrics between brain signal covariation and within brain networks (such as the default mode network). Indeed, in our recent pediatric investigations, we have reported hyperconnectivity patterns in neural networks (Becerra et al., 2014) and amygdala-based covariation (Simons et al., 2014), whereas adult patients are associated with wide-spread hypoconnectivity patterns in the default mode network (Bolwerk, Seifert, & Maihofner, 2013) and insula-centered covariation (Kim, Choi, et al., 2017).

CRPS caused by peripheral nerve injury provides a unique opportunity to investigate specific brain processes involved in disease progression, such as somatotopic specificity of somatosensory changes; chronicity of changes and plasticity of responsivity (resilience to treatment/resistance to disease reversal). While there is a clear distinction in structural and functional metrics across aging populations, it is possible that the reported differential changes in pediatric versus adult CRPS patients reflect age-related effects, or perhaps a transition of circuitry as a consequence of disease duration. Indeed, it is possible that there is a shift from sensory to emotional circuits with pain chronification (Hashmi et al., 2013). However, while this shift is evident in adult populations, it remains unclear whether a similar cascade is present across pediatric and young adult groups. In any case, while differences between patients with CRPS are reported relative to healthy controls, it remains unknown whether a similar set of brain morphometry and resting connectivity circuits are involved during the acute phase of the disease. Furthermore, the relative contribution of the affected limb remains uncertain, as no study to date reports altered structural metrics within brain sites with fine discriminatory somatotopic representations, notably the primary somatosensory cortex (Penfield & Boldrey, 1937) and the ventral posterior thalamic nucleus (Kaas, Nelson, Sur, Dykes, & Merzenich, 1984).

We employed magnetic resonance imaging (MRI) techniques to determine potential brain structural (gray matter density) and subsequent resting functional connectivity strengths in acute (ankle sprain) and chronic (CRPS) pain affecting the leg in pediatric patients and between controls to determine effects of pain "evolution" affecting the same body region. In addition, we evaluated the same metrics in adult chronic (CRPS) pain patients vs. healthy controls affecting the arm to determine the regional somatotopy in sensory regions (viz., thalamus and sensory cortex), as well as to ascertain whether or not if the adult CRPS brain responds differently when compared to the pediatric CRPS brain. Based on previous findings described above, our hypotheses were: (a) alterations within the ventral postero-lateral (VPL) thalamic nucleus or primary somatosensory (SI) cortex should correspond to upper or lower limb somatotopical organization, which would define the specificity of other observed changes; (b) these somatosensory regions would show gray matter atrophy and functional hyperconnectivity patterns in chronic pain patients vs. matched controls in both pediatric and adult patients affecting the same body region; (c) in comparing acute vs. chronic pain cohorts (matched for age and sex) with pain affecting the same body region, the acute group will display less robust gray matter and functional alterations based on the duration of the disease.

2 | MATERIALS AND METHODS

2.1 | Subjects

Fifty-two patients and 52 healthy controls (matched for sex and age) were recruited for the study. The patient group consisted of 32 pediatric patients, equally distributed with unilateral acute or chronic pain of the lower limb extremity (acute, 10 females, 6 males, mean ± *SEM* age: 15.8 ± 0.6 years, range 11–22 years; chronic, 10 females, 6 males, age: 14.3 ± 0.6 years, range 10–17 years; controls, 20 females, 12 males, age: 15 ± 2.7 years, range 10–20 years) and 20 adult patients with unilateral chronic pain of the upper limb extremity (chronic, 15 females, 5 males, age: 57.9 ± 9 years, range 37–71 years; controls, 15 females, 5 males, age: 58.5 ± 10 years, range 37–73 years). Within both the adult and the pediatric patients, there were no significant group differences in age (two-sample *t*-test; $p > .05$) or sex composition (chi-square test, $p > .05$). Here, it is pertinent to note that patients with acute pain were recruited are part of an ongoing longitudinal study, and although one subject slightly exceeded the 3-month reference point, recovery was within a week and as such, we considered it appropriate to include this subject in our acute sample. All chronic pain (3 months or greater) patients were diagnosed with CRPS Type I by Budapest criteria by an experienced pain neurologist (AL, DB) in accordance with a neurological examination and a comprehensive record review. Pediatric patients were recruited from the Pain Treatment Services, Emergency, Orthopedics, and Sports Medicine departments at Boston Children's Hospital. Adult patients were recruited from the Interdisciplinary Pain Unit and Medical Centre of University of Munich, as well as through two internet-based CRPS self-help network sites (crps-netwerk.org; unfallopfer.de). Individual patients reported their average pain intensity from the time of injury on a numerical rating scale (0 = no pain, 1–3 = mild pain, 4–6 = moderate pain, 7–10 = severe pain). Pediatric healthy controls were recruited through flyers on bulletin boards throughout the local community, online list serves (e.g., craigslist, college job boards) and word of mouth.

Participants were excluded from the study if they had any other neurological symptoms, severe medical problems (such as uncontrollable asthma and seizures, cardiac diseases or severe psychiatric disorders), medical implants and/or devices or weighed more than 285 pounds, corresponding to the MRI scanner weight limit. All patients were instructed not to take analgesic medication within at least 4 hr prior to the study scanning session. For the pediatric population, written informed consent and assent were obtained for all procedures, which were conducted under the approval of the Boston Children's Hospital Institutional Review Board. Written consent was

obtained for all procedures, which were conducted under the approval of the Ethics Committee of the Ludwig Maximilian University of Munich and met the Helsinki criteria for the study of pain in humans.

2.2 | MRI acquisition

All subjects were positioned supine in a Siemens 3 T MRI scanner. For image registration, a three-dimension magnetization-prepared rapid gradient-echo (MP-RAGE) sequence was used to acquire a high-resolution T1-weighted anatomical image (raw voxel size = 1.0 mm thick, matrix = 256 × 256 voxels). With the subject still at rest, a series of gradient echo echo-planar image sets with Blood Oxygen Level Dependent contrast were collected as follows: (a) Siemens Magnetom Trio 3 T (image series = 425, axial slices = 51, repetition time = 1,100 ms, echo time = 30 ms, raw voxel size = 3.00 × 3.00 × 3.00 mm thick, matrix = 76 × 76 voxels, 16 acute, 16 controls); (b) Siemens Magnetom Trio 3 T (image series = 150, axial slices = 41, repetition time = 3,000 ms, echo time = 35 ms, raw voxel size = 3.75 × 3.75 × 3.50 mm thick, matrix = 64 × 64 voxels, 8 pediatric chronic, 8 controls); (c) Siemens Magnetom Trio 3 T (image series = 200, axial slices = 41, repetition time = 2,500 ms, echo time = 35 ms, raw voxel size = 3.75 × 3.75 × 3.50 mm thick, matrix = 64 × 64 voxels, 8 pediatric chronic, 8 controls); (d) Siemens Magnetom Skyra 3 T (image series = 250, axial slices = 39, repetition time = 2,500 ms, echo time = 30 ms, raw voxel size = 3.50 × 3.50 × 3.85 mm thick, matrix = 64 × 64 voxels, 20 adult chronic pain, 20 controls). Note that all T1-weighted images were collected on the same scanner as the functional images, that is, as they were all collected on the same session.

2.3 | MRI analysis

2.3.1 | Gray matter density analysis

Using SPM12 (Friston et al., 1994), image preprocessing and gray matter density maps were created with the computational anatomy toolbox (dbm.neuro.uni-jena.de/cat). Here, prior to segmentation, all T1-weighted images in pediatric patients with unilateral left pain were flipped to the right (pediatric acute pain, n = 7; pediatric chronic pain; n = 9). All T1-weighted images were then segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) probability maps. Here, the segmentation approach was based on the Adaptive Maximum A-Posterior technique to model local variations and parameters as slowly varying spatial functions (Rajapakse, Giedd, & Rapoport, 1997) and the partial volume estimation method whereby the fraction of each tissue type within each voxel is estimated to yield more accurate segmentation (Tohka, Zijdenbos, & Evans, 2004). Furthermore, to enhance the quality of the T1-weighted images and further improve the segmentation, we combined two denoising methods: the Spatially Adaptive Non-Local Means (SANLM) filter (Manjon, Coupe, Marti-Bonmati, Collins, & Robles, 2010) and the classical Markov Random Field method (Rajapakse et al., 1997). The segmented images were then registered to the tissue probability map using affine transformation (i.e., linear, preserving proportions), followed by a nonlinear deformation in Montreal Institute Neurological (MNI-152) space. The nonlinear deformation parameters were calculated by using the high-dimensional Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) algorithm (Ashburner, 2007; Ashburner & Friston, 2009). To correct for expansion (or contraction) during the spatial transformation, the normalized images were then "modulated" by multiplying each voxel by the Jacobian determinant (i.e., linear and nonlinear components) derived from the spatial normalization procedure. Finally, the normalized, modulated gray matter images were then smoothed using an isotropic 5 mm full-width-half-maximum (FWHM) Gaussian filter, and the smoothed images were used for subsequent analysis.

Significant differences in gray matter density were determined in pediatric populations between acute and chronic pain patients under the framework of a general linear model using an independent two-sample t-test. To explore gray matter density values in healthy controls, we overlaid the results derived from the between-patients contrasts described above. Here, significant differences between patients (acute and chronic) and healthy controls were then determined using independent two-sample t-tests ($p < .05$). In the adult the population, significant differences between chronic pain patients and healthy controls were determined within a general linear model using an independent two-sample t-test. For the acute and chronic pediatric comparison, the total intracranial volume and age for each subject were factored out as nuisance variables in the modeling. These parameters were also included as nuisance variables in our adult chronic pain and control comparison. Following an a priori primary threshold of $p < .001$, we applied a family-wise error rate (FWE) cluster-level extent threshold to correct for multiple comparisons. We chose this stringent cluster-defined primary threshold based on the simulations of Woo, Krishnan, and Wager (2014) to optimize the control of false positives and to improve overall inferences about specificity. Significant linear relationships between gray matter density values, pain intensity, and disease duration were determined using Pearson (r) correlations.

2.3.2 | Functional connectivity analysis

Using SPM12 (Friston et al., 1994), image preprocessing and seed-based functional connectivity maps were created with the functional connectivity toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). Here, prior to realignment, all functional images in pediatric patients with unilateral left pain were flipped to the right (pediatric acute pain, n = 7; pediatric chronic pain; n = 9).

The first five volumes were then discarded to allow for T1-equilibration effects. All images were subsequently realigned (rigid body translation and rotation) to the first volume as the initial motion correction procedure. The realigned images were then co-registered to the same subjects' T1-weighted anatomical image, and the anatomical images were spatially normalized to MNI-152 space. The deformation field acquired during affine and nonlinear transforms of the T1-weighted anatomical image was then applied to the functional images (i.e., indirect normalization). Finally, the normalized functional images were spatially smoothed with an isotropic 5 mm FWHM Gaussian kernel. Images were linearly detrended to remove global

signal intensity changes and a temporal pass-band filter of 0.01–0.08 Hz applied. To account for nonspecific variance, 18 physiological and motion-related factors were included as nuisance variables, including the first three principle components of the time course derived from separate regions of matter and cerebral spinal fluid (Behzadi, Restom, Liau, & Liu, 2007), and the six body translation and rotation parameters from the realignment procedure. The first temporal derivative of the movement parameters was included to account for temporal shifts in the signal. In recent years, a whole body of literature has established that in-scanner head movement can have substantial influences on resting state functional connectivity (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012; Van Dijk, Sabuncu, & Buckner, 2012), particularly in pediatric populations (Satterthwaite et al., 2012) Here, particular care was taken with examining movement. For all subjects, we set a priori criteria of less than 3 mm cumulative displacement and 3° of angular motion. Three subjects from our sample, made up of two pediatric participants (one chronic, one control) and one adult healthy control, exceeded this criterion and were, therefore, excluded from the functional connectivity analysis.

Using the normalized smoothed images, regions where there were significant contrasts in gray matter density values between the pediatric and adult patients (see above) were used as "seeds" in the subsequent resting state analysis. The seed-based functional connectivity map was generated by computing the correlation coefficient between each voxel's time series with the mean time course of the voxels within the seed region. The individual correlation coefficient maps were then converted to *z*-maps using Fisher's *r*-to-*z* transformation, and these images were used for group-level statistical comparisons. Given the distribution of scanning parameters, significant group differences between pediatric acute and chronic patients were confined to comparisons with healthy controls that were equally matched to the patient's scanning acquisition. We chose this approach to ensure that any significant group differences were not due to differential scanning parameters (such as repetition time), or influenced by factors such as the degrees of freedom due to differences in the number of volumes acquired. Taken together, significant differences between pediatric acute and chronic pain patients as compared with healthy controls were then determined using an independent two-samples *t*-test, under the framework of a general linear model. Significant differences between adult chronic pain patients and controls were determined under the same framework. For all three comparisons, sex and age were factored out as nuisance variables in the modeling. Significant differences were determined using an a priori primary threshold of $p < .001$ with a family-wise error rate cluster-level extent threshold applied to correct for multiple comparisons. Significant linear relationships between seed-based functional connectivity values, pain intensity, and disease duration were determined using Pearson's correlation analysis ($p < .05$). Finally, to determine whether these significant differences in seed-based connectivity strengths were unique or restricted to a particular pain group, we extracted these regions as a masks, and performed a subsequent region of interest-region of interest functional connectivity analysis across subsequent groups. Significant differences in resting connectivity strengths were then determined using independent two-sample *t*-tests ($p < .05$). Of course, given the differences in scanning parameters, the purpose of this additional analyses is to provide some indication of specific regional connectivity across all groups, rather than conclusive outcomes, as with the previous analyses.

3 | RESULTS

3.1 | Psychometrics

Individual pediatric (acute and chronic) and adult chronic subject characteristics, imaging modality inclusion, pain duration and pain intensities are shown in Tables 1 and 2, respectively. Our two pediatric groups and one adult group reported, on average, the following: (a) mean [±*SEM*] pain intensity: pediatric acute, 3.97 ± 0.3; pediatric chronic, 5.91 ± 0.5; adult chronic pain, 4.60 ± 0.5; (b) mean [±*SEM*] pain duration (months): pediatric acute, 1.25 ± 0.2; pediatric chronic, 15.0 ± 5.4; adult chronic pain, 59.67 ± 10.75; and (c) pain distribution: pediatric acute, unilateral lower limb extremity, primarily at the distal leg and foot; pediatric chronic, unilateral lower limb extremity, including thigh, leg and foot; adult chronic pain, unilateral upper limb extremity, primarily at distal forearm and hand, with fewer patients reporting pain in the shoulder.

3.2 | Gray matter density

Within the pediatric sample, gray matter density was significantly different between acute and chronic patients in a number of brain regions (Table 3, Figure 1a). Significantly reduced gray matter density values were observed in chronic patients in the left mid-cingulate cortex (mean [±*SEM*] probability*volume; MCC: acute: 0.598 ± 0.023, chronic: 0.499 ± 0.016; $p = .0013$), right inferior temporal gyrus (ITG: acute: 0.576 ± 0.024, chronic: 0.435 ± 0.018; $p = .00007$), right anterior cingulate cortex (ACC: acute: 0.642 ± 0.022, chronic: 0.543 ± 0.015; $p = .0008$), bilaterally in the orbitofrontal cortex (OFC: acute: 0.681 ± 0.019, chronic: 0.580 ± 0.011; $p = .00008$), bilaterally in the thalamic reticular nucleus (TRN: left, acute: 0.173 ± 0.009, chronic: 0.112 ± 0.005; $p = .000002$; right, acute: 0.183 ± 0.012, chronic: 0.107 ± 0.007; $p = .000006$). In contrast, increased gray matter density in chronic patients was observed exclusively within the left ventral posterolateral nucleus of the thalamus (VPL: acute: 0.484 ± 0.019, chronic: 0.566 ± 0.018; $p = .0035$). Furthermore, we report significant linear relationships between these values and pain intensity, as shown in Figure 1b. Positive correlations indicate that greater gray matter density is associated with greater pain intensity occurring in the VPL ($r = .45$, $p = .0098$), whereas a negative relationship was observed within the left and right TRN suggesting less gray matter being associated with greater pain intensity (left: $r = -.48$, $p = .0054$; right: $r = -.36$, $p = .0430$). Furthermore, a negative relationship was observed between pain duration and gray matter density values within the OFC and right TRN (OFC: $r = -.36$, $p = .043$; right TRN: $r = -.35$, $p = .0496$). No other correlations were observed with pain intensity (ITG: $r = -.28$, $p = 0.12$; OFC: $r = -.24$, $p = 0.19$; ACC: $r = -.19$,

TABLE 1 Clinical characteristics of pediatric patients

Patient (acute)	Age	Gender	Pain intensity (0–10)	Pain duration (months)	Site	Pain distribution	Medication (s)	Patient (chronic)	Age	Gender	Pain intensity (0–10)	Pain duration (months)	Site	Pain distribution	Medication (s)
1	19	M	3.5	0.7	R	Foot	-	17	17	F	7.0	3	R	Leg, foot	Gabapentin
2	19	F	2.0	0.6	R	Foot	Salbutamol	18	11	M	3.0	8	L	Leg, foot	Gabapentincetalopramclonodine
3	17	F	4.0	1.9	R	Foot	-	19[b]	15	M	7.0	12	R	Knee, foot	Amitriptyline
4	15	F	3.5	0.7	L	Distal leg, foot	-	20	16	F	2.5	48	L	Leg, foot	Doxepin
5	12	F	4.0	0.8	L	Foot	Salbutamol	21	17	F	6.0	19	L	Leg, foot	Pregabalin
6	18	M	5.0	0.9	R	Foot	-	22	14	F	7.5	3	L	Leg, foot	Duloxetine
7	22	M	4.5	0.6	L	Foot	-	23	17	F	6.0	85	R	Leg, foot	Gabapentin
8[a]	15	M	6.0	1.7	L	Foot	-	24	13	F	5.5	13	L	Leg, foot	Amitriptyline
9	14	M	4.5	1.5	L	Foot	-	25	10	M	7.0	5	R	Leg, foot	Gabapentin
10	15	F	4.0	1.4	R	Foot	Montelukast	26	15	M	4.0	11	L	Knee	-
11	16	F	2.5	1.1	R	Foot	-	27	14	F	3.5	3	R	Thigh, leg, foot	-
12	15	M	5.5	1.0	L	Foot	-	28	15	F	8.0	9	L	Leg, foot	-
13	15	F	4.5	0.9	R	Foot	-	29	15	F	7.0	10	R	Leg, foot	Gabapentin
14	12	F	4.5	0.9	L	Foot	-	30	11	M	6.0	3	L	Leg, foot	-
15	13	F	3.0	3.1	R	Distal leg, foot	-	31	11	M	7.0	3	R	Leg, foot	-
16	16	F	2.5	2.1	R	Foot	Tetracycline lysine doxycycline	32	17	F	5.5	6	L	Foot	Gabapentinibuprofin Doxepin
Acute mean ± *SEM*	15.8 ± 0.6		3.97 ± 0.3	1.2 ± 0.2				Chronic mean ± *SEM*	14.3 ± 0.6		5.91 ± 0.5	15.0 ± 5.4			

Abbreviations: F, female; L, left; M, male; R, right.

[a]Removed from seed-based connectivity analysis due to failed image acquisition.

[b]Removed from seed-based connectivity analysis due to excessive head motion. Note that brain images for all patients with unilateral left pain were flipped to the right.

TABLE 2 Clinical characteristics of adult patients

Patient (chronic)	Age	Gender	Pain intensity (0–10)	Pain duration (months)	Site	Pain distribution	Medication(s)
1	54	F	1	11	R	Distal hand	Metamizole, tramadol, gabapentin
2	67	F	5	140	R	Hand, forearm	-
3	56	M	4	64	R	Arm, forearm, hand	Metamizole, pregabalin
4	67	M	4	77	R	Distal hand	-
5	59	M	4	50	R	Distal hand	-
6	71	F	4	126	R	Distal hand	Aspirin, metamizole, tramadol
7	46	F	2	80	R	Distal hand	Metamizole
8	62	F	8	7	R	Arm, forearm, hand	Metamizole, tramadol
9	69	F	6	144	R	Distal hand	Ibuprofen, aspirin
10	37	F	3	103	R	Distal hand	Ibuprofen, metamizole
11	47	F	8	57	R	Arm, forearm, hand	Oxycodone
12	55	M	6	109	R	Arm, forearm, hand	Ibuprofen, metamizole
13	67	F	2	105	R	Distal hand	Ibuprofen, pregabalin
14	58	F	5	15	R	Hand, forearm	-
15	57	F	3	8	R	Distal hand	-
16	66	F	4	6	R	Distal hand	Metamizole, tilidine, amitriptyline
17	53	F	9	39	R	Distal hand	-
18	53	M	6	19	R	Arm, forearm, hand	Ibuprofen, metamizole
19	64	F	1	19	R	Distal hand	-
20	51	M	7	13	R	Hand, forearm	Metamizole, tilidine, gabapentin, duloxetine
Mean ± SEM	57.9 ± 9		4.60 ± 0.5	59.7 ± 10.8			

Abbreviations: F, female; M, male; R, right.

p = .30; MCC: r = –.08, p = .66) or disease duration (ITG: r = –.10, p = .59; ACC: r = –.31, p = .08; MCC: r = –.26, p = .15; VPL: r = .16, p = .38; left TRN: r = –.28, p = .12). In summary, compared with pediatric acute pain patients, pediatric chronic pain patients were associated with widespread reduced gray matter density within sensory (VPL)-emotional (MCC, ACC)-descending (OFC) regions, as compared with an increase in sensory modulatory (TRN) region, whereby significant correlations were confined within somatosensory related processing and modulation (VPL and TRN).

Gray matter density values were then extracted in pediatric healthy controls from significant clusters derived from the previous acute and chronic analysis described above. Compared with healthy controls, significant differences in acute and chronic patients were observed in a number of brain regions (Figure 2). Here, compared with acute patients, healthy controls displayed reduced gray matter density within the ITG (mean [±*SEM*] probability*volume; controls: 0.493 ± 0.017; two sample *t*-test, p = .007) and OFC (controls: 0.635 ± 0.010, p = 0.024). No other differences were observed as compared with the acute patient group (ACC: controls: 0.652 ± 0.017, p = .75; MCC: controls: 0.550 ± 0.013, p = .051; VPL: controls: 0.504 ± 0.014, p = .42; left TRN: controls: 0.148 ± 0.009, p = .097; right TRN: controls: 0.162 ± 0.010, p = .23). In contrast, significant differences were observed in all regions when comparing healthy controls with chronic pain patients. Specifically, chronic pain patients displayed reduced gray matter density within the ITG (two sample *t*-test, p = .043), OFC (p = .0014), ACC (p = .0002), MCC (p = .023), left TRN (p = .011) and the right TRN (p = .0008), as compared with controls. Conversely, compared with controls, chronic pain patients were associated with increased gray matter density within the VPL (p = .012).

Within the adult sample, gray matter density differed significantly between chronic patients and healthy controls in sensory and emotional-related brain sites (Table 4, Figure 3a). Specifically, chronic pain patients displayed significantly reduced gray matter density within the left VPL (mean [±*SEM*] probability*volume; chronic: 0.506 ± 0.014, controls: 0.593 ± 0.015; p = .00016), whereas an increase was observed in the left ACC (chronic: 0.354 ± 0.001, controls: 0.328 ± 0.01; p = .0085). Finally, no significant correlations were observed between these values and pain intensity (VPL: r = .02, p = .93; ACC: r = –.33, p = .16) and disease duration (VPL: r = –.03, p = .33; ACC: r = .13, p = .59; Figure 3b).

3.3 | Resting functional connectivity

Using the clusters derived from the gray matter density analysis as "seeds," resting functional connectivity strengths were determined to be significantly different in a number of brain sites across pediatric and adult groups (Table 5, Figure 4). Specifically, in pediatric acute patients,

TABLE 3 Regions in which gray matter density was significantly different in acute compared with chronic pediatric patients

	X	Y	Z	t-statistic value	Cluster size	p-value
Acute > chronic						
Left thalamic reticular nucleus	−25	−20	16	8.42		
	−26	−19	11	6.81	434	.047
	−22	−14	11	6.22		
Left mid-cingulate cortex	−6	14	33	5.01		
	−7	9	35	4.72	930	.001
	−7	17	30	4.62		
Right thalamic reticular nucleus	25	−19	17	6.65	483	.028
	22	−17	−4	6.39		
Right inferior temporal gyrus	55	−30	−27	4.97		
	48	−23	−32	4.70	564	.013
	61	−35	−28	4.24		
Right anterior cingulate nucleus	11	32	22	6.45		
	9	42	23	5.60	942	.0001
	16	36	22	4.45		
Bilateral orbitofrontal cortex	−25	5	−18	9.13		
	3	17	−23	7.92	6,638	.0001
	−4	18	−23	7.42		
Acute < chronic						
Left ventral posterolateral nucleus	−19	−25	5	5.08	503	.046

Locations are in Montreal Neurological Institute space. Significant clusters were from cluster-extent thresholding (family-wise error rate) to correct for multiple comparisons.

significantly greater OFC resting connectivity strength occurred in the right hippocampus (mean [±*SEM*] parameter estimate values; pediatric acute: 0.35 ± 0.02, controls: 0.09 ± 0.05; two-sample *t*-test, $p = .00002$), as compared with healthy controls (Figure 4a). In contrast, pediatric chronic patients had significantly reduced VPL resting connectivity strengths with the left and right primary somatosensory cortex (SI: pediatric chronic: −0.07 ± 0.02, controls: 0.13 ± 0.02; $p = .000001$) (Figure 4b). Compared with controls, adult chronic pain patients exhibited significantly greater ACC connectivity strength with SI (adult chronic pain: 0.08 ± 0.03, controls: −0.08 ± 0.02; $p = .00002$) and reduced VPL connectivity with the posterior cingulate cortex (PCC: adult chronic pain: −0.03 ± 0.03, controls: 0.15 ± 0.03; $p = .00009$; Figure 4C). Extraction of these resting functional connectivity values revealed no significant correlations with pain intensity (OFC-HPC: $r = -.30$, $p = .28$; VPL-SI: $r = -.45$, $p = .09$; ACC-SI: $r = -.04$, $p = .87$; VPL-PCC: $r = -.32$, $p = .17$) or disease duration (OFC-HPC: $r = -.49$, $p = .064$; VPL-SI: $r = .29$, $p = .29$; ACC-SI: $r = .36$, $p = .12$; VPL-PCC: $r = -.05$, $p = .83$). Using the clusters derived from the seed-based functional connectivity strengths, we performed a region of interest-region of interest functional connectivity analysis across all groups and their respective controls. Here, no significant differences were observed across the groups, that is, these significant seed-based functional strengths were restricted to each group in pediatric acute OFC-HPC (pediatric chronic: 0.14 ± 0.04, controls: 0.13 ± 0.05; two-sample *t*-test, $p = .88$; adult chronic pain: 0.11 ± 0.04, controls: 0.10 ± 0.04; $p = .87$), pediatric chronic VPL-SI (pediatric acute: 0.07 ± 0.04, controls: 0.08 ± 0.04; two-sample *t*-test, $p = .95$; adult chronic pain: 0.14 ± 0.04, controls: 0.13 ± 0.04; $p = .81$) and adult chronic pain ACC-SI (pediatric acute: 0.01 ± 0.05, controls: −0.10 ± 0.12; two-sample *t*-test, $p = .12$; pediatric chronic: −0.04 ± 0.04, controls: −0.03 ± 0.05; $p = .88$) and VPL-PCC (pediatric acute: 0.15 ± 0.04, controls: 0.10 ± 0.03; two-sample *t*-test, $p = .34$; pediatric chronic: 0.12 ± 0.04, controls: 0.06 ± 0.04; $p = .33$). In summary, compared with controls: (a) pediatric acute pain patients had greater functional connectivity strengths between the OFC and the HPC; (b) pediatric chronic patients had greater functional connectivity strengths between the VPL and MI-SI; (c) adult chronic patients had greater functional connectivity strengths between the ACC and SI, whereas the VPL had reduced connectivity with the PCC; (d) In all these groups, no significant correlations were observed between functional connectivity strengths and pain intensity or disease duration.

3.4 | Somatotopic representation of upper and lower limb

In this investigation, we report structural and functional alterations in brain sites of pediatric and adult chronic pain patients with a somatotopical representation, notably the thalamus and primary somatosensory cortex (Figure 5). Specifically, we report altered gray matter density in the ventral posterolateral nucleus of the thalamus that is consistent with upper and lower limb somatotopy, that is, lateral to medial, respectively (Kaas et al., 1984). Moreover, we report altered resting functional connectivity with the primary

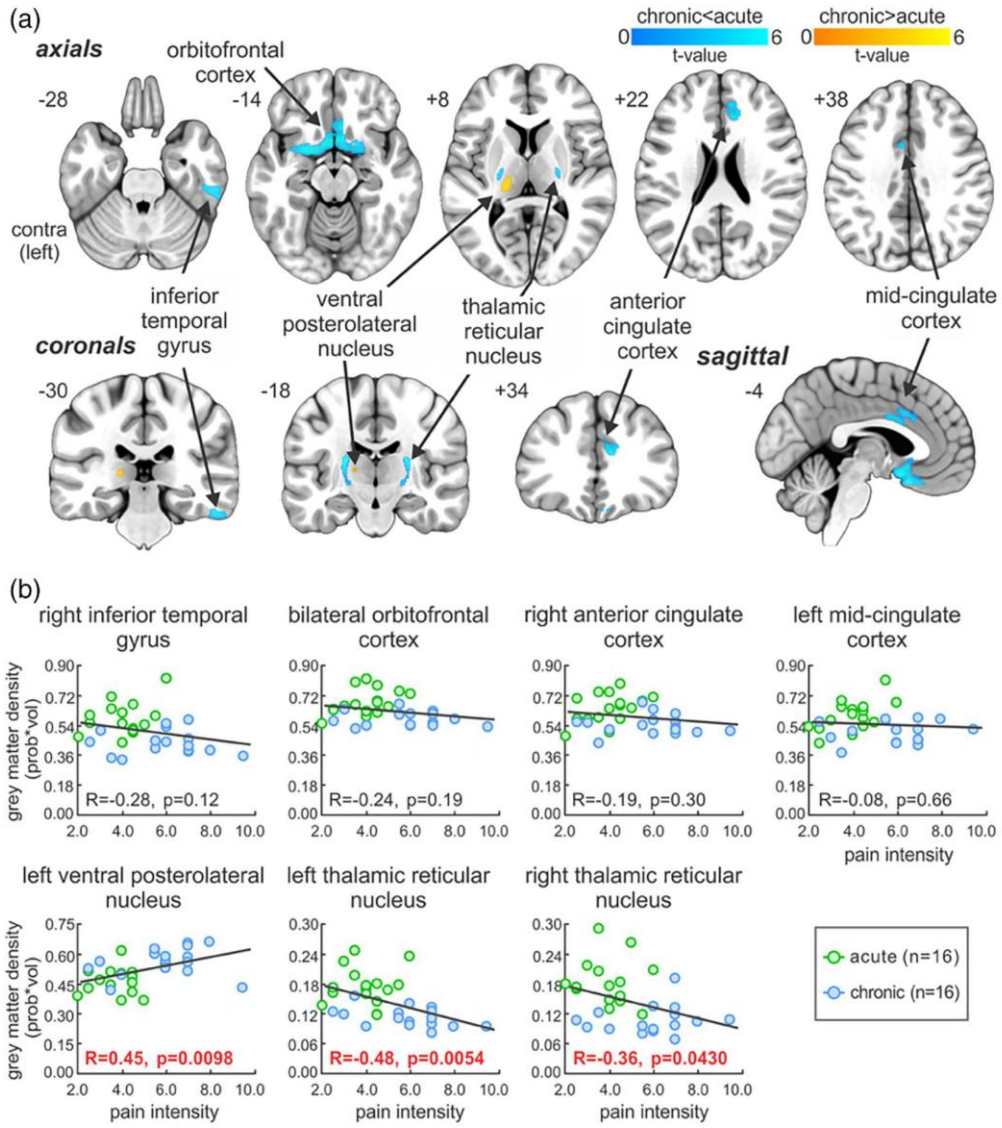

FIGURE 1 Whole brain gray matter density analysis between pediatric chronic versus acute. (a) Brain sites in which significantly reduced (cool color scale) or increased (warm color scale) gray matter density was observed in pediatric chronic pain as compared with patients with acute pain, overlaid onto axial, coronal and sagittal T1-weighted anatomical image set. Slice locations are located on the top left of each image and are in Montreal Neurological Institute space. (b) Correlation between regional gray matter density values within these regions against pain intensity. Note that significant correlations were confined to brain sites associated with somatosensory related processing and modulation, that is, the ventral posterolateral nucleus of the thalamus and the thalamic reticular nucleus [Color figure can be viewed at wileyonlinelibrary.com]

somatosensory cortex, consistent with upper limb (anterolateral) and lower limb (posteromedial) somatotopy (Penfield & Boldrey, 1937).

4 | DISCUSSION

The underlying pathophysiology of the evolution of acute to chronic pain remains unknown. Here we used an approach to evaluate two concepts: (a) is there a difference in brain systems in acute and chronic pain affecting the same region of the body? and (b) is there a difference in the same disease affecting the brain in pediatric vs. adult onset CRPS? In both cases we focused on somatosensory systems for the following reasons: (a) they are well defined and have a known physiology in pain; (b) somatotopy can be relatively easily defined with current imaging technology; (c) comparisons between pediatric and adult region specific areas can be evaluated; (d) these areas are less likely to be influenced by comorbid processes, such as anxiety and depression. Our primary results indicate that there is a shift in gray matter density alterations from pediatric acute (descending modulatory) to pediatric chronic (descending modulatory/sensory-affective) to adult chronic pain (sensory-affective)

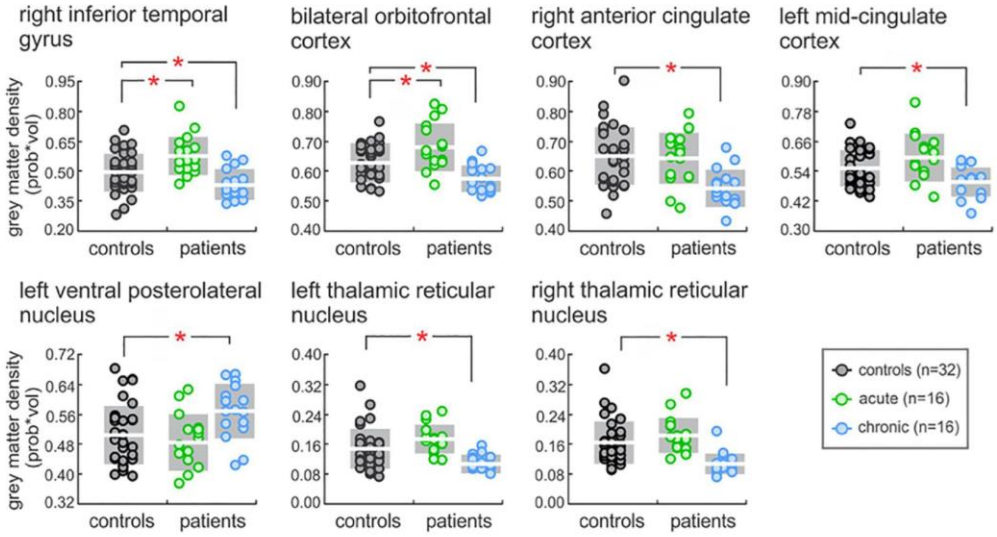

FIGURE 2 Plots of gray matter density values in patients (acute and chronic) and healthy controls. Note that these values were derived from overlaying significant clusters from the patient contrast previously described. Significant between-group differences were determined using independent two-sample *t*-test (*$p < .05$). Note that the white horizontal line reflects the mean, whereas gray shading represents one standard deviation above and below, within each group [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Regions in which gray matter density was significantly different in adult chronic patients as compared with healthy controls

	X	*Y*	*Z*	*t*-statistic value	Cluster size	*p*-value
Chronic > controls						
Left anterior cingulate cortex	−14	15	34	5.44		
	−9	17	39	5.33	1,215	.046
	−14	19	35	5.15		
Chronic < controls						
Left ventral posterolateral nucleus	−12	−17	2	4.66	1,220	.045
	−8	−5	8	3.65		

Note: Locations are in Montreal Neurological Institute space. Significant clusters were from cluster-extent thresholding (family-wise error rate) to correct for multiple comparisons.

across brain sites. In addition, patterns of resting functional connectivity strengths were distinctive across pain conditions and age groups; notably, strengths progressed from descending modulatory memory-related circuits in acute pain to sensory-sensory changes in pediatric chronic to sensory-affective alterations in adult chronic pain. However, it is pertinent to note that we do not argue specific systems (e.g., descending modulatory) exclusively, and instead provide a framework that may correspond to a shift in brain sites from pediatric acute and chronic pain to adult chronic pain. Finally, an overlay of these differences in pediatric (lower limb) and adult (upper limb) chronic pain within VPL and SI revealed differential patterns of structural and functional metrics that were consistent with well-established somatotopical arrangements.

4.1 | Similarities and differences in pediatric and adult CRPS brain

Of the few studies that have explored structural brain metrics in CRPS, a common trend of gray matter atrophy is evident across pediatric and adult populations. Interestingly, compared with controls, comparison with our previous investigation (Erpelding et al., 2016) and others, reveal widespread (sensory, motor, emotional, cognitive, descending modulatory-related) brain alterations in pediatric CRPS (Erpelding et al., 2016), whereas adult CRPS report fewer alterations across sensory, emotional, and descending modulatory-related brain sites (Baliki, Schnitzer, Bauer, & Apkarian, 2011; Barad et al., 2014; Geha et al., 2008). In the present study, we report a similar trend, with sensory (VPL)-emotional (MCC, ACC)-descending (OFC) and sensory modulatory (TRN) alterations in pediatric CRPS, whereas a shift to sensory (VPL)-emotional (ACC) changes in adult CRPS. Finally, it is pertinent to note that although these differences in pediatric CRPS were present when compared with controls, comparison was restricted to those regions that were derived from comparing with acute subjects. This is an important consideration since, it is well established that a number of brain sites are engaged during acute pain perception (Farrell, Laird, & Egan, 2005; Henderson, Bandler, Gandevia, & Macefield, 2006). In any case, comparison within the

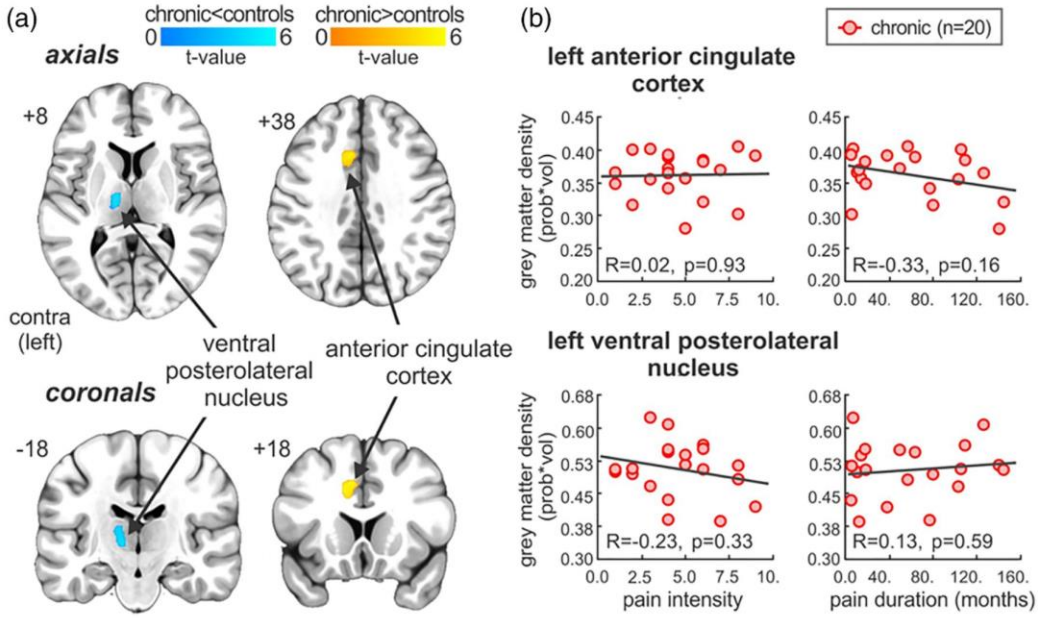

FIGURE 3 Whole brain gray matter density analysis between adult chronic and healthy controls. (a) Regional gray matter density reduced within the ventral posterolateral thalamic nucleus (cool color bar) and increased within the anterior cingulate cortex (warm color bar) in adult chronic as compared with controls, overlaid onto an axial and coronal T1-weighted anatomical image set. Slice locations are located on the top left of each image and are in Montreal neurological institute space. (b) Correlations between regional gray matter density values within these regions against pain intensity and disease duration. Note that no significant correlations were observed [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 5 Regions in which seed-based resting functional connectivity strengths were significantly different across pediatric (acute and chronic) and adult (chronic) groups, as compared with healthy controls

	X	*Y*	*Z*	*t*-statistic value	Cluster size	*p*-value
Pediatric acute > controls						
Orbitofrontal cortex "seed"						
Right hippocampus	28	−28	−16	4.84		
	24	−26	−16	4.80	152	.013
	16	−32	−20	3.95		
Pediatric chronic < controls						
Ventral posterolateral nucleus "seed"						
Bilateral primary motor cortex/primary somatosensory cortex	2	−18	60	6.39		
	0	−32	62	4.74	254	.0001
	−4	−22	68	3.93		
Adult chronic > controls						
Anterior cingulate cortex "seed"						
Left primary somatosensory cortex	−50	−24	62	5.46		
	−36	−26	66	4.61	93	.048
	−34	−30	62	4.38		
Adult chronic < controls						
Ventral posterolateral nucleus "seed"						
Bilateral posterior cingulate cortex	−2	−30	44	4.30		
	0	−24	38	4.00	137	.023
	6	−14	34	3.75		

Note: "seed" clusters were derived from the previous pediatric and adult gray matter density analyses. Locations are in Montreal Neurological Institute space. Significant clusters were from cluster-extent thresholding (family-wise error rate) to correct for multiple comparisons.

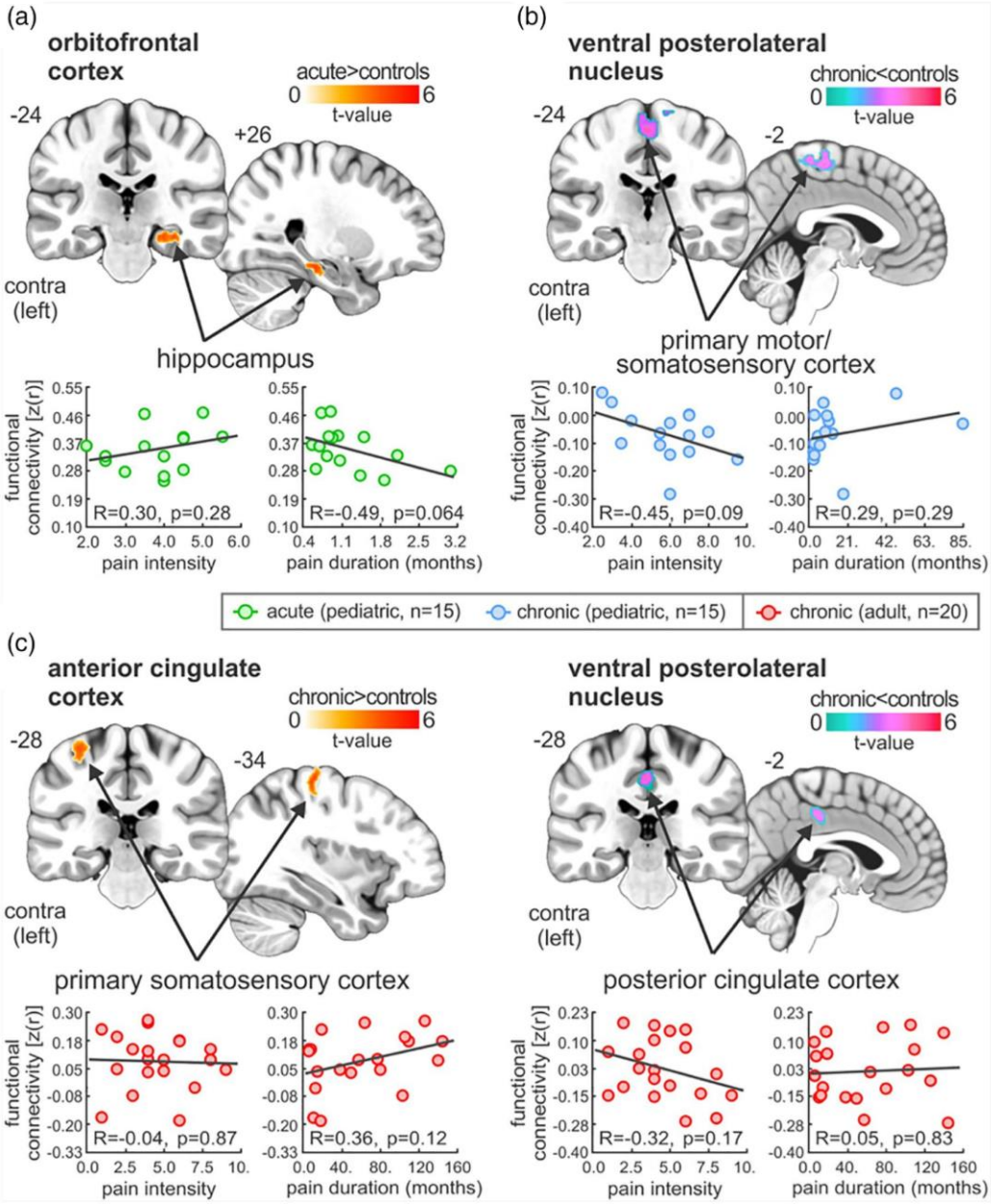

FIGURE 4 Significant differences in seed-based functional connectivity in pediatric (acute and chronic) and adult chronic as compared with controls. (a) Plots of resting seed-based functional connectivity from orbitofrontal cortex cluster, derived from pediatric acute and chronic gray matter density analysis. Note that compared with controls, acute patients have significantly greater functional connectivity strengths between the orbitofrontal cortex and the hippocampus. (b) Plots of resting seed-based functional connectivity from ventral posterolateral thalamic nucleus cluster, derived from pediatric chronic and healthy control gray matter density analysis. Note that compared with controls, pediatric chronic patients have significantly greater functional connectivity strengths between the ventral posterolateral thalamic nucleus and the primary motor and somatosensory cortex. (c) Plots of resting seed-based functional connectivity from anterior cingulate cortex and ventral posterolateral thalamic nucleus clusters, derived from between adult chronic and healthy control gray matter density analysis. Note that compared with controls, adult chronic patients have significantly greater functional connectivity strengths between the anterior cingulate cortex and the primary somatosensory cortex, whereas ventral posterolateral thalamic nucleus had reduced connectivity with the posterior cingulate cortex. Note that no significant correlations were observed between these values and pain intensity or disease duration ($p > .05$) [Color figure can be viewed at wileyonlinelibrary.com]

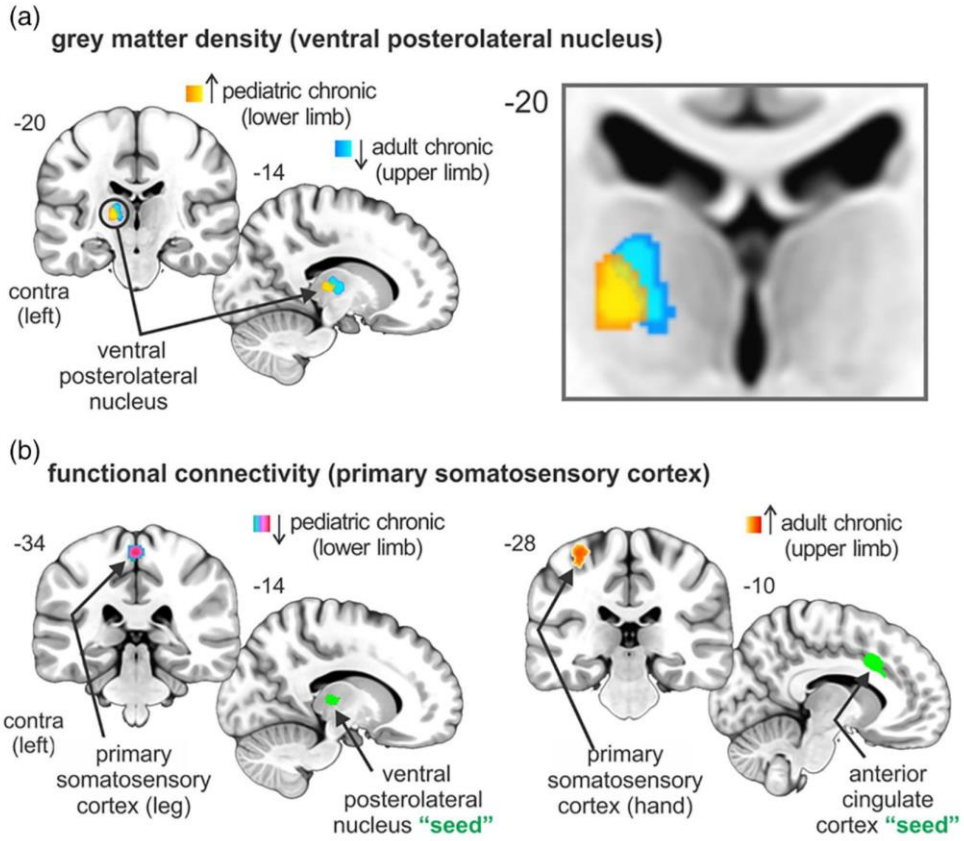

FIGURE 5 Overlay of significant pediatric and adult chronic structural and functional clusters within the ventral posterolateral thalamic nucleus and the primary somatosensory cortex. (a) Altered gray matter density in the ventral posterolateral nucleus of the thalamus that are consistent with upper (adult) and lower (pediatric) limb somatotopy, that is, lateral to medial, respectively. Note that increased gray matter density in pediatric chronic patients was reported relative to both pediatric acute patients and healthy controls. (b) Altered resting functional connectivity strengths within the primary somatosensory cortex, consistent upper limb (anterolateral) and lower limb (posteromedial) somatotopic organization [Color figure can be viewed at wileyonlinelibrary.com]

same brain site in pediatric and adult CRPS revealed a differential pattern of gray matter density alterations in sensory (VPL) and emotional (ACC)-related brain regions.

In addition to structural metrics, differential resting functional connectivity patterns have been reported across age groups. Here, our previous functional brain imaging investigations have revealed widespread hyperconnectivity patterns, notably within fear-related (Simons et al., 2014), pain modulatory circuits (Erpelding et al., 2016), and the default mode network (Becerra et al., 2014), among others. Comparably, in adult CRPS, the inverse pattern of covariation is observed, notably widespread hypoconnectivity patterns have been shown within sensory and affective brain regions (Kim, Choi, et al., 2017) and within the default mode network (Bolwerk et al., 2013). Interestingly, Kim, Choi, et al. (2017) reported altered SI covariation with both affective (anterior insula) and sensory (posterior insula) processing. In our investigation, we report the inverse pattern of connectivity with SI, that is, hyperconnectivity with an affective-motivational (ACC) brain site. Although possible, this is not to suggest that the ACC has a direct integrative role on SI somatotopic processing, since while the VP-SI circuitry is well established, the role of the SI-ACC integration remains relatively unclear. Instead, it is possible that the ACC may integrate SI processing indirectly through VP or TRN alteration, and a lack of observed difference in this study may correspond due to technical limitations such as relatively increased voxel size and smoothing kernel. In any case, it is pertinent to note that in this study patterns of resting functional connectivity were distinctive across pain conditions and age groups. Compared with controls, we report altered resting functional connectivity strengths between descending modulatory (OFC) and memory-related (hippocampus) brain sites in acute pain, within sensory (VPL-SI) regions in pediatric chronic pain, and between sensory-affective (ACC-SI) and sensory-working memory/awareness (VPL-PCC) in adult chronic pain.

4.2 | Somatotopical representation of lower (pediatric) and upper (adult) limb: Fidelity of approach

As noted in the introduction, we utilized the somatotopic organization of the afferent somatosensory pain system to define changes within

the three patient groups and their matched controls. The approach provides a clear fidelity to the data presented. Thalamic somatotopy for pain pathways has been described in rodents (Willis, 1985), non-human primates (Kaas et al., 1984; Ralston 3rd., 2005) and humans (Lenz et al., 1988). SI is one of the most investigated and well-described human brain regions. It encompasses the postcentral gyrus on both the left and right sides of the brain and has a well described somatotopic map, with the leg represented medially in the paracentral lobule and the hand and face more laterally towards the parietal operculum. A few fMRI studies have examined somatotopy of the somatosensory pathway in healthy subjects (DaSilva et al., 2002; Hong, Kwon, & Jang, 2011) and in pain patients (Maleki et al., 2012; Shokouhi et al., 2017) and their connectivity (Yamada et al., 2007). Both these regions show plasticity in pain (Hubbard et al., 2016; Kim, Kim, et al., 2017). Here we report altered gray matter density in the VPL thalamus that is consistent with upper and lower limb somatotopy, that is, lateral to medial, respectively (Kaas et al., 1984).

4.3 | Gray matter changes follow somatotopic organization

In patients with CRPS affecting the foot (pediatric group) and hand (adult group), changes in gray matter show a high degree of somatotopic organization within the somatosensory thalamus (VPL) and somatosensory cortex (SI). Changes in the organization of the ventroposterior lateral thalamus have been reported with sensory deprivation that may induce reorganization in SI (Rasmusson, 1996) with subsequent changes in function. Changes in gray matter volume within the thalamus have been reported in humans with chronic pain (Lutz et al., 2008), but few imaging studies have focused on specificity of somatotopy in chronic pain. Clearly, the thalamus offers an opportunity to define changes in pain within regions that have clearly defined somatotopy. Furthermore, changes within these regions may provide insights into hypersensitivity (Nagasaka, Takashima, Matsuda, & Higo, 2017). The changes have secondary effects on brain connectivity since these areas connect with other brain regions including well-defined areas such as SI and those that are not well recognized, such as the substantia nigra, VPL (Antal, Beneduce, & Regehr, 2014) and superior temporal sulcus (Yeterian & Pandya, 1989). Here, we are the first to report somatotopic alterations within the somatosensory thalamus. It is pertinent to note that thalamic reduction in gray matter density has been reported across a range of chronic pain syndromes (Apkarian et al., 2004). In fact, in neuropathic pain syndromes, decreased blood flow is often exclusively reported in the thalamus (Moisset & Bouhassira, 2007). Furthermore, we report an increase in gray matter density within the ACC, perhaps reflecting increased dominance of the affective-motivational role in our adult population. Specific evaluation of brain regions involved in affective-motivational processing was not the focus of this study. Moreover, it may reflect age-related differences, since our adult population is, on average, 10 years older than previous studies.

4.4 | Acute versus chronic pain in the pediatric brain: Potential insights into somatosensory chronification

Comparison of structural and functional metrics revealed distinct alterations in sensory-discriminative regions with a well-described somatotopical organization (VPL, SI). Moreover, we report alterations within the TRN, a region heavily associated with collateral and terminal branches of the VPL thalamocortical projection neurons, which send collaterals to the TRN and which itself contains GABAergic neurons which project to the VPL thalamus (Lam & Sherman, 2011). As a result, altered VPL-SI, SI-VPL, and TRN-VPL circuitry may result in a disturbance of the normal patterning of activity within the cortex, which may result in the constant perception of pain. Indeed, recent investigations have demonstrated that changes in thalamic function may result in widespread whole brain activity changes and that chronic pain may arise as a consequence of thalamocortical dysrhythmia (Alshelh et al., 2016; Walton, Dubois, & Llinas, 2010). In support, these thalamocortical loops have been shown to involve collaterals to GABAergic neurons in the TRN (Pinault, 2004), and remarkably, investigations have shown reduced thalamic blood flow gray matter density, neural viability and GABAergic content (Gustin et al., 2011; Youssef et al., 2014) and, specifically within the TRN, reduced blood flow and increased infra-slow oscillation power and network connectivity in patients with neuropathic pain (Alshelh et al., 2016; Gustin et al., 2014; Henderson et al., 2013). The reports, in conjunction with our findings, support the idea above, that although there is no obvious nerve injury in Type I patients, one cannot exclude the possibility of a subtle nerve injury that propagates along the ascending tract. Compared with acute, we found gray matter increased within the contralateral (left) VPL in pediatric chronic patients, whereas atrophy was reported bilaterally within the TRN. Furthermore, in this study, we found an association between pain intensity and gray matter density that were exclusive with these somatosensory-related regions, notably within the contralateral VPL and bilateral TRN. Although we did not observe any alterations within SI, it is pertinent to note that given the widespread somatotopic organization, it is possible that a lack of SI alteration may reflect the nature of the group analyses, that is, the fine somatotopic representation of SI between individual subjects may result in no overall SI group difference. Alternatively, it may be that acute subjects are associated with SI gray matter atrophy, or no change, corresponding with pediatric CRPS.

In addition, we reveal differential alterations in resting functional connectivity patterns in the pediatric acute (ankle sprain) versus chronic (CRPS) patients compared with controls. Specifically, pediatric acute had greater rFC strength between the OFC and the hippocampus. In contrast, pediatric CRPS had altered sensory circuit connectivity, that is, reduced rFC between the VPL and SI. Given these differences, the acute stage appears to reflect alterations in pain modulatory and memory-related circuitry, whereas a shift to sensory-circuits appears during the chronic phase. The latter is counter to prior work showing a shift of acute pain from sensory to emotional circuits, previously reported in the adult back pain patients (Hashmi et al., 2013). The shift in rFC from acute to chronic pain in pediatric

populations highlights a trend from initial pain modulatory distribution in acute to sensory-affective circuits in chronic pain. Furthermore, we report an age-related shift in chronic pain, with a sensory-emotional alteration in our adult populations, a finding that is consistent with pain chronification in adults (Hashmi et al., 2013). Indeed, given the age-related shift across the same chronic pain syndrome, our results may contribute to the well-known differences between child and adult pain vulnerability and resilience.

5 | LIMITATIONS

We note the following limitations of this study: (a) *Sex*: Given that our cohort was predominantly female, we did not have the statistical power to explore sex-related differences. We cannot exclude the possibility that some of the observed changes may be related to hormonal/menstrual changes in our subjects; (b) *CRPS subtype*: We did not specify the subtype of CRPS but grouped the patients. Clinically, patients had signs and symptoms of neuropathic involvement. If measures of nerve fiber integrity and function could have been used (including single fiber microneurography), alterations in fiber function could be more fully defined. Thus, is thus a difficult issue to define since while obvious nerve injury is observed in some cases it is not clearly defined in Type 1 CRPS. (c) *Pain intensity and duration*: For the acute and chronic pediatric comparison, the total pain intensity and duration differed between the groups, and therefore our results may, in part, be influenced by baseline differences in these parameters; (d) *Technical*: Given that we used different scanning parameters for group comparisons, particularly in the functional connectivity analyses, it is pertinent to note that for each contrast, group differences were relative to control groups that were sequence matched. Despite this, we acknowledge that we cannot completely exclude influences of parameter factors such as repetition time (signal covariation timeframe) and volume series (influence of degrees of freedom) on the observed results; (e) *Image flip*: Given that we flipped the patients' brain, for those with unilateral left-sided pain, it is pertinent to mention that this may reflect laterality in brain networks (Kucyi, Hodaie, & Davis, 2012; Vukelic et al., 2014). However, given the relatively small sample size of our pediatric patients, and the focus on somatosensory systems, we believe this approach is most appropriate; and (f) *Age and brain development*: we are aware that differential trajectories in gray matter density (Bourisly et al., 2015; Gennatas et al., 2017) and functional connectivity strengths (Chou, Chen, & Madden, 2013; Grady, Sarraf, Saverino, & Campbell, 2016) patterns across brain regions and networks may differ, and that inclusion of age does not completely mitigate this influence.

6 | CONCLUSIONS

These data reveal shift in gray matter density alterations from pediatric acute (descending modulatory) to pediatric chronic (descending modulatory/sensory-affective) to adult chronic pain (sensory-affective) across brain sites. Furthermore, our data reveals a shift in resting functional connectivity circuits, from sensory alterations in pediatric populations to sensory-emotional alterations in adult populations that are consistent with well-established somatotopical arrangements.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests. DB Consults to Biogen unrelated to the material in this manuscript.

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REFERENCES

Alshelh, Z., Di Pietro, F., Youssef, A. M., Reeves, J. M., Macey, P. M., Vickers, E. R., … Henderson, L. A. (2016). Chronic neuropathic pain: It's about the rhythm. *The Journal of Neuroscience*, *36*(3), 1008–1018. https://doi.org/10.1523/jneurosci.2768-15.2016

Antal, M., Beneduce, B. M., & Regehr, W. G. (2014). The Substantia Nigra conveys target-dependent excitatory and inhibitory outputs from the basal ganglia to the thalamus. *The Journal of Neuroscience*, *34*(23), 8032–8042. https://doi.org/10.1523/JNEUROSCI.0236-14.2014

Apkarian, A. V., Sosa, Y., Sonty, S., Levy, R. M., Harden, R. N., Parrish, T. B., & Gitelman, D. R. (2004). Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *The Journal of Neuroscience*, *24*(46), 10410–10415. https://doi.org/10.1523/JNEUROSCI.2541-04.2004

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *NeuroImage*, *38*(1), 95–113. https://doi.org/10.1016/j.neuroimage.2007.07.007

Ashburner, J., & Friston, K. J. (2009). Computing average shaped tissue probability templates. *NeuroImage*, *45*(2), 333–341. https://doi.org/10.1016/j.neuroimage.2008.12.008

Baliki, M. N., Schnitzer, T. J., Bauer, W. R., & Apkarian, A. V. (2011). Brain morphological signatures for chronic pain. *PLoS One*, *6*(10), e26010. https://doi.org/10.1371/journal.pone.0026010

Barad, M. J., Ueno, T., Younger, J., Chatterjee, N., & Mackey, S. (2014). Complex regional pain syndrome is associated with structural abnormalities in pain-related regions of the human brain. *The Journal of Pain*, *15*(2), 197–203.

Becerra, L., Sava, S., Simons, L. E., Drosos, A. M., Sethna, N., Berde, C., … Borsook, D. (2014). Intrinsic brain networks normalize with treatment in pediatric complex regional pain syndrome. *NeuroImage: Clinical*, *6*, 347–369.

Behzadi, Y., Restom, K., Liau, J., & Liu, T. T. (2007). A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *NeuroImage*, *37*(1), 90–101. https://doi.org/10.1016/j.neuroimage.2007.04.042

Bolwerk, A., Seifert, F., & Maihofner, C. (2013). Altered resting-state functional connectivity in complex regional pain syndrome. *The Journal of*

Pain, *14*(10), 1107–1115.e1108. https://doi.org/10.1016/j.jpain.2013.04.007

Bourisly, A. K., El-Beltagi, A., Cherian, J., Gejo, G., Al-Jazzaf, A., & Ismail, M. (2015). A voxel-based morphometric magnetic resonance imaging study of the brain detects age-related gray matter volume changes in healthy subjects of 21-45 years old. *The Neuroradiology Journal*, *28*(5), 450–459. https://doi.org/10.1177/1971400915598078

Bultitude, J. H., Walker, I., & Spence, C. (2017). Space-based bias of covert visual attention in complex regional pain syndrome. *Brain*, *140*(9), 2306–2321. https://doi.org/10.1093/brain/awx152

Chou, Y. H., Chen, N. K., & Madden, D. J. (2013). Functional brain connectivity and cognition: Effects of adult age and task demands. *Neurobiology of Aging*, *34*(8), 1925–1934. https://doi.org/10.1016/j.neurobiolaging.2013.02.012

DaSilva, A. F., Becerra, L., Makris, N., Strassman, A. M., Gonzalez, R. G., Geatrakis, N., & Borsook, D. (2002). Somatotopic activation in the human trigeminal pain pathway. *The Journal of Neuroscience*, *22*(18), 8183–8192.

Erpelding, N., Simons, L., Lebel, A., Serrano, P., Pielech, M., Prabhu, S., … Borsook, D. (2016). Rapid treatment-induced brain changes in pediatric CRPS. *Brain Structure & Function*, *221*(2), 1095–1111. https://doi.org/10.1007/s00429-014-0957-8

Farrell, M. J., Laird, A. R., & Egan, G. F. (2005). Brain activity associated with painfully hot stimuli applied to the upper limb: A meta-analysis. *Human Brain Mapping*, *25*(1), 129–139. https://doi.org/10.1002/hbm.20125

Friston, K. J., Holmes, A. P., Worsley, K. J., Poline, J. P., Frith, C. D., & Frackowiak, R. S. J. (1994). Statistical parametric maps in functional imaging: A general linear approach. *Human Brain Mapping*, *2*(4), 189–210. https://doi.org/10.1002/hbm.460020402

Geha, P. Y., Baliki, M. N., Harden, R. N., Bauer, W. R., Parrish, T. B., & Apkarian, A. V. (2008). The brain in chronic CRPS pain: Abnormal gray-white matter interactions in emotional and autonomic regions. *Neuron*, *60*(4), 570–581. https://doi.org/10.1016/j.neuron.2008.08.022

Gennatas, E. D., Avants, B. B., Wolf, D. H., Satterthwaite, T. D., Ruparel, K., Ciric, R., … Gur, R. C. (2017). Age-related effects and sex differences in gray matter density, volume, mass, and cortical thickness from childhood to young adulthood. *The Journal of Neuroscience*, *37*(20), 5065–5073. https://doi.org/10.1523/jneurosci.3550-16.2017

Grady, C., Sarraf, S., Saverino, C., & Campbell, K. (2016). Age differences in the functional interactions among the default, frontoparietal control, and dorsal attention networks. *Neurobiology of Aging*, *41*, 159–172. https://doi.org/10.1016/j.neurobiolaging.2016.02.020

Gustin, S. M., Peck, C. C., Wilcox, S. L., Nash, P. G., Murray, G. M., & Henderson, L. A. (2011). Different pain, different brain: Thalamic anatomy in neuropathic and non-neuropathic chronic pain syndromes. *The Journal of Neuroscience*, *31*(16), 5956–5964. https://doi.org/10.1523/jneurosci.5980-10.2011

Gustin, S. M., Wrigley, P. J., Youssef, A. M., McIndoe, L., Wilcox, S. L., Rae, C. D., … Henderson, L. A. (2014). Thalamic activity and biochemical changes in individuals with neuropathic pain after spinal cord injury. *Pain*, *155*(5), 1027–1036. https://doi.org/10.1016/j.pain.2014.02.008

Hashmi, J. A., Baliki, M. N., Huang, L., Baria, A. T., Torbey, S., Hermann, K. M., … Apkarian, A. V. (2013). Shape shifting pain: Chronification of back pain shifts brain representation from nociceptive to emotional circuits. *Brain*, *136*(9), 2751–2768. https://doi.org/10.1093/brain/awt211

Henderson, L. A., Bandler, R., Gandevia, S. C., & Macefield, V. G. (2006). Distinct forebrain activity patterns during deep versus superficial pain. *Pain*, *120*(3), 286–296. https://doi.org/10.1016/j.pain.2005.11.003

Henderson, L. A., Peck, C. C., Petersen, E. T., Rae, C. D., Youssef, A. M., Reeves, J. M., … Gustin, S. M. (2013). Chronic pain: Lost inhibition? *The Journal of Neuroscience*, *33*(17), 7574–7582. https://doi.org/10.1523/jneurosci.0174-13.2013

Hong, J. H., Kwon, H. G., & Jang, S. H. (2011). Probabilistic somatotopy of the spinothalamic pathway at the ventroposterolateral nucleus of the thalamus in the human brain. *AJNR. American Journal of Neuroradiology*, *32*(7), 1358–1362. https://doi.org/10.3174/ajnr.A2497

Hubbard, C. S., Becerra, L., Smith, J. H., DeLange, J. M., Smith, R. M., Black, D. F., … Borsook, D. (2016). Brain changes in responders vs. non-responders in chronic migraine: Markers of disease reversal. *Frontiers in Human Neuroscience*, *10*, 497. https://doi.org/10.3389/fnhum.2016.00497

Kaas, J. H., Nelson, R. J., Sur, M., Dykes, R. W., & Merzenich, M. M. (1984). The somatotopic organization of the ventroposterior thalamus of the squirrel monkey, *Saimiri sciureus*. *The Journal of Comparative Neurology*, *226*(1), 111–140. https://doi.org/10.1002/cne.902260109

Kim, J. H., Choi, S. H., Jang, J. H., Lee, D. H., Lee, K. J., Lee, W. J., … Kang, D. H. (2017). Impaired insula functional connectivity associated with persistent pain perception in patients with complex regional pain syndrome. *PLoS One*, *12*(7), e0180479. https://doi.org/10.1371/journal.pone.0180479

Kim, W., Kim, S. K., & Nabekura, J. (2017). Functional and structural plasticity in the primary somatosensory cortex associated with chronic pain. *Journal of Neurochemistry*, *141*(4), 499–506. https://doi.org/10.1111/jnc.14012

Kucyi, A., Hodaie, M., & Davis, K. D. (2012). Lateralization in intrinsic functional connectivity of the temporoparietal junction with salience- and attention-related brain networks. *Journal of Neurophysiology*, *108*(12), 3382–3392. https://doi.org/10.1152/jn.00674.2012

Lam, Y. W., & Sherman, S. M. (2011). Functional organization of the thalamic input to the thalamic reticular nucleus. *The Journal of Neuroscience*, *31*(18), 6791–6799. https://doi.org/10.1523/jneurosci.3073-10.2011

Lenz, F. A., Dostrovsky, J. O., Tasker, R. R., Yamashiro, K., Kwan, H. C., & Murphy, J. T. (1988). Single-unit analysis of the human ventral thalamic nuclear group: Somatosensory responses. *Journal of Neurophysiology*, *59*(2), 299–316. https://doi.org/10.1152/jn.1988.59.2.299

Low, A. K., Ward, K., & Wines, A. P. (2007). Pediatric complex regional pain syndrome. *Journal of Pediatric Orthopedics*, *27*(5), 567–572.

Lutz, J., Jager, L., de Quervain, D., Krauseneck, T., Padberg, F., Wichnalek, M., … Schelling, G. (2008). White and gray matter abnormalities in the brain of patients with fibromyalgia: A diffusion-tensor and volumetric imaging study. *Arthritis and Rheumatism*, *58*(12), 3960–3969. https://doi.org/10.1002/art.24070

Maihofner, C., & Birklein, F. (2007). Complex regional pain syndromes: New aspects on pathophysiology and therapy. *Fortschritte der Neurologie-Psychiatrie*, *75*(6), 331–342. https://doi.org/10.1055/s-2006-944310

Maleki, N., Becerra, L., Brawn, J., Bigal, M., Burstein, R., & Borsook, D. (2012). Concurrent functional and structural cortical alterations in migraine. *Cephalalgia*, *32*(8), 607–620. https://doi.org/10.1177/0333102412445622

Manjon, J. V., Coupe, P., Marti-Bonmati, L., Collins, D. L., & Robles, M. (2010). Adaptive non-local means denoising of MR images with spatially varying noise levels. *Journal of Magnetic Resonance Imaging*, *31*(1), 192–203. https://doi.org/10.1002/jmri.22003

Moisset, X., & Bouhassira, D. (2007). Brain imaging of neuropathic pain. *NeuroImage*, *37*(Suppl 1), S80–S88. https://doi.org/10.1016/j.neuroimage.2007.03.054

Nagasaka, K., Takashima, I., Matsuda, K., & Higo, N. (2017). Late-onset hypersensitivity after a lesion in the ventral posterolateral nucleus of the thalamus: A macaque model of central post-stroke pain. *Scientific Reports*, *7*(1), 10316. https://doi.org/10.1038/s41598-017-10679-2

Penfield, W., & Boldrey, E. (1937). Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain: A Journal of Neurology*, *60*, 389–443.

Pinault, D. (2004). The thalamic reticular nucleus: Structure, function and concept. *Brain Research. Brain Research Reviews*, *46*(1), 1–31. https://doi.org/10.1016/j.brainresrev.2004.04.008

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *NeuroImage*, *59*(3), 2142–2154. https://doi.org/10.1016/j.neuroimage.2011.10.018

Rajapakse, J. C., Giedd, J. N., & Rapoport, J. L. (1997). Statistical approach to segmentation of single-channel cerebral MR images. *IEEE Transactions on Medical Imaging*, *16*(2), 176–186. https://doi.org/10.1109/42.563663

Ralston, H. J., 3rd. (2005). Pain and the primate thalamus. *Progress in Brain Research*, *149*, 1–10. https://doi.org/10.1016/s0079-6123(05)49001-9

Rasmusson, D. (1996). Changes in the organization of the ventroposterior lateral thalamic nucleus after digit removal in adult raccoon. *The Journal of Comparative Neurology*, *364*(1), 92–103. https://doi.org/10.1002/(SICI)1096-9861(19960101)364:1<92::AID-CNE8>3.0.CO;2-N

Satterthwaite, T. D., Wolf, D. H., Loughead, J., Ruparel, K., Elliott, M. A., Hakonarson, H., … Gur, R. E. (2012). Impact of in-scanner head motion on multiple measures of functional connectivity: Relevance for studies of neurodevelopment in youth. *NeuroImage*, *60*(1), 623–632. https://doi.org/10.1016/j.neuroimage.2011.12.063

Shokouhi, M., Clarke, C., Morley-Forster, P., Moulin, D. E., Davis, K. D., & St Lawrence, K. (2017). Structural and functional brain changes at early and late stages of complex regional pain syndrome. *The Journal of Pain*, *19*, 146–157. https://doi.org/10.1016/j.jpain.2017.09.007

Simons, L. E., Pielech, M., Erpelding, N., Linnman, C., Moulton, E., Sava, S., … Borsook, D. (2014). The responsive amygdala: Treatment-induced alterations in functional connectivity in pediatric complex regional pain syndrome. *Pain*, *155*(9), 1727–1742. https://doi.org/10.1016/j.pain.2014.05.023

Tohka, J., Zijdenbos, A., & Evans, A. (2004). Fast and robust parameter estimation for statistical partial volume models in brain MRI. *NeuroImage*, *23*(1), 84–97.

Van Dijk, K. R., Sabuncu, M. R., & Buckner, R. L. (2012). The influence of head motion on intrinsic functional connectivity MRI. *NeuroImage*, *59*(1), 431–438. https://doi.org/10.1016/j.neuroimage.2011.07.044

van Rijn, M. A., Marinus, J., Putter, H., Bosselaar, S. R. J., Moseley, G. L., & van Hilten, J. J. (2011). Spreading of complex regional pain syndrome: Not a random process. *Journal of Neural Transmission*, *118*(9), 1301–1309. https://doi.org/10.1007/s00702-011-0601-1

Verdugo, R. J., & Ochoa, J. L. (2000). Abnormal movements in complex regional pain syndrome: Assessment of their nature. *Muscle & Nerve*, *23*(2), 198–205.

Vukelic, M., Bauer, R., Naros, G., Naros, I., Braun, C., & Gharabaghi, A. (2014). Lateralized alpha-band cortical networks regulate volitional modulation of beta-band sensorimotor oscillations. *NeuroImage*, *87*, 147–153. https://doi.org/10.1016/j.neuroimage.2013.10.003

Walton, K. D., Dubois, M., & Llinas, R. R. (2010). Abnormal thalamocortical activity in patients with complex regional pain syndrome (CRPS) type I. *Pain*, *150*(1), 41–51. https://doi.org/10.1016/j.pain.2010.02.023

Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connectivity*, *2*(3), 125–141. https://doi.org/10.1089/brain.2012.0073

Willis, W. D. (1985). Nociceptive pathways: Anatomy and physiology of nociceptive ascending pathways. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *308*(1136), 253–270.

Woo, C. W., Krishnan, A., & Wager, T. D. (2014). Cluster-extent based thresholding in fMRI analyses: Pitfalls and recommendations. *NeuroImage*, *91*, 412–419. https://doi.org/10.1016/j.neuroimage.2013.12.058

Yamada, K., Nagakane, Y., Yoshikawa, K., Kizu, O., Ito, H., Kubota, T., … Nishimura, T. (2007). Somatotopic organization of thalamocortical projection fibers as assessed with MR tractography. *Radiology*, *242*(3), 840–845. https://doi.org/10.1148/radiol.2423060297

Yeterian, E. H., & Pandya, D. N. (1989). Thalamic connections of the cortex of the superior temporal sulcus in the rhesus monkey. *The Journal of Comparative Neurology*, *282*(1), 80–97. https://doi.org/10.1002/cne.902820107

Youssef, A. M., Gustin, S. M., Nash, P. G., Reeves, J. M., Petersen, E. T., Peck, C. C., … Henderson, L. A. (2014). Differential brain activity in subjects with painful trigeminal neuropathy and painful temporomandibular disorder. *Pain*, *155*(3), 467–475. https://doi.org/10.1016/j.pain.2013.11.008

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5 Bibliography

Ab Aziz, C. B., & Ahmad, A. H. (2006). The Role of the Thalamus in Modulating Pain. *Malaysian Journal of Medical Sciences*, *13*(2), 11–18.

Alexander, G. (1986). Parallel Organization of Functionally Segregated Circuits Linking Basal Ganglia and Cortex. *Annual Review of Neuroscience*, *9*(1), 357–381. doi:10.1146/annurev.neuro.9.1.357

Alshelh, Z., Marciszewski, K. K., Akhter, R., Di Pietro, F., Mills, E. P., Vickers, E. R., Peck, C. C., Murray, G. M., & Henderson, L. A. (2018). Disruption of Default Mode Network Dynamics in Acute and Chronic Pain States. *NeuroImage: Clinical*, *17*, 222–231. doi:10.1016/j.nicl.2017.10.019

Awojoyogbe, B. O., & Dada, M. (2011). Basis for the Application of Analytical Models of the Bloch NMR Flow Equations for Functional Magnetic Resonance Imaging (fMRI): A Review. *Recent Patents on Medical Imaginge*, *1*(1), 33–67. doi:10.2174/1877613211101010033

Azqueta-Gavaldon, M., Schulte-Göcking, H., Storz, C., Azad, S., Reiners, A., Borsook, D., Becerra, L., & Kraft, E. (2017). Basal Ganglia Dysfunction in Complex Regional Pain Syndrome - A Valid Hypothesis? *European Journal of Pain*, *21*(3), 415–424. doi:10.1002/ejp.975

Azqueta-Gavaldon, M., Youssef, A. M., Storz, C., Lemme, J., Schulte-Göcking, H., Becerra, L., Azad, S. C., Reiners, A., Ertl-Wagner, B., Borsook, D., Upadhyay, J., & Kraft, E. (2020). Implications of the putamen in pain and motor deficits in complex regional pain syndrome. *Pain*, *161*(3), 595–608. doi:10.1097/j.pain.0000000000001745

Bailey, J., Nelson, S., Lewis, J., & McCabe, C. S. (2013). Imaging and Clinical Evidence of Sensorimotor Problems in CRPS: Utilizing Novel Treatment Approaches. *Journal of Neuroimmune Pharmacology*, *8*(3), 564–575. doi:10.1007/s11481-012-9405-9

Baker, K. B., Lee, J. Y. K., Mavinkurve, G., Russo, G. S., Walter, B., DeLong, M. R., Bakay, R. A. E., & Vitek, J. L. (2010). Somatotopic Organization in the Internal Segment of the Globus Pallidus in Parkinson's Disease. *Experimental Neurology*, *222*(2), 219–225. doi:10.1016/j.expneurol.2009.12.030

Baliki, M. N., Mansour, A. R., Baria, A. T., & Apkarian, A. V. (2014). Functional Reorganization of the Default Mode Network across Chronic Pain Conditions. *PLOS ONE*, *9*(9), e106133. doi:10.1371/journal.pone.0106133

Barad, M. J., Ueno, T., Younger, J., Chatterjee, N., & Mackey, S. (2014). Complex Regional Pain Syndrome is Associated with Structural Abnormalities in Pain-Related Regions of the Human Brain. *Journal of Pain*, *15*(2), 197–203. doi:10.1016/j.jpain.2013.10.011

Bauer, C. C. C., Barrios, F. A., & Díaz, J.-L. (2014). Subjective Somatosensory Experiences Disclosed by Focused Attention: Cortical-Hippocampal-Insular and Amygdala Contributions. *PLOS ONE*, *9*(8), e104721. doi:10.1371/journal.pone.0104721

Bean, D. J., Johnson, M. H., & Kydd, R. R. (2014). The Outcome of Complex Regional Pain Syndrome Type 1: A Systematic Review. *Journal of Pain*, *15*(7), 677–690. doi:10.1016/j.jpain.2014.01.500

Becerra, L., Sava, S., Simons, L. E., Drosos, A. M., Sethna, N., Berde, C., Lebel, A. A., & Borsook, D. (2014). Intrinsic Brain Networks Normalize with Treatment in Pediatric Complex Regional Pain Syndrome. *NeuroImage. Clinical*, *6*, 347–369. doi:10.1016/j.nicl.2014.07.012

Becerra, L., Schwartzman, R. J., Kiefer, R. T., Rohr, P., Moulton, E. A., Wallin, D., Pendse, G., Morris, S., & Borsook, D. (2015). CNS Measures of Pain Responses Pre- and Post-Anesthetic Ketamine in a Patient with Complex Regional Pain Syndrome. *Pain Medicine*, *16*(12), 2368–2385. doi:10.1111/pme.12939

Beerthuizen, A., Stronks, D. L., Huygen, F. J. P. M., Passchier, J., Klein, J., & Spijker, A. V. T. (2011). The Association between Psychological Factors and the Development of Complex Regional Pain Syndrome Type 1 (CRPS1) - A Prospective Multicenter Study. *European Journal of Pain*, *15*(9), 971–975. doi:10.1016/j.ejpain.2011.02.008

Birklein, F., Ajit, S. K., Goebel, A., Perez, R. S. G. M., & Sommer, C. (2018). Complex Regional Pain Syndrome - Phenotypic Characteristics and Potential Biomarkers. *Nature Reviews. Neurology*, *14*(5), 272–284. doi:10.1038/nrneurol.2018.20

Birklein, F., & Dimova, V. (2017). Complex Regional Pain Syndrome–up-to-Date. *Pain Reports*, *2*(6), e624. doi:10.1097/PR9.0000000000000624

Birklein, F., & Schmelz, M. (2008). Neuropeptides, Neurogenic Inflammation and Complex Regional Pain Syndrome (CRPS). *Neuroscience Letters*, *437*(3), 199–202. doi:10.1016/j.neulet.2008.03.081

Bolwerk, A., Seifert, F., & Maihöfner, C. (2013). Altered Resting-State Functional Connectivity in Complex Regional Pain Syndrome. *The Journal of Pain*, *14*(10), 1107-1115.e8. doi:10.1016/j.jpain.2013.04.007

Borsook, D., Upadhyay, J., Chudler, E. H., & Becerra, L. (2010). A Key Role of the Basal Ganglia in Pain and Analgesia - Insights Gained through Human Functional Imaging. *Molecular Pain*, *6*(1), 1744-8069-6–27. doi:10.1186/1744-8069-6-27

Bosch-Bouju, C., Hyland, B. I., & Parr-Brownlie, L. C. (2013). Motor Thalamus Integration of Cortical, Cerebellar and Basal Ganglia Information: Implications for Normal and Parkinsonian Conditions. *Frontiers in Computational Neuroscience*, *7*, 163. doi:10.3389/fncom.2013.00163

Bruehl, S. (2010). An Update on the Pathophysiology of Complex Regional Pain Syndrome. *Anesthesiology*, *113*(3), 713–725. doi:10.1097/ALN.0b013e3181e3db38

Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. L. (2008). The Brain's Default Network: Anatomy, Function, and Relevance to Disease. *Annals of the New York Academy of Sciences*, *1124*(1), 1–38. doi:10.1196/annals.1440.011

Bultitude, J. H., Walker, I., & Spence, C. (2017). Space-Based Bias of Covert Visual Attention in Complex Regional Pain Syndrome. *Brain*, *140*(9), 2306–2321. doi:10.1093/brain/awx152

Case, L. K., Laubacher, C. M., Olausson, H., Wang, B., Spagnolo, P. A., & Bushnell, M. C. (2016). Encoding of Touch Intensity but not Pleasantness in Human Primary Somatosensory Cortex. *Journal of Neuroscience*, *36*(21), 5850–5860. doi:10.1523/JNEUROSCI.1130-15.2016

Cauda, F., Sacco, K., Duca, S., Cocito, D., D'Agata, F., Geminiani, G. C., & Canavero, S. (2009). Altered Resting State in Diabetic Neuropathic Pain. *PLOS ONE*, *4*(2), e4542. doi:10.1371/journal.pone.0004542

de Mos, M., de Bruijn, A. G. J., Huygen, F. J. P. M., Dieleman, J. P., Stricker, B. H. C., & Sturkenboom, M. C. J. M. (2007). The Incidence of Complex Regional Pain Syndrome: A

Population-Based Study. *Pain*, *129*(1–2), 12–20. doi:10.1016/j.pain.2006.09.008

Del Valle, L., Schwartzman, R. J., & Alexander, G. (2009). Spinal Cord Histopathological Alterations in a Patient with Longstanding Complex Regional Pain Syndrome. *Brain, Behavior, and Immunity*, *23*(1), 85–91. doi:10.1016/j.bbi.2008.08.004

Di Martino, A., Scheres, A., Margulies, D. S., Kelly, A. M. C., Uddin, L. Q., Shehzad, Z., Biswal, B., Walters, J. R., Castellanos, F. X., & Milham, M. P. (2008). Functional Connectivity of Human Striatum: A Resting State fMRI Study. *Cerebral Cortex*, *18*(12), 2735–2747. doi:10.1093/cercor/bhn041

Drummond, P. D. (2010). Sensory Disturbances in Complex Regional Pain Syndrome: Clinical Observations, Autonomic Interactions, and Possible Mechanisms. *Pain Medicine*, *11*(8), 1257–1266. doi:10.1111/j.1526-4637.2010.00912.x

Eickhoff, S. B., Schleicher, A., Zilles, K., & Amunts, K. (2006). The Human Parietal Operculum. I. Cytoarchitectonic Mapping of Subdivisions. *Cerebral Cortex*, *16*(2), 254–267. doi:10.1093/cercor/bhi105

Erpelding, N., Simons, L., Lebel, A., Serrano, P., Pielech, M., Prabhu, S., Becerra, L., & Borsook, D. (2016). Rapid Treatment-Induced Brain Changes in Pediatric CRPS. *Brain Structure & Function*, *221*(2), 1095–1111. doi:10.1007/s00429-014-0957-8

Fallon, N., Chiu, Y., Nurmikko, T., & Stancak, A. (2016). Functional Connectivity with the Default Mode Network is Altered in Fibromyalgia Patients. *PLOS ONE*, *11*(7), e0159198. doi:10.1371/journal.pone.0159198

Fox, M. D., & Greicius, M. (2010). Clinical applications of resting state functional connectivity. *Frontiers in Systems Neuroscience*, *4*, 19. doi:10.3389/fnsys.2010.00019

Fox, M. D., & Raichle, M. E. (2007). Spontaneous Fluctuations in Brain Activity Observed with Functional Magnetic Resonance Imaging. *Nature Reviews Neuroscience*, *8*(9), 700–711. doi:10.1038/nrn2201

Freund, W., Klug, R., Weber, F., Stuber, G., Schmitz, B., & Wunderlich, A. P. (2009). Perception and Suppression of Thermally Induced Pain: A fMRI Study. *Somatosensory & Motor Research*, *26*(1), 1–10. doi:10.1080/08990220902738243

Freund, W., Wunderlich, A. P., Stuber, G., Mayer, F., Steffen, P., Mentzel, M., Weber, F., &

Schmitz, B. (2010). Different Activation of Opercular and Posterior Cingulate Cortex (PCC) in Patients with Complex Regional Pain Syndrome (CRPS I) Compared with Healthy Controls During Perception of Electrically Induced Pain: A Functional MRI Study. *The Clinical Journal of Pain*, *26*(4), 339–347. doi:10.1097/AJP.0b013e3181cb4055

Geha, P. Y., Baliki, M. N., Harden, R. N., Bauer, W. R., Parrish, T. B., & Apkarian, A. V. (2008). The Brain in Chronic CRPS Pain: Abnormal Gray-White Matter Interactions in Emotional and Autonomic Regions. *Neuron*, *60*(4), 570–581. doi:10.1016/j.neuron.2008.08.022

Geyer, S., Schleicher, A., & Zilles, K. (1999). Areas 3a, 3b, and 1 of Human Primary Somatosensory Cortex: Microstructural Organization and Interindividual Variability. *NeuroImage*, *10*(1), 63–83. doi:10.1006/nimg.1999.0440

Gieteling, E. W., van Rijn, M. A., de Jong, B. M., Hoogduin, J. M., Renken, R., van Hilten, J. J., & Leenders, K. L. (2008). Cerebral Activation During Motor Imagery in Complex Regional Pain Syndrome Type 1 with Dystonia. *Pain*, *134*(3), 302–309. doi:10.1016/j.pain.2007.04.029

Harden, R. N., Bruehl, S., Perez, R. S. G. M., Birklein, F., Marinus, J., Maihofner, C., Lubenow, T., Buvanendran, A., Mackey, S., Graciosa, J., Mogilevski, M., Ramsden, C., Chont, M., & Vatine, J.-J. (2010). Validation of Proposed Diagnostic Criteria (the “Budapest Criteria”) for Complex Regional Pain Syndrome. *Pain*, *150*(2), 268–274. doi:10.1016/j.pain.2010.04.030

Hening, W., Harrington, D. L., & Poizner, H. (2009). Basal Ganglia: Motor Functions of. In *Encyclopedia of Neuroscience* (pp. 346–350). Springer Berlin Heidelberg. doi:10.1007/978-3-540-29678-2_561

Hong, J. H., Kwon, H. G., & Jang, S. H. (2011). Probabilistic Somatotopy of the Spinothalamic Pathway at the Ventroposterolateral Nucleus of the Thalamus in the Human Brain. *American Journal of Neuroradiology*, *32*(7), 1358–1362. doi:10.3174/ajnr.A2497

Huge, V., Lauchart, M., Förderreuther, S., Kaufhold, W., Valet, M., Azad, S. C., Beyer, A., & Magerl, W. (2008). Interaction of Hyperalgesia and Sensory Loss in Complex Regional Pain Syndrome Type I (CRPS I). *PLOS ONE*, *3*(7). doi:10.1371/journal.pone.0002742

Jänig, W., & Baron, R. (2003). Complex regional pain syndrome: Mystery explained? *Lancet*

Neurology, *2*(11), 687–697. doi:10.1016/S1474-4422(03)00557-X

Jeon, S. Y., Seo, S., Lee, J. S., Choi, S.-H., Lee, D.-H., Jung, Y.-H., Song, M.-K., Lee, K.-J., Kim, Y. C., Kwon, H. W., Im, H.-J., Lee, D. S., Cheon, G. J., & Kang, D.-H. (2017). [11C]-(R)-PK11195 Positron Emission Tomography in Patients with Complex Regional Pain Syndrome. *Medicine*, *96*(1), e5735. doi:10.1097/MD.0000000000005735

Kim, J.-H., Choi, S.-H., Jang, J. H., Lee, D.-H., Lee, K.-J., Lee, W. J., Moon, J. Y., Kim, Y. C., & Kang, D.-H. (2017). Impaired Insula Functional Connectivity Associated with Persistent Pain Perception in Patients with Complex Regional Pain Syndrome. *PLOS ONE*, *12*(7), e0180479. doi:10.1371/journal.pone.0180479

Kiviniemi, V., Kantola, J. H., Jauhiainen, J., Hyvärinen, A., & Tervonen, O. (2003). Independent Component Analysis of Nondeterministic fMRI Signal Sources. *NeuroImage*, *19*(2), 253–260. doi:10.1016/S1053-8119(03)00097-1

Kriegeskorte, N., Simmons, W. K., Bellgowan, P. S., & Baker, C. I. (2009). Circular Analysis in Systems Neuroscience: The Dangers of Double Dipping. *Nature Neuroscience*, *12*(5), 535–540. doi:10.1038/nn.2303

Kucyi, A., & Davis, K. D. (2015). The Dynamic Pain Connectome. *Trends in Neurosciences*, *38*(2), 86–95. doi:10.1016/j.tins.2014.11.006

Latremoliere, A., & Woolf, C. J. (2009). Central Sensitization: a Generator of Pain Hypersensitivity by Central Neural Plasticity. *Journal of Pain*, *10*(9), 895–926. doi:10.1016/j.jpain.2009.06.012

Lenz, M., Hoffken, O., Stude, P., Lissek, S., Schwenkreis, P., Reinersmann, A., Frettloh, J., Richter, H., Tegenthoff, M., & Maier, C. (2011). Bilateral Somatosensory Cortex Disinhibition in Complex Regional Pain Syndrome Type I. *Neurology*, *77*(11), 1096–1101. doi:10.1212/WNL.0b013e31822e1436

Lewis, J. S., Kersten, P., McCabe, C. S., McPherson, K. M., & Blake, D. R. (2007). Body Perception Disturbance: A Contribution to Pain in Complex Regional Pain Syndrome (CRPS). *Pain*, *133*(1–3), 111–119. doi:10.1016/j.pain.2007.03.013

Lewis, J. S., & Schweinhardt, P. (2012). Perceptions of the Painful Body: The Relationship Between Body Perception Disturbance, Pain and Tactile Discrimination in Complex

Regional Pain Syndrome. *European Journal of Pain*, *16*, 1320–1330. doi:10.1002/j.1532-2149.2012.00120.x

Linnman, C., Becerra, L., Lebel, A., Berde, C., Grant, P. E., & Borsook, D. (2013). Transient and Persistent Pain Induced Connectivity Alterations in Pediatric Complex Regional Pain Syndrome. *PLOS ONE*, *8*(3), e57205. doi:10.1371/journal.pone.0057205

Loher, T. J., Burgunder, J.-M., Weber, S., Sommerhalder, R., & Krauss, J. K. (2002). Effect of Chronic Pallidal Deep Brain Stimulation on off Period Dystonia and Sensory Symptoms in Advanced Parkinson's Disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *73*(4), 395–399. doi:10.1136/jnnp.73.4.395

Lv, H., Wang, Z., Tong, E., Williams, L. M., Zaharchuk, G., Zeineh, M., Goldstein-Piekarski, A. N., Ball, T. M., Liao, C., & Wintermark, M. (2018). Resting-state Functional MRI: Everything that Nonexperts Have Always Wanted to Know. *American Journal of Neuroradiology*, *39*(8), 1390–1399. doi:10.3174/ajnr.A5527

Maihofner, C., Baron, R., DeCol, R., Binder, A., Birklein, F., Deuschl, G., Handwerker, H. O., & Schattschneider, J. (2007). The Motor System Shows Adaptive Changes in Complex Regional Pain Syndrome. *Brain*, *130*(10), 2671–2687. doi:10.1093/brain/awm131

Mansour, A. R., Farmer, M. A., Baliki, M. N., & Apkarian, A. V. (2014). Chronic Pain: The Role of Learning and Brain Plasticity. *Restorative Neurology and Neuroscience*, *32*(1), 129–139. doi:10.3233/RNN-139003

Mercier, C., & Léonard, G. (2011). Interactions Between Pain and the Motor Cortex: Insights from Research on Phantom Limb Pain and Complex Regional Pain Syndrome. *Physiotherapy Canada*, *63*(3), 305–314. doi:10.3138/ptc.2010-08p

Millan, M. J. (2002). Descending Control of Pain. *Progress in Neurobiology*, *66*(6), 355–474. doi:10.1016/S0301-0082(02)00009-6

Munts, A. G., Mugge, W., Meurs, T. S., Schouten, A. C., Marinus, J., Moseley, G. L., van der Helm, F. C. T., & van Hilten, J. J. (2011). Fixed Dystonia in Complex Regional Pain Syndrome: A Descriptive and Computational Modeling Approach. *BMC Neurology*, *11*, 53. doi:10.1186/1471-2377-11-53

Munts, A. G., Van Rootselaar, A.-F., Van Der Meer, J. N., Koelman, J. H. T. M., Van Hilten, J. J.,

& Tijssen, M. A. J. (2008). Clinical and Neurophysiological Characterization of Myoclonus in Complex Regional Pain Syndrome. *Movement Disorders*, *23*(4), 581–587. doi:10.1002/mds.21910

Niazy, R. K., Xie, J., Miller, K., Beckmann, C. F., & Smith, S. M. (2011). Spectral Characteristics of Resting State Networks. *Progress in Brain Research*, *193*, 259–276. doi:10.1016/B978-0-444-53839-0.00017-X

Ogawa, S., Lee, T. M., Kay, A. R., & Tank, D. W. (1990). Brain Magnetic Resonance Imaging with Contrast Dependent on Blood Oxygenation. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(24), 9868–9872. doi:10.1073/pnas.87.24.9868

Pleger, B., Tegenthoff, M., Schwenkreis, P., Janssen, F., Ragert, P., Dinse, H. R., Völker, B., Zenz, M., & Maier, C. (2004). Mean Sustained Pain Levels are Linked to Hemispherical side-to-side Differences of Primary Somatosensory Cortex in the Complex Regional Pain Syndrome I. *Experimental Brain Research*, *155*(1), 115–119. doi:10.1007/s00221-003-1738-4

Reza Daliri, M. (2014). Advantages and Disadvantages of Resting State Functional Connectivity Magnetic Resonance Imaging for Clinical Applications. *OMICS Journal of Radiology*, *3*(1). doi:10.4172/2167-7964.1000e123

Rosazza, C., Minati, M., Ghielmetti, F., Mandelli, M. L., & Bruzzone, M. G. (2012). Functional Connectivity During Resting-State Functional MR Imaging: Study of the Correspondence Between Independent Component Analysis and Region-of-Interest - Based Methods. *American Journal of Neuroradiology*, *33*(1), 180–187. doi:10.3174/ajnr.A2733

Searle, A., & Salibi, A. (2014). Complex Regional Pain Syndrome: A Systemic Review of the Literature. The Past, Present and Future Management. *Journal of Pain & Relief*, *03*(01). doi:10.4172/2167-0846.1000128

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., Reiss, A. L., & Greicius, M. D. (2007). Dissociable Intrinsic Connectivity Networks for Salience Processing and Executive Control. *Journal of Neuroscience*, *27*(9), 2349–2356. doi:10.1523/JNEUROSCI.5587-06.2007

Sesack, S. R., & Grace, A. A. (2010). Cortico-Basal Ganglia Reward Network: Microcircuitry. *Neuropsychopharmacology*, *35*(1), 27–47. doi:10.1038/npp.2009.93

Shroff, S. (2011). Caudate Nucleus. In J. S. Kreutzer, J. DeLuca, & B. Caplan (Eds.), *Encyclopedia of Clinical Neuropsychology* (pp. 504–506). Springer New York, NY. doi:10.1007/978-0-387-79948-3_302

Simons, L. E., Pielech, M., Erpelding, N., Linnman, C., Moulton, E., Sava, S., Lebel, A., Serrano, P., Sethna, N., Berde, C., Becerra, L., & Borsook, D. (2014). The Responsive Amygdala: Treatment-Induced Alterations in Functional Connectivity in Pediatric Complex Regional Pain Syndrome. *Pain*, *155*(9), 1727–1742. doi:10.1016/j.pain.2014.05.023

Tsakiris, M., Hesse, M. D., Boy, C., Haggard, P., & Fink, G. R. (2007). Neural signatures of body ownership: a sensory network for bodily self-consciousness. *Cerebral Cortex*, *17*(10), 2235–2244. doi:10.1093/cercor/bhl131

Upadhyay, J. … Borsook, D. (2012). Modulation of CNS Pain Circuitry by Intravenous and Sublingual Doses of Buprenorphine. *NeuroImage*, *59*(4), 3762–3773. doi:10.1016/j.neuroimage.2011.11.034

van den Heuvel, M. P., & Hulshoff Pol, H. E. (2010a). Specific somatotopic organization of functional connections of the primary motor network during resting state. *Human Brain Mapping*, *31*(4), 631–644. doi:10.1002/hbm.20893

van den Heuvel, M. P., & Hulshoff Pol, H. E. (2010b). Exploring the brain network: A review on resting-state fMRI functional connectivity. *European Neuropsychopharmacology*, *20*(8), 519–534. doi:10.1016/j.euroneuro.2010.03.008

van Hilten, J. J. (2010). Movement Disorders in Complex Regional Pain Syndrome. *Pain Medicine*, *11*(8), 1274–1277. doi:10.1111/j.1526-4637.2010.00916.x

van Rijn, M. A., Marinus, J., Putter, H., Bosselaar, S. R. J., Moseley, G. L., & van Hilten, J. J. (2011). Spreading of Complex Regional Pain Syndrome: Not a Random Process. *Journal of Neural Transmission*, *118*(9), 1301–1309. doi:10.1007/s00702-011-0601-1

Verdugo, R. J., & Ochoa, J. L. (2000). Abnormal Movements in Complex Regional Pain Syndrome: Assessment of their Nature. *Muscle & Nerve*, *23*(2), 198–205. doi:10.1002/(sici)1097-4598(200002)23:2<198::aid-mus9>3.0.co;2-4

Youssef, A. M., Azqueta-Gavaldon, M., Silva, K. E., Barakat, N., Lopez, N., Mahmud, F., Lebel, A., Sethna, N. F., Zurakowski, D., Simons, L. E., Kraft, E., & Borsook, D. (2019). Shifting brain circuits in pain chronicity. *Human Brain Mapping*, *40*(15), 4381–4396. doi:10.1002/hbm.24709

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