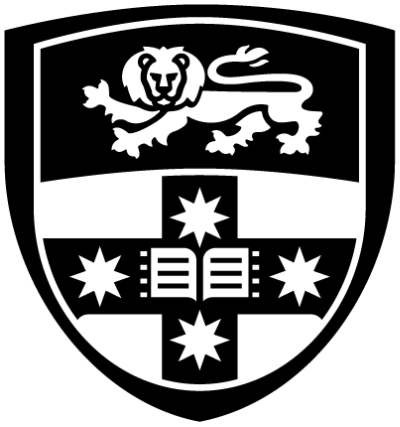

THE UNIVERSITY OF SYDNEY

The Role of the Brain in Complex Regional Pain Syndrome (CRPS) Pain and Motor Dysfunction

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A thesis submitted in to fulfil the requirements for the degree of

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DEDICATION

To my late grandparents Thomas To and Margaret Mak.

STATEMENT OF ORIGINALITY

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Barbara Lee

Date: 30/09/2022

AUTHORSHIP ATTRIBUTION STATEMENT

The work presented in this thesis has been published or submitted for publication in peer-reviewed journals. Author placement of the work presented are listed such that the first author contributed the most to the study and the last author is the principal investigator of the study.

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I collected the data, analysed the data, interpreted the analysis, wrote and edited the manuscript.

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Prof. Caroline Rae: contributed unpublished reagents/analytical tools, analysed the data, interpreted the analysis, and wrote and edited the manuscript.

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ABSTRACT

Background: Complex regional pain syndrome (CRPS) is the most painful disorder known to man and presents with altered sensory perceptions and motor dysfunction. Past neuroimaging studies demonstrate that CRPS is associated with brain changes. The overall aim of this thesis was to investigate the role of the brain in CRPS pain and motor dysfunction.

The sensorimotor cortex is important in pain and motor function. Sensorimotor cortical reorganisation and disinhibition have been identified in CRPS and many have postulated that such changes involve altered gamma-aminobutyric acid (GABA) mechanisms. These sensorimotor changes are thought to be so significant that treatments of CRPS aim to restore sensorimotor cortical reorganisation and disinhibition. And yet despite the postulated GABAergic mechanisms for sensorimotor disinhibition, sensorimotor cortex concentrations of inhibitory and excitatory neurotransmitters have never been evaluated in CRPS.

In addition to the sensorimotor cortex, the basal ganglia also regulates pain. The basal ganglia has separate functional loops involved in motor and non-motor functions. In CRPS, it has been shown that there are changes to basal nuclei such as the putamen and caudate nucleus and such changes have been linked to CRPS pain and motor dysfunction. Further, neuroinflammation by infiltration of activated astrocytes has been found in the basal ganglia of CRPS patients. However, the basal ganglia functional loops have not been systematically evaluated in CRPS.

Finally, the transmission and modulation of pain involves multiple brainstem nuclei such as the periaqueductal gray (PAG), locus coeruleus (LC), and rostral ventromedial medulla (RVM). In other chronic pain conditions, the PAG, LC, and RVM have been found to facilitate pain. Interestingly, many CRPS studies have postulated that pain processing is altered at the brainstem, yet the brainstem has not yet been directly investigated in CRPS.

Methods: A series of three experiments were conducted comparing upper limb CRPS patients with pain-free controls to investigate various brain regions important in pain and motor function. i) In Chapter 2, magnetic resonance spectroscopy (MRS) was used to determine GABA and glutamate concentrations in the sensorimotor cortex of 14 CRPS and 30 pain-free controls. The relationship between GABA and glutamate concentrations and tactile acuity in CRPS was determined using Pearson's correlation. ii) In Chapter 3, resting-state functional magnetic resonance imaging (fMRI) was used to determine infraslow oscillations (ISO) and functional connectivity of the motor and non-motor basal ganglia loops in 15 CRPS and 45 age- and sex-matched pain-free controls. Pearson's correlation was used to determine the relationship between basal ganglia ISO and pain and motor function in CRPS. iii) Finally, in Chapter 4 resting-state fMRI was used to determine the functional connectivity between the PAG, LC, and RVM, and the functional connectivity of the PAG and LC to higher brain areas in 15 CRPS and 30 age and sex-matched pain-free controls. Using Pearson's correlations, the relationship between CRPS functional connectivity changes of brainstem nuclei and pain intensity was determined.

Results: Contrary to our original hypothesis, sensorimotor cortex GABA and glutamate concentrations did not differ between CRPS and controls or between CRPS brain hemispheres

and neither concentration was correlated to tactile acuity in CRPS. Investigations of the basal ganglia circuitry revealed the motor putamen of CRPS patients had increased ISO power and both the putamen and caudate body had increased functional connectivity to the basal ganglia cortical input areas such as the primary motor cortex (M1), cingulate motor area, and orbitofrontal cortex. Increased ISO and functional connectivity of the putamen were correlated to increased perceived pain and motor dysfunction in CRPS. Additionally, functional connectivity between the PAG, LC, and RVM was not different between CRPS and controls. However, compared to controls, the PAG and LC had altered functional connectivity to higher brain areas in CRPS, with decreased PAG to S1 and posterior parietal cortex connectivity and increased LC to the caudate nucleus, anterior cingulate cortex, and hippocampus connectivity. Decreased PAG to S1 functional connectivity correlated to decreased pain in CRPS.

Conclusions: Overall these findings demonstrate that CRPS involves changes to the basal ganglia motor and non-motor loops and brainstem pathways to the higher brain areas but does not involve changes to sensorimotor cortex GABA or glutamate concentration. Increased ISOs in the motor putamen may indicate neuroinflammation via astrogliosis and increased astrocytic calcium wave propagation in CRPS. It is postulated that noradrenaline released by the LC may induce the basal ganglia ISO increases of CRPS by activation of astrocytic α1 receptors. This in turn can potentially decrease $GABA_A$ receptor activity which may explain CRPS sensorimotor reorganisation and disinhibition. Additionally, altered functional connectivity of the PAG and LC to higher brain areas but not the RVM suggests that there are altered ascending brainstem pain pathways but not descending pain modulatory pathways in CRPS. Together with the correlations of brain changes to pain and motor dysfunction, this thesis suggests that basal ganglia and ascending brainstem pain pathways may underpin pain and motor

dysfunction in CRPS. Future CRPS studies could aim to investigate the role of $GABA_A$ and α1 receptors.

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Chapter 1
Introduction

Chapter 1 Introduction

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Raja et al., 2020). Pain becomes chronic when it “persists or recurs for more than 3 months” (Treede et al., 2019). Chronic pain, as the leading cause of disability, affects more than 30% of the world’s population and exerts significant disease and economic burden (Cohen, Vase & Hooten, 2021).

1.1 CRPS

Complex regional pain syndrome (CRPS) is a chronic pain condition that ranks higher on the McGill Pain scale than amputation of a digit and childbirth and is considered to be the most excruciating chronic pain disorder (Abbott-Fleming, 2020; Johnston-Devin et al., 2021). Injury, usually a fracture, is often the precipitating event that results in the development of CRPS, however, CRPS can develop spontaneously without a known predisposing injury (Dijkstra et al., 2003; Marinus et al., 2011). CRPS is characterised by “continuing pain which is disproportionate to any inciting event” and, unlike other chronic pain conditions, often involves profound vasomotor and sudomotor changes characterised by changes in skin colour and temperature as well as oedema and sweating (Harden et al., 2007; Johnston-Devin et al., 2021; Marinus et al., 2011; Ott & Maihöfner, 2018). CRPS most commonly affects the upper limbs with a recent study reporting that of 1,042 CRPS patients, 70% had ongoing pain in the upper limbs, 31% of the lower limbs, and 0.7% had CRPS in more than one location (Ott & Maihöfner, 2018). Further, although CRPS pain is usually located in the distal limb, it can spread

proximally, as well as to the opposite limb (Bruehl, 2015; Forss, Kirveskari & Gockel, 2005; van Rijn et al., 2011).

Throughout the years, CRPS has been referred to by multiple different names including reflex sympathetic dystrophy, causalgia, algoneurodystrophy, and Sudeck's atrophy (Taylor et al., 2021). Although not initially termed CRPS, the pain disorder was first described in 1812 at the Siege of Badajoz where a soldier suffered a bullet injury to his upper limb (Denmark, 1813; Iolascon et al., 2015). While the wound quickly healed, it was documented that the soldier continued to suffer excessive pain of a burning nature (Denmark, 1813; Iolascon et al., 2015). Later, during the American Civil War in 1864, signs and symptoms of CRPS continued to be documented in detail (Mitchell, Morehouse & Keen, 1864). As the syndrome became better documented and defined over time, the nomenclature of the syndrome has been changed to CRPS (Stanton-Hicks et al., 1995; Taylor et al., 2021). Still today, definitive pathophysiological causes of CRPS have not been described, and effective long-term and non-invasive/safe treatments are needed for CRPS (Bruehl, 2015; Marinus et al., 2011).

1.1.1 Epidemiology

1.1.1.1 Prevalence

The prevalence of CRPS in Australia has not yet been investigated. However, in a United States retrospective database analysis of 6,575,999 chronic pain patients, 1.2% (79,025 patients) were diagnosed with CRPS between 2000 and 2012 (Murphy et al., 2017). Further, in another United States retrospective database analysis of 33,406,123 adult hospital inpatients, 0.7% (22,533 patients) were diagnosed with CRPS between 2007 and 2011 (Elsharydah et al., 2017). In both studies, females were more likely to be diagnosed with CRPS (Elsharydah et al., 2017; Murphy

et al., 2017). Other factors that were associated with a higher prevalence of CRPS were Caucasian race, higher income, headache, depression, drug abuse, and having private health insurance (Murphy et al., 2017). Additionally, having long-term disability and multiple pain diagnoses increased the likelihood of CRPS (Elsharydah et al., 2017). Diabetes, obesity, hypothyroidism, and anaemia were associated with lower CRPS prevalence (Murphy et al., 2017).

1.1.1.2 Incidence

As with prevalence, CRPS incidence is greater in females, where there is a 3-4 times greater likelihood that women will be affected by CRPS than men (de Mos et al., 2007; Ott & Maihöfner, 2018; Sandroni et al., 2003). The estimated annual incidence of CRPS ranges from 5.46 to 26.2 cases per 100,000, with peak incidence within the 50–70-year-old age range (Bruehl, 2015; de Mos et al., 2007; Ott & Maihöfner, 2018; Sandroni et al., 2003). Authors from three separate studies identified fracture to be the most common event preceding CRPS development (42-46% of cases) (de Mos et al., 2007; Ott & Maihöfner, 2018; Sandroni et al., 2003). In a 2020 systematic review, it was found that within 4 months of wrist fracture, there was a 3.7 to 14% incidence risk of CRPS development (Rolls et al., 2020). Further, individuals who rated pain greater than 5/10 in the first week following wrist fracture were at a higher risk of developing CRPS than individuals reporting less intense pain (Moseley et al., 2014).

1.1.1.3 Risk factors

Several risk factors have been identified for CRPS onset including being female, sustaining a fracture, and reporting severe pain during the early stages of injury (de Mos et al., 2007;

Elsharydah et al., 2017; Moseley et al., 2014; Murphy et al., 2017; Ott & Maihöfner, 2018; Pons et al., 2015; Sandroni et al., 2003). In addition, Pons et al. (2015) reported that postmenopausal females were more likely to develop CRPS. Interestingly, however, it was found that CRPS development was not related to endogenous estrogen exposure, use of contraceptives, or hormone replacement therapy (de Mos et al., 2009b). Instead, menstrual conditions such as constipation, pain before menstruation, and headaches during menstruation were identified as risk factors for developing CRPS (van den Berg et al., 2009). Additionally, Pons et al. (2015) reported that immobilisation of a fractured limb increased the risk of CRPS. Other conditions such as fibromyalgia and rheumatoid arthritis also increase the risk of CRPS (Taylor et al., 2021).

1.1.1.4 Prognosis

The majority of CRPS cases spontaneously resolve, with a 74% resolution rate in the first year (Sandroni et al., 2003) and a 36% resolution rate after 6 years (de Mos et al., 2009a). Many CRPS patients notice an improvement in their symptoms within 6-13 months (Bean, Johnson & Kydd, 2014). However, patients who do not recover from CRPS often have a poor prognosis of lasting pain and disability (Bean, Johnson & Kydd, 2014; de Mos et al., 2009a). In a study of 102 CRPS patients, 54% of patients self-reported stable CRPS, 30% reported full recovery while 16% continue to report severe and progressive CRPS after 6 years (de Mos et al., 2009a).

As well as dealing with the condition itself, persistent CRPS also adds a significant financial burden (Elsamadicy et al., 2017). In the year of CRPS diagnosis, CRPS patients have an estimated 2.17-fold increase in annual total outpatient-and-inpatient costs and a 2.56-fold increase in annual pain prescription costs, followed by a 1.06-fold annual increase in pain

prescriptions in the years following the initial 12 months after diagnosis (Elsamadicy et al., 2017). In addition, insurance costs of CRPS were 19 times higher and treatment costs 13 times higher than accidents that did not result in an individual developing CRPS (Scholz-Odermatt et al., 2019). Adding to the financial strain is the fact that the majority of CRPS patients are permanently unable to return to work (31-80%), while those that can return to work often require work adjustments such as decreased working hours or reduced physical demands (de Mos et al., 2009a; Kang et al., 2012; Scholz-Odermatt et al., 2019). Overall, CRPS patients experience a profound loss of quality of life (Mouraux et al., 2021; van Velzen et al., 2014).

1.1.2 Diagnostic criteria

The International Association for the Study of Pain (IASP) Budapest Criteria of CRPS is the most preferred diagnostic tool for CRPS in adults and involves clinical evaluation of a set of signs and symptoms (Goebel et al., 2021; Harden et al., 2010; Harden et al., 2007; Mesaroli et al., 2021). There are four categories of signs and symptoms: sensory, vasomotor, sudomotor/oedema, and motor/trophic (Goebel et al., 2021; Harden et al., 2010; Harden et al., 2007). Other than displaying signs and symptoms across the four categories, CRPS diagnosis is also dependent on continuing regional pain that has lasted more than three months, is "disproportionate to any inciting event" and "no other diagnosis can better explain the signs and symptoms" (Goebel et al., 2021; Harden et al., 2010; Harden et al., 2007) (**Table. 1**).

1.1.2.1 CRPS I and CRPS II

There are two main categories of CRPS: CRPS I and CRPS II (Goebel et al., 2021; Marinus et al., 2011; Taylor et al., 2021). These two CRPS categories are defined by the absence (CRPS

I) or presence (CRPS II) of a defined peripheral nerve injury (Marinus et al., 2011; Taylor et al., 2021). Therefore, CRPS that has occurred spontaneously without injury would be classified as CRPS I, while CRPS following injury or trauma to a nerve, such as radial nerve damage, would be classified as CRPS II. However, there is evidence that CRPS I is associated with small fibre peripheral nerve damage (Geertzen et al., 2015; Oaklander & Fields, 2009). Hence, both CRPS I and CRPS II are likely associated with some form of peripheral nerve damage. Furthermore, both CRPS I and CRPS II have identical diagnostic signs and the clinical significance of dividing CRPS into CRPS I and II are unclear (Goebel et al., 2021). Thus, for this thesis, there is no differentiation between CRPS I and II, and the syndrome will be referred to as CRPS.

Table. 1 IASP diagnostic criteria for complex regional pain syndrome: The Budapest Criteria (Goebel et al., 2021)

All the following criteria (A-D) must be met: A. The patient has continuing pain which is disproportionate to any inciting event. B. The patient reports at least one symptom in 3 or more of the categories. C. The patient displays at least one sign in 2 or more of the categories. D. No other diagnosis can better explain the signs and symptoms.	
Category	**Sign/Symptom**
1 “Sensory”	*Allodynia* (to light touch/brush stroke and/or temperature sensation and/or deep somatic pressure and/or joint movement), and/or *hyperalgesia* (to pinprick). Reported *hyperesthesia* also qualifies as a symptom.
2 “Vasomotor”	Temperature asymmetry and/or skin colour changes and/or skin colour asymmetry.
3 “Sudomotor/oedema”	Oedema and/or sweating changes and/or sweating asymmetry.
4 “Motor/trophic”	Decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair/nail/skin).

1.2 CRPS and the nervous system

1.2.1 Autonomic dysfunction

Autonomic abnormalities such as vasomotor and sudomotor changes, as well as pain in the affected limb of CRPS are thought to be at least partially driven by imbalances in sympathetic output (Knudsen et al., 2019). Sympathetic arousal and startle in healthy controls have been demonstrated to result in symmetrical vasoconstriction of the digits and decreased pain (Drummond et al., 2001). However, in CRPS, startle has instead been associated with increased perceived pain intensity and greater vasoconstriction on the affected CRPS hand compared to the unaffected (Drummond et al., 2001). It is therefore thought that increased responsiveness to sympathetic activity may drive pain in CRPS (Drummond et al., 2001; Knudsen et al., 2019). Indeed, increased sympathetic output such as stress has been shown to contribute to pain (Hannibal & Bishop, 2014). Interestingly, in CRPS, injection of phenylephrine, an α1 adrenergic receptor agonist, was associated with prolonged pain and pinprick hyperalgesia in patients with upregulated expression of α1 adrenergic receptors in peripheral nociceptors and keratinocytes (Drummond et al., 2018b). It has been postulated that upregulated α1 receptors increase the responsiveness of peripheral nociceptors to noradrenaline that is released during sympathetic activity, thus inducing pain and hyperalgesia in CRPS (Knudsen et al., 2019). Further, autonomic changes as well as pain and motor dysfunction in CRPS are thought to involve changes to not only the periphery but also the brain (e.g., hypothalamus) (Barad et al., 2014; Lebel et al., 2008).

1.2.2 CRPS and the brain

To date, most research exploring the underlying mechanisms responsible for the development and maintenance of CRPS has primarily focused on the brain (Jänig & Baron, 2002). This is likely because neither the presence nor severity of an injury can account for the development of CRPS (Goebel et al., 2021; Harden et al., 2010; Harden et al., 2007). Indeed, there have been instances where CRPS has developed without injury, while individuals with severe compound wrist fractures did not develop CRPS (Moseley et al., 2014). Given this, the development and maintenance of CRPS likely involves changes within the central nervous system at either the dorsal horn and/or within the brain itself (Bruehl, 2015). The involvement of the brain is consistent with the fact that CRPS pain continues long after injury healing, and that ongoing pain can spread in a non-dermatomal pattern to another limb (van Rijn et al., 2011). In fact, the spreading of CRPS pain to the contralateral side of the body is commonly reported and it is thought that such mirror-image pain distributions results from changes at the spinal or cortical level (van Rijn et al., 2011). Indeed, the mirror-image spread of neuropathic pain and allodynia have been associated with increased proinflammatory cytokines and phosphorylation of the NMDA receptor 1 subunit in the spinal cord of rats (Kwak et al., 2009; Milligan et al., 2003). More importantly, in a single CRPS case with mirror-like spread of CRPS pain and motor dysfunction, there was abnormal bilateral cortical activation to unilateral stimulation (Forss, Kirveskari & Gockel, 2005). It was hence suggested that mirror-image spread of CRPS was associated with the abnormal interhemispheric spread of cortical activation (Forss, Kirveskari & Gockel, 2005). Further supporting the role of the brain in CRPS is that higher brain areas are responsible for sensory perceptions such as body representation and precision of touch, and such sensory perceptions are also altered in CRPS (Halicka et al., 2020; Maihöfner et al., 2003; Pleger et al., 2006).

1.2.2.1 Altered sensory perceptions

As well as commonly reported sensory abnormalities such as hyperalgesia (increased sensitivity to painful stimuli such as pinprick) and allodynia (pain caused by non-painful stimuli such as brush stroke), CRPS patients' perceptions of their painful limb are disturbed and most likely reflect changes to the central nervous system. For instance, CRPS patients have described distorted body perceptions of their affected limb. It has been reported that CRPS patients perceive their affected limbs to be larger than they are in reality (Moseley, 2005) and some CRPS patients also experience neglect-like symptoms where their affected limb feels "alien", "foreign" or like it is not a part of their body (Galer & Jensen, 1999; Lewis & McCabe, 2010). Sensory perceptions such as tactile processing are also disturbed in CRPS and such disturbances are associated with the side or space of the affected limb rather than the affected limb itself (Moseley, Gallace & Spence, 2009). In a tactile temporal-order judgement task, CRPS patients perceived tactile information from the unaffected space before the CRPS affected space even when CRPS patients cross their affected limb to the unaffected space (Moseley, Gallace & Spence, 2009). Additionally, when CRPS patients have their eyes closed, they experience referred sensations where touch is mislocalised to body areas adjacent to the touch-stimulated area on the cortical body map (Maihöfner et al., 2006; McCabe et al., 2003).

1.2.2.2 Tactile acuity

Poor tactile acuity or perceived precision of touch in CRPS is another manifestation of impaired CRPS limb perception (Catley et al., 2014; David et al., 2015; Maihöfner & DeCol, 2007; Peltz et al., 2011; Pfannmöller et al., 2019; Pleger et al., 2006; Reiswich et al., 2012). Tactile acuity can be measured using two-point discrimination (TPD), where two points are applied to the

skin and the participant is asked if they feel one or two points. A TPD threshold is the minimum distance at which a subject may accurately perceive two points on their skin instead of one (Moberg, 1990). Thus, an individual with good tactile acuity has a low TPD threshold, and poor tactile acuity has a high TPD threshold. Many studies have demonstrated that CRPS patients have high TPD thresholds on their affected limbs and hence have poor tactile acuity (Catley et al., 2014; David et al., 2015; Maihöfner & DeCol, 2007; Peltz et al., 2011; Pleger et al., 2006; Reiswich et al., 2012).

1.3 Cortical circuits

1.3.1 Sensorimotor cortex

Poor tactile acuity in CRPS is thought to be associated with alterations in the function of the sensorimotor cortex and in particular, the primary somatosensory cortex (S1) (Maihöfner et al., 2003; Pleger et al., 2006). The sensorimotor cortex is composed of the primary somatosensory cortex (S1) and the primary motor cortex (M1) and it is theorised that this brain region is critical for the maintenance of pain in CRPS (Vittersø et al., 2022). Indeed, sensory and motor signs and symptoms are most likely to persist with ongoing CRPS (Bean, Johnson & Kydd, 2014), thus the sensorimotor cortex is of interest in CRPS.

1.3.2 The primary somatosensory cortex (S1)

The S1 is the primary cortical region responsible for the processing of somatosensory information including tactile acuity (Borich et al., 2015; Haag et al., 2015). The S1 is located on the postcentral gyrus and is somatotopically organised (Borich et al., 2015). The S1 somatotopic map or representative field of specific body parts was defined through electrical stimulation of different points along the length of the S1 during open brain surgery which elicited sensation in different body regions in awake humans (Penfield & Boldrey, 1937). The S1 is somatotopically organised such that each specific body part is spatially mapped onto distinct representative fields of the contralateral cortex, with the medial superior part of the S1 representing the lower limbs followed by the trunk, upper limbs, and face while moving laterally and inferiorly along S1 (Borich et al., 2015; Penfield & Boldrey, 1937). The S1 representative field size of the hands is disproportionately larger than less innervated body parts such as the trunk and legs (Penfield & Boldrey, 1937). Further, nociceptive inputs of individual

digits were found to be somatotopically organised in the contralateral S1 with comparable cortical representations for non-painful tactile stimuli (Mancini et al., 2012). Given this, it is generally accepted that one of the primary functions of S1 is the conscious perception and spatial localisation of pain (Backonja, 1996; Bushnell et al., 1999; Mancini et al., 2012; Ohara, Vit & Jasmin, 2005).

1.3.2.1 CRPS and S1

Several investigations have found the S1 representative field size of the affected hand to be altered in CRPS and this alteration has often been termed "cortical reorganisation" (Di Pietro et al., 2013b; Juottonen et al., 2002; Maihöfner et al., 2003; Maihöfner et al., 2004; Pfannmöller et al., 2019; Pleger et al., 2004; Vartiainen et al., 2009); although a recent study reported no such S1 reorganisation in CRPS (Mancini et al., 2019). Studies using functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and magnetoencephalography (MEG) have reported that the S1 representation of the affected hand in CRPS is smaller than the unaffected hand (Di Pietro et al., 2013b; Juottonen et al., 2002; Maihöfner et al., 2003; Maihöfner et al., 2004; Pfannmöller et al., 2019; Pleger et al., 2004; Vartiainen et al., 2009). Interestingly, in contrast to the smaller representation of the CRPS affected hand, it has also been found that the S1 cortical representation of the CRPS unaffected hand was larger than healthy individuals (Di Pietro et al., 2015). This remapping of S1 in CRPS has been linked to the greater severity of CRPS pain and mechanical hyperalgesia (Maihöfner et al., 2003). Given this, the S1 cortical reorganisation of the "shrunken hand" is thought to be so significant that treatments for CRPS currently include cortically-targeted therapies that aim to "train the brain" and "restore" somatotopic S1 representations (Catley et al., 2014; Moseley, Gallace & Spence,

2008; O'Connell et al., 2013). Indeed, upon CRPS recovery, the S1 cortical reorganisation has been reported to resolve (Maihöfner et al., 2004).

1.3.3 The primary motor cortex (M1)

CRPS is often associated with changes in motor function, such as decreased range of movement, weakness, tremor, and even dystonia (abnormal and involuntary muscle contractions, spasms, and posture). It is known that both the S1 and primary motor cortex (M1) activation are required for movement control and lesions confined to either cortical region can lead to impaired movement (Bashir et al., 2012; Borich et al., 2015; Murata et al., 2008; Shibasaki et al., 1993). The M1 is located on the precentral gyrus and, similar to the S1, is somatotopically organised with the lower body represented medially and the upper body and head more laterally (Penfield & Boldrey, 1937). The M1 also works in conjunction with other motor areas to plan, execute and control movement (Shibasaki et al., 1993). Movement abnormalities such as weakness, tremor, and dystonia of the CRPS affected limb become increasingly common as CRPS worsens and is a considerable part of CRPS disease burden (Bean, Johnson & Kydd, 2014; van Hilten, 2010). Furthermore, CRPS patients may have a reduced range of motion and a loss of fine motor control (van Hilten, 2010).

1.3.3.1 CRPS and M1

As with the S1, there is evidence of cortical reorganisation of the M1 where Maihöfner et al. (2007) found enlarged fMRI activation signals in the M1 contralateral to the CRPS affected side compared to the unaffected side and controls during finger tapping. Further, M1 function and excitability are altered in CRPS patients (Di Pietro et al., 2013a). Electrical stimulation of

the M1 in CRPS patients has been reported to increase the range of motion and decrease the perceived pain intensity of the affected limb (Fonoff et al., 2011; Velasco et al., 2009). Furthermore, in individuals affected by dystonia, M1 excitability is increased compared to healthy controls (Gilio et al., 2003; Ikoma et al., 1996), and in CRPS, decreased strength and range of movement of the affected limb are associated with decreased reactivity of M1 contralateral to the affected limb (Kirveskari et al., 2010). Importantly, this decreased reactivity in CRPS is associated with excitation or lack of inhibition (disinhibition) of the M1 (Kirveskari et al., 2010) and is correlated with the pain intensity of CRPS patients, where a greater decrease in M1 reactivity (and hence increased disinhibition) is associated with greater pain intensity (Kirveskari et al., 2010). Moreover, a meta-analysis of the M1 in CRPS reveals evidence, although limited, for bilateral M1 disinhibition in upper limb CRPS (Di Pietro et al., 2013a).

1.3.4 Disinhibition of the sensorimotor cortex

It has been proposed that disinhibition of the sensorimotor cortex in CRPS patients drives S1 cortical reorganisation (Di Pietro et al., 2013a; Di Pietro et al., 2013b; Eisenberg et al., 2005; Lenz et al., 2011; Pfannmöller et al., 2019; Schwenkreis et al., 2003). In the study of S1 disinhibition in CRPS, a paired-pulse stimulation paradigm was used to elicit somatosensory evoked potentials (Lenz et al., 2011). During paired-pulse stimulation, two consecutive electrical stimuli are applied to the median nerve, with a short time between the application of the first and second stimuli. In pain-free controls, this paired stimulation results in the inhibition of the S1 somatosensory evoked potential generated by the second stimulus (Lenz et al., 2011). However, since there was reduced inhibition of the second stimuli somatosensory evoked potentials in the left and right S1 in CRPS patients compared to controls, Lenz et al. (2011) concluded that there was bilateral S1 disinhibition in CRPS. Meanwhile, studies exploring M1

disinhibition in CRPS used transcranial magnetic stimulation (TMS) to deliver two consecutive stimuli of different thresholds over the M1 hand area to elicit a motor-evoked potential response in peripheral muscles. A short time interval between the pair of TMS stimuli over the M1 elicits short intracortical inhibition of the motor-evoked potential response (Klomjai, Katz & Lackmy-Vallée, 2015). In CRPS, there was reduced short intracortical inhibition, that is, disinhibition of the M1 (Eisenberg et al., 2005; Pfannmöller et al., 2019; Schwenkreis et al., 2003). Both bilateral M1 disinhibition (Schwenkreis et al., 2003) and M1 disinhibition exclusively of the CRPS affected side have been reported (Eisenberg et al., 2005; Pfannmöller et al., 2019). Therefore, the sensorimotor cortex of CRPS is disinhibited compared to healthy individuals, and it has been postulated that abnormal mechanisms involving gamma-aminobutyric acid (GABA) may account for this sensorimotor disinhibition in CRPS (Eisenberg et al., 2005; Lenz et al., 2011; Schwenkreis et al., 2003).

1.4 GABA

1.4.1 GABA and cortical representative fields

GABA is the primary inhibitory neurotransmitter in the brain. Experimental animal studies demonstrate that cortical representative field sizes are controlled by GABAergic inhibition, as sensorimotor representative field sizes expanded with the application of pharmacological antagonists of the $GABA_A$ and $GABA_B$ receptor but reduced in size when $GABA_B$ receptor agonists are applied (Capaday & Rasmusson, 2003; Chowdhury & Rasmusson, 2002; Dykes et al., 1984; Garraghty, Lachica & Kaas, 1991b; Tremere, Hicks & Rasmusson, 2001a). In monkeys, peripheral nerve transection resulted in S1 cortical reorganisation and decreased histological antibody staining of GABA (Garraghty, Lachica & Kaas, 1991a). More importantly, in humans, sensory input interruption by deafferentation can lead to sensorimotor cortical reorganisation, and decreased sensorimotor GABA concentration (Levy et al., 2002). Given this, the altered cortical reorganisation reported in CRPS stimulation studies may be due to alterations in GABAergic processes.

1.4.2 GABA and Sensory function

In vivo chemical compositions in regions throughout the brain can be measured using magnetic resonance spectroscopy (MRS), including the quantification of GABA content. In healthy pain-free individuals, greater tactile acuity has been correlated with greater in vivo sensorimotor GABA concentrations (Kolasinski et al., 2017; Puts & Edden, 2012). Furthermore, individuals with greater sensorimotor GABA are more likely to improve their tactile acuity with tactile acuity training (Heba et al., 2016). In CRPS and some other chronic pain conditions, tactile

acuity training has been shown to reduce the intensity of ongoing pain (Kälin, Rausch-Osthoff & Bauer, 2016; Schmid et al., 2017). However, on the CRPS affected side, improvements in tactile acuity following training were significantly less pronounced than in controls (Maihöfner & DeCol, 2007). Thus, the impaired sensory functions of CRPS and the reduced ability to improve tactile acuity through training may be caused by a reduction in sensorimotor GABA concentration.

1.4.3 GABA and Motor function

As with sensory function, sensorimotor GABA concentration in healthy individuals is also associated with motor function. In pain-free individuals, transient decreases in M1 GABA concentrations evoked by the application of transcranial direct current stimulation (tDCS) resulted in slower reaction times but faster short-term motor learning (Stagg, Bachtiar & Johansen-Berg, 2011). Furthermore, pain-free individuals with greater in vivo M1 GABA concentrations display reduced motor learning ability (Kolasinski et al., 2019). Thus, lower M1 GABA concentrations are associated with better motor learning outcomes. Further, motor learning is a key component of graded motor imagery (GMI), a commonly used treatment for CRPS that aims to restore the altered cortical reorganisation and sensorimotor disinhibition in CRPS (Guillot et al., 2008; Lotze et al., 1999; Strauss et al., 2021). However, CRPS patients may have poorer motor learning capacity than healthy controls as the imagined movement of the CRPS affected limb displayed decreased cortical activation of motor control areas such as the S1 and premotor cortex (Gieteling et al., 2008). Therefore, from a motor learning perspective, there may be increased M1 GABA concentration in CRPS. This is in direct contrast to the hypothesised decreased CRPS sensorimotor GABA concentration from a

sensory function and tactile acuity point of view. However, given the vast evidence of sensorimotor disinhibition in CRPS, it is more plausible that CRPS patients may have decreased sensorimotor GABA concentrations, but this remains to be investigated.

1.5 Glutamate

Glutamate is the main excitatory neurotransmitter in the brain and increased levels of glutamate have been associated with chronic pain conditions such as fibromyalgia (Peek et al., 2020; Pereira & Goudet, 2018). Studies using TMS have shown that in healthy controls, glutamate is associated with primarily intracortical facilitation and silent period duration (Liepert et al., 1997; Tremblay et al., 2013b). However, intracortical facilitation and silent period in CRPS are not different between affected and unaffected sides or in comparison with pain-free controls (Krause, Foerderreuther & Straube, 2005; Schwenkreis et al., 2003). Therefore, glutamate on its own is unlikely to be responsible for CRPS sensorimotor disinhibition. However, given that glutamate and GABA maintain the excitatory and inhibitory balance within the brain, it may be that this excitatory-inhibitory balance is altered in CRPS sensorimotor disinhibition. Thus, glutamate content is important to investigate.

1.6 Neuroinflammation and CRPS

In addition to sensorimotor disinhibition, neuroinflammation has been postulated to be a central mechanism in CRPS pathophysiology (Cooper & Clark, 2013). It has been suggested in chronic pain conditions and CRPS that neuroinflammation may be initiated by peripheral release of cytokines in response to peripheral tissue or nerve damage (Austin & Fiore, 2019; Cooper & Clark, 2013). Peripheral cytokines can mediate the activation of glial cells such as microglia and astrocytes in the dorsal horn to further mediate the release of pro-inflammatory cytokines and subsequent activation of ascending neurons (Austin & Fiore, 2019). Following, pro-inflammatory cytokines are released in the brain by glial cells neighbouring the ascending neurons, thus leading to neuroinflammation in chronic pain conditions (Austin & Fiore, 2019). Indeed, astrogliosis, the activation and change in phenotype and function of astrocytes, has been found in the dorsal horn of CRPS subjects (Del Valle, Schwartzman & Alexander, 2009). Astrocytes actively maintain homeostasis in the central nervous system (Li et al., 2019) and respond to neuronal activity with increases in intracellular calcium levels that occur in waves (Scemes & Giaume, 2006). Increases in intracellular calcium levels trigger the release of gliotransmitters (Scemes & Giaume, 2006). These astrocytic calcium levels are transmitted as intercellular calcium waves and are a form of intercellular communication (Scemes & Giaume, 2006). The astrocytic calcium waves propagate at a frequency of 0.03-0.06 Hz (Cornell-Bell et al., 1990; Crunelli et al., 2002). Infraslow oscillations (ISO) are electrical brain rhythms between the frequencies of 0.01 and 0.1 Hz (Watson, 2018). ISOs at the 0.03-0.06 Hz range are therefore thought to reflect astrocytic calcium wave propagation and increased ISOs at 0.03-0.06 Hz are thought to reflect astrogliosis (Henderson & Di Pietro, 2016). Astrogliosis can occur during neuroinflammation and it is thought that astrogliosis is involved in the development and maintenance of chronic pain (Li et al., 2019). Of interest, increased ISOs at

the 0.03-0.06 Hz range have been found in the thalamus and in a cluster comprised of the putamen and insular cortex in CRPS patients compared to controls (Di Pietro, Lee & Henderson, 2020) (

). It has been postulated that astrogliosis is responsible for the increased ISOs found in CRPS and other types of chronic pain (Di Pietro, Lee & Henderson, 2020; Henderson & Di Pietro, 2016).

1.7 Sub-cortical circuits

1.7.1. Basal ganglia

In addition to the M1, the control of motor function also involves processing within the basal ganglia, which consists of the striatum, globus pallidus, substantia nigra, and subthalamic nucleus. This group of nuclei has a well-described set of circuits that receive inputs from the sensorimotor and other cortical regions (Alexander, DeLong & Strick, 1986; Di Martino et al., 2008; Künzle, 1975; Künzle, 1977). While the basal ganglia is well known for its role in motor control and regulation, the basal ganglia also plays a role in cognition, reward/motivation, visual processing, and sensory integration (Alexander, DeLong & Strick, 1986; Da Cunha, Gomez-A & Blaha, 2012; Middleton & Strick, 1996; Redgrave et al., 2010). Each basal ganglia loop involves cortical input to the striatum (putamen and caudate) which, via the globus pallidus and substantia nigra, communicates with the thalamus which in turn loops back to the cortex (Alexander, DeLong & Strick, 1986) (**Figure 1.1**). It has been proposed that different basal ganglia-thalamocortical loops comprise different regions of these subcortical nuclei and are involved in different brain functions.

1.7.2 Basal ganglia neuroinflammation

Neuroinflammation of the basal ganglia is present in CRPS (Jeon et al., 2017). Jeon et al. (2017) used [^{11}C]-(R)-PK11195, a marker of activated microglia and astrocytes (Cosenza-Nashat et al., 2009), and positron emission tomography (PET) to investigate neuroinflammation in CRPS. Compared to healthy controls, CRPS patients had increased [^{11}C]-(R)-PK11195 distribution volume ratio (DVR) in areas of the basal ganglia including the caudate nucleus, putamen,

nucleus accumbens, and thalamus (Jeon et al., 2017). This is consistent with a more recent [^{11}C]-(R)-PK11195 PET study, where there was greater [^{11}C]-(R)-PK11195 binding in the putamen and caudate nucleus in CRPS than in healthy controls (Seo et al., 2021). Interestingly, the higher the [^{11}C]-(R)-PK11195 DVR in the caudate nucleus, the greater the pain intensity in CRPS (Jeon et al., 2017). Additionally, as mentioned above, CRPS subjects have been found to have increased ISOs in a cluster composed of the putamen and insular cortex compared to controls (Di Pietro, Lee & Henderson, 2020) (

). Therefore, given the PET evidence of basal ganglia neuroinflammation and increased ISOs in the putamen and insular cluster, astrogliosis may be involved in the development and persistence of CRPS pain. Although there is evidence of increased ISO in the putamen, it is unknown whether the putamen or insular cortex contributed more towards the finding of increased ISO in the putamen/insular cortex cluster in CRPS. Additionally, ISOs have not yet been investigated in different basal ganglia-thalamocortical loops.

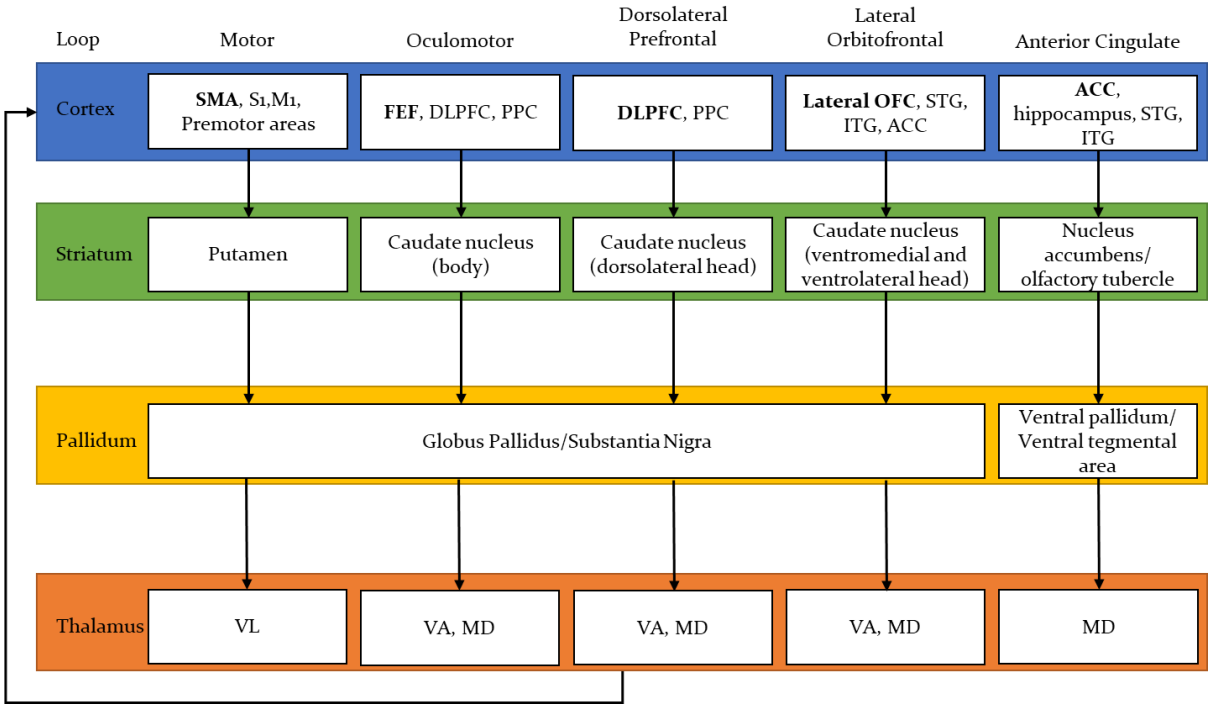

Figure 1.1. Schematic of basal ganglia thalamocortical loops. Thalamocortical connections of the motor, oculomotor, dorsolateral prefrontal, lateral orbitofrontal, and anterior cingulate basal ganglia functional loops. Each basal ganglia loop involves separate regions of the cortex, striatum, pallidum, and thalamus. Thalamocortical feedback for each loop is restricted to the cortical regions in **bold**. Abbreviations: ACC = anterior cingulate cortex, DLPFC = dorsolateral prefrontal cortex, FEF = frontal eye fields, GPi = internal globus pallidus, ITG = inferior temporal gyrus, M1 = primary motor cortex, MD = medial dorsal thalamus, OFC = orbitofrontal cortex, PPC = posterior parietal cortex, S1 = primary somatosensory cortex, SMA = supplementary motor area, SNr = substantia nigra pars reticulata, STG = superior temporal gyrus, VA = ventral anterior thalamus, VL = ventrolateral thalamus. Adapted from Alexander, DeLong and Strick (1986).

1.7.3 Motor loop

The basal ganglia motor loop receives information from cortical areas involved in motor control such as the supplementary motor area (SMA) and sensorimotor cortex. Cortical information enters the basal ganglia via the putamen which in turn projects to the globus pallidus, thalamus, and eventually the information projects back to the cortex (Alexander, DeLong & Strick, 1986) (**Figure 1.1**). This motor basal ganglia thalamocortical loop can facilitate or inhibit movement, and dysfunction of this loop can lead to motor dysfunction (Albin, Young & Penney, 1989).

One form of motor dysfunction that results from a disorder of the basal ganglia is dystonia. Dystonia is a common debilitating motor impairment in CRPS, and approximately 20% of CRPS patients are affected by dystonia (van Hilten, 2010). Indeed, in a retrospective study of CRPS, up to 91% of CRPS patients were found to have dystonia (van Rijn et al., 2007). Yet despite the prevalence of dystonia in CRPS, treatment options for CRPS dystonia are limited and there is little evidence that these treatments are effective (Birklein & Dimova, 2017). Given that dystonia is a disorder of the basal ganglia, dystonia in CRPS individuals may result from altered function of the motor basal ganglia loop. However, little research has been done on the basal ganglia in CRPS, hence this hypothesis has not yet been proven.

1.7.3.1 Putamen and CRPS

Consistent with the hypothesis that dystonia in CRPS is caused by altered motor basal ganglia loop function, it has been demonstrated that the putamen, an essential part of the motor loop,

is altered in CRPS. Compared to pain-free controls, Barad et al. (2014) found that the dorsal putamen contralateral to the CRPS affected side had an increased gray matter volume, while Azqueta-Gavaldon et al. (2020) conversely found an overall bilaterally decreased putamen gray matter volume in CRPS patients. Further, paediatric CRPS patients display increased putamen functional connectivity with the sensorimotor and salience networks at rest (Becerra et al., 2014) and increased putamen functional connectivity with the thalamus during cold-induced pain (Linnman et al., 2013). Additionally, greater putamen functional connectivity has been associated with greater motor impairment in CRPS (Azqueta-Gavaldon et al., 2020). Interestingly, while motor inputs to the putamen are somatotopically organised (Choi, Yeo & Buckner, 2012; Nambu et al., 2002), to our knowledge, there has been no CRPS study that has systematically investigated the putamen with respect to its somatotopic organisation.

1.7.4 Non-motor loops

As mentioned above, in addition to a basal ganglia motor loop, there exists several non-motor basal ganglia loops, that is, the oculomotor, dorsolateral prefrontal, lateral orbitofrontal, and anterior cingulate loops (**Figure 1.1**) (Alexander, DeLong & Strick, 1986). These non-motor loops of the basal ganglia are involved in visual attention and eye movement planning (oculomotor), cognition and executive function (dorsolateral prefrontal), emotional integration for contextually appropriate behaviour (lateral orbitofrontal), and motivated behaviours (anterior cingulate) (Alexander, DeLong & Strick, 1986; Leisman, Braun-Benjamin & Melillo, 2014; Seger, 2013). With exception of the anterior cingulate loop which projects to the nucleus accumbens and olfactory tubercle (ventral striatum), the non-motor basal ganglia loops involve cortical projection to the caudate nucleus: the oculomotor loop involves the body and tail of

the caudate nucleus; the dorsolateral prefrontal loop involves the dorsolateral caudate nucleus head; and the lateral orbitofrontal loop involves the ventromedial and ventrolateral caudate nucleus head (Alexander, DeLong & Strick, 1986; Öngür & Price, 2000; Seger, 2013) (**Figure 1.1**). The caudate body and dorsolateral caudate head of the oculomotor and dorsolateral prefrontal loops receive cortical inputs from the parietal cortex, an area important for spatial attention and integration (Alexander, DeLong & Strick, 1986; Seger, 2013). Thus, it is postulated that altered non-motor basal ganglia loops may be associated with distorted body perceptions of CRPS such as neglect-like syndrome and referred sensations.

1.7.4.1 Caudate nucleus and CRPS

Similar to the putamen, several studies have reported altered structure and function of the caudate nucleus in CRPS. The caudate nucleus contralateral to the CRPS affected side displayed increased gray matter volume compared to the other CRPS side (Azqueta-Gavaldon et al., 2020). Furthermore, different regions of the caudate nucleus have increased and decreased functional connectivity to other cortical regions such as the intraparietal sulcus in CRPS compared to controls (Bolwerk, Seifert & Maihöfner, 2013). Further, during cold-induced pain, paediatric CRPS patients displayed increased caudate nucleus functional connectivity to the superior temporal gyrus, which persisted even after CRPS recovery (Linnman et al., 2013). Additionally, despite CRPS recovery, the caudate nucleus had persistent increased functional connectivity to other cortical areas such as the M1, thalamus, cingulate and parahippocampal gyrus (Linnman et al., 2013). Moreover, in recovered CRPS paediatric patients, caudate activity during brush stimulation remained increased in the CRPS affected side compared to the unaffected side (Lebel et al., 2008). Despite the previous

structural and functional studies of the whole caudate in CRPS, no study has systematically investigated discrete non-motor basal ganglia loops within caudate sub-regions in CRPS.

1.7.5 Pain and basal ganglia

In addition to its involvement with motor dysfunction and distorted body perceptions, the basal ganglia is involved in the processing of noxious information. This processing is separated based on body location, with nociceptive information being somatotopically represented within the putamen (Bingel et al., 2004). Consistent with putamen activation during painful stimuli, individuals with putamen lesions display reduced sensitivity to heat pain (Starr et al., 2011), and in individuals with CRPS, increased putamen to sensorimotor cortex functional connectivity is associated with higher perceived pain intensity (Azqueta-Gavaldon et al., 2020). The caudate nucleus is also activated by noxious stimuli and in the anticipation of pain (Freund et al., 2009; Freund et al., 2010; Keltner et al., 2006; Wunderlich et al., 2011). Further, not only is the basal ganglia activated during acute pain and chronic pain states such as CRPS, the basal ganglia is thought to be involved in pain modulation and analgesia (Borsook et al., 2010). The basal ganglia is thus involved in motor dysfunction, distorted body perceptions, and pain, and as such, is of considerable interest in uncovering the pathophysiology of CRPS.

1.8 Pain pathways and modulation

1.8.1 Ascending pain pathway

Whilst the basal ganglia is likely involved in many aspects of CRPS, other brain regions such as the brainstem are also likely to be critical for the persistence of pain. The transmission of noxious information from peripheral nociceptors to the brain involves multiple ascending pain pathways (Yam et al., 2018) (**Figure 1.2**). Noxious information is detected by peripheral primary afferent nociceptors and transmitted to the dorsal horn in the spinal cord (Yam et al., 2018). Following this, second-order nociceptive neurons from the dorsal horn cross the midline and ascend the spinal cord via the lateral spinothalamic tract to transmit the noxious information to the thalamus (Yam et al., 2018). Third-order nociceptive neurons in the thalamus then project to cortical areas such as the S1 where an individual may perceive the intensity and location of pain (Yam et al., 2018). Second-order nociceptive neurons of the ascending pain pathways also project to several regions located in the brainstem including the brainstem reticular formation, midbrain periaqueductal gray matter (PAG), and the locus coeruleus (Boadas-Vaello et al., 2016). These brainstem regions are thought to be involved in fundamental survival mechanisms such as fight or flight behavioural responses and the overall sensitivity of the brain to incoming sensory information (Keay & Bandler, 2008; Kozlowska et al., 2015; Ross & Van Bockstaele, 2020). In addition, it is well known that several brainstem regions, such as the PAG are involved in the modulation of noxious information, and dysfunction of these regions may be involved in the maintenance of pain following injury.

Beginning at the periphery, maladaptive changes occur to the ascending pain pathway during neuropathic pain. During neuropathic pain, primary afferent nociceptive neurons release

inflammatory mediators such as substance P and glutamate which not only contribute to the excitation of neighbouring nociceptive neurons but also the excitation of second-order neurons of the spinothalamic tract (Boadas-Vaello et al., 2016). Indeed, Wesseldijk et al. (2008) found that plasma levels of inflammatory mediators such as glutamate were increased in CRPS compared to controls. Further of interest, Leis et al. (2003) found that CRPS subjects had increased sensitivity to substance P.

Additionally, inflammatory mediators in neuropathic pain induce phenotypic changes to primary nociceptors, such that there is an increased expression of voltage-gated ion channels for sodium and calcium, and ligand-gated ion channels such as purinergic P2X receptors (Boadas-Vaello et al., 2016). Interestingly, Zhao et al. (2008) found that the biopsied skin of CRPS subjects have increased immunolabeling of sodium channels such as Na(v)1.7 and Na(v)1.8 compared to controls. Zhao et al. (2008) further proposed that CRPS pain may be partly maintained by the increased sodium channel expression on keratinocytes and potential subsequent increased epidermal adenosine triphosphate (ATP) and hence excessive P2X receptor activation on primary sensory axons. The increase in expression of these ion channels contributes to nociceptor hyperexcitability, leading to the further release of inflammatory mediators and hence an increase in excitability of the spinothalamic tract (Boadas-Vaello et al., 2016).

Moreover, the inflammatory mediators released by primary nociceptors in the dorsal horn interact with microglia and astrocytes in the spinal cord (Boadas-Vaello et al., 2016; Pocock & Kettenmann, 2007; Schomberg & Olson, 2012). Then, in an almost self-propagating manner, microglia and astrocytes release more inflammatory mediators, which in turn further activate

more microglia and astrocytes and further contribute to the hyperexcitability and sensitisation of the spinothalamic tract and hence neuropathic pain (Boadas-Vaello et al., 2016; Ji et al., 2013; Shi et al., 2012). Thus, astrogliosis found in the dorsal horn of CRPS subjects (Del Valle, Schwartzman & Alexander, 2009) may partly be resultant of changes to the ascending pain pathway. Further, using PET and radioligand ^{11}C-PBR28, increased glial activation has been found in higher brain areas of chronic back pain subjects such as the thalamus and S1 (Loggia et al., 2015). Indeed, increased ISOs which are suggestive of astrogliosis have been found throughout the ascending pain pathway, including the thalamus and S1, in chronic orofacial pain (Alshelh et al., 2016). Additionally, studies have found evidence of astrogliosis in brainstem areas such as the PAG and rostral ventral medulla (RVM) in rat models of neuropathic pain (Ni et al., 2019; Wei et al., 2008a). Thus, CRPS pain may be maintained by astrocytic changes throughout the ascending pain pathway. Moreover, given that the higher order and brainstem areas such as the S1, thalamus, PAG and RVM are also part of the descending pain pathways, descending pain modulation may also be altered in CRPS.

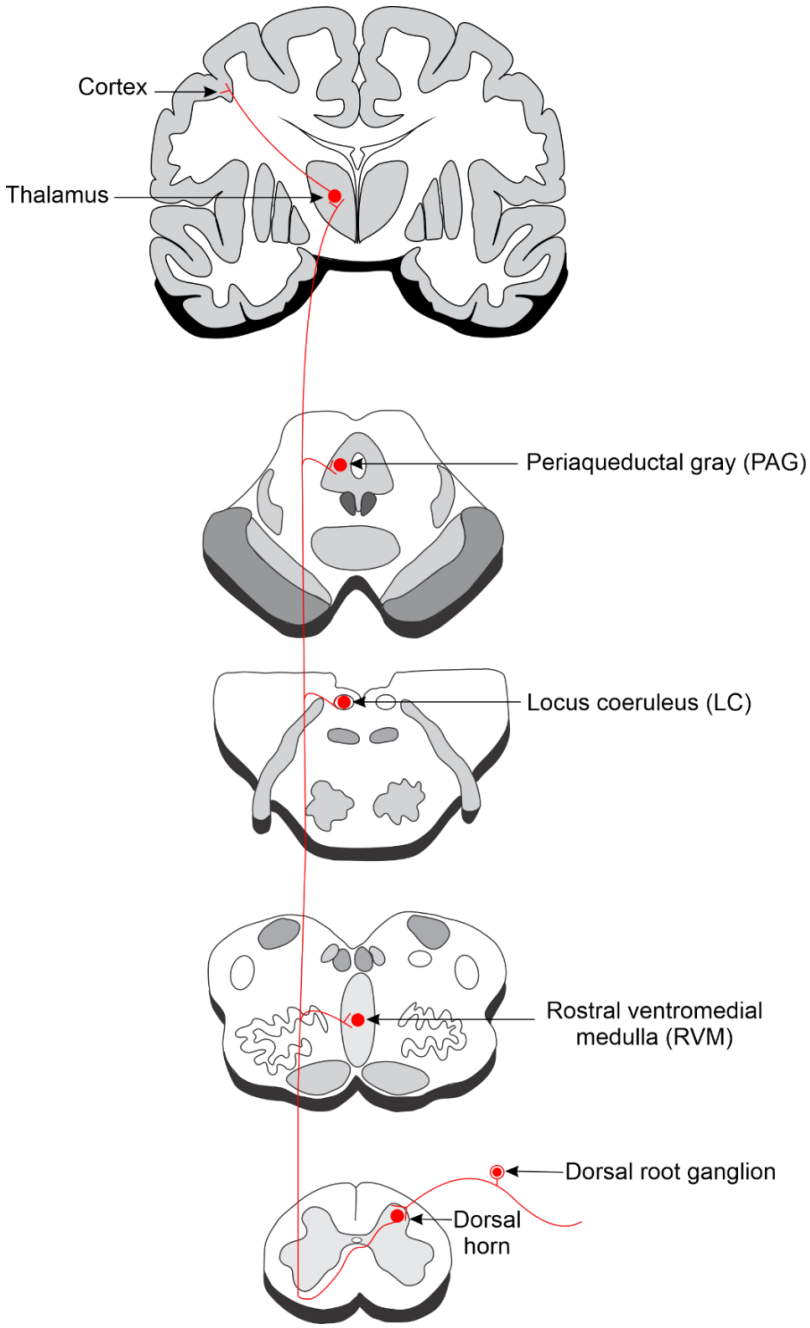

Figure 1.2. Ascending pain pathway. Nociceptive information is transmitted by primary afferent neurons, whose cell bodies are located in the dorsal root ganglion, to the dorsal horn. The axons of second-order nociceptive neurons cross the midline and ascend to project to the thalamus, as well as brainstem structures; rostral ventral medulla (RVM), locus coeruleus (LC), and periaqueductal gray (PAG). Third-order neurons from the thalamus project to different cortical areas for pain processing, for example, to the primary somatosensory cortex for sensory discrimination of pain (Boadas-Vaello et al., 2016; Yam et al., 2018).

1.8.2 Pain modulation via brainstem circuits

1.8.2.1 PAG and RVM

One of the best described pain modulatory circuits in the brain involves the PAG-rostral ventromedial medulla (RVM)-dorsal horn (DH) pathway (Basbaum & Fields, 1984). The PAG surrounds the cerebral aqueduct of the midbrain and serves as the main control centre for descending pain modulation (Boadas-Vaello et al., 2016). The PAG is divided into four longitudinal columns along the rostro-caudal axis: dorsomedial (dm), dorsolateral (dl), lateral (l), and ventrolateral (vl) (Carrive, 1993; Keay & Bandler, 2008). In particular, the three lateral (dl, l, and vl) PAG columns are associated with emotional coping strategies to pain, "fight or flight" behaviours (dlPAG and lPAG) or quiescent and hyporeactivity (vlPAG) (Keay & Bandler, 2008). Importantly, the dlPAG and lPAG produce non-opioid-mediated analgesia while the vlPAG produces opioid-mediated analgesia (Keay & Bandler, 2008). In mice, pharmacogenetic inhibition of the lateral (l) and ventrolateral (vl) PAG increases nociceptive behaviours to mechanical stimuli (Li et al., 2021). In humans, deep brain stimulation of the dlPAG and lPAG decreased pain intensity, that is, produced an analgesic effect and triggered the release of endogenous opioids in patients with deafferentation pain (Sims-Williams et al., 2017).

The PAG does not directly project to the spinal cord, rather, the RVM acts as the primary relay station between the PAG and dorsal horn for descending pain modulation (Boadas-Vaello et al., 2016; WeiWei et al., 2021) (**Figure 1.3**). The RVM lies in the midline of the medulla and contains three sets of neurons involved in descending pain modulation: ON, OFF, and NEUTRAL (Fields et al., 1983). RVM ON and OFF cells send projections to the dorsal horn

where afferent nociceptors synapse (Fields, Malick & Burstein, 1995). PAG neurons projecting to the RVM release neurotransmitters and endogenous opioid neuropeptides on RVM ON and OFF cells to modulate descending pain modulation (Boadas-Vaello et al., 2016).

Whilst the main focus of the PAG-RVM-DH pathway has been on pain relief, the RVM can also facilitate noxious information. RVM ON and OFF action potentials modify the excitability of spinal nociceptive neurons to increase and decrease nociceptive transmission, respectively (Fields et al., 1983; Salas et al., 2016; WeiWei et al., 2021). NEUTRAL cells are not associated with pain regulation (Fields, Barbaro & Heinricher, 1988; WeiWei et al., 2021). During noxious stimulation, there is an increase in action potential firing of spinal nociceptive neurons, which is correlated with increased ON cell and decreased OFF cell firing in rats (Salas et al., 2016). Further, local morphine microinjection to the RVM decreased ON cell firing but also nociceptive neuronal firing at the dorsal horn despite noxious stimulation, hence it is suggested that RVM ON cells facilitates pain transmission (Salas et al., 2016). In contrast, increased OFF cell activity by selective μ-opioids produced antinociception (Heinricher et al., 1994).

It is suggested that increased activation of ON cells and decreased activation of OFF cells, results in increased facilitation and decreased inhibition of pain transmission and is responsible for hyperalgesia and allodynia in chronic pain (Boadas-Vaello et al., 2016; Carlson et al., 2007; Neubert, Kincaid & Heinricher, 2004). Indeed, PAG and RVM activity are altered in chronic pain conditions compared to healthy controls (Bosma et al., 2016; Ioachim et al., 2022; Mills et al., 2018). Further, during voluntary suppression of pain in CRPS, the PAG is activated significantly less compared to healthy controls (Freund et al., 2011). Given this, it is likely that

persistent pain in CRPS involves alterations in the PAG-RVM-DH pathway that ultimately results in the facilitation of the dorsal horn and increased activity of ascending pain pathways.

Interestingly, in neuropathic pain, maladaptive changes of the ascending pain pathway can contribute to changes to the descending PAG-RVM-DH pathway. Indeed, peripheral inflammatory signals of the ascending pain pathway can facilitate pain via the RVM ON and OFF cells. In mice, single-unit recordings demonstrated that intradermal injection of pruritogens such as histamine or chloroquine, or algogens such as capsaicin, excited RVM ON cells but inhibited OFF cells (Follansbee et al., 2018). In addition, under physiological conditions, RVM ON cells typically facilitate pain, where β-endorphin released by the PAG will interact with μ-opioid receptors on RVM ON cells to hyperpolarise and hence inhibit the facilitation of pain (Carlson et al., 2007). However, in neuropathic pain, peripheral inflammation and inflammatory mediators from the ascending pain pathway such as substance P result in a decreased expression of μ-opioid receptors, but increased expression of other receptors such as NMDA/AMPA, Trk-B and NK1 that facilitate depolarisation of RVM ON cells and hence lead to increased pain (Boadas-Vaello et al., 2016; Guo et al., 2006; Lagraize et al., 2010; Miki et al., 2002). Similarly, there are also molecular changes with RVM OFF cells, such that there are increased $GABA_A$ and κ-opioid receptors (Boadas-Vaello et al., 2016; Gutstein et al., 1998). Ascending spino-PAG and spino-RVM pathways respectively stimulate the release of β-endorphin by PAG neurons, and GABA by RVM interneurons, resulting in hyperpolarised RVM OFF cells and hence reduced anti-nociception (Boadas-Vaello et al., 2016). Thus, given the interplay between ascending and descending pain pathways in neuropathic pain, it is important to investigate both pathways in CRPS to better understand pain in CRPS.

Astrogliosis in brainstem areas such as the PAG and RVM in neuropathic pain may further facilitate pain in the descending pathway (Ni et al., 2019; Wei et al., 2008a). Indeed, proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were elevated in a rat chronic constriction injury (CCI) model of neuropathic pain, and intra-RVM injection of astrocytic inhibitors propentofylline, fluorocitrate and minocycline attenuated mechanical allodynia and hyperalgesia in rats (Wei et al., 2008b). Similarly in another rat study, intra-plantar injection of carrageenan into the hindpaw induced peripheral inflammation, and increased immunolabelling of activated astrocytes and phosphorylated p38 MAPK, a proinflammatory cytokine promotor, in the RVM (Roberts, Ossipov & Porreca, 2009). Further, injection of fluorocitrate or minocycline attenuated astrocytic activation, tactile allodynia and thermal hyperalgesia (Roberts, Ossipov & Porreca, 2009). Hence, in neuropathic pain, inflammatory mediators released by activated astrocytes may also facilitate pain via RVM cells in the descending pain pathway.

In neuropathic pain, activated glial cells cause a change in the chloride anionic gradient in nociceptive neurons in the spinal dorsal horn and it has been speculated that spinal chloride dysregulation is important in neuropathic pain (Beggs, Trang & Salter, 2012; Coull et al., 2005). Within the RVM, the nucleus raphe magnus (RMg) is the primary source of the neurotransmitter serotonin (5-HT) (Boadas-Vaello et al., 2016). Descending 5-HT neurons from the RMg project onto inhibitory $GABA_A$-ergic interneurons in the dorsal horn. In mice, optogenetic stimulation of these 5-HT RMg fibres inhibited spinal nociception, was associated with decreased action potential frequency of nociceptive C-fibres in the dorsal horn and increased pain sensitivity to mechanical and thermal stimulation (Aby et al., 2022). In contrast,

when K+-Cl– cotransporter 2 (KCC2), a cell membrane transporter that maintains intracellular chloride concentration, was blocked in dorsal horn projection neurons, optogenetic stimulation of 5-HT RMg fibres significantly decreased paw withdrawal threshold and hence, increased pain sensitivity (Aby et al., 2022; Wilke et al., 2020). Similarly, in a murine spared nerve injury (SNI) model of neuropathic pain, optogenetic stimulation of 5-HT RMg fibres was associated with increased mechanical and thermal hypersensitivity and increased C-fibre action potential frequency in the dorsal horn (Aby et al., 2022). Further, Aby et al. (2022) found that pharmacologically enhancing KCC2 by CLP290 attenuated the mechanical and thermal hypersensitivity in SNI mice. Aby et al. (2022) therefore proposed the potential use of KCC2 enhancers for neuropathic pain relief in combination with selective serotonin reuptake inhibitors. A greater understanding of the involvement of the descending pain pathways in CRPS may therefore offer newer therapeutic options for CRPS.

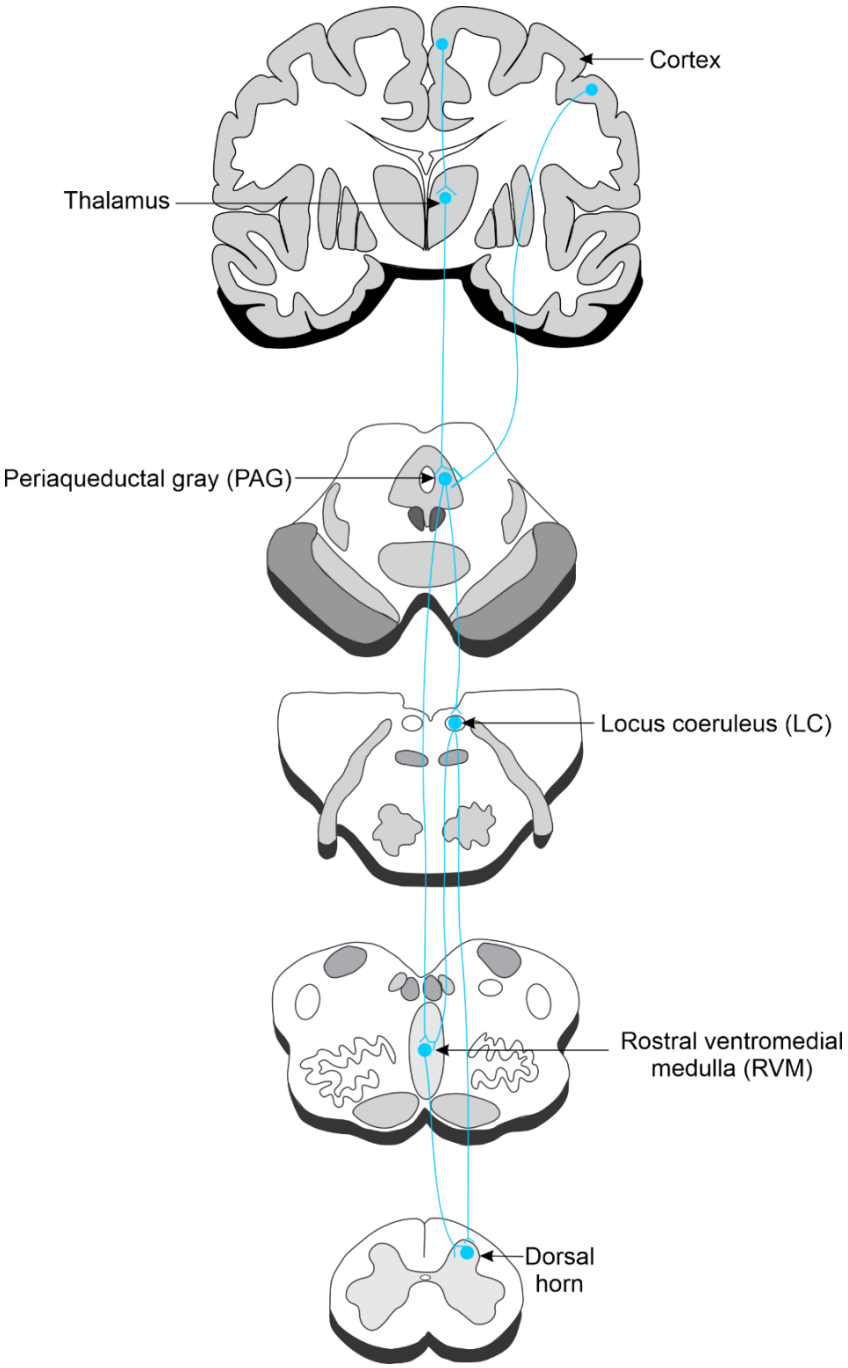

Figure 1.3. Descending pain modulatory pathways. Descending pain modulation from the cortex projects directly or indirectly via the thalamus to the periaqueductal gray (PAG). The PAG then projects its axons to the rostral ventromedial medulla (RVM) and the locus coeruleus (LC). Descending pain modulation is sent directly to the dorsal horn from the LC and RVM. In addition, the RVM also receives projections from the LC (Boadas-Vaello et al., 2016; WeiWei et al., 2021).

1.8.2.2 LC and noradrenaline

The LC is located in the pons and is the principal source of noradrenaline (NA) in the brain (Taylor & Westlund, 2017). The LC projects to the RVM (Clark & Proudfit, 1991) and directly to the spinal cord dorsal horn where it can modulate incoming noxious information (Bajic & Proudfit, 1999; Boadas-Vaello et al., 2016; Bruinstroop et al., 2012) (**Figure 1.3**). Indeed, descending noradrenergic projections to the spinal cord dorsal horn are important in descending pain modulation and primarily arise from the LC (Ossipov, Morimura & Porreca, 2014). In rats, optogenetic activation of specific subpopulations of LC noradrenergic neurons produced both antinociceptive and pronociceptive effects (Hickey et al., 2014). Furthermore, in the dorsal horn, α1 and α2 adrenergic receptors can facilitate or inhibit pain when activated by NA respectively (Boadas-Vaello et al., 2016; Holden, Schwartz & Proudfit, 1999; Ossipov, Morimura & Porreca, 2014).

The LC and NA system has been implicated in chronic pain. For example, in rodent models of neuropathic pain, lidocaine microinjection to the LC attenuated signs of allodynia and hyperalgesia (Brightwell & Taylor, 2009). Further, in a mouse model of neuropathic pain, pharmacological activation of noradrenergic neurons of the LC reduced pain (Li et al., 2022) and in humans, α2 adrenergic receptor agonists have been used to treat chronic pain conditions (Smith & Elliott, 2001). In animal models of nerve injury and neuropathic pain, there is upregulated expression and increased α1 adrenergic receptor activity, thereby facilitating pain (Drummond, 2012). It has been postulated that changes in the LC are associated with CRPS and chronic pain conditions (Drummond, 2012; Drummond & Finch, 2022; Taylor & Westlund, 2017).

1.8.2.3 Brainstem pain modulation nuclei and higher brain areas

Whilst the PAG and LC can individually modulate pain, they are also under the influence of higher brain regions (Coulombe et al., 2016; Song, Neal & Lee, 2021) (**Figure 1.3**). Indeed, connections between pain modulatory areas of the brainstem and higher brain areas may modulate pain in an ascending (bottom-up) or descending (top-down) direction. For example, during distraction from noxious stimulation, the orbitofrontal and anterior cingulate cortices (ACC) exert top-down modulation on the PAG such that there was a reduction in pain intensity and unpleasantness (Tracey et al., 2002; Valet et al., 2004). Top-down cortical inputs from the ACC to the PAG-RVM and ACC to LC have been shown to produce attentional analgesia (Oliva et al., 2021). The medial prefrontal cortex receives ascending nociceptive input where emotional and cognitive components of pain are processed (Kummer et al., 2020). Electrical stimulation of the LC suppresses nociceptive-evoked activity in the medial prefrontal cortex, suggesting the LC-NA system may modulate pain in an ascending and cortical manner (Condés-Lara, 1998). Indeed, optogenetic stimulation of noradrenergic projections from the LC to the ACC enhanced pain behaviours in mice (Koga et al., 2020).

In chronic pain, there is altered activity of the PAG, RVM, and LC and engaged higher brain areas (Mills et al., 2018). Many studies have postulated that CRPS is associated with and possibly maintained by changes at the level of the brainstem and brainstem regions associated with pain modulation (Drummond, 2012; Drummond & Finch, 2021; Drummond & Finch, 2022; Drummond et al., 2018a; Seifert et al., 2009; Thoma et al., 2022). However, to our knowledge, no study of CRPS has simultaneously investigated the PAG, RVM, and LC to

elucidate possible changes in pain modulatory pathways within the level of the brainstem and with higher brain areas in CRPS.

1.9 Neuroimaging Techniques

1.9.1 Magnetic Resonance Imaging (MRI)

One of the tools used to investigate brain function in CRPS is magnetic resonance imaging (MRI). This non-invasive imaging technique uses strong magnetic fields and radiofrequency pulses to produce images of the internal organs of the body including the brain. Different MRI sequences of magnetic fields, magnetic gradients, and radiofrequency pulses allow for the investigation of in vivo chemical concentration, structure, and activity of the brain.

1.9.2 Magnetic Resonance Spectroscopy (MRS)

Magnetic resonance spectroscopy (MRS) is a technique that can non-invasively detect neurochemicals in vivo (Mullins et al., 2014; Puts & Edden, 2012). Neurochemicals are, in part, structurally composed of hydrogen atoms. The MRI machine applies an external magnetic field to make hydrogen atoms spin at higher energy levels in MRS. As the hydrogen atoms relax, energy is released as radiofrequency signals which are measured in parts per million (ppm). The radiofrequency signals of hydrogen nuclear spins depend on neighbouring atoms (chemical environment) and adjacent spinning molecules (spin-spin coupling or J-coupling). The magnetic resonance spectrum is formed by the collection of signals from different compounds and contains a series of peaks where the compound with the highest proton concentration dominates (Puts & Edden, 2012; Rae, 2014). Radiofrequency signals for neurotransmitters such as GABA and glutamate can be isolated from the spectrum, identified and concentration quantified because of radiofrequency signal specificity for different neurochemicals (Mullins et al., 2014; Puts & Edden, 2012).

1.9.2.1 MEscher-Garwood Point RESolved Spectroscopy (MEGA-PRESS) and GABA

The concentration of GABA is difficult to quantify because GABA concentration is relatively low in the brain and the radiofrequency signature of GABA coincides with neurochemicals of greater concentration (especially creatine). Further, the fact that GABA is affected by spin-spin coupling, which divides the GABA signal into approximately 10 sub-peaks of lower peak intensity distributed over the spectrum, makes GABA especially hard to detect and quantify (Puts & Edden, 2012). The MEscher-Garwood Point RESolved Spectroscopy (MEGA-PRESS) is an editing technique that involves taking the difference between two interleaved datasets (ON and OFF-edited sequences) to quantify GABA concentration (Mullins et al., 2014). The MEGA-PRESS method utilises spin-spin coupling in GABA, where GABA has signals at 1.9 ppm and 3.0 ppm. GABA spins at 1.9 ppm are coupled to GABA spins at 3.0 ppm, but not all GABA spins at 3.0 ppm are coupled to 1.9 ppm. The ON editing pulse selectively targets the GABA spins at 1.9 ppm where the GABA spins at 3.0 ppm that are not coupled to 1.9 ppm are refocused. The OFF pulse allows hydrogens to spin freely. The difference between ON and OFF spectra selectively retains signals affected by the ON editing pulse, that is GABA at 3.01 ppm (Mullins et al., 2014).

1.9.2.2 Glutamate

Like GABA, glutamate is also affected by overlapping neurochemical signals and by spin-spin coupling making glutamate difficult to detect and quantify (Puts & Edden, 2012; van Veenendaal et al., 2018). In addition, glutamate and glutamine, the precursor of glutamate, have such similar molecular structures that it is difficult to separate glutamate and glutamine

and as such are often quantified together as Glx (Dou et al., 2015; Puts & Edden, 2012; van Veenendaal et al., 2018). The MEGA-PRESS difference spectrum produces an additional Glx peak that is not overlapped by other neurochemical signals, hence making Glx easier to quantify at 3.75 ppm (van Veenendaal et al., 2018). There are relatively higher concentrations of glutamate than glutamine in the brain, and although both glutamate and glutamine contribute to the MEGA-PRESS edited Glx peak, the MEGA-PRESS edited Glx peak reflects glutamate more than glutamine concentration (Cleve, Gussew & Reichenbach, 2015; Rae, 2014; Shungu et al., 2013; van Veenendaal et al., 2018).

1.9.3 Functional magnetic resonance imaging (fMRI)

Neural activity can be investigated using blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI). Regional blood flow increases and oversupplies an activated brain region with oxygenated blood (Bandettini, 2012). Oxygenated haemoglobin is not magnetic (diamagnetic), while deoxygenated blood is magnetic (paramagnetic) (Pauling & Coryell, 1936). fMRI detects the changes in magnetism of blood. Whilst fMRI can identify neural activity changes when individuals perform a task, it is a relative measure and thus cannot indicate absolute ongoing neural activity. It can however be used at rest to explore signal covariations between regions and thus provide an indication of region-to-region interactions. Brain regions that have simultaneous BOLD signal changes are said to be functionally connected.

1.9.3.1 Infraslow oscillations (ISOs)

fMRI signals can also be decomposed to explore patterns of resting signals and thus can be used to explore more subtle aspects of ongoing signals such as the power of infraslow (<0.1Hz) signal oscillations (Cordes et al., 2001). Low-frequency fluctuations contribute to the functional connectivity of brain regions at rest (Cordes et al., 2001). Amplitudes of low-frequency fluctuations (ALFF) is the total power of BOLD signal within the infraslow oscillation (ISO) range of 0.01-0.1 Hz and is proportional to neural activity (Lv et al., 2018). Increased ISOs are found in CRPS and other chronic pain conditions and are postulated to be involved in the development and maintenance of chronic pain (Di Pietro, Lee & Henderson, 2020; Henderson & Di Pietro, 2016).

1.10 Overall Thesis Aim

The overall thesis aim was to investigate the role of interconnected brain networks, including cortical, basal ganglia, and brainstem structures, in Complex Regional Pain Syndrome (CRPS) pain and motor dysfunction.

1.10.1 Rationale and Aims

The thesis consists of three experimental chapters in the form of three manuscripts, Chapters 2 and 3 are published, and Chapter 4 is currently under review.

Chapter 2 Rationale: The sensorimotor cortex is critical in pain and motor function and is thought to be responsible for altered sensory perceptions such as tactile acuity in CRPS. Sensorimotor disinhibition and sensorimotor cortical reorganisation, both of which are present in CRPS, are considered to be critical to the pathophysiology of CRPS. As a result, treatments for CRPS like GMI try to restore these abnormalities. It is thought that both sensorimotor cortical reorganisation and disinhibition may be due to changes in inhibitory balance via altered GABAergic function in CRPS. Both glutamate and GABA maintain the excitatory-inhibitory balance of the brain, however, to our knowledge, neither GABA nor glutamate concentration of the sensorimotor cortex of CRPS patients has been studied.

Chapter 2 Aim: The first experimental chapter uses magnetic resonance spectroscopy to determine brain biochemistry in CRPS. More specifically, this chapter explores whether there

are differences in the biochemical content of the sensorimotor cortex, that represents the most affected limb, of individuals with CRPS compared with pain-free controls. Furthermore, the relationship between this biochemistry and sensory function is determined.

Chapter 3 Rationale: The basal ganglia receive cortical input from the sensorimotor cortex and are important in pain and motor function. The basal ganglia consist of motor and non-motor functional loops that regulate motor function as well as cognition, reward, visual processing, and sensory integration. The function of key areas of the basal ganglia such as the putamen, which is somatotopically organised, and caudate nucleus are altered, and such changes have been correlated to pain and motor dysfunction in CRPS. In addition, neuroinflammation has been found in the basal ganglia of CRPS patients with infiltration of activated astrocytes and it is thought that astrogliosis that occurs with neuroinflammation may be involved in the development and maintenance of chronic pain in CRPS. Increased ISOs are thought to reflect astrogliosis and increased ISOs have been found in CRPS and other chronic pain conditions. However, to our knowledge, the putamen has not been somatotopically evaluated nor have the non-motor basal ganglia loops been systematically evaluated.

Chapter 3 Aim: The second experimental chapter explores the basal ganglia in CRPS. More specifically, it details the use of resting-state functional magnetic resonance imaging to systematically investigate the patterns of resting signal intensity fluctuations (infraslow oscillations) and the functional connectivity of motor (putamen) and non-motor basal ganglia loops in CRPS compared to age- and sex-matched pain-free controls.

Chapter 4 Rationale: Transmission and modulation of pain through ascending and descending pain pathways both involve synapses onto brainstem nuclei such as the PAG, LC, and RVM. In other chronic pain conditions, functional changes to the PAG, LC, and RVM have been observed where it has been suggested that pain is facilitated by descending modulatory circuits. Many studies have postulated that CRPS may have altered pain processing at the level of the brainstem, however, there is a lack of direct evidence to support this postulation. To our knowledge, no study has concurrently investigated the PAG, LC, and RVM to understand potential changes in pain pathways at the level of the brainstem and with higher brain areas.

Chapter 4 Aim: The third and final experimental chapter uses resting-state functional magnetic resonance imaging to investigate the functional connectivity of brainstem pain modulatory pathways in CRPS. It assesses resting functional connectivity strengths between the PAG, LC, and RVM in CRPS compared to age- and sex-matched pain-free controls. In addition, it assesses connectivity between the PAG and LC with higher brain regions in CRPS.

Chapter 2

CRPS Is Not Associated with Altered Sensorimotor Cortex GABA or Glutamate

eNeuro

Research Article: Negative Results

Disorders of the Nervous System

CRPS Is Not Associated with Altered Sensorimotor Cortex GABA or Glutamate

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Abstract

Complex regional pain syndrome (CRPS) is a debilitating chronic pain disorder typically in the upper or lower limbs. While CRPS usually develops from a peripheral event, it is likely maintained by CNS changes. Indeed, CRPS is reported to be associated with sensorimotor cortex changes, or functional "reorganization," as well as deficits such as poor tactile acuity. While the mechanisms underpinning cortical reorganization in CRPS are unknown, some have hypothesized that it involves disinhibition (i.e., a reduction in GABA activity). In this study, we addressed this hypothesis by using edited magnetic resonance spectroscopy to determine sensorimotor GABA and glutamate concentrations in 16 humans with CRPS and 30 matched control subjects and the relationship of these concentrations with tactile acuity. We found that individuals with upper limb CRPS displayed reduced tactile acuity in the painful hand, compared with the nonpainful hand and pain-free control subjects. Despite this acuity deficit, CRPS was not associated with altered GABA or glutamate concentrations within the sensorimotor cortex on either the side that represents the affected or unaffected hand. Furthermore, there was no significant relationship between sensorimotor GABA or glutamate concentrations and tactile acuity in CRPS subjects or control subjects. Although our sample was small, these data suggest that CRPS is not associated with altered total sensorimotor GABA or glutamate concentrations. While these results are at odds with the sensorimotor cortex disinhibition hypothesis, it is possible that GABAergic mechanisms other than total GABA concentration may contribute to such disinhibition.

Key words: chronic pain; cortical reorganization; GABA; glutamate; inhibition; sensorimotor cortex

Significance Statement

Complex regional pain syndrome is a debilitating chronic pain disorder that usually affects the limbs. It is associated with altered sensorimotor cortex function including reorganization and reduced tactile acuity, which are thought to result from reduced ongoing inhibition. However, we found that this pain condition is not associated with reduced ongoing sensorimotor inhibition in the form of GABA concentration, the major inhibitory neurotransmitter in the brain. These findings strongly suggest that changes in sensorimotor function in individuals with complex regional pain syndrome are explained by factors other than neurotransmitter concentration.

Introduction

Complex regional pain syndrome (CRPS) is a debilitating chronic pain disorder characterized by spontaneous or regionally evoked pain lasting for more than 3 months that typically affects the distal extremities, particularly the upper limbs (Marinus et al., 2011). CRPS often develops following an injury such as a fracture, but it can develop

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spontaneously (Marinus et al., 2011). While CRPS usually develops from a peripheral event, it is thought to be maintained by changes in the CNS since it often spreads to other body regions in a nondermatome fashion (van Rijn et al., 2011). There is no relationship between the severity of an injury and the development of CRPS; however, fracture patients who perceive excessive pain [visual analog scale (VAS) score, ≥5 of 10] in the first week after fracture are more likely to develop CRPS than patients who report lower pain intensity (Moseley et al., 2014). Further evidence of CNS involvement is the finding that CRPS patients often exhibit perceptual deficits such as a neglect-like syndrome (Galer and Jensen, 1999), distorted mental perceptions of their affected limbs (Moseley, 2005), and frequent mislocalized tactile perceptions and referred sensations (McCabe et al., 2003). Despite evidence of higher neural involvement, the underlying mechanisms responsible for the development of CRPS remain unknown, and there is no definitive treatment or cure (Marinus et al., 2011).

There is growing evidence that CRPS is associated with changes in primary sensorimotor (S1/M1) cortex function. Functional neuroimaging investigations have demonstrated that CRPS patients display primary somatosensory cortex (S1) "reorganization," whereby the S1 region responsible for the CRPS-affected hand is smaller than that of the unaffected hand (Juottonen et al., 2002; Maihöfner et al., 2003, 2004; Pleger et al., 2004; Vartiainen et al., 2009; Di Pietro et al., 2013a). This is thought to underlie the perceptual abnormalities seen in these patients; the most commonly reported is tactile acuity via two-point discrimination testing (TPD), which is simply the smallest distance between two points that is correctly perceived as two points, rather than one, touching the skin. CRPS patients have poor tactile acuity on their affected limbs (i.e., a high TPD threshold), and this has been associated with the degree of cortical reorganization (Pleger et al., 2006; Maihöfner and DeCol, 2007; Peltz et al., 2011; Reiswich et al., 2012; Catley et al., 2014; David et al., 2015). The primary motor cortex (M1) is also altered in CRPS, with reported changes in M1 function and excitability in CRPS patients (Di Pietro et al., 2013b). Although the fundamental mechanisms underpinning cortical reorganization in CRPS are unknown, some have suggested that a change in ongoing inhibition, particularly disinhibition, may underlie the S1/M1 changes in CRPS. Indeed, both S1 and M1 disinhibition have been detected in CRPS (Schwenkreis et al., 2003; Eisenberg et al., 2005; Lenz et al., 2011; Di Pietro et al., 2013a,b). Evidence suggests that S1 and M1 exhibit reduced intracortical inhibition compared with healthy control subjects, hence CRPS patients have been proposed to have bilateral disinhibition of the sensorimotor cortex. While studies postulate that the disinhibition in CRPS is associated with changes in GABA activity, no study has investigated GABA concentration in the sensorimotor cortex of CRPS patients or the balance between GABA and glutamate. This is surprising and somewhat of an oversight given that there are treatments that have been developed and adopted clinically that are theoretically aimed at restoring CNS inhibition (Moseley et al., 2012).

The aim of this study was to use magnetic resonance spectroscopy (MRS) to investigate sensorimotor GABA concentration in CRPS subjects. We hypothesized that sensorimotor GABA concentration in CRPS subjects would be decreased compared with healthy control subjects. Furthermore, since GABA and glutamate work together to maintain excitatory/inhibitory balance, we also investigated sensorimotor glutamate concentration and hypothesized that it would remain at control levels in CRPS. Finally, since greater sensorimotor GABA concentration has been found to predict better tactile acuity performance (Puts et al., 2011; Kolasinski et al., 2017) and individuals with CRPS have poor tactile acuity (Catley et al., 2014), we hypothesized that CRPS subjects would have reduced tactile acuity and that this would be correlated with reduced sensorimotor GABA concentration.

Materials and Methods

Because of the challenging nature of recruiting an eligible and willing sample of participants with upper limb CRPS, no sample size calculation was performed; the study recruited a convenience sample. Sixteen subjects with CRPS (12 females; mean ± SEM age, 48 ± 3 years) and 30 pain-free healthy control subjects (17 females; mean ± SEM age, 34 ± 2 years) were recruited for the study. CRPS subjects were recruited and their condition diagnosed in accordance with the International Association for the Study of Pain "Budapest" diagnostic criteria (Harden et al., 2007) and had ongoing pain for at least 3 months. For each subject, handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Subjects were excluded if they did not meet standard MRI safety criteria and healthy control subjects were excluded if they experienced any chronic pain condition. Informed written consent was obtained for all procedures, which were conducted under the approval by local Institutional Human Research Ethics Committees and consistent with the Declaration of Helsinki.

For the CRPS subjects, sensory signs of hyperalgesia and allodynia were assessed via pinprick on the dorsal web space of the hand and light brush strokes on the dorsum of the hands/forearms respectively. Vasomotor signs of skin temperature asymmetry were assessed through touch, skin color changes/asymmetry through visual observation and sudomotor/edema signs of sweating via touch, and edema was determined using a tape measure.

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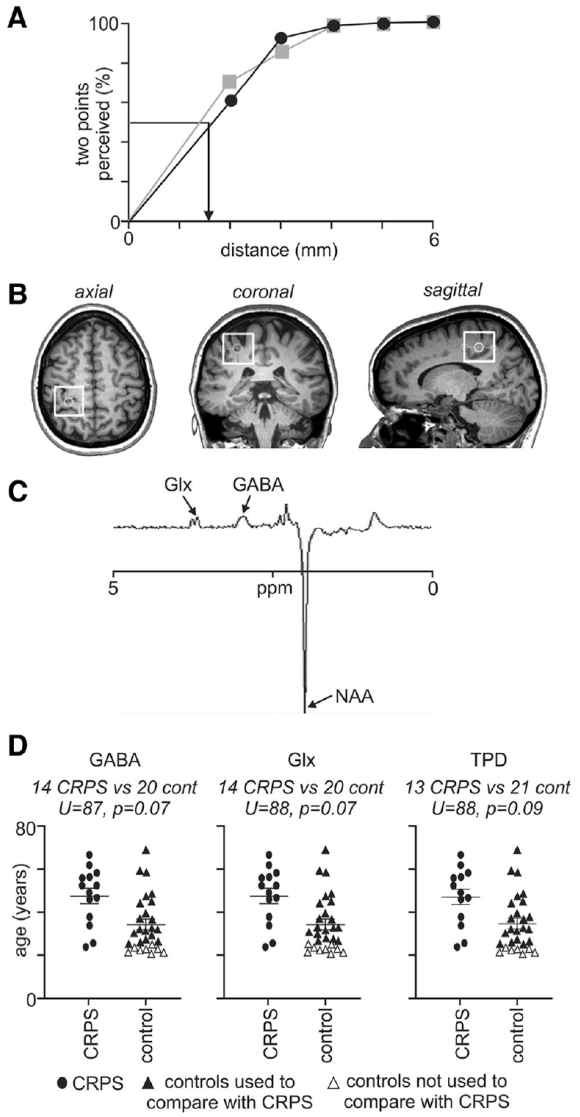

Figure 1. ***A***, A psychometric function of two-point discrimination threshold for a single subject's index finger. The percentage of two-point perception was plotted against the different point-to-point distances tested in the two-point discrimination task. The gray curve with gray squares indicates the subject's percentage of correct responses for each distance, and the black curve with circles is the fitted binary logistic regression. The two-point discrimination threshold is the distance at which correct two-point perception was at 50% (black arrow). ***B***, MRS voxel placement on the sensorimotor cortex. Voxel placement over the left sensorimotor cortex of a single subject indicated by the white square outline. The voxel was guided by the "hand hook" in the sagittal plane and the "hand knob" in the axial plane. ***C***, A typical MEGA-PRESS spectrum obtained from the sensorimotor cortex. NAA, *N*-acetyl aspartate. ***D***, Plots demonstrating the age distribution of the two groups. The left plot shows subjects used for GABA, the middle plot for Glx, and the right plot for TPD. While there were significant differences between groups if all control and CRPS subjects were used for the analyses, restriction of the control group numbers allowed for group comparisons in which there was no overall significant difference in age (Mann–Whitney *U* test, $p > 0.05$), sex (χ^2 test, $p > 0.05$), or handedness (Mann–Whitney *U* test, $p > 0.05$).

Motor signs were assessed by visual observation of finger, hand, and wrist movement. Trophic changes to hair, nail, and skin were visually assessed. CRPS subjects assessed the intensity of their ongoing pain using a VAS (0 = no pain to 10 = worst pain imaginable) three times per day for 7 consecutive days during the week of the scanning session. The average of these pain ratings was taken as a measure of "diary pain" intensity. Subjects also described their pain distribution by outlining the area of their chronic pain on a standard drawing of the body and assessed their pain on the day of the scanning session on a 10 cm VAS (i.e., "scan pain" intensity).

Tactile acuity

Tactile acuity was measured with a TPD assessment. TPD was assessed following the MRS scan to prevent possible influences of tactile acuity training on neurochemistry (Heba et al., 2016; Schmid et al., 2017). Subjects were instructed to rest their hand in a supine position and to keep their eyes closed, with the order of testing randomized. A TPD wheel (Exacta) was applied longitudinal to the surface of the distal pulp of the index finger until the first skin blanching around the points. Each distance was presented seven times in a randomized order, resulting in a total of 35 trials. The subjects reported whether they felt one point or two points touching the skin. When the subject could not feel two points at 5 mm spacing, then the distance was gradually increased until two points could be felt. The percentage of two-point perception was plotted against the distance between the points and fitted by a binary logistic regression (SPSS Statistics for Windows, version 24.0, IBM), resulting in a psychometric function of absolute threshold. From the binary logistic regression fit, the threshold was determined as the distance at which the chance level (50%) of correct two-point perception was reached (Fig. 1*A*).

MRI scans

Each subject was placed in the supine position on the MRI scanner bed in a 3 Tesla MRI scanner (Achieva TX, Philips Medical Systems), with their head in a 32-channel head coil to which padding was added to prevent head movement. With the subject relaxed, a high-resolution T1-weighted anatomical image of the whole brain was collected (288 axial slices, repetition time = 5600 ms; raw voxel size = 0.87 × 0.87 × 0.87 mm thick). Using this T1-weighted anatomical image set, a 30 × 30 × 30 mm voxel was placed over the right and then separately over the left sensorimotor cortex over the region that represents the hand (Fig. 1*B*). All three planes were referenced to ensure that the voxel position minimized the inclusion of

cerebrospinal fluid (CSF), did not incorporate dura mater, and did not enter the ventricles.

The GABA-edited Mescher-Garwood Point Resolved Spectroscopy (MEGA-PRESS) method of MRS was then performed on the 30 × 30 × 30 mm voxel, on the left and right sides individually (repetition time = 1800 ms; echo time = 68 ms; 2048 data points, acquisition time = ~8 min). The GABA resonance at 3.01 ppm was acquired by applying the MEGA-PRESS edited pulse at 1.9 ppm ("ON" spectra) and at 7.46 ppm ("OFF") in interleaved scans.

MRS analysis

Because of the similar chemical structure of glutamate and glutamine, the separation of glutamate and glutamine is problematic at 3 T field strength (Govindaraju et al., 2000). The combination of glutamate and glutamine is referred to as Glx; Glx and glutamate are used interchangeably here. The Glx peak in edited MEGA-PRESS spectra is largely proportional to the concentrations of glutamate and glutamine (Shungu et al., 2013), and, as glutamate concentrations are generally reported to be approximately threefold to fourfold higher than glutamine, the Glx peak can be considered to be derived proportionately more from glutamate than from glutamine (Rae, 2014), although relative changes in either metabolite cannot reliably be determined from the Glx resonance (Sanaei Nezhad et al., 2017). All MRS data were processed using the Java-based Magnetic Resonance User's Interface version 3 (jMRUI 3.0, MRUI Consortium). The ON and OFF spectral subsets were separately summed to produce single ON and OFF subspectra for each spectral dataset. The ON and OFF subspectra were then subtracted, resulting in GABA-edited difference spectra to measure GABA concentration at 3.01 ppm and Glx concentration at 3.75 ppm (Fig. 1C). The dominant water resonance was removed from the difference spectra using the Hankel–Lanczos Singular Values Decomposition Filter tool. GABA and Glx concentrations were quantified using the Advanced Method for Accurate, Robust and Efficient Spectral fitting (AMARES), a nonlinear least-squares fitting algorithm operating in the time domain. Peak fitting for GABA and Glx was performed after manually defining the center frequency and line width of GABA and Glx peaks. Lorentzian curves were used to obtain the peak amplitudes for GABA and Glx.

The OFF spectra subsets were summed to produce a single OFF subspectra to measure creatine (Cr) concentration at 3.02 ppm. Cr concentration was quantified using QUEST (QUantification ESTimation), a time-domain algorithm that fits a weighted metabolite basis set to the spectra acquired (Ratiney et al., 2005). GABA and Glx concentrations were expressed relative to creatine (i.e., GABA/Cr and Glx/Cr ratios). To ensure that there were no tissue fraction differences between hemispheres and groups (e.g., CRPS vs control subjects), the fraction of gray matter (GM), white matter (WM) and CSF was measured for each MEGA-PRESS 30 × 30 × 30 mm voxel. The GM, WM, and CSF fractions were acquired using MATLAB (MathWorks) and the partial volume code obtained from the Bangor Imaging Unit website (Gasparovic et al., 2006; Goulden and Mullins, 2020). The signal-to-noise ratio was defined in the frequency domain as the maximum height of the largest metabolite peak divided by the root mean square (rms) amplitude of noise in a signal-free and artifact-free part of the spectrum. The mean (±SEM) signal-to-noise ratio for GABA was 0.47 ± 0.01 (range, 0.29–0.64), and for Glx was 1.34 ± 0.11 (range, 0.18–2.72).

Statistical analysis

All statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software). All data were first tested for normality via the D'Agostino and Pearson normality test. For normally distributed data, parametric tests were used; paired *t* tests were used for within-subject comparisons and unpaired *t* tests were used for between-group comparisons. Nonparametric tests were used for non-normally distributed data; Wilcoxon matched-pairs signed rank test (W) for within-subject and Mann–Whitney *U* test (U) for between-group comparisons. To account for GM and WM differences, SPSS Statistics (version 24.0, IBM) was used to run ANCOVA for MRS GABA and Glx comparisons with GM/WM ratios as covariates. There was no difference in significance with GM and WM as covariates (not reported here).

Results

Two of the 16 CRPS subjects were excluded from the MRS analysis, and 2 of the control subjects were excluded from the GABA and 1 from the glutamate analysis due to technical difficulties and claustrophobia. The demographics and clinical characteristics of the remaining 14 CRPS subjects are shown in Table 1. All 14 CRPS subjects reported ongoing pain in the upper limb and 7 also reported pain in the lower limb. Eleven of the 14 subjects reported pain in the upper limb restricted to one side of the body, 9 on the right and 2 on the left, and the remaining 3 reported bilateral pain, although pain was greater on the right side in 2 and on the left in 1 of these subjects. For CRPS subjects, the S1/M1 contralateral to the greatest ongoing upper limb pain was considered to be the "affected" side and vice versa for the "unaffected" side (i.e., there were 11 right-sided pain and 3 left-sided pain).

GABA

In the 11 subjects with unilateral pain, there was no difference in GABA concentration between the hemispheres representing the painful (affected) and non-painful (unaffected) limbs (mean ± SEM $\times 10^{-1}$ GABA/Cr ratio: affected, 2.82 ± 0.12; unaffected, 2.91 ± 0.13; $p = 0.49$). Similarly, for the whole group of 14 CRPS subjects, we found no significant differences in S1/M1 GABA between the right and left hemispheres (right, 2.86 ± 0.11; left, 2.96 ± 0.10; $p = 0.32$) or between the dominant (handedness) and nondominant hemispheres (dominant, 2.91 ± 0.11; nondominant, 2.92 ± 0.11; $p = 0.94$; Fig. 2A). For the 28 control subjects, we

Table 1: Demographics and clinical characteristics of patients with CRPS

									Signs (symptoms)					
Subject	Age	Sex	EHI score (handedness)	Pain duration (years)	CRPS affected region	Inciting event	Medications	Comorbidity	Sensory	Vasomotor	Sudomotor/edema	Motor/trophic	Pain intensity (diary VAS)	Pain intensity (day VAS)
1	49	M	100.0 (R)	7.0	**R UL**, R LL, L LL, face, abdomen	Pain in R hand	Turmeric tablets	None	+ (+)	+ (+)	+ (+)	+ (+)	4.5	4.0
2	56	F	100.0 (R)	4.2	**L UL**, R UL, R LL, R and L chest	L humerus fracture	Duloxetine, gabapentin, oxycodone, quetiapine, tapentadol	L radial nerve palsy, Triangular fibrocartilage complex of R hand	+ (+)	+ (+)	+ (+)	+ (+)	8.1	7.8
3	56	F	60.0 (R)	0.9	**R UL**, R neck, R chest	Spontaneous onset	Ashwagandha, budesonide, cannabis, codeine, formoterol, oxycodone, paracetamol, salbutamol	Back pain, COPD, fibromyalgia, osteoarthritis, peptic ulcer, radiculopathy, Raynaud's disease, spinal disc herniation	+ (+)	+ (+)	– (+)	+ (+)	8.3	7.9
4	62	F	100.0 (R)	6.2	**L UL**, *R UL*[2]	R hand tendon release surgery	Amitriptyline, cannabidiol drops, codeine, levothyroxine, magnesium, paracetamol, topiramate, tramadol, valerian	Diverticulitis, gastroesophageal reflux disease, Graves' disease (thyroidectomized)	+ (+)	+ (+)	– (+)	+ (+)	5.8	4.3
5	58	F	–20.0 (A)	8.7	**R UL**, R LL, R face	R arm surgery	Codeine, duloxetine, linagliptin, meloxicam, metformin, paracetamol	Diabetes	+ (+)	+ (+)	+ (+)	+ (+)	4.7	4.1
6	67	F	100.0 (R)	9.5	**R UL**, L UL, R LL, L LL	R radius fracture	Amlodipine, gabapentin, ketamine in lipoderm cream, metformin, metoprolol, pantoprazole, salbutamol	Asthma, diabetes, gastric reflux, hypertension, osteoarthritis, pubic symphysitis, supraventricular tachycardia	+ (+)	– (+)	– (+)	+ (+)	3.7	5.0
7	47	M	44.4 (R)	1.5	**R UL**	Spontaneous onset	Amlodipine, atorvastatin, ibuprofen, paracetamol, perindopril, pregabalin	Hyperlipidemia, hypertension	+ (+)	+ (+)	– (–)	+ (+)	6.8	7.1
8	34	F	–23.1 (A)	5.3	**R UL**, R LL, R hip	R wrist fracture	Amitriptyline, buprenorphine patch	Migraine, R hip bursitis	+ (+)	+ (+)	+ (+)	+ (+)	4.3	2.4
9	26	F	–40.0 (A)	1.3	**R UL**, L and R neck, spine, L LL	R hand nerve damage	None	Endometriosis, polycystic ovarian syndrome	+ (+)	+ (+)	+ (+)	+ (+)	5.4	6.8
10	46	F	80.0 (R)	3.9	**L UL**	L hand carpal tunnel release surgery	Amitriptyline, betahistine, duloxetine, naproxen, pantoprazole, rizatriptan, tapentadol, valaciclovir	Carpal tunnel of R hand, fibromyalgia, herpes, migraine, polycystic ovarian syndrome with insulin resistance, vertigo	+ (–)	+ (+)	+ (+)	– (+)	0.6	5.6
11	24	F	70.0 (R)	2.6	**R UL**, L UL	Overload	Amitriptyline, gabapentin, levothyroxine	Hashimoto's disease	– (–)	– (+)	+ (+)	+ (+)	4.5	4.4

(Continued)

Table 1: Continued

Subject	Age	Sex	EHI score (handedness)	Pain duration (years)	CRPS affected region	Inciting event	Medications	Comorbidity	Signs (symptoms) Sensory	Vasomotor	Sudomotor/edema	Motor/trophic	Pain intensity (diary VAS)	Pain intensity (day VAS)
12	52	F	40.0 (A)	2.9	**R UL**, R torso	Broke tailbone	Ashwagandha, fish oil, ibuprofen, magnesium, mega B, melatonin, paracetamol, tapentadol, vitamin C, vitamin D	Endometriosis	+ (+)	+ (+)	− (+)	− (+)	3.8	3.5
13	38	F	17.6 (A)	12.7	**R UL**, R neck, L LL	Spontaneous onset	Duloxetine, gabapentin, naloxone, oxycodone, palmitoylethanolamide (PEA)	Endometriosis, endosalpingiosis, Raynaud's disease	+ (+)	+ (+)	+ (+)	+ (+)	0.0	5.9
14	52	M	−88.9 (L)	1.9	**R UL**, L and R neck, back	R scaphoid fusion surgery	Cholecalciferol, ibuprofen, magnesium, oxycodone, paracetamol, pregabalin, tramadol, venlafaxine, zopiclone	L shoulder bursitis, Sleep apnea	+ (+)	+ (+)	+ (+)	+ (+)	7.0	7.6

R, Right; L, left; A, ambidextrous; UL, upper limb; LL, lower limb; +, present; −, absent. Bold type indicates the CRPS region with the most severe pain. Italic type indicates remission of the CRPS region. Underline indicates medication taken in the last 24 h of the day of testing. The presence (+) or absence (-) of CRPS signs and symptoms are presented as signs (symptoms).

also found no significant differences between the right and left hemispheres (right, 3.07 ± 0.06; left, 3.17 ± 0.09; $p = 0.51$) or between the dominant and nondominant hemispheres (dominant, 3.06 ± 0.05; nondominant, 3.18 ± 0.09; $p = 0.36$; Fig. 2*B*).

To address the primary aim of characterizing GABA concentration in CRPS, a group of 20 control subjects was selected such that there was no significant difference between control subjects and CRPS subjects with respect to age (Fig. 1*D*; Mann–Whitney *U* test, $p > 0.05$), sex (χ^2 test, $p > 0.05$) and handedness (Mann–Whitney *U* test, $p > 0.05$). We found no significant difference in S1/M1 GABA when comparing the CRPS-affected with control subjects' dominant (affected, 2.87 ± 0.10; dominant, 2.95 ± 0.11; $p = 0.36$), CRPS-affected versus control subjects' nondominant (affected, 2.87 ± 0.10; nondominant, 3.14 ± 0.14; $p = 0.15$), CRPS-unaffected versus control subjects' dominant (unaffected, 2.95 ± 0.11; dominant, 2.95 ± 0.11; $p = 0.99$), or CRPS-unaffected versus control subjects' nondominant (unaffected, 2.95 ± 0.11; nondominant, 3.14 ± 0.14; $p = 0.64$; Fig. 2*C*).

Glx

Similar to the findings for GABA, in the 11 subjects with unilateral pain, we found no difference between the hemispheres representing the affected and unaffected limbs (mean ± SEM × 10^{-1} Glx/Cr ratio: affected, 1.88 ± 0.10; unaffected, 1.90 ± 0.08; $p = 0.79$; Fig. 3*A*). In the group of 14 CRPS subjects, we found no significant differences in S1/M1 Glx between the right and left hemispheres (right, 1.90 ± 0.06; left, 1.83 ± 0.08; $p = 0.30$) or between the dominant and nondominant hemispheres (dominant, 1.83 ± 0.08; nondominant, 1.89 ± 0.07; $p = 0.45$). For the 29 control subjects, we also found no significant differences between the right and left hemispheres (right, 1.93 ± 0.03; left, 1.86 ± 0.03; $p = 0.07$) or between the dominant and nondominant hemispheres (dominant, 1.89 ± 0.04; nondominant, 1.90 ± 0.03; $p = 0.65$; Fig. 3*B*).

To determine the differences between CRPS and control subjects, we analyzed all 14 CRPS subjects against a group of 20 control subjects so that there was no significant difference between control subjects and CRPS subjects with respect to age (Fig. 1*D*; Mann–Whitney *U* test, $p > 0.05$), sex (χ^2 test, $p > 0.05$), and handedness (Mann–Whitney *U* test, $p > 0.05$). We found no significant difference in S1/M1 Glx when comparing the CRPS-affected subjects to control subjects' dominant (affected, 1.85 ± 0.08; dominant, 1.89 ± 0.04; $p = 0.72$), the CRPS-affected subjects to control subjects' nondominant (affected, 1.85 ± 0.08; nondominant, 1.88 ± 0.03; $p = 0.69$), the CRPS-unaffected subjects to control subjects' dominant (unaffected, 1.87 ± 0.07; dominant, 1.89 ± 0.04; $p = 0.88$), or the CRPS-unaffected subjects to control subjects' nondominant (unaffected, 1.87 ± 0.07; nondominant, 1.88 ± 0.03; $p = 0.87$; Fig. 3*C*).

Tactile acuity

Three of the 16 CRPS subjects were excluded from TPD analysis due to ongoing pain preventing testing, and 2 of the 30 control subjects due to the perception of two points during one-point stimuli runs. As hypothesized, in CRPS patients, the affected hand had a significantly

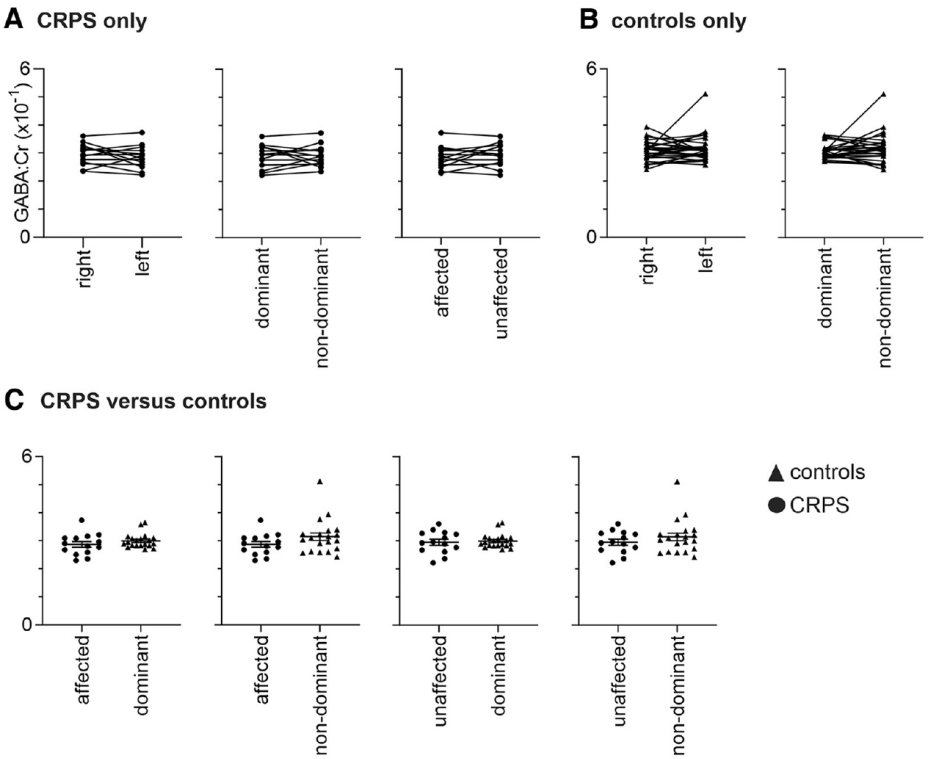

Figure 2. Plots of GABA/Cr ratios ($\times 10^{-1}$) of a 30 × 30 × 30 mm voxel placed over the hand representation of the sensorimotor cortex. ***A***, Values for individual subjects with upper limb CRPS for the right and left, dominant and nondominant hemispheres and also for the affected (hemisphere representing the side of ongoing pain) and unaffected hemispheres. Plots show pairwise connections for each individual subject. ***B***, Values for individual control subjects. ***C***, Values comparing individual CRPS and control subjects. Horizontal lines indicate the mean ± SEM for each group. Note there are no significant differences between any hemisphere in the CRPS or control subjects alone or between CRPS and control groups.

greater TPD threshold (i.e., reduced tactile acuity) than the less/unaffected hand (mean ± SEM TPD in millimeters; affected, 3.09 ± 0.23; unaffected, 2.35 ± 0.16; $p = 0.004$), and, given that most CRPS subjects had right side pain in the upper limb, there was a significant TPD threshold difference between the right and left hands (right, 3.02 ± 0.25; left, 2.41 ± 0.14; $p = 0.04$), but no difference between the dominant and nondominant hands (dominant, 2.74 ± 0.24; nondominant, 2.69 ± 0.20; $p = 0.87$; Fig. 4*A*). In control subjects, we found no significant difference between the right and left hands (right, 2.45 ± 0.13; left, 2.27 ± 0.12; $p = 0.11$) or the dominant and nondominant hands (dominant, 2.26 ± 0.13; nondominant, 2.45 ± 0.11; $p = 0.08$; Fig. 4*B*).

To determine the differences between CRPS and control subjects, we analyzed all 13 CRPS subjects against a group of 21 control subjects so that there was no significant difference between control subjects and CRPS subjects with respect to age (Fig. 1*D*; Mann–Whitney *U* test, $p > 0.05$), sex (χ^2 test, $p > 0.05$), and handedness (Mann–Whitney *U* test, $p > 0.05$). This comparison revealed that the CRPS-affected hand had significantly greater TPD threshold, and hence poorer tactile acuity, than the dominant hand of the control subjects (affected, 3.09 ± 0.23; dominant, 2.23 ± 0.17; $p = 0.007$) and the nondominant hand of the control subjects (affected, 3.09 ± 0.23; nondominant, 2.49 ± 0.13; $p = 0.04$; Fig. 4*C*). However, tactile acuity for the CRPS-unaffected hand was not different from the dominant hand of the control subjects (unaffected, 2.35 ± 0.16; dominant, 2.23 ± 0.17; $p = 0.32$) or the nondominant hand of control subjects (unaffected, 2.35 ± 0.16; nondominant, 2.49 ± 0.13; $p = 0.24$).

Although we found a significant difference in TPD threshold in CRPS subjects between the affected and unaffected hands, there were no significant correlations between TPD in the affected hand and GABA or Glx in the affected S1/M1 (GABA: $r = 0.49$, $p = 0.06$; $r = 0.17$, $p = 0.59$), or between TPD in the unaffected hand and GABA or Glx in the unaffected S1/M1 (GABA: $r = 0.12$, $p = 0.35$; Glx: $r = -0.09$, $p = 0.77$). Similarly in control subjects, there were no significant correlations between TPD in the dominant hand and GABA or Glx in the dominant S1/M1 (GABA: $r = 0.09$, $p = 0.32$; Glx: $r = 0.24$, $p = 0.12$) or between TPD in the nondominant hand and GABA or Glx in the nondominant S1/M1 (GABA: $r = 0.24$, $p = 0.12$; Glx: $r = 0.01$, $p = 0.99$).

Discussion

Contrary to our hypothesis, we found no significant difference in primary sensorimotor cortex GABA concentration in

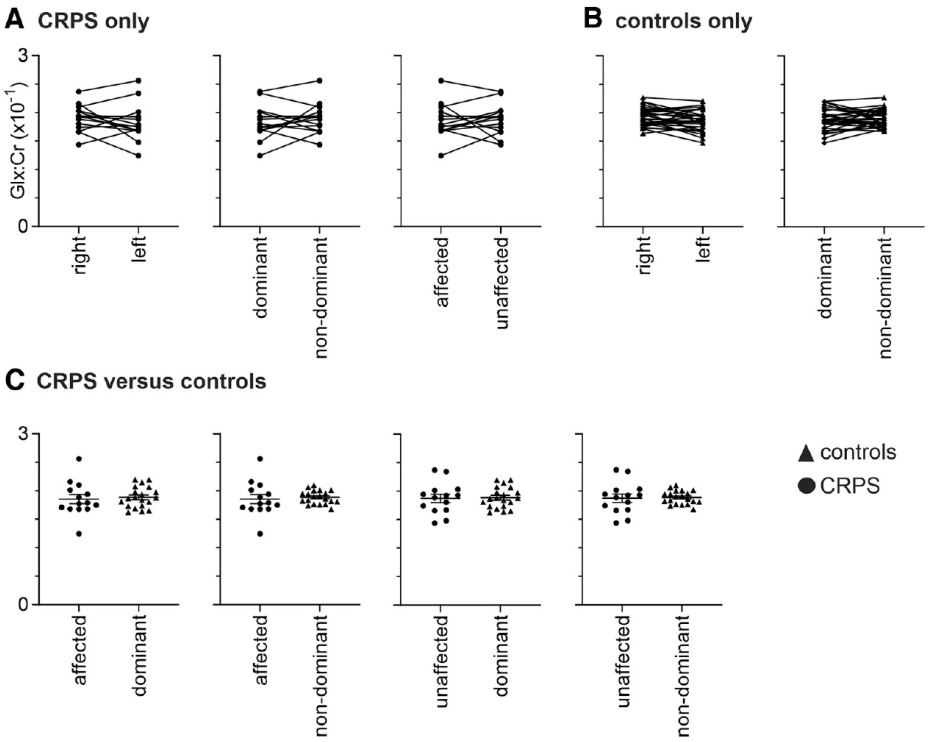

Figure 3. Plots of Glx/Cr ratios ($\times 10^{-1}$) of a 30 × 30 × 30mm voxel placed over the hand representation of the sensorimotor cortex. ***A***, Values for individual subjects with upper limb CRPS for the right and left hands, dominant and nondominant hemispheres, and also for the affected (hemisphere representing the side of ongoing pain) and unaffected hemispheres. Plots show pairwise connections for each individual subject. ***B***, Values for individual control subjects. ***C***, Values comparing individual CRPS subject and control subject groups. Horizontal lines indicate the mean ± SEM for each group. Note that there are no significant differences between any hemisphere in the CRPS or control subjects alone or between CRPS and control groups.

CRPS subjects compared with control subjects. Furthermore, although the affected hand displayed poor tactile acuity relative to the unaffected hand, there was no difference in GABA concentration in the S1/M1 region representing the affected compared with the unaffected hand in CRPS subjects. As hypothesized, we found no significant difference in sensorimotor cortex glutamate concentration in CRPS subjects compared with control subjects or in CRPS subjects between the affected and unaffected hemispheres. For the first time in the field, these findings suggest that ongoing pain and reduced tactile acuity in CRPS subjects may not result from altered GABA or glutamate concentrations in the primary sensorimotor cortex.

Transcranial magnetic stimulation (TMS) studies have identified reduced short intracortical inhibition in the sensorimotor cortex in individuals with CRPS, and it has been postulated that this sensorimotor disinhibition results from reduced sensorimotor cortex GABAergic inhibition (Schwenkreis et al., 2003; Eisenberg et al., 2005; Lotze and Moseley, 2007; Lenz et al., 2011). While Lenz et al. (2011) and Schwenkreis et al. (2003) reported bilateral sensorimotor cortex disinhibition, Eisenberg et al. (2005) reported more disinhibition in the sensorimotor cortex representing the affected limb than the unaffected one. In any case, we found no difference in GABA concentration in CRPS subjects relative to control subjects or between the hemispheres representing the affected limb compared with the unaffected limb. Furthermore, although we found that CRPS subjects displayed reduced tactile acuity in the affected hand, this altered acuity was not associated with altered contralateral sensorimotor GABA concentration. Consistent with this lack of difference, we also found no difference in sensorimotor glutamate concentrations and no relationship to tactile acuity in control subjects or CRPS subjects. While the lack of difference in GABA is surprising, the lack of change in glutamate is not. It has been reported that glutamate activity is positively correlated with intracortical facilitation (Liepert et al., 1997) and cortical silent period duration (Tremblay et al., 2013) in healthy control subjects. Given that in CRPS patients, intracortical facilitation and cortical silent period durations are not different from healthy control subjects (Schwenkreis et al., 2003; Krause et al., 2005), as we hypothesized, sensorimotor glutamate concentration was not different between CRPS subjects and healthy control subjects.

It is possible that a reduction in inhibition is not reflected in “ongoing” levels of GABA. A recent investigation found that TMS physiological disinhibition was not significantly

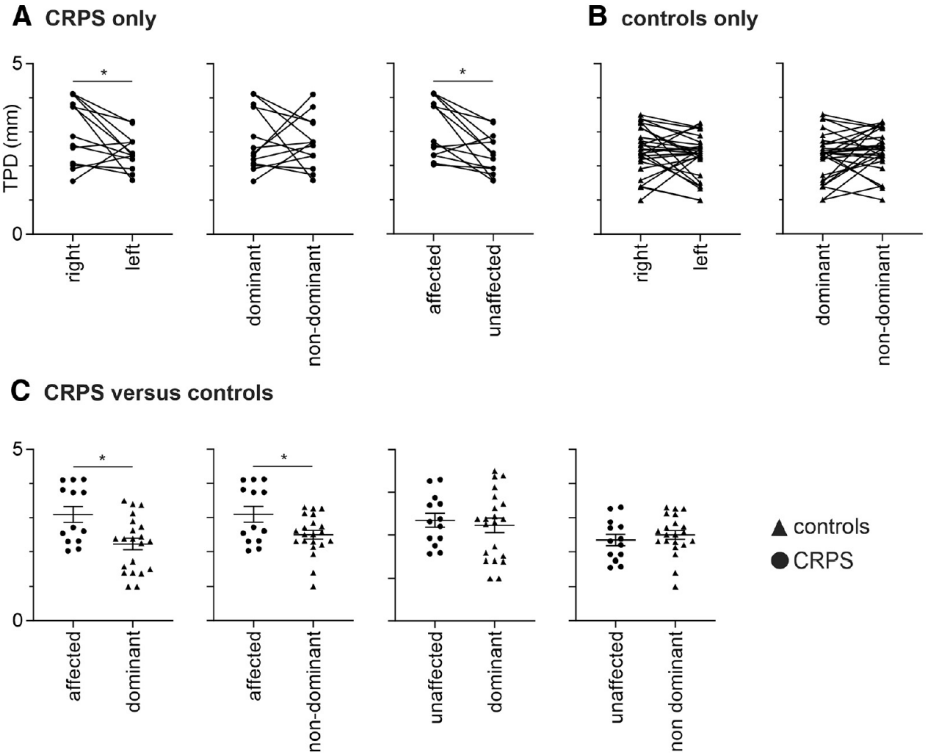

Figure 4. Plots of TPD in millimeters. ***A***, Values for individual subjects with upper limb CRPS for the right and left hands, dominant and nondominant hands, and also for the affected (hand with ongoing pain) and unaffected hands. Plots show pairwise connections for each individual subject. ***B***, Values for individual control subjects. ***C***, Values comparing individual CRPS subject and control subject groups. Horizontal lines indicate the mean ± SEM for each group. Note that CRPS subjects displayed reduced tactile acuity (increased TPD) in the affected hand compared with the unaffected hand and the dominant and nondominant hands in control subjects ($*p < 0.05$).

correlated to MRS measures of sensorimotor GABA concentration (Dyke et al., 2017), and it has been proposed that TMS-related inhibition may better reflect transient phasic GABAergic signaling dependent on $GABA_A$ receptor activity (Stagg et al., 2011a). Indeed, it has been previously proposed that MRS is a measure of tonic inhibition and that the GABA concentration measured by MRS is largely extrasynaptic GABA, which better reflects the amount of GABA that can be released for tonic inhibition (Nasrallah et al., 2011; Ziemann, 2013; Rae, 2014; Stagg, 2014). Hence, it is possible that the disinhibition in CRPS examined using TMS reflects phasic disinhibition and not tonic inhibition as measured by MRS in this study. Consistent with this idea, experimental animal investigations have shown that blocking $GABA_A$ receptor activity results in an expansion of the size of cortical representation within S1 and M1 (Tremere et al., 2001; Chowdhury and Rasmusson, 2002; Capaday and Rasmusson, 2003), and the short intracortical inhibition revealed using TMS in CRPS may reflect reduced activation of synaptic $GABA_A$ receptors on GABAergic interneurons (Eisenberg et al., 2005; Ziemann et al., 2015).

It is generally accepted that CRPS is associated with S1 reorganization, with the hemisphere receiving input from the painful limb displaying a smaller functional representation (Juottonen et al., 2002; Maihöfner et al., 2003, 2004; Pleger et al., 2004; Vartiainen et al., 2009; Di Pietro et al., 2013a). In addition, in a recent investigation of upper limb CRPS, it was reported that, as well as reduced S1 representation of the affected hand, there was a significant expansion of the unaffected hand representation compared with control subjects (Di Pietro et al., 2015). Interestingly, this expansion of the healthy hand representation did not appear to relate to overall hand use or the severity of dysfunction of the painful hand (Di Pietro et al., 2016). Given these findings, it is difficult to reconcile how bilateral short intracortical inhibitory changes in CRPS and the proposed role for $GABA_A$ in cortical organization relate to such opposing changes in S1 cortical organization in the affected compared with the unaffected hemispheres. It is possible that disinhibition in CRPS may be associated with differences in synaptic $GABA_A$ receptor activity rather than total GABA concentration, but how this is related to S1 organization remains unknown.

Although it is possible that differences in synaptic $GABA_A$ receptor activity may account for TMS-associated phasic disinhibition in CRPS, it is noted that extrasynaptic GABA, when released by neural activation (Nasrallah et

al., 2011) can act on extrasynaptic $GABA_A$ receptors, which are thought to mediate more tonic changes in activity (Semyanov et al., 2003; Yeung et al., 2003; Farrant and Nusser, 2005). MRS measures total GABA concentration and does not separate GABA into functional pools (e.g., extracellular, vesicular, cytoplasmic; Stagg et al., 2011a). Thus, while total GABA concentration of CRPS patients may not differ from healthy control subjects, the ratio of GABA in each pool may differ. Increased extracellular GABA can increase the tonic inhibition of neurons, making neurons less likely to activate and potentially resulting in disinhibition, and thus it is possible that both synaptic $GABA_A$ receptor activity and extracellular GABA concentration contribute to disinhibition in CRPS (Farrant and Nusser, 2005).

As hypothesized and consistent with previous literature, the CRPS-affected hand displayed poor tactile acuity relative to the CRPS-unaffected hand and pain-free control subjects (Pleger et al., 2006; Maihöfner and DeCol, 2007; Peltz et al., 2011; Lewis and Schweinhardt, 2012; Reiswich et al., 2012; Catley et al., 2014; David et al., 2015). While in healthy control subjects, one might hypothesize that increased use of the dominant hand would result in better tactile acuity than the nondominant hand, we found no difference in dominant and nondominant tactile acuity in control subjects, a result consistent with findings of previous investigations (Gelber et al., 1995; Ozcan et al., 2004; Pleger et al., 2006; Tustumi et al., 2015). Despite some past studies showing an increased sensorimotor GABA concentration correlating with better tactile acuity performance (Puts et al., 2011; Kolasinski et al., 2017), there is also evidence that lower M1 GABA concentration correlates with better motor learning capabilities in a healthy sample (Stagg et al., 2011b). Our results did not show any correlation between sensorimotor GABA concentration and tactile acuity in either healthy control subjects or CRPS patients. This difference with previous studies may result from the fact that we measured tactile acuity using a two-point discrimination test, whereas in previous studies subjects were asked to either discriminate whether two different vibration frequencies were the same or to identify which finger was stimulated first. We found a ceiling effect in the performance of the tactile acuity task in our healthy control subjects however we justify our choice of test; two-point discrimination has been more widely used to measure tactile acuity in CRPS patients than other techniques, and the method of two-point discrimination testing has largely been standardized in pain studies (save for the type of tool; e.g., callipers or wheels; Cashin and McAuley, 2017). Nevertheless, future studies should evaluate the difference in tactile acuity assessment via discrimination and temporal order judgment tasks to understand whether different tactile assessments can lead to different tactile acuity outcomes and relationships with GABA. This is important in a clinical setting, given that tactile acuity training reportedly helps to improve tactile acuity as well as reduce pain intensity in CRPS (David et al., 2015; Schmid et al., 2017).

There are several limitations needing discussion. First, we investigated a limited number of CRPS subjects, although our sample size is considerable given the relatively rare nature of this disorder, and this sample is larger than most previous neuroimaging studies. Given the negative results presented in this study, it is possible that a type 2 error has occurred (i.e., that our sample size is underpowered and that differences may exist with larger numbers). However, there were no signs of trends in the main analyses, suggesting that if there are such differences in total neurotransmitter content they are likely to be small and therefore perhaps unlikely to explain CRPS pathophysiology. Second, as with other neuroimaging studies in CRPS, our investigation had a cross-sectional design. We did not assess individuals before they developed CRPS and so cannot determine whether S1/M1 biochemical changes occur throughout the course of CRPS, for example during the establishment of CRPS, and whether they may then return to control levels in the long term. A larger sample of patients, recruited early in the course of the disorder and followed longitudinally, would ideally shed light on this. Of course it would be ideal to recruit only unilateral or bilateral CRPS patients, rather than both. However, not only would this be challenging to recruit, it would not reflect the reality of the clinical setting. Furthermore, in this study we were still able to demonstrate the difference in TPD between hands across the CRPS group despite mixed presentations. Finally, it is crucial to take into account methodological considerations that may impact on our findings. For instance, measurements of sensorimotor biochemical concentrations are affected by the size of the area investigated, which, because of the low signal-to-noise ratio of GABA, was large ($30 \times 30 \times 30$ mm) in this investigation (Mullins et al., 2014). While this relatively large voxel size provides better signal quality, it may encompass brain areas outside S1/M1, and, furthermore, our results may have been different if we were able to restrict our investigation to the area of specifically S1 or M1. In addition, the large voxel size covers areas of different tissues, that is, the voxel includes gray matter, white matter, and CSF, tissues that have different biochemical concentrations. While it is likely that there were differences between subjects with respect to the proportions of different tissue types with the region sampled, we limited such effects on GABA and glutamate concentrations by performing voxel parcellations to confirm that fractions of brain tissue were not different between control subjects and CRPS subjects. Movement is an important methodological consideration with MRS. Ideally, we would have acquired a second anatomical scan after the MRS sequence to determine whether participants had moved in the scan; however, we could not practically achieve this. Finally, it is important to note that the experimenter testing tactile acuity was not blinded to participant grouping, and this inherently poses a risk of inflation of effect size. What we can say is that the administration of this test was delivered consistently across all participants.

As with any clinical disorder, we recruited a group taking a variety of medications. The research suggests that the effect of medications on GABA levels in the brain is specific to brain region and to pathology. There is not an

extensive amount of literature on medication effects on GABA in the sensorimotor cortex in chronic pain, as there is for areas such as the occipital cortex in disorders such as epilepsy (Puts and Edden, 2012). Of the drugs most commonly used over the past 24 h in our sample, the two most worthy of comment are gabapentin and pregabalin, both GABA-derivative drugs. These two are known to exert an effect on the GABAergic system and have been found to decrease pain levels. Their mechanism of action is not fully understood, and they are not universally effective (Enna and McCarson, 2006). These drugs were used by less than half of CRPS participants in the last 24 h, and thus we think it unlikely that our negative findings are due to any masking of results by medications.

In conclusion, our findings show that individuals with upper limb CRPS display reduced tactile acuity in the painful hand compared with the nonpainful hand and pain-free control subjects. Despite this acuity difference, CRPS was not associated with altered GABA or glutamate concentrations within the sensorimotor cortex on either the side that represents the affected hand or the side that represents the unaffected hand. While these results appear to be at odds with the sensorimotor cortex disinhibition reported in investigations using TMS, it is possible that GABAergic mechanisms other than total GABA concentration, as measured here with MRS, may contribute to such disinhibition. Clearly, further work with larger samples would confirm these findings. More importantly, future studies should investigate sensorimotor GABA in relation to other GABAergic mechanisms, such as $GABA_A$ receptor activity, to determine whether other GABAergic mechanisms can explain disinhibition of CRPS and in turn provide direction for targeted restoration of inhibition.

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Chapter 3

Altered basal ganglia infraslow oscillation and resting functional connectivity in complex regional pain syndrome

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RESEARCH ARTICLE

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Altered basal ganglia infraslow oscillation and resting functional connectivity in complex regional pain syndrome

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Abstract

Complex regional pain syndrome (CRPS) is a painful condition commonly accompanied by movement disturbances and often affects the upper limbs. The basal ganglia motor loop is central to movement, however, non-motor basal ganglia loops are involved in pain, sensory integration, visual processing, cognition, and emotion. Systematic evaluation of each basal ganglia functional loop and its relation to motor and non-motor disturbances in CRPS has not been investigated. We recruited 15 upper limb CRPS and 45 matched healthy control subjects. Using functional magnetic resonance imaging, infraslow oscillations (ISO) and resting-state functional connectivity in motor and non-motor basal ganglia loops were investigated using putamen and caudate seeds. Compared to controls, CRPS subjects displayed increased ISO power in the putamen contralateral to the CRPS affected limb, specifically, in contralateral putamen areas representing the supplementary motor area hand, motor hand, and motor tongue. Furthermore, compared to controls, CRPS subjects displayed increased resting connectivity between these putaminal areas as well as from the caudate body to cortical areas such as the primary motor cortex, supplementary and cingulate motor areas, parietal association areas, and the orbitofrontal cortex. These findings demonstrate changes in basal ganglia loop function in CRPS subjects and may underpin motor disturbances of CRPS.

KEYWORDS
basal ganglia, chronic pain, complex regional pain syndrome, infraslow oscillations, motor dysfunction, putamen, resting-state fMRI

1 | INTRODUCTION

Complex regional pain syndrome (CRPS) is a chronic pain disorder of the limbs, most often affecting the upper limbs (Marinus et al., 2011). The development of CRPS is usually precipitated by an injury such as a fracture but can also occur spontaneously and is characterized by pain, sensory disturbances, motor dysfunction, and autonomic dysregulation (Harden et al., 2007; Marinus et al., 2011). Motor dysfunction in CRPS can involve a decreased range of motion, muscle weakness, tremor, and dystonia (Harden et al., 2007). Symptoms of CRPS can persist for many years, and the most persistent sign is motor dysfunction (Bean et al., 2014).

To date, cortical circuits have been the primary focus of studies examining motor dysfunction in CRPS, with functional magnetic resonance imaging (fMRI) studies showing altered activation

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patterns in the primary motor cortex (M1) and supplementary motor area (SMA) during finger tapping or action observation (Hotta et al., 2017; Maihöfner et al., 2007). Subcortical structures, such as the basal ganglia, are also crucial in motor function, including action selection and voluntary movement (Alexander et al., 1986; Redgrave et al., 2011). Basal ganglia motor loops, via the putamen, are somatotopically organized (Nambu et al., 2002), with thalamocortical projections determining whether a movement in a particular body region is facilitated or suppressed (Albin et al., 1989). It is well established that basal ganglia pathology results in movement disorders (e.g., Parkinson's disease), and specific putamen lesions can lead to dystonia (Neychev et al., 2011), as well as changes in pain perception (Borsook et al., 2010; Starr et al., 2011).

Altered thalamocortical rhythm is associated with neuropathic pain and burst firing of infraslow oscillations (ISOs) (Gerke et al., 2003; Iwata et al., 2011; Sarnthein et al., 2006). In a recent series of studies, we reported differences in resting infraslow oscillations (ISOs) in pain processing regions in individuals with CRPS and other chronic neuropathic pain conditions (Alshelh et al., 2016; Di Pietro et al., 2020). We suggested that these differences may underpin altered thalamocortical rhythm and ultimately in the persistence of pain. Altered thalamocortical rhythm has not only been reported in neuropathic pain. Putamen projections mediate thalamocortical rhythm regulating movement (Opri et al., 2019) and altered thalamocortical rhythm is also found in Parkinson's disease (Vanneste et al., 2018). Furthermore, in Parkinson's disease, increased ISO power is associated with increased motor dysfunction (Hou et al., 2014; Wang et al., 2020). Interestingly, in CRPS subjects, we also found increased ISOs in the region of the putamen with extension into the insula cortex contralateral to the affected limb (Di Pietro et al., 2020). The frequency range of ISO increase in CRPS as well as Parkinson's disease is indicative of astrogliosis as it coincides with the range of astrocytic calcium waves (0.03–0.06 Hz) and release of gliotransmitters (Crunelli et al., 2002; Henderson & Di Pietro, 2016). Indeed, increased ISO and increased astrocyte activation have been found in the same brain regions in neuropathic pain (Alshelh et al., 2016; Okada-Ogawa et al., 2009). Furthermore, optimal astrocytic calcium levels are needed as either attenuating or elevating astrocytic calcium levels lead to motor impairments (Agulhon et al., 2013; Padmashri et al., 2015). Thus, a detailed investigation of ISOs in multiple putamen and caudate seeds in CRPS may reveal underlying astrocytic calcium differences compared to controls that may be related to altered motor function.

Several studies have reported structural and functional connectivity changes in the putamen in adult and pediatric cases of CRPS (Azqueta-Gavaldon et al., 2020; Becerra et al., 2014; Linnman et al., 2013), however, these studies did not evaluate the putamen's somatotopic organization. In addition, functional connectivity changes in non-motor cortico-basal ganglia re-entrant loops that regulate cognition, reward/motivation, visual processing, and sensory integration (Alexander et al., 1986; Choi et al., 2012; Da Cunha et al., 2012; Middleton & Strick, 1996; Redgrave et al., 2010) have been reported in CRPS (Becerra et al., 2014; Geha et al., 2008; Lebel et al., 2008). However, again, these studies did not systematically evaluate each basal ganglia functional loop, nor did they determine if changes were related to sensory, motor, or non-motor disturbances.

Significance

Complex regional pain syndrome is a chronic pain disorder affecting the limbs and is associated with motor dysfunction. The basal ganglia are critical in regulating movement but also non-motor functions such as sensory integration, visual processing, and cognition. No previous study has systematically evaluated the functional connectivity of basal ganglia motor and non-motor territories in CRPS. We found that CRPS participants have greater connectivity in cortico-basal ganglia loops specific to motor function and visuospatial integration. Changes in basal ganglia connectivity in CRPS likely underlie motor disturbances like dystonia and tremor and altered visuospatial perception of the CRPS affected limb.

The aim of this resting-state fMRI study was to systematically investigate if adult CRPS subjects have altered ISOs and functional connectivity in motor (putamen) and non-motor basal ganglia loops, compared to pain-free control subjects. We hypothesized that CRPS subjects would have altered basal ganglia ISOs and connectivity, and that changes in motor loop activity and connectivity would be associated with motor dysfunction of CRPS.

2 | MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee of the University of Sydney (HREC reference number 2018/073) and was conducted in accordance with the Declaration of Helsinki. Due to the uncommon nature of CRPS and difficulty in recruiting eligible and willing upper limb CRPS participants, no sample size calculation was performed, hence the study recruited a convenience sample. Prior to study participation, informed written consent was obtained from all participants. Data from the CRPS participants and a subset of the pain-free healthy control participants have been published in prior studies (Di Pietro et al., 2020; Lee et al., 2020).

2.1 | Study participants

Sixteen eligible individuals with CRPS gave consent to participate in the study. Imaging data were not obtained from one CRPS subject due to claustrophobia in the MRI scanner. Thus, imaging data from 15 upper limb CRPS subjects (11 females; mean ± SEM age: 47.5 ± 3.2 years) and 45 age- and sex-matched pain-free healthy controls (33 females; 47.3 ± 1.9 years) were collected. CRPS subjects were diagnosed following the International Association for the Study

of Pain "Budapest" diagnostic criteria (Harden et al., 2007) and had ongoing pain for at least 3 months. CRPS subjects were eligible for the study if they reported other regions of pain or CRPS, but upper limb CRPS was required to be their primary complaint. Exclusion criteria included any MRI contraindications such as cardiac pacemakers and metal implants, or any significant mental health disorders, developmental delays, or neurological disorders that would prevent safe participation.

2.2 | CRPS assessment

The researcher assessed CRPS signs in both upper limbs of each CRPS subject. (i) *Sensory*: Hyperalgesia and allodynia were assessed by pinprick on the dorsal webspace of the hand and light brush strokes on the dorsum of the hands, respectively. (ii) *Vasomotor*: Skin temperature asymmetry was assessed through touch, and skin color changes/asymmetry was assessed visually. (iii) *Sudomotor/edema*: Sweating (sudomotor) was assessed by touching the subject's palms of both hands. Edema was assessed through measurement of the circumference of the wrist and proximal phalanx of the middle finger with a tape measure. Signs of sudomotor function and edema were recorded as present if there was asymmetry between the upper limbs. (iv) *Motor/Trophic*: We observed for motor signs such as tremor and dystonia. Motor weakness was assessed through a power grip test on the researcher's index and middle fingers. Hair, nail, and skin changes/asymmetry between the upper limbs (trophic changes) were assessed visually.

2.3 | Questionnaires

2.3.1 | Each CRPS subject completed several questionnaires

Pain

CRPS participants rated their pain intensity on the day of the study ("day pain") on a 10 cm visual analogue scale (VAS) (0 = no pain to 10 = worst imaginable pain). Using the 10 cm VAS, CRPS subjects were also asked to record their ongoing pain intensity three times a day for 7 days before or following the scanning session. The mean "diary pain" score was obtained by averaging the 21 pain intensity scores.

Functional assessment

The patient-rated Wrist and Hand Evaluation (PRWHE) assessed task-associated pain intensity and functional difficulty of the CRPS affected limb (MacDermid, 1996). The PRWHE is divided into pain and function subscores, as well as total score. The PRWHE pain score ranges from 0 to 50, the function score 0 to 50, and the total score 0 to 100. Higher scores indicate more pain and functional disability of the CRPS affected limb. The shortened 11-item Disabilities of the Arm, Shoulder and Hand (QuickDASH) Outcome Measure (Beaton et al., 2005) assessed the overall function of the upper limbs irrespective of CRPS affected side, and the score ranges from 0 to 100. A higher QuickDASH score indicates greater disability of the upper limbs.

Body perception and motor dysfunction

The Bath CRPS Body Perception Disturbance Scale (Lewis & McCabe, 2010) and Foreign limb Feelings (FLF) questionnaire (Galer & Jensen, 1999) assessed self-perception of the CRPS affected limb. The Bath and FLF questionnaires scores range from 0 to 57 and 0 to 20, respectively. In addition, the FLF questionnaire assesses aspects of motor dysfunction such as involuntary movement. For both scales, a higher score indicates greater disturbance to self-perception of the CRPS affected limb.

2.4 | Tactile acuity (two-point discrimination)

Tactile acuity data of CRPS participants in this study have previously been published elsewhere (Di Pietro et al., 2020; Lee et al., 2020). Tactile acuity measures were not obtained from 3 of the 15 CRPS subjects due to extreme hand pain. The 2-point discrimination (TPD) test assessed tactile acuity and was performed immediately following the MRI. TPD is the ability to discriminate two distinct points touching the skin as two points and not one. The researcher applied a TPD wheel (Exacta™, CA) longitudinally to the distal pulp of the subject's index finger and asked the subject to report if they felt 1 point or 2 points touching their skin with each stimulation. The distances of 0 (i.e., 1 point), 2, 3, 4, and 5 mm between two points were applied seven times in a pseudo-randomized order, resulting in 35 trials per hand. The percentage of correct two-point perception versus distance between the points was fitted by a binary logistic regression (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) to obtain individual psychometric functions of TPD for each subject's hand. The discrimination distance was determined at a 50% (chance) threshold of correct two-point perception. A higher discrimination distance indicates poorer tactile acuity.

2.5 | MRI data collection

All MRI data were collected in a 3 Tesla MRI scanner (Achieva TX, Philips Medical Systems) at Neuroscience Research Australia in Sydney, Australia. Each subject lay supine on the MRI scanner bed with their head immobilized in a padded 32-channel head coil. A high-resolution T1-weighted anatomical image of the whole brain in the sagittal plane was obtained (repetition time = 5600 ms; echo time = 2.5 ms, flip angle = 8°, raw voxel size 0.87 mm^3). Subjects were then asked to relax with their eyes closed as resting-state fMRI (rsfMRI) images were collected (series of 180 fMRI image volumes, gradient-echo echo-planar sequence with blood oxygen level-dependent contrast; repetition time = 2000 ms; echo time = 30 ms, flip angle = 90°, 37 axial slices, raw voxel size 3 × 3 × 4 mm).

2.6 | MRI data analysis

Image analysis was performed using SPM12 (Friston et al., 1995) and custom software. The T1 and rsfMRI images of the CRPS subjects with pain restricted to the left upper limb (or the more intense pain in the left upper limb) were left–right reflected across the midline on the *y*-axis before data processing. Hence, the brain's left hemisphere was contralateral to the CRPS affected limb in all 15 subjects. All fMRI images were then slice-time corrected, realigned and the Dynamic Retrospective Filtering (DRIFTER) toolbox (Särkkä et al., 2012) used to model and remove cardiac (frequency band of 60–120 beats per minute +1 harmonic) and respiratory (frequency band of 8–25 breaths per minute +1 harmonic) noise. LMRP detrending was used to remove movement-related signal changes. The linear model of the global signal (LMGS) method (Macey et al., 2004) was then used to remove global drifts in fMRI signal intensity. Each subject's fMRI images were then co-registered to the subject's own T1-weighted anatomical image. The T1 images were spatially normalized to Montreal Neurological Institute (MNI) space, and the normalization parameters were applied to the fMRI images sets to place them into MNI space. The resulting fMRI images were then smoothed using a 6 mm full-width at half-maximum (FWHM) Gaussian filter.

2.7 | Seeds

To explore regional specificity, we created multiple basal ganglia seeds (Figure 1a). First, we refined a left putamen seed (putamen ISO seed) from a previous study where we showed that ISO power was significantly greater in CRPS compared with control participants (Di Pietro et al., 2020). The putamen ISO seed for this current study was refined to only include the putamen portion of a larger seed that originally encompassed the putamen and extended into the insular cortex. Second, we created four putamen seeds, each a 3 mm radius sphere and representative of: the motor hand (center *X*, *Y*, *Z* MNI coordinates: −28, −7, 2), motor foot (−28, −8, 6), motor tongue (−32, −8, −5), and SMA hand (−25, −7, 5) (Choi et al., 2012). Third, to elucidate the involvement of non-motor basal ganglia circuitry, we created six more seeds (3 mm radius spheres) representing the left caudate tail (visual processing loop; −29, −8, −17), caudate body (oculomotor loop; −15, −1, 18), ventrolateral (vl) caudate head (lateral orbitofrontal loop; −9, 10, 1), dorsolateral (dl) caudate head (dorsolateral prefrontal loop; −12, 10, 8), ventral putamen (default network loop; −29, −11, −10), and ventral striatum (limbic loop; −12, 11, −8) (Alexander et al., 1986; Choi et al., 2012; Middleton & Strick, 1996). In total, we created and assessed 11 basal ganglia seeds. In addition, to investigate basal ganglia functional connectivity, the fMRI analysis was restricted to cortical areas that receive input from the basal ganglia seeds (Alexander et al., 1986; Middleton & Strick, 1996) by applying a mask of the frontal lobe, anterior cingulate cortex (ACC), middle temporal gyrus, and parietal lobe (Figure 1b). All seeds were created in the left hemisphere; hence all seeds are contralateral to the CRPS affected limb.

2.8 | ISO analysis

We used the SPM Data Processing Assistant for Resting-State fMRI (DPARSF) toolbox (Chao-Gan & Yu-Feng, 2010) to calculate the amplitudes of low-frequency fluctuations (ALFFs), that is, power, of three standard ISO frequency ranges (slow-5: 0.01–0.027 Hz, slow-4: 0.027–0.073 Hz and slow-3: 0.073–0.198 Hz) defined by Buzsaki and Draguhn (2004) on the preprocessed fMRI image sets of control

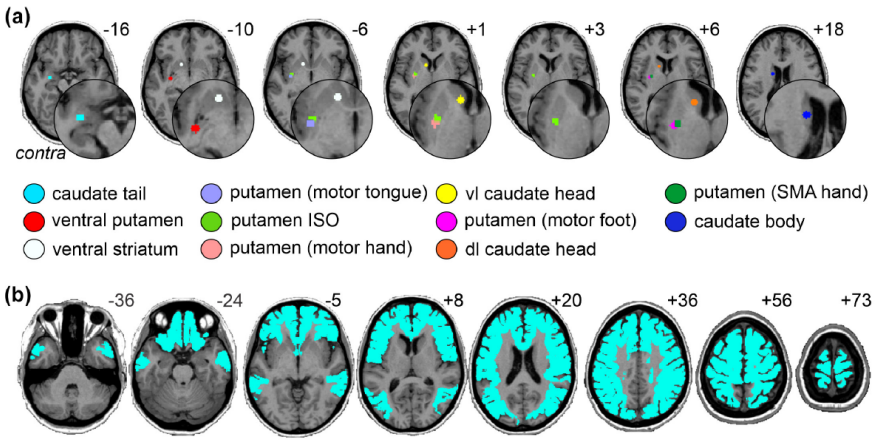


FIGURE 1 (a) Locations of the basal ganglia seeds used in the infraslow oscillation (ISO) and resting functional connectivity analysis. The seed regions are located contralateral to the CRPS affected limb. (b) The mask of the frontal lobe, anterior cingulate cortex, middle temporal gyrus, and parietal lobe used to restrict the functional connectivity analysis. Slice locations in Montreal neurological institute space are indicated on the top right of each axial slice. Contra, contralateral to affected limb; dl, dorsolateral; SMA, supplementary motor area; vl, ventrolateral

and CRPS subjects. The analysis was restricted to the 11 basal ganglia seeds to investigate ISO differences within the basal ganglia. Two-sample *t* tests were used to determine any differences between controls and CRPS subjects' slow-5, slow-4, and slow-3 ALFF power ($p < 0.05$, two-tailed). The slow-5, slow-4, and slow-3 mean ± SEM ALFF power for controls and CRPS subjects were plotted for each seed. For seeds that were significantly different between controls and CRPS subjects, Pearson's correlations ($p < 0.05$, two-tailed) were performed between CRPS subjects' ALFF power and pain duration, pain intensity, questionnaire scores, and tactile acuity, using GraphPad Prism 9.1 (GraphPad Software, Inc., San Diego, CA). The D'Agostino-Pearson normality test confirmed the normal distribution of pain, questionnaire, and tactile acuity data before correlation analysis. To account for multiple comparisons for each seed and each ISO frequency range, the Benjamini and Hochberg method (Benjamini & Hochberg, 1995) was used to calculate false discovery rate (FDR)-adjusted *p* values at 5% FDR.

2.9 | Seed-based connectivity analysis

For each of the 11 basal ganglia seeds, the mean signal intensity at each time point was calculated and averaged over the entire seed. Relationships between signal intensity changes and the signal intensity changes in each voxel of the brain were then determined for each seed in each subject. The resultant resting-state connectivity strength brain maps were then smoothed using a 6 mm FWHM Gaussian filter and second-level random-effects analysis was performed to determine significant differences between CRPS and control groups ($p < 0.05$, FDR corrected for multiple comparisons, minimum contiguous cluster size of 20 voxels). For the resting-state connectivity analysis of the putamen ISO seed, the beta value (connectivity strength effect size) of each significant cluster was extracted, and the mean ± SEM plotted. In addition, for the CRPS subjects, linear relationships between these beta values and pain duration, pain intensity, questionnaire scores, and tactile acuity data were determined using Pearson's correlations ($p < 0.05$, two-tailed) and the Benjamini and Hochberg method (Benjamini & Hochberg, 1995) was used to calculate FDR-adjusted *p* values at 5% FDR. One-sample one-sided *t* tests were performed within groups for each basal ganglia seed (see Supporting Information).

3 | RESULTS

3.1 | CRPS subject characteristics

Individual CRPS subject characteristics are reported in Table 1. The CRPS subjects' average pain duration was 4.6 ± 0.9 years (mean ± SEM). All 15 CRPS subjects reported ongoing pain in the upper limb, with 12 of the 15 subjects reporting upper limb pain restricted to one side (10 right, 2 left). Three subjects reported bilateral upper limb pain (2 reporting greater pain in the right upper limb and 1 on the left). Seven CRPS subjects also reported pain in the lower limb. CRPS subjects' motor signs, questionnaire scores, and tactile acuity data are reported in Table 2. Thirteen CRPS subjects displayed motor signs on the day of the study;10 CRPS subjects presented with weakness, 2 with dystonia, 1 with tremor, and 1 with rigidity. CRPS subjects reported an average pain intensity of 5.3 ± 0.5 for day pain and 4.6 ± 0.6 for diary pain.

3.2 | ISOs

As expected, for the putamen ISO seed, the CRPS group displayed significantly greater slow-4 ALFF (0.027–0.073 Hz) power than controls (mean ± SEM, CRPS: 1.06 ± 0.06, controls: 0.85 ± 0.03, $p < 0.001$) (Figure 2a). The putamen ISO seed also displayed significantly greater slow-3 ALFF (0.073–0.198 Hz) power in the CRPS group (CRPS: 1.11 ± 0.09, controls: 0.88 ± 0.03, $p = 0.003$). This result was regionally specific; only 3 of the remaining 10 basal ganglia seeds displayed significant differences in ALFF power between groups. Namely, the putamen SMA hand, motor hand, and motor tongue area seeds demonstrated greater CRPS slow-4 ALFF power in CRPS than controls (SMA hand: CRPS: 0.93 ± 0.06, controls: 0.81 ± 0.03, $p = 0.046$; motor hand: CRPS: 1.06 ± 0.08, controls: 0.86 ± 0.03, $p = 0.005$; motor tongue: CRPS: 1.03 ± 0.07, controls: 0.84 ± 0.03, $p = 0.004$) (Figure 2b). There were no significant differences in slow-4 ALFF power for the remaining seven seeds or for either slow-3 or slow-5 (0.01–0.027 Hz) frequency bands in any basal ganglia seed, apart from the putamen ISO seed.

In CRPS subjects, increased ISO power of the putamen was correlated with pain and functional disability (Table 3). A lower PRWHE function score, indicating better wrist function of the CRPS affected hand, correlated with increased slow-4 ALFF power of the putamen ISO seed (Figure 3a) and putamen motor hand (Figure 3b). A lower QuickDASH score, indicating less functional disability of the upper limbs, correlated with increased slow-4 putamen motor hand (Figure 3c), putamen motor tongue, and slow-3 putamen motor hand (Figure 3d) ALFF power.

3.3 | Functional connectivity

For each of the 11 basal ganglia seeds, significant differences in resting-state functional connectivity strengths between CRPS and control groups were determined. In no region was connectivity strength greater in controls than in CRPS subjects. For the putamen ISO seed, CRPS subjects displayed significantly greater connectivity strengths compared with controls in the ipsilateral and contralateral primary motor cortices (M1) in the region innervating the lower limbs (mean ± SEM beta values: ipsilateral M1: CRPS 0.13 ± 0.02, controls 0.02 ± 0.02; contralateral M1: CRPS 0.15 ± 0.02, controls 0.03 ± 0.01), contralateral M1 in the upper limb and hand region (CRPS: 0.13 ± 0.02, controls 0.02 ± 0.01), contralateral cingulate motor area (CMA; CRPS 0.16 ± 0.02, controls 0.05 ± 0.02),

TABLE 1 CRPS subject demographics, medical history, and medication

Subject	Age	Sex	Pain duration (years)	CRPS affected region(s)	Inciting event	Medications	Comorbidities	
							Pain-related	Non-pain-related
1	49.2	M	7.0	**R UL**, R LL, L LL, face, abdomen	Pain in R hand	Turmeric tablets	None	None
2	56.4	F	4.2	**L UL**, R UL, R LL, R and L chest	L humerus fracture	Duloxetine, gabapentin, oxycodone, quetiapine, tapentadol	L radial nerve palsy, triangular fibrocartilage complex degeneration of R hand	None
3	55.8	F	0.9	**R UL**, R neck, R chest	Spontaneous onset	Ashwagandha, budesonide, cannabis, codeine, formoterol, oxycodone, paracetamol, salbutamol	Back pain, fibromyalgia, osteoarthritis, radiculopathy, spinal disc herniation	COPD, peptic ulcer, Raynaud's disease
4	61.7	F	6.2	**L UL**, *R UL*	R hand tendon release surgery	Amitriptyline, cannabidiol drops, codeine, levothyroxine, magnesium, paracetamol, topiramate, tramadol, valerian	None	Diverticulitis, gastro-esophageal reflux disease, Graves' disease (thyroidectomized)
5	58.1	F	8.7	**R UL**, R LL, R face	R arm surgery	Codeine, duloxetine, linagliptin, meloxicam, metformin, paracetamol	None	Diabetes
6	66.6	F	9.5	**R UL**, L UL, R LL, L LL	R radius fracture	Amlodipine, gabapentin, ketamine in lipoderm cream, metformin, metoprolol, pantoprazole, salbutamol	Osteoarthritis	Asthma, diabetes, gastric reflux, hypertension, pubic symphysitis, supraventricular tachycardia
7	46.8	M	1.5	**R UL**	Spontaneous onset	Amlodipine, atorvastatin, ibuprofen, paracetamol, perindopril, pregabalin	None	Hyperlipidemia, hypertension
8	34.2	F	5.3	**R UL**, R LL, R hip	R wrist fracture	Amitriptyline, buprenorphine patch	Migraine, R hip bursitis	None
9	25.9	F	1.3	**R UL**, L and R neck, spine, L LL	R hand nerve damage	None	Endometriosis	Polycystic ovarian syndrome
10	45.2	M	1.2	**R UL**	R wrist injury	Codeine, ibuprofen, magnesium, pregabalin, topical rub (containing copaiba, frankincense, peppermint, coconut oil), vitamin B, vitamin C	Bell's palsy, bulging discs	None
11	45.9	F	3.9	**L UL**	L hand carpal tunnel release surgery	Amitriptyline, betahistine, duloxetine, naproxen, pantoprazole, rizatriptan, tapentadol, valaciclovir	Carpal tunnel of R hand, fibromyalgia, migraine	Herpes, polycystic ovarian syndrome with insulin resistance, vertigo
12	23.8	F	2.6	**R UL**, L UL	Overload	Amitriptyline, gabapentin, levothyroxine	None	Hashimoto's disease

TABLE 1 (Continued)

Subject	Age	Sex	Pain duration (years)	CRPS affected region(s)	Inciting event	Medications	Comorbidities	
							Pain-related	Non-pain-related
13	52.3	F	2.9	**R UL**, R torso	Broke tailbone	Ashwagandha, fish oil, ibuprofen, magnesium, mega B, melatonin, paracetamol, tapentadol, vitamin C, vitamin D	Endometriosis	None
14	37.8	F	12.7	**R UL**, R neck, L LL	Spontaneous onset	Duloxetine, gabapentin, naloxone, oxycodone, palmitoylethanolamide (PEA)	Endometriosis, endosalpingiosis	Raynaud's disease
15	52.3	M	1.9	**R UL**, L and R neck, back	R scaphoid fusion surgery	Cholecalciferol, ibuprofen, magnesium, oxycodone, paracetamol, pregabalin, tramadol, venlafaxine, zopiclone	L shoulder bursitis	Sleep apnea
Mean (±SEM)	47.5 (±3.2)		4.6 (±0.9)					

Note: The CRPS region with the most severe pain is in bold. CRPS regions that are in remission are in *italics*. Medication taken in the last 24 h of the day of testing are underlined.

Abbreviations: L, left; LL, lower limb; R, right; SEM, standard error of mean; UL, upper limb.

ipsilateral primary somatosensory cortex (S1) in the hand region (CRPS 0.11 ± 0.02, controls 0.004 ± 0.01), ipsilateral supramarginal gyrus (CRPS 0.17 ± 0.02, controls 0.04 ± 0.01), ipsilateral pars opercularis (CRPS 0.19 ± 0.02, controls 0.04 ± 0.02), ipsilateral orbitofrontal cortex (CRPS 0.14 ± 0.02, controls 0.02 ± 0.01), and in the contralateral middle temporal gyrus (CRPS 0.13 ± 0.03, controls 0.01 ± 0.01) (Figure 4 and Table 4). In CRPS subjects, the connectivity between the putamen ISO seed and the ipsilateral OFC was positively correlated with day pain ($r = 0.60$) and putamen ISO and contralateral M1 hand area was positively correlated with a higher foreign limb feelings (FLF) questionnaire score ($r = 0.62$), however, neither correlations were significant after multiple comparisons adjustment.

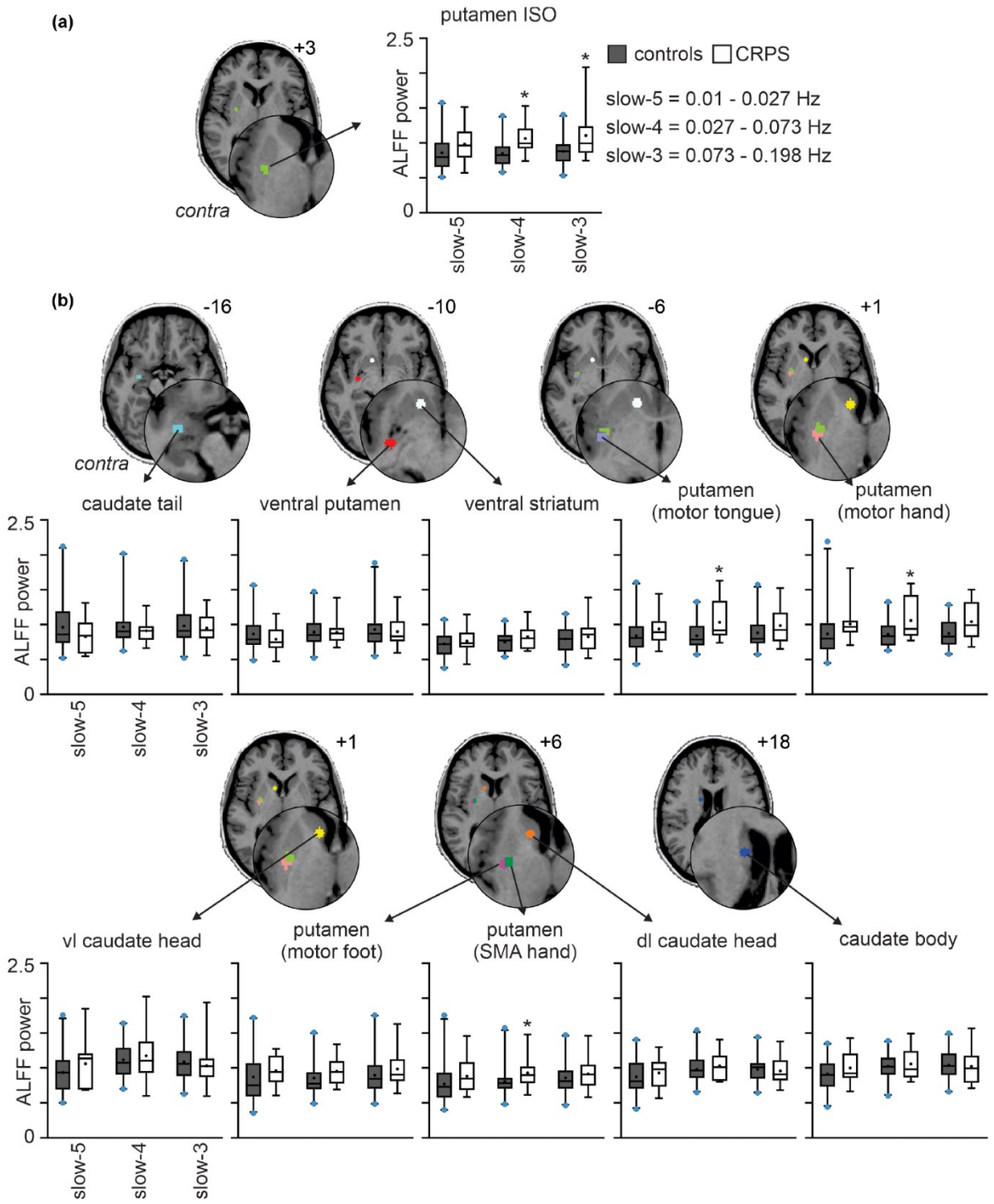
Analysis of the remaining 10 basal ganglia seeds revealed that 5 seeds displayed significant connectivity increases in the CRPS group, 4 of which were putamen motor loop seeds. Similar to the putamen ISO seed, the putamen motor hand seed in CRPS subjects displayed greater resting connectivity strengths than controls with: the ipsilateral M1 lower limb region (mean ± SEM beta values: CRPS 0.14 ± 0.02, controls 0.04 ± 0.01), ipsilateral and contralateral M1 hand region (ipsilateral: CRPS 0.16 ± 0.02, controls 0.05 ± 0.01; contralateral: CRPS 0.16 ± 0.03, controls 0.04 ± 0.01), contralateral CMA (CRPS 0.17 ± 0.03, controls 0.05 ± 0.01), ipsilateral OFC (CRPS 0.13 ± 0.03, controls 0.02 ± 0.01), and the ipsilateral angular gyrus (CRPS 0.16 ± 0.02, controls 0.04 ± 0.01) (Figure 5a, Table 4). Similarly, for the putamen motor foot seed analysis, CRPS subjects also displayed significantly greater connectivity strengths in the ipsilateral M1 hand region (CRPS 0.17 ± 0.03, controls 0.04 ± 0.01), contralateral M1 hand region (CRPS 0.17 ± 0.04, controls 0.04 ± 0.02), ipsilateral M1 face region (CRPS 0.08 ± 0.01, controls 0.01 ± 0.01), ipsilateral and contralateral CMA (ipsilateral: CRPS 0.13 ± 0.03, controls 0.01 ± 0.01; contralateral: CRPS 0.20 ± 0.03, controls 0.06 ± 0.01), ipsilateral angular gyrus (CRPS 0.18 ± 0.04, controls 0.01 ± 0.02), and in the ipsilateral supramarginal gyrus (CRPS 0.18 ± 0.03, controls 0.04 ± 0.02) (Figure 5b, Table 4). In contrast, connectivity strength for the putamen motor tongue seed was only greater in CRPS subjects compared with controls in a discrete region of the ipsilateral frontal cortex (CRPS 0.18 ± 0.02, controls 0.01 ± 0.02) (Figure 5c, Table 4). For the putamen SMA hand seed, CRPS subjects displayed greater connectivity than controls in the contralateral CMA (CRPS 0.14 ± 0.02, controls 0.03 ± 0.01), ipsilateral OFC (CRPS 0.11 ± 0.03, controls 0.004 ± 0.01), contralateral M1 hand area (CRPS 0.15 ± 0.03, controls 0.05 ± 0.01), ipsilateral M1 tongue area (CRPS 0.13 ± 0.03, controls 0.01 ± 0.01), ipsilateral angular gyrus (CRPS 0.16 ± 0.03, controls 0.05 ± 0.01), ipsilateral and contralateral supramarginal gyrus (ipsilateral: CRPS 0.16 ± 0.02, controls 0.03 ± 0.01; contralateral: CRPS 0.13 ± 0.03, controls 0.01 ± 0.02), contralateral frontal cortex (CRPS 0.11 ± 0.03, controls 0.01 ± 0.02), and the contralateral middle temporal gyrus (CRPS 0.11 ± 0.02, controls 0.03 ± 0.01) (Figure 5d, Table 4).

The caudate body seed was the only non-motor region in which resting functional connectivity was significantly different between

TABLE 2 CRPS subjects' motor signs, questionnaire scores, and tactile acuity

Subject	Motor signs	Pain intensity		Questionnaire scores						TPD (mm)	
				PRWHE							
		Day pain	Diary pain	Pain score	Function score	Total score	QuickDASH	Bath	FLF	Affected hand	Unaffected hand
1	Tremor, dystonia	4.0	4.5	32	30.5	62.5	81.8	34	18	3.8	1.6
2	Rigidity	7.8	8.1	45	30.5	75.5	75.0	29	16	–	–
3	Weakness	7.9	8.3	46	38	84	75.0	39	12	3.7	3.3
4	Weakness	4.3	5.8	42	46	88	54.5	3	6	2.7	1.6
5	Weakness	4.1	4.7	43	40.5	83.5	75.0	32	19	4.1	2.3
6	Weakness	5.0	3.7	26	26.5	52.5	56.8	10	5	4.1	3.3
7	Weakness	7.1	6.8	41	37	78	75.0	12	4	2.1	1.8
8	Weakness	2.4	4.3	28	33.2	61.2	31.8	19	15	3.7	2.4
9	Weakness	6.8	5.4	40	36.5	76.5	75.0	18	2	2.6	2.2
10	Weakness	3.0	2.0	38	38	76	79.5	18	11	–	–
11	–	5.6	0.6	5	8.5	13.5	25.0	29	3	2.3	1.9
12	Weakness	4.4	4.5	28	16.5	44.5	47.7	21	0	2.5	2.7
13	–	3.5	3.8	28	15.5	43.5	54.5	30	11	4.1	2.7
14	Weakness	5.9	0.0	23	21	44	54.5	25	7	2.0	1.9
15	Dystonia	7.6	7.0	46	47	93	88.6	26	16	–	–
Mean (±SEM)		5.3 (±0.5)	4.6 (±0.6)	34.1 (±2.9)	31.0 (±2.9)	65.1 (±5.6)	63.3 (±4.8)	23.0 (±2.5)	9.7 (±1.6)	3.1 (±0.2)	2.3 (±0.2)

Abbreviations: Bath, bath CRPS body perception disturbance scale; FLF, foreign limb feelings; PRWHE, patient-rated wrist and hand evaluation; QuickDASH, shortened disabilities of the arm, shoulder and hand; SEM, standard error of mean; TPD, two-point discrimination.



CRPS subjects and controls (Figure 6, Table 4). CRPS subjects displayed greater connectivity strength than controls in several brain regions, including the ipsilateral OFC (CRPS 0.13±0.03, controls 0.02±0.01), ipsilateral and contralateral dorsolateral prefrontal cortex (dlPFC; ipsilateral: CRPS 0.15±0.01, controls 0.04±0.01; contralateral: CRPS 0.17±0.02, controls 0.06±0.02), ipsilateral and contralateral mid-cingulate cortex (MCC; ipsilateral: CRPS 0.17±0.01, controls 0.07±0.01; contralateral: CRPS 0.15±0.02, controls 0.02±0.01), ipsilateral and contralateral superior parietal cortex (ipsilateral: CRPS 0.16±0.04, controls 0.03±0.02;

FIGURE 2 Increased infraslow oscillations (ISO) in CRPS subjects as compared with matched healthy controls. Box and whisker plots of mean amplitude of low-frequency fluctuations (ALFF) power over three standard ISO frequency bands: Slow-5: 0.01–0.027 Hz, slow-4: 0.027–0.073 Hz and slow-3: 0.073–0.198 Hz in the region of the (a) putamen ISO and (b) caudate tail, ventral putamen, ventral striatum, motor tongue area of the putamen, motor hand area of the putamen, ventrolateral (vl) caudate head, motor foot area of the putamen, supplementary motor hand area of the putamen (SMA hand), dorsolateral (dl) caudate head and caudate body seeds. The seed regions are located contralateral to the CRPS affected limb. The box indicates the interquartile range: The median is indicated by the solid line inside the box, the 25th percentile by the bottom line of the box and 75th percentile by the top line of the box. The mean is represented by the black dot within the box. The whiskers extend from the 2.5th to 97.5th percentile. The blue dots above and below the whiskers represent data points that lie outside the 2.5–97.5 percentile range. Slice locations in Montreal Neurological Institute space are indicated on the top right of each axial slice. Contra: Contralateral to affected limb. ($^{*}p < 0.05$ significantly different to controls; two-sample *t* test)

TABLE 3 Correlations between infraslow oscillation power and questionnaire scores

Seed	Correlated questionnaire scores	Pearson *r*	*p* value
Slow 4			
Putamen ISO	PRWHE pain score	−0.7289	0.0137
	PRWHE function score	−0.6728	0.0260
	PRWHE total score	−0.7275	0.0137
Putamen motor hand	PRWHE pain score	−0.7593	0.0130
	PRWHE function score	−0.6406	0.0328
	PRWHE total score	−0.7264	0.0143
	QuickDASH	−0.7023	0.0152
Putamen motor tongue	PRWHE pain score	−0.7436	0.0098
	PRWHE total score	−0.6604	0.0321
	QuickDASH	−0.7901	0.0065
Slow 3			
Putamen motor hand	PRWHE pain score	−0.6951	0.0442
	PRWHE total score	−0.5993	0.0789
	QuickDASH	−0.6656	0.0442

Note: All seeds are contralateral to the CRPS affected limb. The Pearson's correlation coefficient (*r*) and the FDR-adjusted significance of correlation (*p*) are reported.

Abbreviations: PRWHE, patient-rated wrist and hand evaluation; QuickDASH, shortened disabilities of the arm, shoulder and hand.

contralateral: CRPS 0.19 ± 0.02, controls 0.08 ± 0.02), contralateral superior frontal cortex (CRPS 0.15 ± 0.02, controls 0.04 ± 0.01), contralateral anterior cingulate cortex (ACC; CRPS 0.17 ± 0.02, controls 0.06 ± 0.01), contralateral supramarginal gyrus (CRPS 0.17 ± 0.04, controls 0.05 ± 0.02), ipsilateral and contralateral M1 (ipsilateral: CRPS 0.13 ± 0.03, controls 0.02 ± 0.02; contralateral: CRPS 0.13 ± 0.03, controls 0.01 ± 0.02), ipsilateral and contralateral angular gyrus (ipsilateral: CRPS 0.17 ± 0.03, controls 0.05 ± 0.02; contralateral: CRPS 0.16 ± 0.04, controls 0.03 ± 0.02), ipsilateral and contralateral piriform cortex (ipsilateral: CRPS 0.14 ± 0.02, controls 0.04 ± 0.01; contralateral: CRPS 0.12 ± 0.02, controls 0.02 ± 0.01), ipsilateral and contralateral middle temporal gyrus (ipsilateral: CRPS 0.13 ± 0.02, controls 0.04 ± 0.01; contralateral: CRPS 0.12 ± 0.02, controls 0.03 ± 0.01). No significant functional connectivity strength differences were found for the non-motor regions, caudate tail, dl caudate head, vl caudate head, ventral striatum, and ventral putamen seeds.

4 | DISCUSSION

Consistent with our hypothesis, we report significant differences in both basal ganglia ISO power and basal ganglia functional connectivity between individuals with CRPS and pain-free controls. These differences are primarily restricted to the motor basal ganglia loops, particularly regions that represent the upper limb, with one exception being the caudate body oculomotor loop. More specifically, we found increased ISO power in CRPS subjects in putamen divisions representative of the SMA hand, motor hand, and motor tongue. In addition to the putamen SMA hand, motor hand, motor tongue seeds, the putamen motor foot, and caudate body seeds displayed significantly greater (than controls) resting connectivity strength to multiple basal ganglia-cortical input areas such as M1, SMA, CMA, and OFC in CRPS subjects. These results highlight alterations in basal ganglia function at rest in CRPS and likely underpin the alterations in motor control commonly seen in this condition.

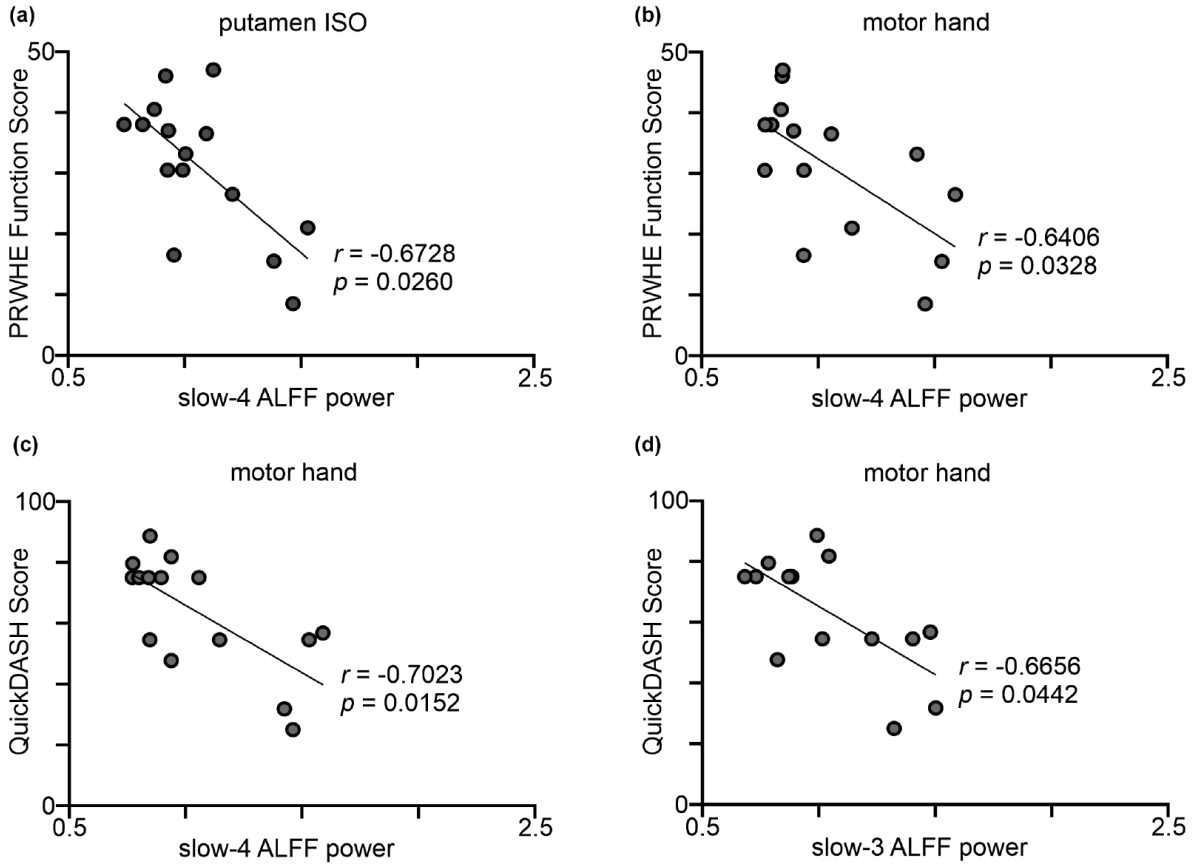


FIGURE 3 Increased infraslow oscillations (ISO) power in CRPS subjects correlated with less disability. Scatter plots showing Pearson correlations; the line represents the best fit for the correlations. The Pearson's correlation coefficient (*r*) and the FDR-adjusted significance of correlation (*p*) are displayed on the plots. Patient-rated wrist and hand evaluation (PRWHE) correlated with slow-4 ALFF power of the (a) putamen ISO seed and (b) putamen motor hand area. Shortened disabilities of the arm, shoulder and hand (QuickDASH) outcome measure correlated with (c) slow-4 ALFF power, and (d) slow-3 ALFF power of the putamen motor hand area

We found greater putaminal ISO power, primarily of the slow-4 bandwidth, which includes the 0.03–0.06 Hz range, a range we have previously reported on (Di Pietro et al., 2020). This range coincides with the astrocytic calcium wave propagation frequency range and subsequent astrocytic gliotransmitter release (Cornell-Bell et al., 1990; Crunelli et al., 2002) and we have hypothesized that increased ISOs found in CRPS and other forms of chronic neuropathic pain may be due to chronic astrogliosis (Henderson & Di Pietro, 2016). While no investigation has explored chronic astrogliosis in the putamen of CRPS patients, a postmortem study reported chronic astrogliosis in the spinal cord dorsal horn in CRPS (Del Valle et al., 2009). Furthermore, two human positron emission tomography studies have reported increased putaminal translocator protein binding in CRPS, a marker that binds to microglia and potentially also astrocytes (Jeon et al., 2017; Seo et al., 2021). Given this, we now propose that astrogliosis may also contribute to motor dysfunction in CRPS. Indeed, in mice, artificial elevation of calcium through activation of GFAP+ glial cells (astrocytes) in the brain and spinal cord resulted in motor coordination impairment (Agulhon et al., 2013). From the results of this current study, we speculate that astrogliosis within the basal ganglia is specific to the areas of greater slow-4 oscillations found in CRPS subjects, namely putamen SMA hand, motor hand, and motor tongue areas contralateral to the CRPS affected limb. Supporting this idea is evidence of elevated pro-inflammatory cytokine levels in the striatum of rats with chronic neuropathic pain (Al-Amin et al., 2011; Apkarian et al., 2006; Fiore & Austin, 2016). We also identified greater CRPS slow-3 oscillations in the putamen and motor hand area of the putamen contralateral to the CRPS affected limb. This suggests that in addition to calcium wave elevation and astrogliosis, there are other aberrant mechanisms that contribute to the motor dysfunction seen in CRPS.

In addition to altered resting oscillation patterns, we report significant increases in resting putamen connectivity. The putamen is the input region of the basal ganglia motor loop; it is organized somatotopically and has segregated inputs from M1 and SMA (Alexander et al., 1986; Nambu et al., 2002). Activity fluctuations in

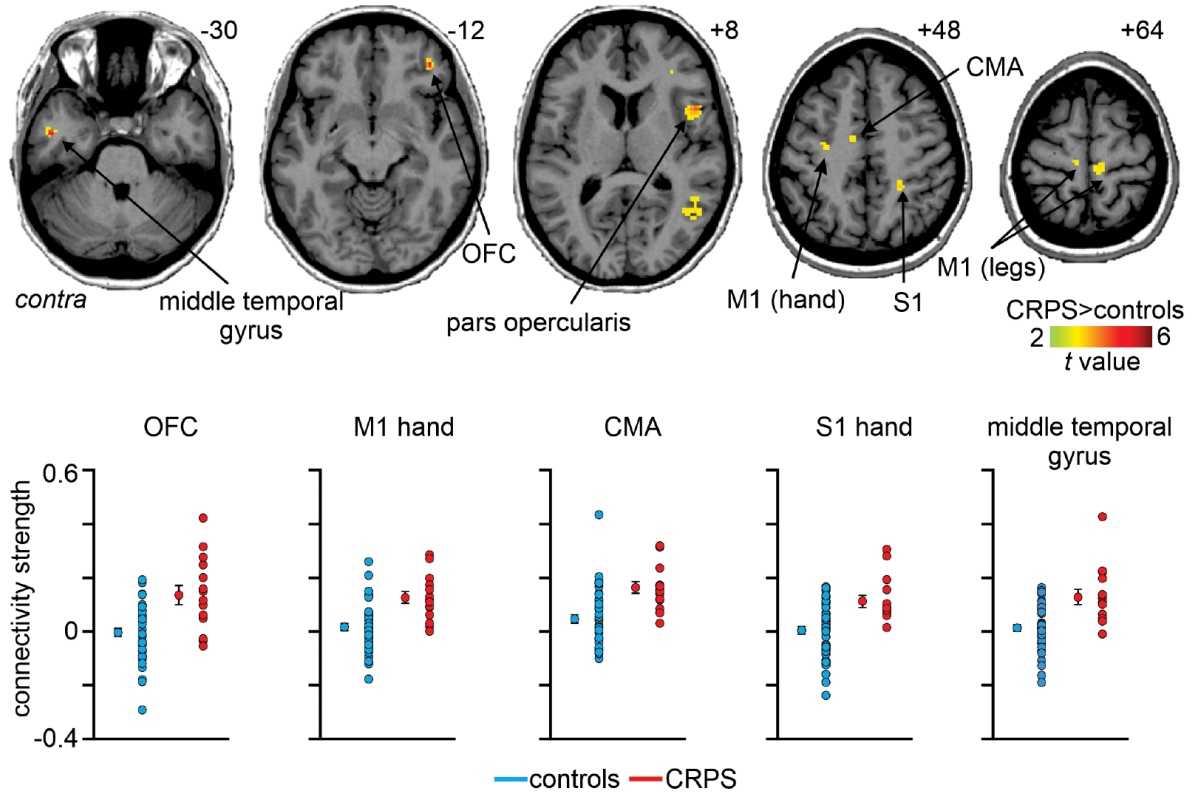


FIGURE 4 Significantly greater functional connectivity strength of the putamen infraslow oscillations (ISO) seed in CRPS subjects as compared to controls ($p < 0.05$; false discovery rate corrected for multiple comparisons, hot color scale). Slice locations in Montreal neurological institute space are indicated on the top right of each axial slice. Lower panel are plots of individual subject and mean ± SEM beta values of putamen ISO seed connectivity to the orbitofrontal cortex (OFC), hand area of the primary motor cortex (M1 hand), cingulate motor area (CMA), hand area of the somatosensory cortex (S1 hand), and middle temporal gyrus. Contra, contralateral to affected limb

the putamen motor hand, motor foot and SMA hand areas displayed greater synchrony with cortical areas in CRPS subjects. Overall, these areas showed stronger coupling with other motor related cortical regions such as the M1 region innervating the body as well as the CMA. Our findings of altered bilateral putamen-M1 connectivity are consistent with the bilateral nature of M1 changes in CRPS (Di Pietro et al., 2013a; Maihöfner et al., 2007). Contralateral changes are hypothesized to be due to altered functioning of the affected limb and ipsilateral changes related to compensatory use of the unaffected limb (Azqueta-Gavaldon et al., 2020). Consistent with this, we found that increased contralateral putamen-M1 hand functional connectivity was correlated with higher FLF scores in CRPS, indicating a greater self-perceived disturbance in motor function and neglect-like feelings of the CRPS affected limb. In Parkinson's disease and CRPS, greater putamen-M1 connectivity predicts poorer motor performance in a pegboard task that requires fast and accurate motor coordination (Azqueta-Gavaldon et al., 2020; Simioni et al., 2015). Furthermore, in CRPS, decreased putamen-M1 functional connectivity correlated with reduced forearm range of motion (Azqueta-Gavaldon et al., 2020). Thus, the increased putamen-M1 hand functional connectivity in CRPS subjects may underlie motor dysfunction.

As well as altered connectivity to well-described motor cortical regions, we found increased putamen connectivity with frontal and parietal association cortices, consistent with putamen anatomical connectivity to those areas (Jarbo & Verstynen, 2015). We found increased connectivity of the putamen ISO and putamen SMA hand seeds with the OFC, an important brain area in emotion and reward. Increased OFC connectivity is associated with non-reward, punishment, and depression (Rolls et al., 2020) and the increased putamen-OFC connectivity found in the current study may underpin the aversion to movement commonly seen in CRPS. Our study demonstrated increased putamen motor hand, motor foot, and SMA hand connectivity to the parietal cortex, mainly the inferior parietal lobule (IPL; formed by the supramarginal and angular gyri). The IPL's role involves sensorimotor integration, motor planning, spatial and nonspatial attention, and motor preparation (Caspers et al., 2013). In CRPS, finger tapping on the CRPS affected side increased IPL activation compared to the unaffected cerebral hemisphere and also to healthy controls (Schwenkreis et al., 2009), and in CRPS

TABLE 4 Montreal neurological institute (MNI) coordinates, *t*-scores, cluster size, and beta values for regions with significant functional connectivity differences between CRPS subjects and healthy controls

Region	Side	MNI coordinates			*t*-score	Cluster size	Beta values (mean ± SEM)	
		X	*Y*	*Z*			CRPS	Controls
Putamen ISO seed CRPS > Controls								
Primary motor cortex (M1) leg area	ipsi	8	−28	64	3.75	21	0.13 ± 0.02	0.02 ± 0.02
Primary motor cortex (M1) leg area	contra	−10	−24	58	4.33	53	0.15 ± 0.02	0.03 ± 0.01
Primary motor cortex (M1) hand area	contra	−30	−18	54	4.37	59	0.13 ± 0.02	0.02 ± 0.01
Cingulate motor area (CMA)	contra	−4	−8	54	3.95	31	0.16 ± 0.02	0.05 ± 0.02
Primary somatosensory (S1) hand area	ipsi	24	−42	48	3.83	23	0.11 ± 0.02	0.004 ± 0.01
Supramarginal gyrus	ipsi	46	−42	38	4.86	555	0.17 ± 0.02	0.04 ± 0.01
Pars opercularis	ipsi	48	10	8	4.30	156	0.19 ± 0.02	0.04 ± 0.02
Orbitofrontal cortex (OFC)	ipsi	30	34	0	4.66	26	0.14 ± 0.04	−0.003 ± 0.02
Orbitofrontal cortex (OFC)	ipsi	40	42	−12	4.60	38	0.14 ± 0.02	0.02 ± 0.01
Middle temporal gyrus	contra	−46	−6	−30	4.84	23	0.13 ± 0.03	0.01 ± 0.01
Putamen (motor hand) seed CRPS > Controls								
Primary motor cortex (M1) leg area	ipsi	6	−30	62	4.25	26	0.14 ± 0.02	0.04 ± 0.01
Primary motor cortex (M1) hand area	ipsi	36	−8	50	4.75	35	0.16 ± 0.02	0.05 ± 0.01
Primary motor cortex (M1) hand area	contra	−32	−10	48	4.50	93	0.16 ± 0.03	0.04 ± 0.01
Cingulate motor area (CMA)	contra	−8	−8	50	4.34	59	0.17 ± 0.03	0.05 ± 0.01
Angular gyrus	ipsi	34	−70	22	4.75	82	0.16 ± 0.02	0.04 ± 0.01
Angular gyrus	ipsi	42	−62	8	4.01	81	0.18 ± 0.03	0.05 ± 0.02
Orbitofrontal cortex (OFC)	ipsi	38	42	−14	4.32	32	0.13 ± 0.03	0.02 ± 0.01
Putamen (motor foot) seed CRPS > Controls								
Primary motor cortex (M1) hand area	ipsi	22	−16	62	3.74	41	0.16 ± 0.03	0.02 ± 0.02
Primary motor cortex (M1) hand area	ipsi	38	−8	50	4.58	82	0.17 ± 0.03	0.04 ± 0.01
Primary motor cortex (M1) hand area	contra	−32	−10	46	3.57	26	0.17 ± 0.04	0.04 ± 0.02
Primary motor cortex (M1) face area	ipsi	62	10	18	3.50	21	0.08 ± 0.01	0.01 ± 0.01
Cingulate motor area (CMA)	ipsi	20	−4	54	3.81	26	0.13 ± 0.03	0.01 ± 0.01
Cingulate motor area (CMA)	contra	−10	−8	50	4.60	89	0.20 ± 0.03	0.06 ± 0.01
Supramarginal gyrus	ipsi	54	−36	30	4.01	64	0.18 ± 0.03	0.04 ± 0.02
Angular gyrus	ipsi	34	−70	22	4.21	54	0.19 ± 0.03	0.05 ± 0.02
Angular gyrus	ipsi	54	−66	18	4.10	24	0.12 ± 0.03	−0.003 ± 0.02
Angular gyrus	ipsi	46	−50	10	4.23	81	0.18 ± 0.04	0.01 ± 0.02
Putamen (motor tongue) seed CRPS > Controls								
Frontal cortex	ipsi	26	8	48	5.76	25	0.18 ± 0.02	0.01 ± 0.02
Putamen (SMA hand) seed CRPS > Controls								
Cingulate motor area (CMA)	contra	−10	−10	48	4.31	33	0.14 ± 0.02	0.03 ± 0.01
Superior frontal cortex	contra	−6	48	38	3.76	24	0.11 ± 0.03	0.01 ± 0.02
Primary motor cortex (M1) hand area	contra	−30	−10	46	4.13	33	0.15 ± 0.03	0.05 ± 0.01

(Continues)

TABLE 4 (Continued)

Region	Side	MNI coordinates			t-score	Cluster size	Beta values (mean ± SEM)	
		X	Y	Z			CRPS	Controls
Primary motor cortex (M1) tongue area	ipsi	50	−6	30	3.94	42	0.13 ± 0.03	0.01 ± 0.01
Supramarginal gyrus	contra	−40	−60	26	3.60	20	0.13 ± 0.03	0.01 ± 0.02
Supramarginal gyrus	ipsi	52	−36	22	4.98	294	0.16 ± 0.02	0.03 ± 0.01
Angular gyrus	ipsi	36	−70	24	4.08	35	0.16 ± 0.03	0.05 ± 0.01
Orbitofrontal cortex (OFC)	ipsi	32	18	−18	4.04	39	0.11 ± 0.03	0.004 ± 0.01
Middle temporal gyrus	contra	−52	−2	−32	4.21	38	0.11 ± 0.02	0.03 ± 0.01
Caudate body seed CRPS > Controls								
Superior parietal cortex	ipsi	22	−54	68	3.18	51	0.10 ± 0.03	0.01 ± 0.01
Superior parietal cortex	ipsi	4	−52	62	3.36	31	0.16 ± 0.03	0.04 ± 0.02
Superior parietal cortex	ipsi	18	−56	58	2.97	25	0.11 ± 0.03	0.01 ± 0.02
Superior parietal cortex	contra	−28	−56	40	3.07	80	0.19 ± 0.02	0.08 ± 0.02
Superior parietal cortex	contra	−14	−78	34	3.15	27	0.17 ± 0.03	0.06 ± 0.02
Superior parietal cortex	ipsi	18	−82	20	4.40	337	0.16 ± 0.04	0.03 ± 0.02
Middle cingulate cortex (MCC)	contra	−14	−10	42	4.84	6886	0.15 ± 0.02	0.02 ± 0.01
Middle cingulate cortex (MCC)	ipsi	12	26	42	3.31	93	0.17 ± 0.01	0.07 ± 0.01
Superior frontal cortex	contra	−20	30	32	3.18	67	0.19 ± 0.03	0.07 ± 0.02
Superior frontal cortex	contra	−12	56	32	3.12	48	0.17 ± 0.03	0.07 ± 0.01
Superior frontal cortex	contra	−16	66	6	3.71	135	0.15 ± 0.02	0.04 ± 0.01
Anterior cingulate cortex (ACC)	contra	−12	32	28	3.45	131	0.17 ± 0.02	0.06 ± 0.01
Supramarginal gyrus	contra	−46	−36	38	3.22	60	0.17 ± 0.04	0.05 ± 0.02
Primary motor cortex (M1)	contra	−40	−6	20	3.74	84	0.13 ± 0.03	0.01 ± 0.02
Primary motor cortex (M1)	ipsi	50	6	10	3.18	124	0.13 ± 0.03	0.02 ± 0.02
Angular gyrus	contra	−26	−88	14	3.88	216	0.16 ± 0.04	0.03 ± 0.02
Angular gyrus	ipsi	48	−52	14	3.30	80	0.17 ± 0.03	0.05 ± 0.02
Angular gyrus	contra	−38	−66	8	2.97	37	0.14 ± 0.04	0.02 ± 0.02
Dorsolateral prefrontal cortex (dlPFC)	contra	−36	36	6	3.38	21	0.17 ± 0.02	0.06 ± 0.02
Dorsolateral prefrontal cortex (dlPFC)	ipsi	44	48	−2	4.00	837	0.15 ± 0.01	0.04 ± 0.01
Orbitofrontal cortex (OFC)	ipsi	22	56	−16	4.32	88	0.13 ± 0.03	0.02 ± 0.01
Piriform cortex	ipsi	20	4	−16	3.75	66	0.14 ± 0.02	0.04 ± 0.01
Piriform cortex	contra	−22	8	−24	4.15	76	0.12 ± 0.02	0.02 ± 0.01
Middle temporal gyrus	contra	−48	−8	−26	3.62	91	0.12 ± 0.02	0.03 ± 0.01
Middle temporal gyrus	ipsi	46	−4	−30	4.70	173	0.13 ± 0.02	0.04 ± 0.01

Note: All seeds are contralateral to the CRPS affected limb.

subjects with dystonia, imagined movements of the affected limb resulted in reduced IPL activation compared to control subjects (Gieteling et al., 2008). In Parkinson's disease subjects with tremor, IPL-M1 connectivity was increased compared to controls (Vervoort et al., 2016). While our study did not test the connectivity between IPL and M1, both IPL and M1 had increased connectivity to the putamen seeds contralateral to the CRPS affected limb. Thus, it is probable that increased contralateral putamen-IPL connectivity indicates dysfunction in IPL in CRPS subjects and may underlie motor dysfunction such as tremor.

Most basal ganglia seeds that displayed significant connectivity differences were motor areas; however, we also found alterations in the caudate body, which represents the striatal portion of the oculomotor loop (Alexander et al., 1986). A recent study identified this basal ganglia region as a convergence zone of corticostriatal projections with integration of reward, executive control and spatial attention during spatial reinforcement learning thought to be associated with the caudate body (Jarbo & Verstynen, 2015). In our study, the caudate body seed displayed the greatest degree of increased functional connectivity to cortical areas including M1,

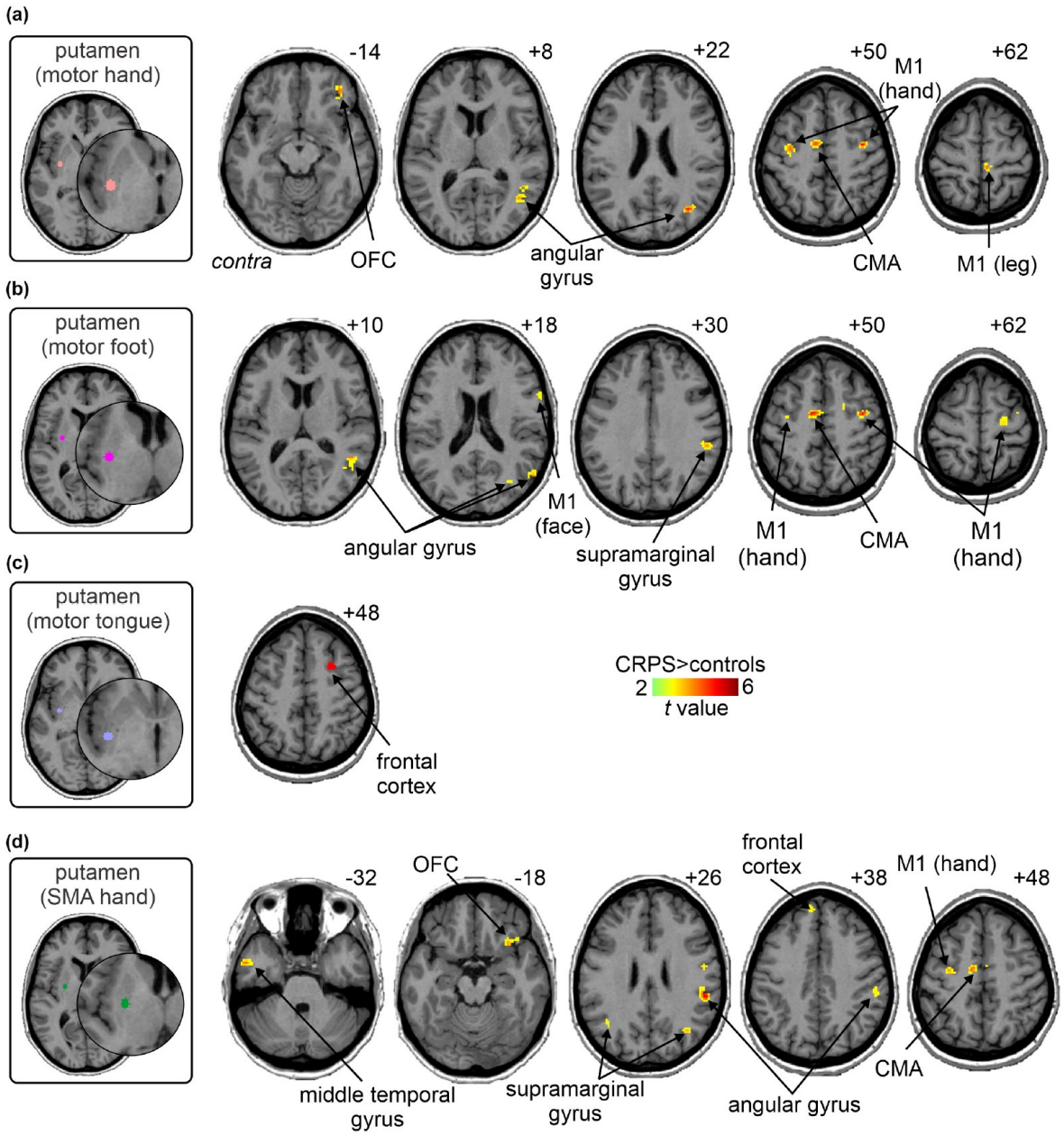


FIGURE 5 Increased functional connectivity of putamen motor loop seeds in CRPS subjects as compared with matched healthy controls. Increased functional connectivity of the putaminal (a) motor hand area, (b) motor foot area, (c) motor tongue area, and (d) the supplementary motor hand area of the putamen (SMA hand) ($p < 0.05$; false discovery rate corrected for multiple comparisons, hot color scale). Slice locations in Montreal neurological institute space are indicated on the top right of each axial slice. CMA, cingulate motor area; contra, contralateral to affected limb; M1, primary motor cortex; OFC, orbitofrontal cortex

prefrontal and parietal association cortices in CRPS subjects compared to controls. Perceptual disturbances such as neglect-like syndrome and a foreign feeling of the CRPS affected limb are common features of CRPS (Galer & Jensen, 1999; Kuttikat et al., 2016). The caudate body showed increased connectivity with cortical regions governing visual attention (middle temporal gyrus), spatial attention (superior frontal cortex) and visuospatial perception (superior parietal cortex) and thus, may relate to the visuospatial issues in CRPS. Indeed, lesions to the caudate and the IPL can lead to deficits in visuospatial perception that present as neglect (Karnath et al., 2005; Kumral et al., 1999). However, in the current study, we did not perform correlation analysis of caudate body functional connectivity to

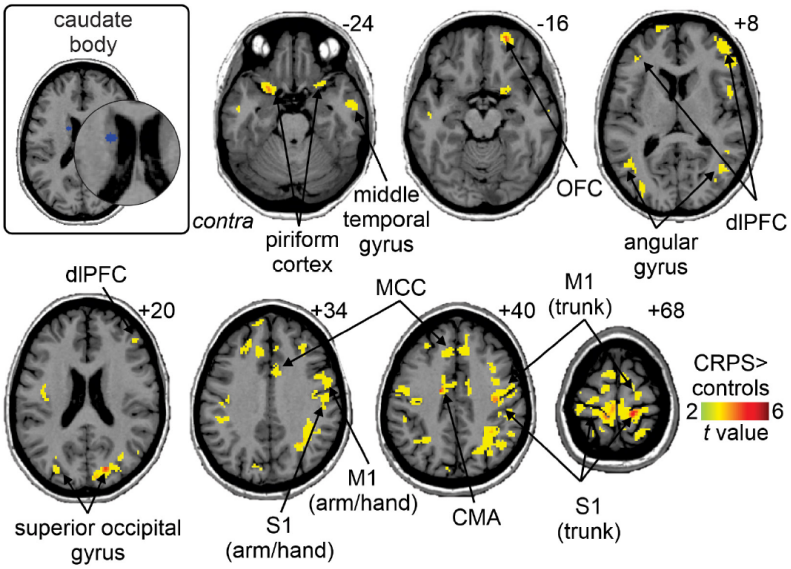


FIGURE 6 Increased functional connectivity of the caudate body seed in CRPS subjects as compared with matched healthy controls ($p < 0.05$; false discovery rate corrected for multiple comparisons, hot color scale). Slice locations in Montreal neurological institute space are indicated on the top right of each axial slice. CMA, cingulate motor area; contra, contralateral to affected limb; dlPFC, dorsolateral prefrontal cortex; M1, primary motor cortex; MCC, mid-cingulate cortex; OFC, orbitofrontal cortex; S1, primary somatosensory cortex

body perception. Thus, it is unknown to what degree visuospatial perception may be related to caudate body functional connectivity in CRPS.

There are several limitations to this study. This study had a limited sample of 15 CRPS subjects. However, given the rare nature of CRPS, it is comparable to previous studies (Di Pietro et al., 2013a, 2013b). It is noted, however, the sample size of this study is a statistically limits correlation interpretations (even when adjusted for FDR), and a much larger sample size is needed for greater reliability on correlation interpretations. The longitudinal following of subjects recruited before CRPS development would identify whether brain differences are true changes, and whether they are pathophysiological or adaptive. Furthermore, it is unknown whether the findings in our study are specific to CRPS or are also present in other pain or motor dysfunction conditions as we did not compare changes in sensory and motor disabilities between different conditions. Future studies should aim to compare changes in sensory and motor disabilities between different conditions. Nonetheless, it is clear that the sensory and motor disturbances are a result of reorganization of the sensory and motor cortices and related cortico-basal ganglia loops and are part of the CRPS disease pathology. The resting-state nature of our scanning and the lack of quantitative assessment of motor dysfunction are also weaknesses. Future studies should include quantitative assessment of motor dysfunction (e.g., pegboard task, Unified Parkinson Disease Rating Scale) or examine functional connectivity during movement tasks (e.g., finger tapping, wrist extension)—although this would present a practical challenge. Medications may have had a potential effect on results as 13 of the 15 CRPS subjects had taken medication before the study; however, it is not easy or necessarily ethical to exclude medicated CRPS subjects or include a washout period before the study. Ideally, the study would have recruited CRPS participants with no comorbidities, however, given the rare nature of CRPS and multi-system dysfunction following CRPS (Schwartzman, 2012), this was difficult. Hence, it cannot be fully excluded that multiple comorbidities may have also had a potential effect in this study's findings. However, all CRPS participants had been diagnosed with CRPS, and the list of comorbidities varies greatly between each CRPS participant. Given the great variability, collectively the multiple comorbidities are unlikely to contribute significantly to the overall changes reported in this study.

5 | CONCLUSION

Our study is the first to systematically evaluate infraslow oscillations and resting-state functional connectivity in motor and non-motor basal ganglia functional loops in CRPS subjects. Compared to controls, we identified increased ISOs in the putamen contralateral to the CRPS affected side, but not in non-motor basal ganglia regions in CRPS subjects. Moreover, putaminal ISOs correlated with wrist function and disability scores. We demonstrated increased functional connectivity in the contralateral putamen (especially the motor hand region) and caudate body seeds, with a network of cortical structures including the M1 hand region, SMA, and other frontal

and parietal association cortices. There were no changes in striatal seeds representing cognitive and limbic basal ganglia loops. In summary, the recruitment of anatomically specific motor basal ganglia-cortical networks likely underlies motor symptoms, such as dystonia and tremor, while the caudate body network may be related to altered visuospatial integration, in CRPS.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, F.D.P., L.A.H., and P.J.A.; *Methodology*, F.D.P., L.A.H., and P.J.A.; *Investigation*, B.L. and F.D.P.; *Formal Analysis*, B.L., L.A.H., and P.J.A.; *Writing – Original Draft*, B.L., L.A.H., and P.J.A.; *Writing – Review & Editing*, B.L., F.D.P., L.A.H., and P.J.A.; *Visualization*, B.L., F.D.P., L.A.H., and P.J.A.; *Supervision*, F.D.P., L.A.H., and P.J.A.; *Funding Acquisition*, F.D.P.

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DATA AVAILABILITY STATEMENT

Data sharing not available due to privacy/ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

FIGURE S1 One-sample *t*-test within group results for putamen seeds; a: putamen ISO, b: putamen (motor hand), c: putamen (motor foot), d: putamen (motor tongue), and e putamen (SMA hand). Figures were masked using frontal lobe, anterior cingulate cortex (ACC), middle temporal gyrus and parietal lobe and visualised at a threshold of $p = 0.05$ FDR and 20 voxel minimum cluster size

FIGURE S2 One-sample *t*-test within group results for non-motor basal ganglia seeds; a: caudate body, b: caudate tail, c: ventrolateral (vl) caudate head, d: dorsolateral (dl) caudate head, e: ventral striatum, and f: ventral putamen. Figures were masked using frontal lobe, anterior cingulate cortex (ACC), middle temporal gyrus and parietal lobe and visualised at a threshold of $p = 0.05$ FDR and 20 voxel minimum cluster size. The ventral striatum CRPS (e) did not have any contrast at $p = 0.05$ FDR, 20 voxel minimum cluster size when masked with the frontal lobe, anterior cingulate cortex (ACC), middle temporal gyrus and parietal lobe mask

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Chapter 4

Altered resting state functional connectivity of the midbrain periaqueductal gray matter and locus coeruleus in Complex Regional Pain Syndrome

Chapter 4 is currently under review by eNeuro.

Chapter 4 Altered resting state functional connectivity of the midbrain periaqueductal gray matter and locus coeruleus in Complex Regional Pain Syndrome

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The authors have no conflicts of interest to declare.

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Research data are not shared due to human ethics requirements.

4.1 Abstract

Complex regional pain syndrome (CRPS) is a chronic pain condition that can develop following minor trauma. Emerging evidence indicates that CRPS is associated with altered processing of pain modulatory regions within the brainstem, although direct evidence of this is lacking. Previous human brain imaging investigations in other chronic pain conditions have reported functional changes in brainstem regions such as the midbrain periaqueductal gray matter (PAG), locus coeruleus (LC), and rostral ventromedial medulla (RVM), however, no study has investigated connectivity of these brainstem regions or between these regions and higher brain centres in CRPS. In this study, we used resting state functional magnetic resonance imaging to investigate functional connectivity changes of the PAG, LC, and RVM within the brainstem and with higher brain areas in 15 CRPS and 30 matched pain-free controls. Using PAG, LC, and RVM seeds, we found that resting functional connectivity within the brainstem was not different in CRPS compared with control participants. However, CRPS participants displayed decreased left caudal and mid PAG functional connectivity with higher brain areas such as the primary somatosensory cortex (S1) and posterior cingulate cortex (PCC), and increased functional connectivity between the LC and the caudate nucleus, anterior cingulate cortex (ACC), and hippocampus. Further, decreased left caudal PAG-S1 functional connectivity was negatively correlated to pain in CRPS. These findings demonstrate that while resting functional connectivity between brainstem pain modulatory pathways is not altered in CRPS, connections between the brainstem and higher brain centres may play a role.

4.2 Significance Statement

This is the first study to evaluate resting-state functional connectivity of pain modulatory pathways within the brainstem and with higher brain areas in CRPS. Contrary to speculation that brainstem modulatory pathway connectivity between the PAG, LC and RVM would be increased in CRPS participants compared to controls, we found no changes in functional connectivity between these brainstem nuclei at rest. In contrast, we demonstrate for the first time, alterations in brainstem to higher brain area functional connectivity, with PAG to S1 and PCC connectivity being decreased, and LC to caudate, ACC and hippocampus connectivity being increased. We therefore postulate that changes in ascending brainstem projections to higher brain regions may contribute to pain in CRPS.

4.3 Introduction

Complex regional pain syndrome (CRPS) is a chronic pain condition that can develop following minor limb trauma, with recent evidence indicating CRPS onset in 4 – 14% of wrist fractures (Rolls et al., 2020). Presenting similarly to neuropathic pain, CRPS is characterised by pain that is disproportionate to the injury, hyperalgesia and allodynia, motor impairments, and co-morbid depression. Unfortunately, the efficacy of anti-neuropathic agents (e.g., Gabapentin) and opioids are poor. However, opioids are still widely prescribed and contribute to increasing opioid misuse (Roxburgh & L, 2017). The pathophysiology of CRPS and indeed neuropathic pain in general is not well understood and this lack of understanding underlies our inability to develop more effective treatment regimens.

Some studies have begun to explore the pathophysiology of CRPS and many have begun to elucidate the role of the brainstem in the development of on-going pain. Whilst there is little direct data exploring the brainstem itself, Seifert et al. (2009) reported decreased adaptation to noxious electrical stimuli and increased hyperalgesia in CRPS and suggested that these findings indicate that descending modulatory pain pathways are facilitating nociceptive input. More recently, it was reported that CRPS is associated with altered nociceptive blink reflex excitability and habituation, also indicative of altered brainstem pain processing (Thoma et al., 2022). Furthermore, it has been suggested that the presence of photophobia in individuals with CRPS results from abnormal brainstem nociceptive processing (Drummond & Finch, 2021). While these studies indicate a pathophysiological role of the brainstem in CRPS, few have investigated functional changes in brainstem pain processing regions themselves.

It is well established that the brainstem contains a number of regions that modulate incoming noxious information (Crawford et al., 2022). The most well-described is that of the midbrain periaqueductal gray matter (PAG) - rostral ventromedial medulla (RVM) – dorsal horn (DH) circuit which can either inhibit or facilitate incoming noxious information (Basbaum & Fields, 1984; Ghazni, Cahill & Stroman, 2010). Other brainstem pain modulatory sites include the locus coeruleus (LC) which has been implicated in placebo and conditioned pain modulation analgesia (Crawford et al., 2022; Crawford et al., 2021; Youssef, Macefield & Henderson, 2016a; Youssef, Macefield & Henderson, 2016b). Importantly, it has been previously shown that individuals with chronic orofacial neuropathic pain display increased resting connectivity strengths between these brainstem sites (Mills et al., 2018). Furthermore, these brainstem sites, particularly the PAG and LC, are thought to be modulated by descending inputs from higher brain regions such as the prefrontal and cingulate cortices (Boadas-Vaello et al., 2016; Heinricher et al., 2009; Knudsen et al., 2018; Youssef, Macefield & Henderson, 2016a). Whilst it has been suggested that brainstem pain modulatory sites are altered in CRPS, no study has directly investigated these regions in human participants. Furthermore, no study has investigated potential descending influences over these brainstem circuits in CRPS.

The aim of this study was to use resting state functional magnetic resonance imaging (fMRI) to determine if CRPS participants have altered functional connectivity in brainstem pain modulatory pathways, compared to pain-free control participants. We hypothesised that there would be significantly greater resting functional connectivity between the PAG, LC, and RVM in individuals with CRPS. Furthermore, we hypothesised altered connectivity strengths between higher brain centres, the prefrontal and cingulate cortices, and the PG and LC in CRPS.

4.4 Materials and Methods

4.4.1 Study participants

Sixteen eligible participants with upper limb CRPS consented to participate in the study, however, 1 CRPS participant was excluded due to claustrophobia. Data from 15 upper-limb CRPS participants (11 females; mean ± SEM age: 47.5 ± 3.2 years) and 30 age- and sex-matched pain-free controls (22 females; 47.4 ± 2.3 years) were analysed. CRPS participants were diagnosed in accordance with the International Association for the Study of Pain "Budapest" diagnostic criteria (Harden et al., 2007). All CRPS participants had upper limb CRPS as their primary complaint and reported ongoing pain for at least 3 months. Participants were excluded if they had any MRI contraindications (e.g. pacemakers, metal implants), neurological disorders, or significant mental health disorders or developmental delays that would impact participation. Pain-free control participants were excluded if they had any chronic pain condition. Informed written consent was obtained from all participants. This study was conducted with the approval of the Human Research Ethics Committee of the University of Sydney and conducted in accordance with the Declaration of Helsinki. No sample size calculation was performed; hence, a convenience sample was recruited due to the low incidence of CRPS and difficulty in recruiting eligible and willing CRPS participants, particularly for an MRI study.

4.4.2 CRPS assessment

CRPS signs were assessed in both upper limbs of each CRPS participant. i) Sensory: Hyperalgesia and allodynia were assessed via pinprick of the dorsal webspace of the hand and

light brush strokes on the dorsum of the hand, respectively. ii) Vasomotor: Skin temperature and skin colour asymmetry were determined by touch and visual assessment. iii) Sudomotor/Oedema: Sweating (sudomotor) of the participant's palms was assessed by touch; oedema of the wrist and proximal phalanx of the middle finger was assessed with circumferential measurement with a tape measure. iv) Motor/Tropic: Tremor and dystonia were visually assessed. Motor weakness was determined by a power grip test on the researcher's index and middle fingers. Trophic changes were visually assessed by noting hair, nail, and skin asymmetry between upper limbs.

4.4.3 Pain assessment

For all CRPS participants, pain intensity on the day of the study ("day pain") was rated on a 10 cm visual analogue scale (VAS) (0 = no pain to 10 = worst imaginable pain). In addition, ongoing pain intensity was rated three times a day for seven days before or following the scanning session on the 10 cm VAS. The mean pain rating of the seven days was taken as the "diary pain" score. Task-associated pain intensity in the affected limb was assessed using the Patient-rated Wrist and Hand Evaluation (PRWHE) pain subsection (MacDermid, 1996). The PRWHE pain score ranges from 0 to 50, with a higher score indicating greater task-associated pain.

4.4.4 MRI data collection

Participants lay supine on the MRI scanner bed in a 3 Tesla MRI scanner (Achieva TX, Philips Medical Systems) with their head immobilised in a padded 32-channel head coil. For each

participant, a high-resolution T1-weighted anatomical image of the whole brain in the sagittal plane was collected (repetition time = 5600 ms; echo time = 2.5 ms, flip angle = 8°, raw voxel size 0.87mm3). Following this, participants were asked to relax with their eyes closed as a series of 180 gradient-echo echo-planar resting-state fMRI volumes with blood oxygen level-dependent contrast (BOLD) were collected (repetition time = 2000 ms; echo time = 30 ms, flip angle = 90°, 37 axial slices, raw voxel size 3×3×4mm, phase encoding along the anterior to posterior direction).

4.4.5 MRI data analysis

SPM12 (Friston et al., 1995) and custom software was used to perform MRI data analysis. To ensure that the brain's left hemisphere was contralateral to the CRPS-affected limb for all CRPS participants, the T1 and rsfMRI images of left-side restricted upper limb CRPS participants (n = 3) were left-right reflected across the midline on the y-axis before data processing. Thus, results restricted to the right side of the brain are ipsilateral to the most severe on-going pain. All fMRI images were realigned and the Dynamic Retrospective Filtering toolbox (Särkkä et al., 2012) was used to model and remove cardiac and respiratory noise. To remove global drifts in fMRI signal intensity the images were linear detrended and to remove movement-related signal changes the images were detrended using a linear modelling of realignment parameters procedure. Each participant's fMRI image set was co-registered to their own T1-weighted anatomical image. The T1 images were then spatially normalised into Montreal Neurological Institute (MNI) space, and the normalisation parameters were applied to the fMRI images sets to place them into MNI space. This procedure resulted in the reslicing of images into 2x2x2mm voxels. The resulting fMRI images were then smoothed using a 6mm

full-width at half-maximum (FWHM) Gaussian filter. For brainstem analyses, prior to spatial normalisation, the SUIT (A Spatially Unbiased Atlas Template of the Cerebellum and Brainstem) toolbox (Diedrichsen, 2006) was used to isolate and create binary masks for the brainstem of each participant's T1 and fMRI image sets. Using the brainstem masks, the brainstem of T1 and fMRI image sets were spatially normalised to the SUIT brainstem template in MNI space.

4.4.6 Connectivity analysis

4.4.6.1 Brainstem

Nine brainstem seeds, each consisting of 6 contiguous voxels, were used to explore connectivity within brainstem pain modulatory pathways and between the brainstem and higher brain regions. To explore the PAG, we divided it into caudal (z = -12 to -10 in MNI space), mid (z = -9 to -7) and rostral (z = -6 to -4) segments. Left and right PAG seeds were made for each rostro-caudal level, totalling 6 PAG seeds (left caudal PAG, right caudal PAG, left mid PAG, right mid PAG, left rostral PAG and right rostral PAG). Separate rostro-caudal seeds were created since experimental animal studies have shown a crude somatotopic organization of afferent inputs to the PAG, with inputs from the spinal system terminating primarily in the contralateral caudal PAG whereas those from the orofacial system terminate primarily in the contralateral rostral PAG (Keay et al., 1997; Wiberg, Westman & Blomqvist, 1987). Two seeds, one encompassing the left and the other the right LC (z = -26 to -17) and a single seed encompassing the RVM (z = -51 to -47) were also created (**Figure 4.1A**).

For each of the 9 brainstem seeds, a voxel-by-voxel analysis was performed over the brainstem to determine the relationships between the mean resting signal intensity changes within each seed region and that of each voxel of the brainstem in each participant. The resultant brainstem connectivity maps were smoothed using a 2 mm FWHM Gaussian filter. To determine significant differences between CRPS and pain-free controls, second-level, two-group random-effects analysis was performed for each of the 9 seeds ($p<0.001$ uncorrected for multiple comparisons, minimum contiguous cluster size of 10 voxels). Further, connectivity strengths (beta values) between seeds on the same side (i.e., left PAG to left LC, right PAG to right LC) and between the PAG, LC, and RVM were extracted, individual and mean ± standard error of mean (SEM) connectivity strength (beta values) plotted, and significance between groups determined ($p<0.05$, 2-sample, 2 tailed t-tests).

4.4.6.2 Whole brain

For 8 brainstem seeds (i.e. all seeds except the RVM, which does not have direct cortical projections), a voxel-by-voxel analysis was performed over the remainder of the brain (whole brain not including the brainstem) to determine the relationships between the mean resting signal intensity changes within each seed region and that of each voxel of the brain in each participant. Significant differences between CRPS and pain-free controls were determined by performing a second-level, two-group random-effects analysis for each seed ($p<0.001$ uncorrected, minimum contiguous cluster size of 10 voxels). A gray matter mask was applied to the analysis. For significant clusters, individual and mean±SEM connectivity strength values (beta values) were plotted. In addition, for CRPS participants, linear relationships between connectivity strength of significant clusters and pain duration, day pain, diary pain, and PRWHE pain score were determined using Pearson's correlations (Pearson's r, $p<0.05$, two-

tailed). The Benjamini-Hochberg method (Benjamini & Hochberg, 1995) was used to adjust correlation p values at 5% false discovery rate.

4.5 Results

4.5.1 Participant characteristics

Of the 15 upper limb CRPS participants, three reported upper limb pain primarily on the left side, while the remaining participants' pain was primarily in the right upper limb. Most participants reported pain elsewhere in addition to their upper-limb CRPS and 7 participants reported lower limb pain (**Figure 4.*1*B, Table 4.1**). The mean CRPS duration was 4.6±0.9 years, day pain 5.3±0.5, diary pain 4.6±0.6, and PRWHE pain score 34.1±2.9.

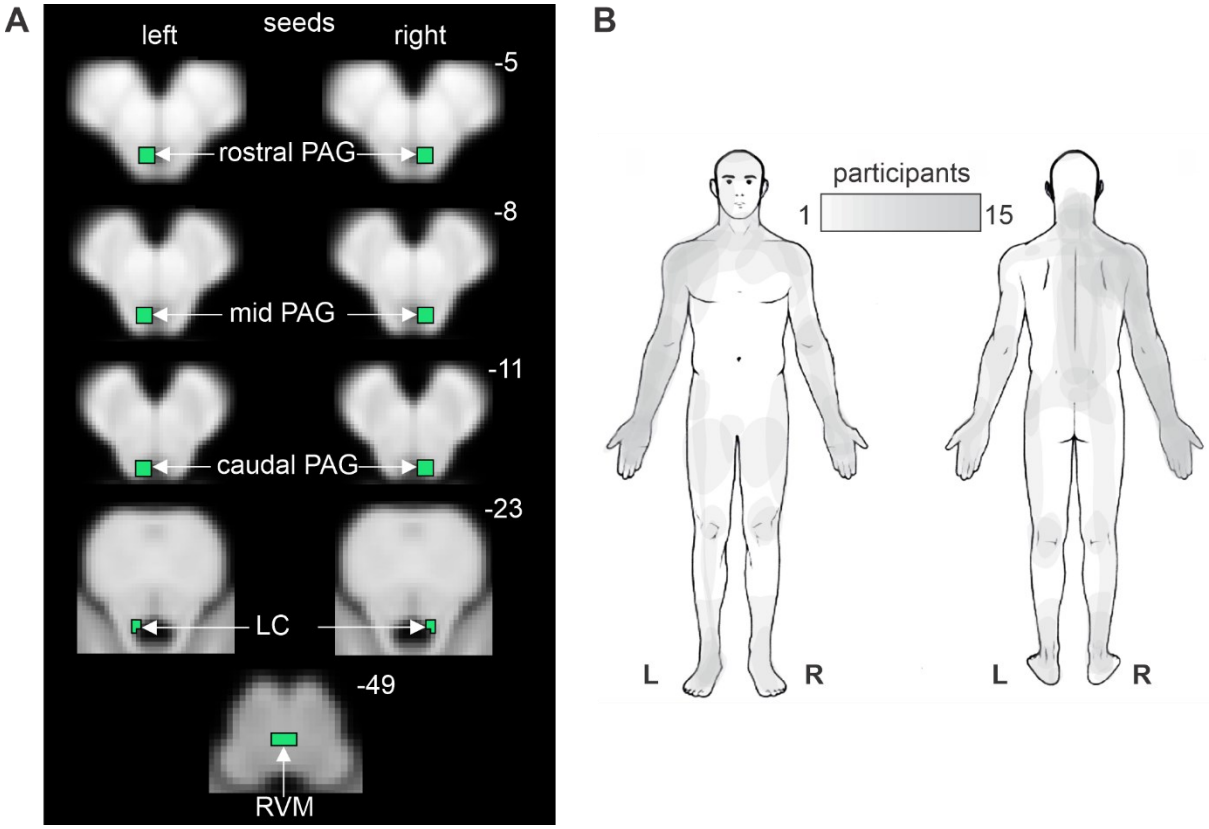

Figure 4.1. (A) Locations of the 9 brainstem functional connectivity seeds overlaid onto a series of axial T1-weighted anatomical slices. Slice locations in Montreal Neurological Institute space are indicated on the top right of each slice. **(B)** Body maps showing the distribution of on-going pain in all 15 CRPS participants. LC: locus coeruleus; PAG: midbrain periaqueductal gray matter; RVM: rostral ventromedial medulla.

Table 4.1. CRPS participant demographics, medical history, medication, and pain intensity scores.

Partic-ipant	Age	Sex	Pain duration (years)	CRPS Affected Region(s)	Inciting Event	Medications	Comorbidities		Pain Intensity		
							Pain-related	Non-pain-related	Day Pain	Diary Pain	PRWHE pain score
1	49.2	M	7.0	**R UL**, R LL, L LL, face, abdomen	Pain in R hand	None	None	None	4.0	4.5	32.0
2	56.4	F	4.2	**L UL**, R UL, R LL, R and L chest	L humerus fracture	Duloxetine, Gabapentin, Oxycodone, Quetiapine, Tapentadol	L radial nerve palsy, fibrocartilage complex degeneration of R hand	None	7.8	8.1	45.0
3	55.8	F	0.9	**R UL**, R neck, R chest	Spontaneous onset	Ashwagandha, Budesonide, Cannabis, Codeine, Formoterol, Oxycodone, Paracetamol, Salbutamol	Back pain, Fibromyalgia, Osteoarthritis, Radiculopathy, Spinal disc herniation	COPD, Peptic ulcer, Raynaud's disease	7.9	8.3	46.0
4	61.7	F	6.2	**L UL**, *R UL*	R hand tendon release surgery	Amitriptyline, Cannabidiol drops, Codeine, Levothyroxine, Paracetamol, Topiramate, Tramadol, Valerian	None	Diverticulitis, Gastro-oesophageal reflux disease, Graves' disease (thyroidectomised)	4.3	5.8	42.0
5	58.1	F	8.7	**R UL**, R LL, R face	R arm surgery	Codeine, Duloxetine, Linagliptin, Meloxicam, Metformin, Paracetamol	None	Diabetes	4.1	4.7	43.0
6	66.6	F	9.5	**R UL**, L UL, R LL, L LL	R radius fracture	Amlodipine, Gabapentin, Ketamine in lipoderm cream, Metformin, Metoprolol, Pantoprazole, Salbutamol	Osteoarthritis	Asthma, Diabetes, Hypertension, Pubic symphysitis, Supraventricular tachycardia	5.0	3.7	26.0

7	46.8	M	1.5	**R UL**	Spontaneous onset	Amlodipine, Atorvastatin, Ibuprofen, Paracetamol, Perindopril, Pregabalin	None	Hyperlipidaemia, Hypertension	7.1	6.8	41.0
8	34.2	F	5.3	**R UL**, R LL, R hip	R wrist fracture	Amitriptyline, Buprenorphine patch	Migraine, R hip bursitis	None	2.4	4.3	28.0
9	25.9	F	1.3	**R UL**, L and R neck, spine, L LL	R hand nerve damage	None	Endometriosis	Polycystic ovarian syndrome	6.8	5.4	40.0
10	45.2	M	1.2	**R UL**	R wrist injury	Codeine, Ibuprofen, Pregabalin,	Bell's palsy, Bulging discs	None	3.0	2.0	38.0
11	45.9	F	3.9	**L UL**	L hand carpal tunnel release surgery	Amitriptyline**,** Betahistine, Duloxetine, Naproxen, Pantoprazole, Rizatriptan, Tapentadol, Valaciclovir	Carpal tunnel of R hand, Fibromyalgia, Migraine	Herpes, Polycystic ovarian syndrome, Vertigo	5.6	0.6	5.0
12	23.8	F	2.6	**R UL**, L UL	Overload	Amitriptyline, Gabapentin, Levothyroxine	None	Hashimoto's Disease	4.4	4.5	28.0
13	52.3	F	2.9	**R UL**, R torso	Fractured coccyx	Ibuprofen, Melatonin, Paracetamol, Tapentadol	Endometriosis	None	3.5	3.8	28.0
14	37.8	F	12.7	**R UL**, R neck, L LL	Spontaneous onset	Duloxetine, Gabapentin, Naloxone, Oxycodone, Palmitoylethanolamide	Endometriosis, Endosalpingiosis	Raynaud's disease	5.9	0.0	23.0
15	52.3	M	1.9	**R UL**, L and R neck, back	R scaphoid fusion surgery	Ibuprofen, Oxycodone, Paracetamol, Pregabalin, Tramadol, Venlafaxine, Zopiclone	L shoulder bursitis	Sleep apnoea	7.6	7.0	46.0
Mean (±SEM)	**47.5 (±3.2)**		**4.6 (±0.9)**						**5.3 (±0.5)**	**4.6 (±0.6)**	**34.1 (±2.9)**

Abbreviations: R, right; L, left; UL, upper limb; LL, lower limb; SEM, standard error of mean. The CRPS region with the most severe pain is in **bold**. CRPS regions that are in remission are in *italics*. Medication taken in the last 24 hours of the day of testing are underlined.

4.5.2 Brainstem resting functional connectivity

Direct comparison of resting connectivity between CRPS and control participants revealed no significant differences in functional connectivity strength for any of the 9 brainstem seeds. To verify that there were no significant group differences we also extracted connectivity strength (beta values) on the left and right sides between the PAG and LC, the PAG and RVM, and the LC and RVM seeds and assessed significance (**Figure 4.2, Table 4.2**). Consistent with the voxel-by-voxel analysis we found no significant group differences between groups for any of these connectivity strengths.

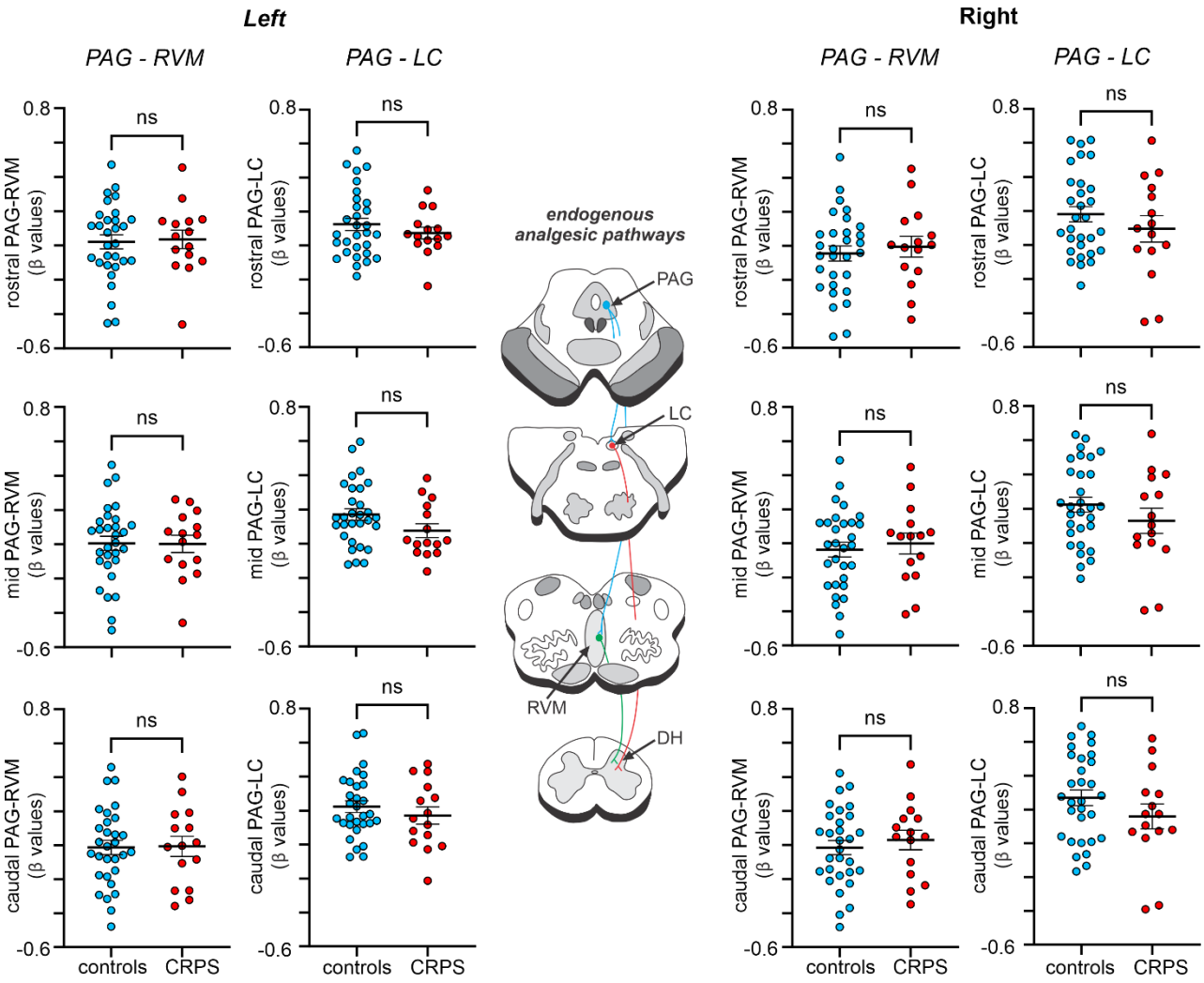

Figure 4.2. No significant connectivity strength group differences between brainstem seeds. The middle diagram shows the descending pain modulatory pathway with coloured lines indicating projections. Plots of individual participant connectivity strengths (beta values), with horizontal lines indicating the mean±SEM for each group. Left side plots are group connectivity strength comparisons between seeds on the left side and right side plots for comparisons between seeds on the right side. Ns: not significant; DH: dorsal horn. LC: locus coeruleus; PAG: midbrain periaqueductal gray matter; RVM: rostral ventromedial medulla.

Table 4.2. Connectivity strength (beta values) between brainstem seeds.

Seed-to-seed comparison	Beta values (β; mean ± SEM)	
	CRPS	Controls
Rostral PAG		
Left rostral PAG - left LC	0.07 ± 0.03	0.12 ± 0.04
Left rostral PAG - RVM	0.04 ± 0.05	0.02 ± 0.04
Right rostral PAG - right LC	0.10 ± 0.08	0.18 ± 0.05
Right rostral PAG - RVM	-0.005 ± 0.06	-0.05 ± 0.04
Mid PAG		
Left mid PAG - left LC	0.08 ± 0.04	0.17 ± 0.03
Left mid PAG - RVM	0.002 ± 0.05	0.005 ± 0.04
Right mid PAG - right LC	0.13 ± 0.07	0.22 ± 0.04
Right mid PAG - RVM	-0.001 ± 0.06	-0.04 ± 0.04
Caudal PAG		
Left caudal PAG - left LC	0.17 ± 0.05	0.22 ± 0.03
Left caudal PAG - RVM	-0.007 ± 0.06	-0.01 ± 0.04
Right caudal PAG - right LC	0.16 ± 0.07	0.27 ± 0.05
Right caudal PAG - RVM	0.03 ± 0.06	-0.01 ± 0.04
LC		
Left LC - RVM	0.07 ± 0.04	0.02 ± 0.04
Right LC - RVM	0.003 ± 0.06	0.02 ± 0.04

Abbreviations: PAG, midbrain periaqueductal gray matter; LC, locus coeruleus; RVM, rostral ventromedial medulla.

4.5.3 Whole brain resting functional connectivity

4.5.3.1 PAG

Whole brain voxel-by-voxel analysis of the PAG revealed significant functional connectivity differences for the left (contralateral to pain) caudal PAG and left mid PAG seed only. There were no significant functional connectivity differences between CRPS participants and controls for the left rostral PAG or any of the right PAG seeds. For the left caudal PAG seed, CRPS participants displayed significantly decreased functional connectivity strengths with the left primary somatosensory cortex (S1) in the region representing the upper body (mean±SEM beta values; CRPS: -0.02±0.01, controls: 0.04±0.01), right posterior cingulate cortex (PCC) (CRPS: -0.01±0.02, controls: 0.09±0.01), right caudate nucleus (CRPS -0.03±0.03, controls:

0.08±0.01), and the left cerebellar cortex (CRPS: -0.03±0.03, controls: 0.09±0.02; **Figure 4.3A**). In CRPS participants, the left caudal PAG to left S1 functional connectivity was negatively correlated to diary pain (r=-0.61, p=0.03) and PRWHE pain score (r=-0.75, p=0.006). That is, the greater the left caudal PAG to left S1 functional connectivity, the lower the diary pain and PRWHE score (reflecting low pain level) in CRPS participants. There were no other significant correlations between left caudal PAG connectivity strength and pain duration, day pain, diary pain, or PRWHE pain score (**Table 4.3**).

For the left mid PAG seed, compared to controls, CRPS participants also had significantly decreased connectivity strength with two clusters in the right PCC regions (CRPS: -0.01±0.02, controls: 0.09±0.01 and CRPS: -0.02±0.02, controls: 0.06±0.01) and one in the right caudate nucleus (CRPS: -0.04±0.02, controls: 0.06±0.01) (**Figure 4.3B**). In none of these clusters was connectivity significantly correlated with pain duration, day pain, diary pain or PRWHE pain score (**Table 4.3**).

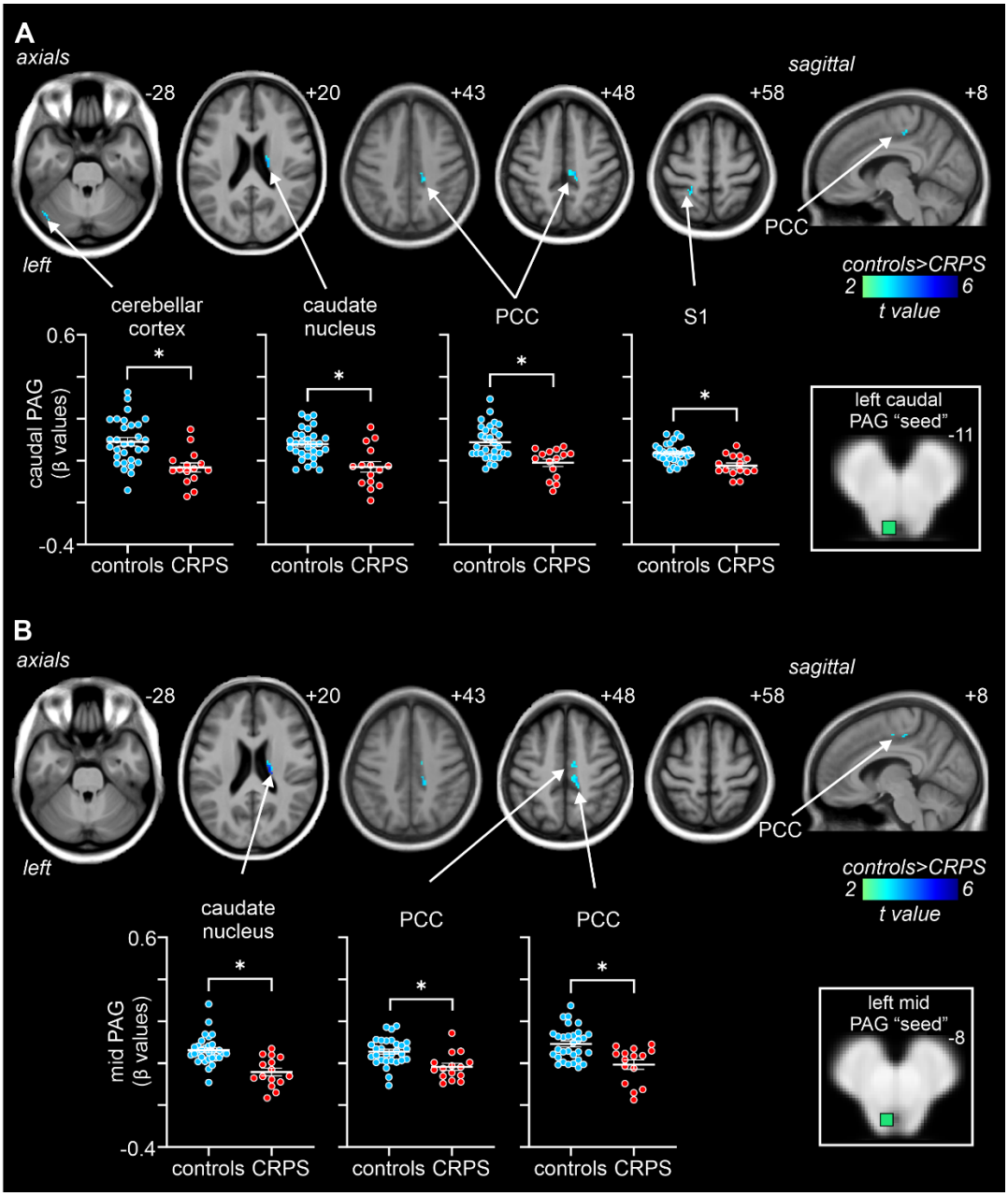

Figure 4.3. Decreased functional connectivity strength of the **(A)** left caudal midbrain periaqueductal gray matter (PAG) and **(B)** left mid PAG seed in CRPS participants compared to controls. ($p < 0.001$; uncorrected, minimum contiguous cluster size of 10 voxels, cool colour scale). Slice locations in Montreal Neurological Institute space are indicated on the top right of each axial and sagittal slice. Plots of individual participant connectivity strength (beta values), with horizontal lines indicating the mean ± SEM for each group. *, significant difference between groups determined by voxel-by-voxel analysis. PCC: posterior cingulate cortex; S1: primary somatosensory cortex.

4.5.3.2 LC

Whole brain voxel-by-voxel of the LC revealed significant functional connectivity differences for both the left and right LC seeds. CRPS participants displayed significantly greater left LC connectivity than controls with the left hippocampus (CRPS: 0.10±0.03, controls: -0.03±0.02), two left caudate nucleus clusters (CRPS: 0.13±0.03, controls: -0.02±0.02 and CRPS: 0.08±0.02, controls: -0.00002±0.01), left anterior insula (CRPS: 0.09±0.03, controls: -0.001±0.01) and the left posterior insula (CRPS: 0.09±0.02, controls: -0.01±0.02) (**Figure 4.4A**).

Analysis of the right LC seed revealed that CRPS participants displayed greater connectivity strength than controls in the right anterior cingulate cortex (ACC) (CRPS: 0.16±0.03, controls: 0.03±0.02), the right hippocampus (CRPS: 0.17±0.05, controls: -0.02±0.03) and the right parahippocampus (CRPS: 0.18±0.03, controls: 0.07±0.01; **Figure 4.4B**). There were no significant correlations between any of the significantly different clusters derived from the left or right LC seeds with pain duration, day pain, diary pain, or PRWHE pain score (**Table 4.3**).

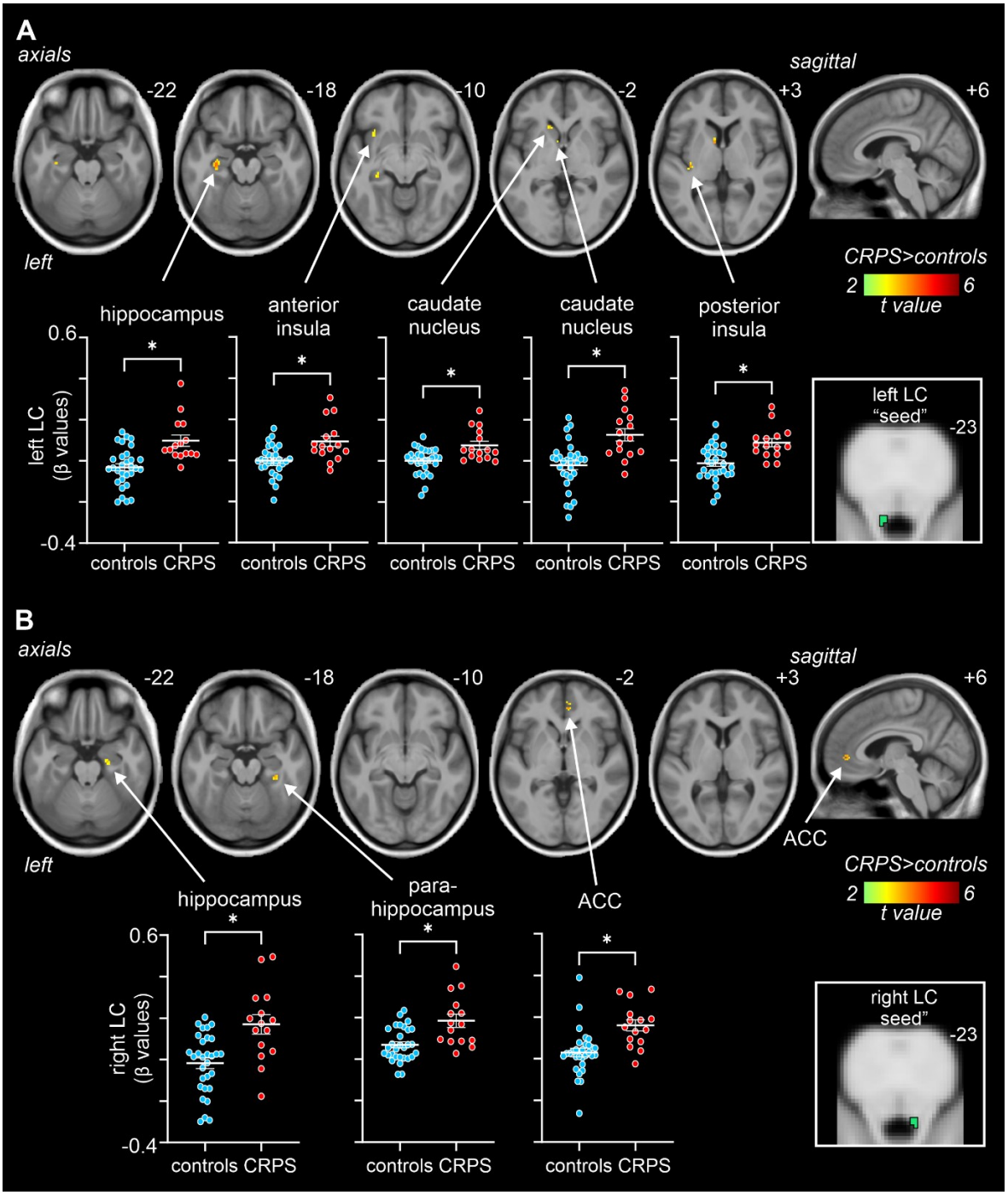

Figure 4.4. Increased functional connectivity strength of the **(A)** left locus coeruleus (LC) and **(B)** right LC seed in CRPS participants compared to controls. Slice locations in Montreal Neurological Institute space are indicated on the top right of each axial and sagittal slice. Plots of individual participant connectivity strength (beta values), with horizontal lines indicating the mean ± SEM for each group. *, significant difference between groups determined by voxel-by-voxel analysis. ACC: anterior cingulate cortex.

Table 4.3. Montreal neurological institute (MNI) coordinates, t-scores, cluster size, beta values, and correlations between beta values and pain for regions with significant functional connectivity differences between CRPS participants and healthy controls.

Region	MNI Coordinates			*t*-score	Cluster size	Correlation to pain of CRPS participants (Pearson's *r*)			
	X	Y	Z			Pain duration	Day Pain	Diary Pain	PRWHE pain score
Left caudal PAG seed									
CRPS < Controls									
left S1	-20	-44	58	3.85	34	-0.13	-0.19	-0.61*	-0.75*
right PCC	14	-28	46	3.95	46	0.11	-0.07	-0.52	-0.42
right caudate nucleus	16	-14	18	4.16	16	0.30	-0.41	-0.11	-0.14
left cerebellar cortex	-44	-70	-28	3.96	11	0.11	-0.26	-0.27	-0.25
Left mid PAG seed									
CRPS < Controls									
right PCC	14	-32	48	3.93	45	0.08	-0.17	-0.47	-0.33
	10	-12	48	3.84	15	-0.26	-0.36	-0.26	0.07
right caudate nucleus	18	-20	20	4.51	31	0.06	-0.11	0.08	0.11
Left LC seed									
CRPS > Controls									
left posterior insula	-34	-22	4	3.96	13	-0.08	-0.11	-0.52	-0.45
left caudate nucleus	-8	4	2	4.18	13	0.14	-0.36	-0.48	-0.49
	-14	20	-2	3.68	10	0.05	-0.09	-0.33	-0.57
left anterior insula	-32	14	-10	3.54	10	-0.16	-0.14	-0.32	-0.37
left hippocampus	-32	-20	-18	4.20	69	-0.27	-0.37	-0.56	-0.36
Right LC seed									
CRPS > Controls									
right ACC	6	42	-2	4.04	14	-0.37	0.08	-0.16	-0.004
right parahippocampus	32	-32	-18	3.87	14	-0.47	0.05	0.02	-0.14
right hippocampus	22	-16	-24	3.84	11	-0.54	0.02	-0.13	-0.02

* significant correlation between CRPS participant beta values and pain score (FDR-adjusted significance of correlation, $p < 0.05$). ACC: anterior cingulate cortex; LC: locus coeruleus; PAG: midbrain periaqueductal gray matter; S1: primary somatosensory cortex; PCC: posterior cingulate cortex; PRWHE, Patient-Rated Wrist and Hand Evaluation.

4.6 Discussion

Contrary to our hypothesis, CRPS participants did not display altered functional connectivity within the brainstem compared to pain-free controls. Specifically, there was no functional connectivity difference between PAG and LC, PAG and RVM, or LC and RVM in CRPS compared to pain-free controls. In contrast, consistent with our hypothesis, CRPS participants displayed altered functional connectivity between brainstem pain pathway regions and higher brain areas compared to controls. CRPS participants displayed decreased functional connectivity between the PAG and higher brain areas such as the S1 and PCC, and increased connectivity between the LC and areas including the caudate nucleus, hippocampus, and ACC. These results suggest that at rest, CRPS is associated with altered cortical influences over the PAG and LC, although PAG and LC descending connections to the RVM appear to be unaffected.

A number of previous investigations have suggested that CRPS is associated with altered pain processing at the level of brainstem. For example, Thoma et al. (2022) reported increased nociceptive blinking reflex excitability and decreased habituation in CRPS and Seifert et al. (2009) reported decreased habituation to painful electrical stimulation of the painful hand of CRPS participants. Additionally, Drummond and Finch (2021) suggested that photophobia experienced by CRPS participants is likely due to abnormal processing in brainstem pain processing regions. Whilst these studies have speculated brainstem involvement, they did not measure brainstem function directly. Our results suggest that at least at rest, connectivity between the major brainstem pain modulatory regions is not different in CRPS participants compared with controls.

This is in striking contrast to our previous study in which we reported significant differences in resting connectivity between the brainstem pain modulatory regions in individuals with chronic orofacial neuropathic pain (Mills et al., 2018). In our previous investigation, we found that chronic orofacial neuropathic pain was associated with significantly greater connectivity strengths between the RVM and the PAG, LC and RVM; here we found no such connectivity differences in individuals with CRPS. It should be noted that we used the same fMRI sequence in this study as that of this previous study which suggests the disparity does not stem from differences in image sensitivity or spatial resolution. Experimental animal investigations have revealed that the RVM contains "on" and "off" cells that can inhibit and facilitate the primary nociceptive synapse, respectively (Fields & Heinricher, 1985; Heinricher et al., 2009). In pain-free individuals, it has been proposed that this descending system is finely balanced whereas in individuals with chronic neuropathic pain, the system shifts to a pro-nociception state (Burgess et al., 2002; Heinricher, Barbaro & Fields, 1989b). Indeed, it has been previously reported that PAG-RVM connectivity increases during spontaneous increases in pain in individuals with orofacial neuropathic pain (Mills et al., 2020). Whilst we did not find any overall connectivity strength differences within the brainstem in CRPS, it is possible that the balance between PAG regulation of RVM on and off cells has shifted in equal amounts to increase "off" and decrease "on" cell inputs resulting a no net change in fMRI signal intensity. It is unlikely that the PAG controls noxious inputs via direct projections to the dorsal horn, since although tract tracing studies in primates and cats have shown some projects to the spinal cord, they primarily terminate in deep laminae (Mantyh, 1983; Mouton & Holstege, 1994).

Although we did not find alterations in connectivity within the brainstem, we did find decreased functional connectivity in both the caudal PAG and mid PAG contralateral to the CRPS affected side with higher brain areas such as the S1, PCC, and caudate nucleus in CRPS

participants. Interestingly, these connectivity differences only occurred in the contralateral caudal-mid levels of the PAG that receive inputs from the spinal cord and not from the more rostral PAG levels (Keay et al., 1997; Wiberg, Westman & Blomqvist, 1987) and where arm stimulation produces the greatest somatosensory evoked potential responses (Pereira et al., 2013). We found decreased functional connectivity between the contralateral caudal PAG seed and contralateral S1 as compared to pain-free controls. It is hypothesised that S1 is responsible for the conscious perception and spatial localisation of pain (Backonja, 1996; Bushnell, Ceko & Low, 2013) and that S1 stimulation can modulate pain (Xie, Huo & Tang, 2009). In a previous investigation, it was reported that CRPS is associated with increased resting oscillation power in the thalamic region that receives somatosensory inputs and CRPS participants displayed increased connectivity strengths between this thalamic region and the same S1 region reported in this study (Di Pietro, Lee & Henderson, 2020). It might be that the interactions between ascending information to S1 via both the PAG and thalamus are ultimately responsible for the intensity of on-going pain in CRPS. Indeed, we found that diary pain and PRWHE pain scores were negatively correlated to the functional connectivity between the left caudal PAG and S1, that is, the lower the connectivity between left caudal PAG and S1, the greater the pain intensity.

Both the left caudal and left mid PAG also displayed decreased functional connectivity to the PCC, a brain area also implicated in pain control. Whilst the ACC likely codes the affective component of pain, the PCC is involved in sensory orientation (Vogt, 2005). Most investigations linking the cingulate cortex with analgesia report changes in the ACC and its influence over the PAG-RVM circuitry (Xie, Huo & Tang, 2009). However, PCC fMRI signal changes are associated with conditioned pain modulation analgesia and PAG-PCC functional connectivity increases during electroacupuncture (Youssef, Macefield & Henderson, 2016a;

Zyloney et al., 2010). In addition, given the role of the PCC role in sensory orientation, changes in LC-PCC connectivity in CRPS may be related to avoidance or aversion to pain (Nielsen, Balslev & Hansen, 2005; Rolls, 2019).

In contrast to PAG seeds, in which connectivity with higher brain regions was reduced in CRPS participants, LC seeds displayed greater functional connectivity with higher brain centres including the caudate nucleus, ACC, and hippocampus. The caudate nucleus has been shown to be involved in pain modulation as it is activated during noxious stimulation as well as pain anticipation (Freund et al., 2009; Keltner et al., 2006; Wunderlich et al., 2011). In monkeys, electrical stimulation of the caudate decreased pain reactivity (Lineberry & Vierck, 1975). Whilst tract tracing studies have revealed that the LC sends projections directly to the spinal cord, these terminate primarily in deep laminae and the ventral horn, with only very sparse innervation of the superficial dorsal horn (Proudfit & Clark, 1991). Given this, it is likely that the differences in LC connectivity with higher brain regions in CRPS shown here represent ascending influences and not direct descending control over incoming nociceptive information. The LC has major ascending projections including towards areas such as the ACC and hippocampus as part of the LC-noradrenaline system (Schmidt, Bari & Chokshi, 2020; Taylor & Westlund, 2017). It has been postulated that shifts in this ascending system may develop and maintain allodynia and hyperalgesia through pain facilitation in chronic pain since stimulation of the noradrenergic LC-ACC projection in rodents produces nociceptive paw-wiping behaviours as well as decreased mechanical pain thresholds (Koga et al., 2020; Taylor & Westlund, 2017). Further, destruction of LC neurons reduces the development of and reverses once developed, the behavioural signs of pain in an animal model of neuropathic pain (Brightwell & Taylor, 2009). It is possible that ascending projections from the LC to higher

brain structures are at least partly responsible for the development and maintenance of pain in individuals with CRPS.

There are several limitations to this study worth noting. The PAG can be separated into anatomically and functionally distinct longitudinal columns (dorsomedial, dorsolateral, lateral, and ventrolateral) (Carrive, 1993; Keay & Bandler, 2008). However, due to the spatial resolution constraints at 3T, seed placements of the PAG into distinct longitudinal columns was not possible without considerable overlap of neighbouring PAG columns (Ezra et al., 2015). Higher resolution fMRI images at 7T are recommended for seed placements in distinct PAG longitudinal columns (Ezra et al., 2015; Faull et al., 2015). While we were able to determine changes in PAG and LC functional connectivity, we are unable to determine the direction of signal transmission, i.e., whether signals may be part of the ascending or descending pain pathways. Although resting state connectivity within brainstem pain modulatory regions were unaltered in CRPS, we do not know if activating the system by, for example, the application of noxious stimulus or an analgesic paradigm such as conditioned pain modulation, would reveal significant functional differences within this brainstem system. In addition, the use of relatively large voxel sizes limits our ability to explore precise regional variations in areas such as the PAG. Given this structure has a detailed organization with different roles in pain processing (Bandler & Keay, 1996), it may be the case that greater spatial resolution might reveal regionally specific differences, particularly in small brainstem structures. Finally, the statistical threshold of the 2-sample t-tests remains uncorrected for multiple comparisons. While we have increased the threshold to 0.001 and 10 minimum contiguous voxels, there is still the possibility of type 2 errors. Although difficult to achieve practically, future studies with larger sample sizes may overcome this limitation.

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Chapter 5
Discussion

Chapter 5 Discussion

Pain and motor dysfunction are primary contributors to disability in longstanding CRPS (Bean, Johnson & Kydd, 2014). The overall aim of this thesis was to investigate the role of the interconnected brain networks, including the cortical, basal ganglia, and brainstem structures, in CRPS pain and motor dysfunction. To achieve this, a series of three studies have been performed and presented in this thesis. The key research findings of each study will be summarised, and their collective implications and contribution to CRPS pathogenesis will be discussed (see **Figure 5.2**). Limitations and potential future research will be identified.

5.1 Summary of key research findings

The sensorimotor cortex is involved in pain and motor control. Consistently, sensorimotor cortical reorganisation and disinhibition have been found in CRPS and GABAergic mechanisms are thought to underlie these CRPS changes (Di Pietro et al., 2013a; Di Pietro et al., 2013b; Eisenberg et al., 2005; Lenz et al., 2011; Pfannmöller et al., 2019; Schwenkreis et al., 2003). These changes are thought to be so central to CRPS that therapies have been designed and implemented to restore sensorimotor cortical reorganisation and disinhibition (Guillot et al., 2008; Lotze et al., 1999; Strauss et al., 2021). Chapter 2 was the first study to directly investigate GABA and glutamate concentration in the sensorimotor cortex of CRPS using magnetic resonance spectroscopy (MRS). In contrast to the hypothesised decrease in sensorimotor GABA concentration in CRPS, no differences were found in sensorimotor cortex GABA or glutamate concentration between CRPS and controls or between CRPS affected and unaffected representative hemispheres. Chapter 2, therefore, reveals that pain and motor

dysfunction in CRPS are likely unrelated to GABA or glutamate concentrations in the sensorimotor cortex.

Like the sensorimotor cortex, the basal ganglia are also important in motor dysfunction as well as pain (Alexander, DeLong & Strick, 1986; Borsook et al., 2010; Starr et al., 2011). It has been determined that the basal ganglia are organised into specific functional loops responsible for motor and non-motor roles (Alexander, DeLong & Strick, 1986). However, in CRPS, the basal ganglia have not been systematically evaluated. To address this, resting-state functional magnetic imaging (fMRI) was used to systematically investigate infraslow oscillations (ISOs) and functional connectivity of motor (putamen) and non-motor basal ganglia loops in CRPS compared to pain-free controls (Chapter 3). In Chapter 3, it was found that CRPS patients had increased ISO power in the motor putamen representing the hand, SMA hand, and tongue compared to controls, which may indicate astrogliosis and increased astrocytic calcium waves in the CRPS basal ganglia. Additionally, in CRPS, the same motor putamen regions had increased resting functional connectivity to the M1, cingulate motor areas (CMA), parietal association areas, and orbitofrontal cortex (OFC) compared to controls. Increased motor putamen ISO power and functional connectivity in CRPS were correlated to greater pain ratings and perceived motor dysfunction. Except for increased functional connectivity of the caudate nucleus body to areas such as the sensorimotor cortex, CMA and OFC, the non-motor basal ganglia regions did not display ISO or functional connectivity differences between CRPS and controls. Chapter 3, therefore, reveals that there is altered ISO power and functional connectivity of the basal ganglia, and these changes may be involved in pain and possibly motor dysfunction in CRPS.

Additionally, like the sensorimotor cortex and basal ganglia, the brainstem is involved in pain, and it has been postulated that brainstem function is altered in CRPS (Drummond & Finch, 2021; Drummond & Finch, 2022; Drummond et al., 2018a; Seifert et al., 2009; Thoma et al., 2022). Further, the facilitation of pain via brainstem nuclei and its descending pathways has been identified in other chronic pain conditions (Mills et al., 2018). However, in CRPS, no study had investigated the modulatory pain pathways involving the PAG, RVM, and LC brainstem nuclei. The aim of Chapter 4, therefore, was to use resting-state fMRI to investigate the functional connectivity of brainstem pain modulatory pathways involving the PAG, RVM, and LC in CRPS compared to pain-free controls. Compared to controls, in Chapter 4, CRPS patients had no functional connectivity differences between brainstem nuclei. However, in CRPS, there is altered brainstem to higher brain area functional connectivity, with decreased functional connectivity of the contralateral PAG to S1, and posterior parietal cortex, and increased functional connectivity of the LC to the caudate nucleus, anterior cingulate cortex, and hippocampus, as compared to controls. Further, decreased functional connectivity between the contralateral PAG and S1 was related to increased pain intensity in CRPS. Chapter 4, therefore, demonstrates that altered connectivity of brainstem nuclei involved in modulatory pain pathways, specifically the PAG and LC, may be involved in pain in CRPS.

5.2 Implications

5.2.1 CRPS cortical reorganisation, sensorimotor disinhibition, and the $GABA_A$ receptor

Despite the lack of differences in CRPS sensorimotor GABA compared to pain-free controls, altered GABAergic mechanisms are still likely to be involved in the pathology of CRPS pain and motor dysfunction. Sensorimotor disinhibition studies in CRPS have mostly been conducted with transcranial magnetic stimulation (TMS), which has indicated reduced short intracortical inhibition (Eisenberg et al., 2005; Lenz et al., 2011; Schwenkreis et al., 2003). Further, the authors of these TMS studies have proposed that sensorimotor disinhibition in CRPS may involve abnormal GABA activity. Importantly, however, TMS measures of inhibition do not correlate with MRS measures of GABA concentration (Dyke et al., 2017; Tremblay et al., 2013a). Rather, it is proposed that TMS measures of inhibition reflect a transient response or "phasic" inhibition to a stimulus, while MRS measures of GABA are linked to "tonic" sustained inhibition (Dyke et al., 2017). Further, increased short intracortical inhibition in TMS is mediated by increased $GABA_A$ receptor activity (Di Lazzaro et al., 2006; Ilić et al., 2002). Therefore, since there is reduced short intracortical inhibition (Eisenberg et al., 2005; Lenz et al., 2011; Schwenkreis et al., 2003), there may be decreased $GABA_A$ receptor activity in CRPS. Further supporting altered $GABA_A$ receptor activity in CRPS is the cortical reorganisation of the sensorimotor cortex in CRPS (Juottonen et al., 2002; Krause, Förderreuther & Straube, 2006; Maihöfner et al., 2007; Maihöfner et al., 2003; Maihöfner et al., 2004; Pfannmöller et al., 2019; Pleger et al., 2004; Vartiainen et al., 2009). In animal studies, cortical reorganisation is partly controlled by $GABA_A$ receptors where $GABA_A$ receptor antagonists increase sensorimotor somatotopic representative field sizes, and following neuropathic injury, $GABA_A$ receptor binding is reduced in the S1 (Mowery, Walls & Garraghty, 2013; Tremere, Hicks & Rasmusson, 2001a; Tremere, Hicks & Rasmusson, 2001b; Wellman

et al., 2002). Thus, it is postulated that altered $GABA_A$ receptor activity is involved with CRPS cortical reorganisation and sensorimotor disinhibition.

Interestingly, more recent studies challenge the view of cortical S1 reorganisation in CRPS as no differences in the S1 hand representation size were identified between CRPS hemispheres or between CRPS and healthy controls (Mancini et al., 2019; Strauss et al., 2021). Strauss et al. (2021) and Mancini et al. (2019) argue that such contradictory results are due to improvements in methodological approach, where Strauss et al. (2021) and Mancini et al. (2019) use fMRI which has higher spatial resolution than previous EEG/MEG studies of CRPS S1 cortical reorganisation (Juottonen et al., 2002; Maihöfner et al., 2003; Pleger et al., 2004; Vartiainen et al., 2009). The contrasting findings of S1 cortical reorganisation in CRPS imply that potential GABAergic differences may be less involved in CRPS than first postulated in Chapter 2.

Potential GABAergic mechanisms, however, remain important in CRPS. Pharmacological treatment of CRPS such as ketamine produces its analgesic effects primarily through increased GABAergic transmission (Sorel et al., 2018), which is mainly mediated by $GABA_A$ receptor activity (Tabata & Kano, 2010). Interestingly, $GABA_B$ receptor agonists, like gabapentin and baclofen are used to treat CRPS, where gabapentin has a small effect on pain and baclofen improves pain and motor dysfunction in difficult-to-treat CRPS only (Bertrand et al., 2001; van de Vusse et al., 2004). Additionally, following GMI, an intervention theoretically aimed at restoring CRPS S1 somatotopic representations and disinhibition (O'Connell et al., 2013), there was increased short intracortical inhibition along with improvements in pain and motor function (Strauss et al., 2021). Thus, while sensorimotor GABA concentration is less likely to

be involved, GABAergic mechanisms particularly involving GABA receptors in the sensorimotor cortex may be involved in CRPS pain and motor dysfunction. Future CRPS research into GABA receptors is warranted for a better understanding of CRPS pathophysiology and development of more efficacious treatments.

5.2.2 Sensorimotor cortex functional connectivity in CRPS

Many resting-state fMRI studies have found altered functional connectivity of the sensorimotor cortex in CRPS (Azqueta-Gavaldon et al., 2020; Bolwerk, Seifert & Maihöfner, 2013; Di Pietro, Lee & Henderson, 2020; Kim et al., 2017; Shokouhi et al., 2018). Compared to controls, Bolwerk, Seifert and Maihöfner (2013) found greater sensorimotor cortex functional connectivity with the thalamus, cingulate, parietal, temporal, and temporal cortices in CRPS, while Shokouhi et al. (2018) found reduced M1 connectivity to the superior parietal lobule. Further, it has been found that there is decreased insular to S1 (Kim et al., 2017), increased thalamus to S1 (Di Pietro, Lee & Henderson, 2020), and increased putamen to S1 functional connectivity (Azqueta-Gavaldon et al., 2020). This thesis, consistent with the broader literature, also found altered functional connectivity to the sensorimotor cortex in CRPS compared to controls. The motor (putamen) and non-motor (caudate body) loops of the basal ganglia displayed increased functional connectivity to the hand and arm representative area of the S1 and M1 (Chapter 3), while in brainstem modulatory pain pathways, there was decreased PAG to S1 functional connectivity (Chapter 4). More importantly, altered sensorimotor functional connectivity correlated to pain intensity and motor dysfunction (Chapters 3 and 4). In CRPS decreased functional connectivity between the sensorimotor and insular cortex is associated with pain severity (Bolwerk, Seifert & Maihöfner, 2013; Kim et al., 2017), and increased

putamen to sensorimotor cortex functional connectivity is correlated to increased motor impairment and pain intensity (Azqueta-Gavaldon et al., 2020). Further, increased thalamus to S1 connectivity is correlated to decreased pain intensity (Di Pietro, Lee & Henderson, 2020) (

). Indeed, this thesis also found that sensorimotor functional connectivity correlated to pain and motor dysfunction, with increased putamen-M1 functional connectivity correlated to increased motor dysfunction perception (Chapter 3), and decreased PAG-S1 functional connectivity correlated to greater pain intensity in CRPS (Chapter 4). Despite CRPS investigations of different brain regions and neural networks including the basal ganglia and modulatory pain pathways, functional connectivity to the sensorimotor cortex is consistently altered, and these alterations correlate to CRPS pain and motor dysfunction. Therefore, altered sensorimotor cortex connectivity may contribute to pain and motor dysfunction in CRPS.

5.2.3 Basal ganglia, CRPS, and Parkinson's disease

Basal ganglia dysfunction has been found in dystonia as well as Parkinson's disease (Wichmann & Delong, 2011). In Chapter 3, basal ganglia dysfunction involving motor and non-motor loops was identified in CRPS. CRPS and Parkinson's disease share some similar motor disturbances, such as tremor and dystonia (Bruehl, 2015; Magrinelli et al., 2016) as well as altered thalamocortical rhythm (Di Pietro, Lee & Henderson, 2020; Vanneste, Song & De Ridder, 2018). Further, compared to controls, CRPS patients have greater putamen to M1 functional connectivity (Chapter 3) and in both CRPS and Parkinson's greater putamen to M1 functional connectivity is associated with poorer motor function (Azqueta-Gavaldon et al.,

2020; Simioni, Dagher & Fellows, 2015). Given the similarities in disease presentation and brain changes, treatments used for Parkinson's disease may help in CRPS.

5.2.4 Basal ganglia, GABA, and glutamate

The basal ganglia facilitates (direct pathway) and inhibits (indirect pathway) movement via a series of inhibitory GABAergic and excitatory glutamatergic and dopaminergic transmissions between basal nuclei, the thalamus, and the cortex (Fiore et al., 2016; Svensson et al., 2019) (**Figure 5.1**). Under physiological conditions, glutamatergic neurons from the cortex (SMA, S1, M1, premotor) projects to the striatum (caudate and putamen). From the striatum, GABAergic projections of the direct pathway terminates at the internal globus pallidus (GPi) and the indirect pathway terminates at the external globus pallidus (GPe). The subthalamic nucleus (STN) receives glutamatergic projections from the GPe and via GABAergic neurons projects to the GPi. GABAergic neurons from the GPi then project to the thalamus, and the thalamus projects back onto the cortex via glutamatergic neurons, completing the basal ganglia loop (Fiore et al., 2016; Svensson et al., 2019). Both the direct and indirect pathways are modulated at the striatum by dopaminergic transmission from the substantia nigra pars compacta (SNpc) (Filippo et al., 2008; Fiore et al., 2016; Svensson et al., 2019) (**Figure 5.1**).

The balance of inhibitory and excitatory transmission within the basal ganglia are critical to motor function as an imbalance is implicated in diseases with motor dysfunction. For example, in Huntington's disease, hypokinesia or slowed movement can be induced by the loss of striatal GABAergic projection neurons of the direct pathway, leading to increased GPi GABA release and subsequent inhibition of the thalamus and decreased thalamic glutamatergic release onto the cortex (André, Cepeda & Levine, 2010; Blumenstock & Dudanova, 2020). In addition, in

Parkinson's disease hypokinesia and tremor are a result of the loss of dopaminergic neurons in the SNpc which ultimately leads to increased GPi GABAergic transmission to the thalamus and decreased cortical excitation by thalamic glutamate (Filippo et al., 2008; Jankovic & Tan, 2020). It could therefore be suggested that CRPS motor dysfunction may involve neurotransmitter imbalances within in the basal ganglia pathways.

However, any neurotransmitter change in the motor basal ganglia would likely lead to a change in cortical glutamate concentrations due to the looped architecture of basal ganglia transmission. Given that the motor basal ganglia loop projects to the SMA, within the sensorimotor cortex, and that there were no glutamate differences in the sensorimotor cortex of CRPS (Chapter 2), it remains unlikely that there are neurotransmitter concentration differences within the thalamocortical projection of the motor basal ganglia loop in CRPS. Having said that, it should be noted that whilst the motor basal ganglia loop receives input from large areas of the sensorimotor cortex, output via thalamocortical projections is restricted to the SMA and not the sensorimotor cortex as a whole (see **Chapter 1**, **Figure 1.1**). Hence, measuring glutamate concentrations of the SMA would better reflect any neurotransmitter differences within the motor basal ganglia loop of CRPS rather than the glutamate concentration of the sensorimotor cortex. Future studies of CRPS should investigate SMA glutamate concentrations using MRS.

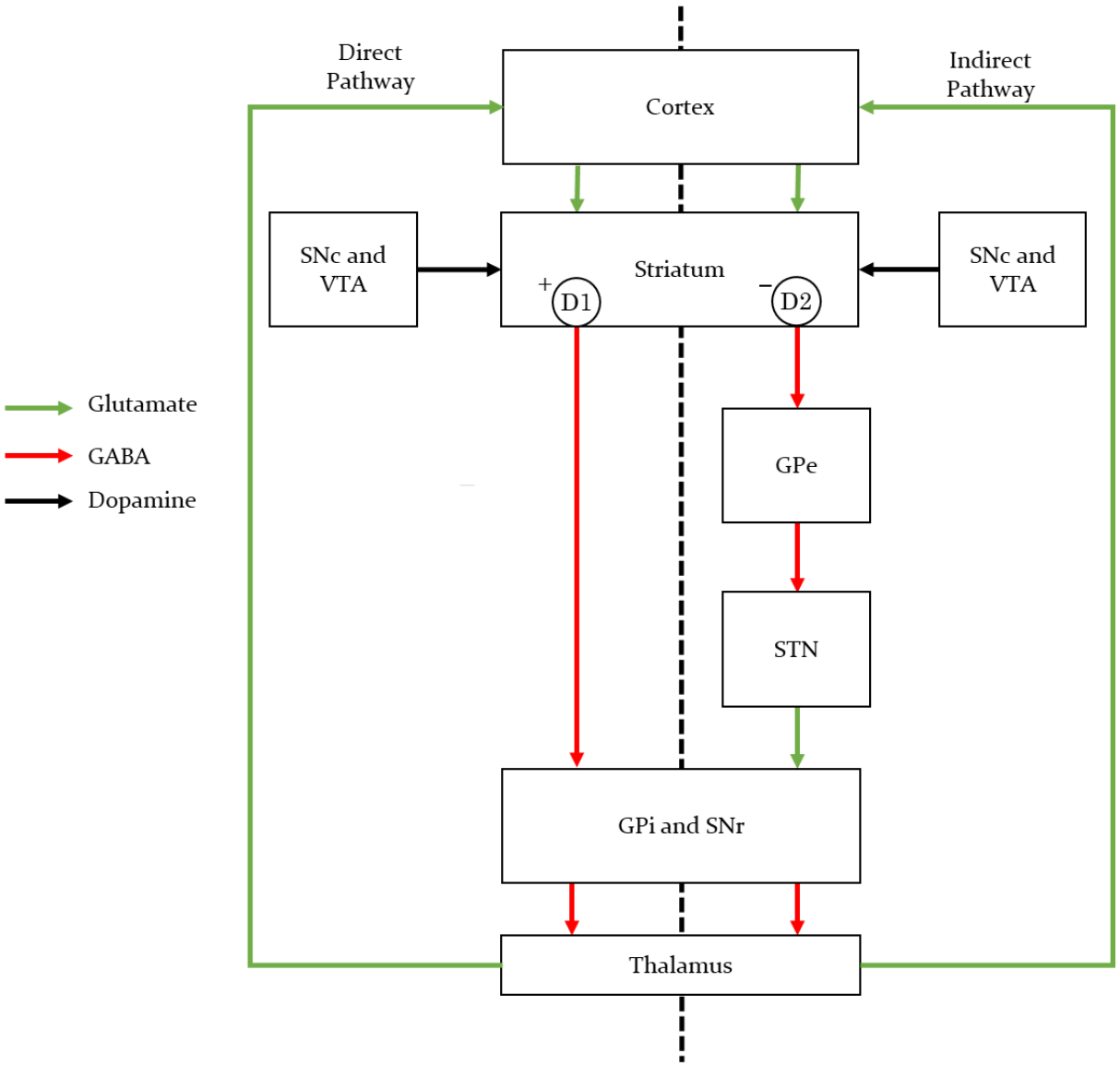

Figure 5.1. Neurotransmitters and the direct and indirect pathways of the basal ganglia. The direct and indirect pathways of the basal ganglia are respectively responsible for initiating and inhibiting movement. Dopaminergic signals from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) activates D1 receptors in the direct pathway and inhibits D2 receptors in the indirect pathway (Filippo et al., 2008; Fiore et al., 2016; Svensson et al., 2019). Abbreviations: GPe = external globus pallidus, GPi = internal globus pallidus, SNc = substantia nigra pars compacta, SNr = substantia nigra pars reticulata, STN = subthalamic nucleus, VTA = ventral tegmental area

5.2.5 CRPS treatments targeting the basal ganglia

5.2.5.1 Levodopa

In Parkinson's disease, levodopa is the most used pharmacological treatment to improve motor dysfunction and acts by replacing dopaminergic transmission of the nigrostriatal pathway (Armstrong & Okun, 2020; Bloem, Okun & Klein, 2021; Gao et al., 2017). Interestingly, treatment targeting the basal ganglia inhibitory/excitatory balance has been effective in treating motor dysfunction in a single case study of CRPS, where levodopa was able to improve signs of CRPS-associated tremor (Navani et al., 2003). While this may suggest the possibility of dopaminergic basal ganglia changes in CRPS, the authors suggested that the patient may have had another underlying movement disorder (Navani et al., 2003). Further, given that this was a single patient, and that there were no glutamate differences in a sample of fourteen CRPS patients (Chapter 2), it is unlikely that there are dopamine concentration changes in CRPS.

5.2.5.2 Deep brain stimulation

Given the basal ganglia changes in CRPS found in Chapter 3, treatments targeting the basal ganglia may be beneficial to CRPS pain and motor function. Deep brain stimulation involving the implantation of electrodes into basal ganglia areas such as the internal globus pallidus, has also been used in Parkinson's disease as well as dystonia with improvements in quality of life as well as motor function (Wichmann & Delong, 2011). Of interest, in a CRPS case study deep brain stimulation of the internal globus pallidus not only significantly alleviated dystonic motor impairment but also pain and allodynia in two patients with CRPS (Javed et al., 2011). Additionally, deep brain stimulation of M1, which is a part of the motor basal ganglia loop,

reduces pain and resolves signs of allodynia and hyperalgesia (Lopez et al., 2016; Velasco et al., 2009). Chapter 3 findings of basal ganglia dysfunction in CRPS may therefore provide a basis for treatment strategies that target the basal ganglia to alleviate pain and motor dysfunction in CRPS.

5.2.6 Altered ascending pain pathways in CRPS involve the LC

CRPS involves changes to the ascending rather than descending pain pathways. This thesis presents the first study to directly investigate the PAG, LC, and RVM brainstem nuclei of the modulatory pain pathways. Descending modulatory pain projections from the PAG and LC project to the RVM (Boadas-Vaello et al., 2016; Clark & Proudfit, 1991), with sparse LC projections directly to the dorsal horn (Proudfit & Clark, 1991). However, in comparison to controls, there were no functional connectivity differences between the PAG or LC with the RVM, instead, functional connectivity of the PAG and LC to higher brain areas was altered in CRPS (Chapter 4). Hence, rather than altered descending pain pathways, CRPS may involve altered ascending pain pathways.

Changes to the ascending pain pathways in CRPS may involve the LC-noradrenaline system. In Chapter 4, it was found that there was increased LC-ACC functional connectivity in CRPS compared to controls. Interestingly, in mice, stimulation of the LC-ACC pathway induced nociceptive behaviour changes such as paw scratching and wiping, and decreased pain threshold to mechanical stimulation (Koga et al., 2020). Further, electrical stimulation of the LC enhanced ACC neural activity via noradrenaline and α1 adrenergic receptors (Koga et al., 2020). Given that the LC is the primary producer of NA and that the ACC receives major

ascending projections from the LC (Taylor & Westlund, 2017), enhanced LC-ACC connectivity in CRPS may reflect enhanced ascending LC-NA projections to the ACC which may be involved with pain in CRPS.

5.2.7 Basal ganglia ISO, LC-NA system, and α1 adrenergic receptor on astrocytes

ISO changes in CRPS may be related to the LC-NA system. In addition to the enhanced LC-ACC functional connectivity, CRPS was associated with enhanced LC-caudate nucleus functional connectivity compared to controls (Chapter 4). Further, the caudate nucleus had increased functional connectivity to higher brain regions such as the sensorimotor cortex (S1 and M1 hand area) (Chapter 3). The caudate, as well as the putamen and thalamus, receives noradrenergic innervation from the LC (Mason & Fibiger, 1979). Compared to controls, in CRPS there was increased ISO power in the putamen (Chapter 3) and thalamus (Di Pietro, Lee & Henderson, 2020) at the 0.03-0.06 Hz range which corresponds to astrocytic calcium wave propagation and astrocytic gliotransmitter release. It is hypothesised that such increases in ISO power reflect chronic astrogliosis (Henderson & Di Pietro, 2016). Indeed, compared to controls, the caudate, putamen, and thalamus in CRPS had increased [11C]-(R)-PK11195 binding, a marker of activated astrocytes and microglia (Jeon et al., 2017; Seo et al., 2021; Yao et al., 2020). Of interest, noradrenaline activation of α1 adrenergic receptors on astrocytes increases astrocytic intracellular calcium levels and leads to subsequent gliotransmitter release (Oe et al., 2020; Wahis & Holt, 2021). In addition, increased putamen ISO power is negatively correlated to pain in CRPS (Chapter 3), and in mice, increasing intracellular astrocytic calcium leads to impaired motor coordination (Agulhon et al., 2013). Therefore, changes to ISO power at the

0.03-0.06 Hz range may be mediated by the α1 adrenergic receptor and the LC-NA system and may maintain pain and motor dysfunction in CRPS.

Interestingly, similar NA astrocytic- and nociceptor/keratinocyte-α1 receptor interactions in the spinal cord and peripheral nerves may drive CRPS pain. In a post-mortem immunohistochemical study of the spinal cord, astrogliosis was identified in the dorsal horn of CRPS subjects (Del Valle, Schwartzman & Alexander, 2009). Further, it has been reported that α1 receptor expression in peripheral nociceptors and keratinocytes is upregulated in CRPS and activation of α1 receptors using α1-agonist phenylephrine was associated with prolonged pain and pinprick hyperalgesia in CRPS (Drummond et al., 2018b). Additionally, NA is increased in the blood plasma of CRPS patients (Harden et al., 2004) (although it should be noted that NA in the bloodstream is unlikely to be derived from the LC as NA cannot cross the blood-brain barrier). Thus, NA- astrocytic- and nociceptor/keratinocyte- α1 receptor interactions in the spinal cord and periphery may contribute to pain in CRPS. Importantly, such peripheral interactions may lead to central changes in CRPS. Indeed, in chronic pain conditions, immune and glial activation in the periphery can result in neuroinflammation in the brain via immune-to-brain transmission routes (Austin & Fiore, 2019; Capuron & Miller, 2011). Hence, it is possible that neuroinflammation of the thalamus and basal ganglia via astrogliosis may be in part driven by peripheral changes in CRPS. It should be noted, however, that peripheral-driven neuroinflammatory changes may potentially only occur during the acute phase of CRPS as skin blister fluid and serum cytokine levels after 6 months of CRPS were demonstrated to be comparable with healthy controls (Lenz et al., 2013). Therefore, while CRPS pain may be mediated by astrocyte- and keratinocyte/nociceptor-α1 receptor interactions in the brain and periphery, respectively, the maintenance of CRPS chronic pain may primarily involve changes in the brain.

5.2.8 The caudate nucleus, basal ganglia, and brainstem

The caudate nucleus is important in the integration of spatial information during motor preparation and lesions to the caudate are associated with spatial neglect (Karnath, Himmelbach & Rorden, 2002; Karnath & Rorden, 2012; Postle & D'Esposito, 1999). Of interest, the caudate nucleus is also involved in acute pain and chronic pain conditions (Borsook et al., 2010) and the spatial discrimination of pain involves the caudate nucleus (Oshiro et al., 2007). More importantly, it has been suggested that the caudate nucleus plays a role in the pain modulatory system as the caudate nucleus is activated during the suppression and anticipation of thermal and electrical pain in healthy individuals (Freund et al., 2009; Freund et al., 2007; Keltner et al., 2006; Wunderlich et al., 2011). In addition, together with the caudate nucleus, the insular cortex and dorsolateral prefrontal cortex (DLPFC) are also simultaneously activated during the suppression of pain (Freund et al., 2009; Freund et al., 2007; Keltner et al., 2006; Wunderlich et al., 2011). Indeed, in CRPS, painful electrical stimulation displayed stronger caudate nucleus and insular cortex activation than controls (Freund et al., 2010). Therefore, given that Chapter 3 found increased caudate to DLPFC functional connectivity, and Chapter 4 found increased LC to caudate and insular connectivity, changes in caudate nucleus connectivity may underlie aspects of CRPS motor dysfunction as well as symptoms of neglect-like syndrome and mislocalised sensation of pain. Additionally, although, there was no overlap between Chapter 3 and 4 caudate regions, increased connectivity of the LC and caudate to the insular and DLPFC, regions associated with pain modulation, suggest that there may be some overlapping disruptions of the basal ganglia and brainstem pain modulatory circuits in CRPS. Thus, not only is the caudate nucleus involved in altered motor planning and spatial discrimination in CRPS but also altered pain modulation potentially through interaction with brainstem pain pathways.

5.2.9 GABA, basal ganglia and brainstem: Astrocytic α1-NA receptor signaling and $GABA_A$ receptors

The CRPS mechanisms proposed in this thesis are highly likely to be interconnected. The thesis has postulated that CRPS cortical reorganisation and sensorimotor disinhibition may be due to altered $GABA_A$ receptor activity and CRPS pain and motor dysfunction may be driven by LC-derived NA activation of astrocytic α1 adrenergic receptors (**Figure 5.2**). Of interest, GABAergic inhibition is modulated by astrocytes (Wahis & Holt, 2021). As a result of NA activation of astrocytic α1 adrenergic receptors, intracellular calcium levels of astrocytes increase, and subsequently, adenosine triphosphate (ATP) is exocytosed and released (Gordon et al., 2005; Oe et al., 2020; Wahis & Holt, 2021). The ATP released by astrocytes not only reduces the amplitude of $GABA_A$-mediated currents but also downregulates synaptic and extrasynaptic $GABA_A$ receptors via neuronal P2X purinergic receptors (Gordon et al., 2005; Wahis & Holt, 2021). Given that the motor basal ganglia circuit tightly regulates inhibitory/excitatory transmission, the motor basal ganglia may be affected by downregulated $GABA_A$ current amplitudes. Therefore, in CRPS as well as mediating pain and motor dysfunction, α1-NA receptor signalling in astrocytes may mediate cortical reorganisation and sensory disinhibition via downregulation of $GABA_A$ receptor (**Figure 5.2**).

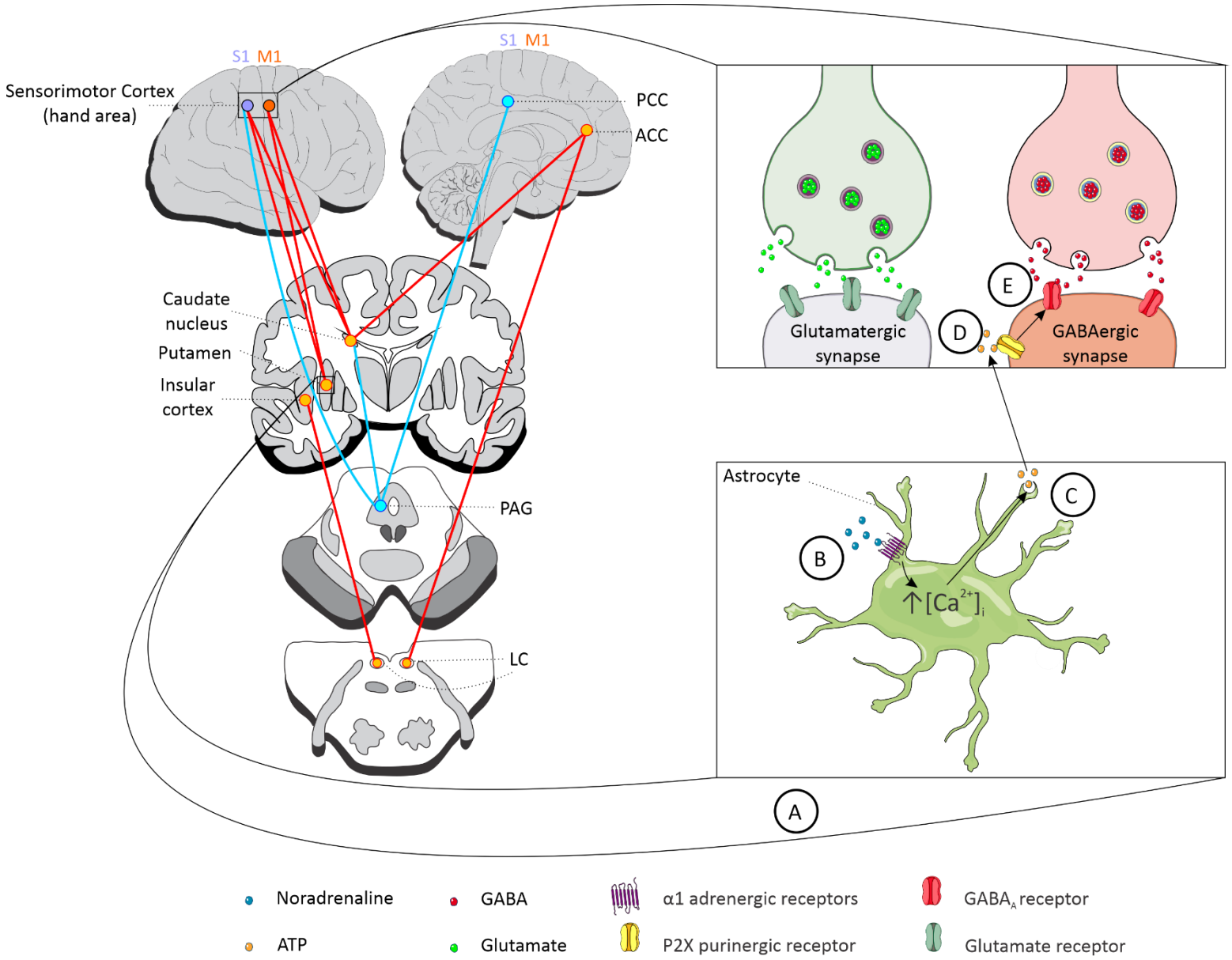
S1 M1
S1 M1
Sensorimotor Cortex
(hand area)
PCC
ACC
Caudate
nucleus
Putamen
Insular
cortex
PAG
LC
Glutamatergic
synapse
GABAergic
synapse
D
E
Astrocyte
B
C
↑[Ca2+]i
A
Noradrenaline
ATP
GABA
Glutamate
α1 adrenergic receptors
P2X purinergic receptor
GABAA receptor
Glutamate receptor

Figure 5.2. Summary of key findings and potential mechanisms. In CRPS compared to controls, increased (red lines) and decreased (blue lines) functional connectivity between brain regions are indicated on the left (Chapters 3 and 4). Potential brain mechanisms of CRPS pathogenesis are depicted on the right. Basal ganglia and brainstem functional connectivity to the sensorimotor cortex and caudate nucleus was altered indicating possible interaction between the basal ganglia and brainstem in CRPS pain and motor dysfunction. CRPS pain intensity was positively correlated with **A)** increased ISO power at the 0.03-0.06Hz range found in the putamen contralateral to the CRPS affected side (Chapter 3), which may indicate increased astrocytic calcium wave propagation and astrogliosis. The putamen receives noradrenergic innervation from the locus coeruleus (LC). **B)** Noradrenaline activation of α1 adrenergic receptors on astrocytes increases astrocytic intracellular calcium levels, which may account for the increased ISO power at 0.03-0.06 Hz. **C)** Increased astrocytic intracellular calcium levels can lead to exocytosis of adenosine triphosphate (ATP). **D)** ATP can act on neuronal P2X purinergic receptors which then **E)** downregulate $GABA_A$ receptors and $GABA_A$ current amplitudes, which may potentially explain CRPS cortical reorganisation and sensorimotor disinhibition in CRPS, despite CRPS sensorimotor glutamate and GABA concentrations remaining unchanged compared to controls (Chapter 2). Abbreviations: ACC = anterior cingulate cortex, LC = locus coeruleus, M1 = primary motor cortex, PAG = periaqueductal gray, PCC = posterior cingulate cortex, S1 = primary somatosensory cortex. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

5.3 Limitations and Future Research

The majority of CRPS patients investigated in this thesis were taking medications for their pain and other comorbidities. Medications may have exerted an effect on the results presented in this thesis. Indeed, using MRS, GABAergic drugs such as gabapentin have been found to increase in vivo GABA concentrations (Cai et al., 2012). Additionally, CRPS patients were using μ-opioid drugs such as codeine and noradrenaline uptake inhibitors such as duloxetine, which can affect pain modulation pathways (Fornasari, 2014; Snyder, 2014; Taylor & Westlund, 2017). However, while medications may have had a potential effect, it was not feasible or ethical to exclude CRPS patients that were taking medications due to the rarity of the condition. Future studies could include a washout period before investigation or exclude certain types of medications such as GABAergic or μ-opioid drugs.

Comorbidities and other pain conditions may also have influenced the results. Some of the CRPS patients who participated in these studies had multiple comorbidities and CRPS patients were not excluded if they had painful comorbidities such as fibromyalgia, osteoarthritis, and migraine (see **Chapter 3, Table 1**). However, comorbidities between individual CRPS patients varied substantially and therefore are less likely to contribute to the results of this thesis. Further, although the CRPS population of this thesis had comorbidities, all patients had upper limb CRPS in common, which was confirmed according to the Budapest criteria of CRPS at the time of the study (Harden et al., 2010; Harden et al., 2007). Future studies could aim to exclude painful comorbidities to better identify brain changes that are unique to CRPS pain and not any other potential painful comorbidity.

Critically, because CRPS is an uncommon condition, only a small number of CRPS patients were able to be recruited for this thesis. However, the sample size of CRPS patients in this thesis is comparable to other CRPS studies (Di Pietro et al., 2013a; Di Pietro et al., 2013b; Mancini et al., 2019; Reid et al., 2017; Thoma et al., 2022). Still, to reduce the type 2 error rate, future studies should aim to recruit more CRPS patients. However, this may prove difficult if excluding medication usage and comorbidities.

The same CRPS cohort was used for Chapters 2, 3 and 4, where subjects underwent multiple scanning sequences in a single testing session and the data were then divided into Chapters 2, 3 and 4 following separate analyses of the different sequences. The use of the same CRPS cohort throughout all three chapters gives the potential opportunity for exploratory correlative analysis, perhaps through multivariate regression, of the GABA, glutamate, basal ganglia fMRI, and brainstem fMRI data. Further, the single testing session for each CRPS subject is particularly advantageous for between data set comparisons as within-subject variability such as anxiety, pain, and CRPS stage/development on the day of testing will be minimal compared to testing on separate days. Exploratory correlative analyses between data sets may reveal certain endotypes of CRPS and may show a more global and realistic picture of CRPS brain pathology than looking only at single data sets. However, it is to be noted that a larger CRPS cohort will be required for exploratory correlative analysis such as multivariate regression, as the current small CRPS sample may yield results with high standard errors and hence low confidence results. Further, although normality was determined for each data set and appropriate statistical analyses were performed based on normality, the small CRPS sample size may not be truly reflective of the larger CRPS population. Therefore, while there is potential to perform exploratory correlative analysis between the Chapter 2, 3 and 4's data sets,

a larger CRPS sample should be used to reveal any potential endotypes such that it may be reflective of the broader CRPS population.

The cross-sectional nature of the studies presented in this thesis makes it difficult to determine whether basal ganglia and brainstem (PAG and LC) changes in CRPS are the pathological causes or maladaptive effects of CRPS. Longitudinal studies with neural imaging of patients before CRPS development, potentially just after wrist fracture, during CRPS, and after CRPS resolution may help to demonstrate whether brain changes in CRPS are pathological or adaptive. However, longitudinal studies are difficult to perform as they require a long period of time, are expensive, and possibly face high dropout rates. Alternatively, future studies could investigate the sensorimotor cortex, basal ganglia, and brainstem in CRPS patients compared with wrist fracture subjects, resolved CRPS, and pain-free controls.

The lack of GABA and glutamate differences may potentially be improved by better spatial sampling. In Chapter 2, spatial sampling of the hand area of the sensorimotor cortex was anatomically guided by the sagittal "hand hook" and axial "hand knob", landmarks of the motor hand area (Yousry et al., 1997). Yousry et al. (1997) identified the anatomical "hand knob" to be a reliable anatomical landmark for the motor hand area on the precentral gyrus where increased fMRI signals were consistently located in the anatomical "hand knob" during a motor task in 12 out of 14 hemispheres of 10 healthy individuals. However, more recent studies have found that the sensorimotor hand area functionally mapped by TMS or fMRI is not always located in the anatomical "hand knob" and "hand hook" landmark areas (Bonzano et al., 2022; Hamidian et al., 2018; Hou, Bhatia & Carpenter, 2016). Indeed, in 10 healthy individuals, the average distance between the anatomical landmark and functional motor hand area determined

by finger tapping during fMRI was 10.46 mm and varied between 5.45 and 20.40 mm (Hou, Bhatia & Carpenter, 2016). However, given the large voxel size (30 x 30 x 30 mm^3) used in Chapter 2 to sample the sensorimotor hand area, it is likely that the voxel would have encompassed the functional sensorimotor hand area. Thus, the lack of differences in GABA and glutamate between CRPS and healthy individuals most likely reflects a physiological lack of difference rather than a spatial sampling issue. Nonetheless, given that there are location variations between anatomical and functional identification of the sensorimotor cortex hand area, it is suggested that future CRPS studies of neurochemistry in the sensorimotor hand area functionally identify the sensorimotor cortex hand area using TMS motor and somatosensory evoked potentials or task-based fMRIs such as finger-tapping for voxel placement (Bonzano et al., 2022; Hamidian et al., 2018; Hou, Bhatia & Carpenter, 2016).

Repetition of the experiments at higher magnetic field strengths may yield better results. All experiments in this thesis were performed at 3T. In MRS, the voxel volume has a linear relationship with the signal-to-noise ratio (SNR) per unit time (Li, Regal & Gonen, 2001). In Chapter 2, a large voxel (30 x 30 x 30 mm^3) that covered the sensorimotor cortex was used to detect neurochemicals over 8 minutes. Ideally, a smaller voxel size would be used to separately investigate S1, M1, and SMA neurochemical concentrations and also to minimise neurochemical detection in unwanted neighbouring brain areas. However, to use a smaller MRS voxel at 3T, acquisition time would potentially have to increase to 17-27 minutes (Di Costanzo et al., 2007). Instead, 7T can provide greater SNR in smaller voxel volumes without compromising acquisition time and provide greater precision in the detection and measurement of neurochemicals (Pradhan et al., 2015; Ryan et al., 2018). Additionally, the experiment in Chapter 2 was unable to separate glutamate and glutamine due to their similar molecular structures, however, glutamate and glutamine can be reliably detected and separated at 7T (Dou

et al., 2015). Further, the PAG can be divided into distinct longitudinal columns with each column having different functions (Keay & Bandler, 2008), however, in Chapter 4, due to spatial resolution constraints, the division of the PAG in fMRI images could not be performed without significant overlap of adjacent PAG columns (Ezra et al., 2015). The resolution necessary for PAG division using fMRI images is best achieved at 7T and is not yet achievable at 3T (Ezra et al., 2015; Faull et al., 2015). Repetition of experiments at 7T could potentially reveal glutamate and glutamine differences in CRPS and better reveal CRPS differences in distinct PAG columns, which are not achievable at 3T.

The studies in this thesis primarily investigate CRPS brain changes during rest. Interestingly, in both Chapter 3 and Chapter 4, basal ganglia and brainstem changes have been correlated to PRWHE pain. The PRWHE pain score assesses pain associated with tasks and activities. Future CRPS studies on the basal ganglia and brainstem involving fMRI with a movement paradigm such as clenching, or noxious stimulation of the CRPS affected and unaffected hand may reveal additional brain changes associated with CRPS pain and motor dysfunction.

The CRPS brain changes found in this thesis may not be entirely specific to CRPS. Indeed, basal ganglia and brainstem changes have been found in other neuropathic pain and motor disorders. Comparing CRPS with other chronic pain conditions and basal ganglia disorders such as fibromyalgia and Parkinson's disease may help to reveal distinct brain changes associated with CRPS pain and motor dysfunction. Further, comparing CRPS patients with and without motor dysfunction may reveal brain changes associated with CRPS motor dysfunction only.

Given the implication of possible $GABA_A$ and α1 receptor changes in CRPS, future studies could investigate these receptors. Positron emission tomography (PET) with specific markers binding $GABA_A$, α1 receptors, and dopamine transporter could confirm or disprove the hypothesis of possible receptor or neurotransmitter differences between CRPS and pain-free controls. Additionally, although there have been previous CRPS PET studies using [^{11}C]-(R)-PK11195, a marker for both activated microglia and astrocytes, more astrocyte-specific PET ligands could be used to investigate astrogliosis in CRPS (Jeon et al., 2017; Seo et al., 2021). Future CRPS astrogliosis studies could be investigated with PET ligands such as ^{11}C-deuterium-L-deprenyl (^{11}C-DED) and ^{11}C-BU99008 which target binding sites (monoamine oxidase-B and imidazoline$_2$ respectively) on the outer mitochondrial membrane of activated α1 astrocytes (Harada et al., 2022; Liu et al., 2022). If receptor or biochemical changes are indeed found, this could lead to potential new therapeutics for CRPS.

5.4 Summary and significance

In summary, this thesis demonstrates that CRPS pain and motor dysfunction involve changes to multiple neural networks and brain areas. Specifically, for the first time, this thesis presents evidence that CRPS pain and motor dysfunction involves functional connectivity and ISO changes of the basal ganglia motor (putamen) and non-motor (caudate oculomotor) loops. Further, this thesis shows that CRPS pain involves functional connectivity changes of the PAG and LC to higher brain areas which reflect changes in CRPS ascending pain transmission. Additionally, for the first time, this thesis demonstrates that CRPS pain and motor dysfunction are unrelated to sensorimotor GABA concentration. The research has implications for understanding the mechanisms of CRPS as it suggests that brain changes involving the basal ganglia and pain pathway brainstem nuclei may underlie pain and motor dysfunction maintenance in CRPS. Additionally, given the findings, it is implicated that changes to $GABA_A$ and astrocytic α1 receptor activity may underlie some of the observed brain changes associated with CRPS pain and motor dysfunction. NA-activated astrocytic α1 receptor signalling may be responsible for the increased basal ganglia ISO power and may drive CRPS sensorimotor disinhibition through postulated $GABA_A$ receptor activity decreases in CRPS. However, further work such as PET studies on $GABA_A$ and α1 receptors in CRPS are required to determine this. Future studies should aim to compare CRPS patients with and without motor dysfunction as well as to other chronic pain and motor conditions, to provide further clarity on brain changes associated with CRPS-specific pain and motor dysfunction.

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Appendix I

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RESEARCH ARTICLE

WILEY

Altered resting activity patterns and connectivity in individuals with complex regional pain syndrome

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Abstract

Complex regional pain syndrome (CRPS) is a chronic neuropathic pain disorder that typically occurs in the limbs, usually the upper limb. CRPS usually develops from a peripheral event but its maintenance relies on changes within the central nervous system. While functional abnormalities in the thalamus and primary somatosensory cortex (S1) of the brain are some of the most consistently reported brain findings in CRPS, the mechanisms are yet to be explored in full, not least of all how these two regions interact and how they might relate to clinical deficits, such as the commonly reported poor tactile acuity in this condition. This study recruited 15 upper-limb CRPS subjects and 30 healthy controls and used functional magnetic resonance imaging (fMRI) to investigate infra-slow oscillations (ISOs) in critical pain regions of the brain in CRPS. As hypothesised, we found CRPS was associated with increases in resting signal intensity ISOs (0.03–0.06 Hz) in the thalamus contralateral to the painful limb in CRPS subjects. Interestingly, there was no such difference between groups in S1, however CRPS subjects displayed stronger thalamo-S1 functional connectivity than controls, and this was related to pain. As predicted, CRPS subjects displayed poor tactile acuity on the painful limb which, interestingly, was also related to thalamo-S1 functional connectivity strength. Our findings provide novel evidence of altered patterns of resting activity and connectivity in CRPS which may underlie altered thalamocortical loop dynamics and the constant perception of pain.

KEYWORDS
chronic pain, CRPS, infra-slow oscillations, primary somatosensory cortex, resting state fMRI, tactile acuity, thalamus

1 | INTRODUCTION

Complex regional pain syndrome (CRPS) is a chronic pain disorder characterised by spontaneous or regionally evoked pain and other signs and symptoms typically affecting the distal extremities, particularly the upper limbs (Marinus et al., 2011). Whilst CRPS usually develops after a peripheral event, it is likely maintained by changes in the central nervous system (Marinus et al., 2011; van Rijn et al., 2011). There are several lines of evidence of central nervous system involvement. These include the observation that the pain often spreads in a non-dermatome fashion (van Rijn et al., 2011), that pain intensity of the initial injury, but not injury severity, predicts the development of CRPS (Moseley et al., 2014), as well as the presence of perceptual deficits including altered two-point discrimination ability (Galer & Jensen, 1999a; McCabe, Haigh, Halligan, & Blake, 2003; Moseley, 2005). Despite evidence

of higher changes, the neural mechanisms responsible for CRPS remain unknown.

Multiple human brain imaging studies have shown that chronic neuropathic pain is not associated with ongoing increases in activity in the classic 'pain pathways', but instead a reduction in thalamic blood flow and functional 'cortical reorganisation' in the primary somatosensory cortex (S1) are arguably the most consistent findings (Di Pietro et al., 2013; Hsieh, Belfrage, Stone-Elander, Hansson, & Ingvar, 1995; Iadarola et al., 1995; Youssef et al., 2014). Furthermore, in addition to chronic neuropathic pain being associated with altered thalamic burst firing and altered thalamocortical rhythm (Di Pietro et al., 2018; Jones, 2010; Sarnthein, Stern, Aufenberg, Rousson, & Jeanmonod, 2006; Walton, Dubois, & Llinas, 2010; Walton & Llinás, 2010), we have recently shown that neuropathic pain in the orofacial region is characterised by significantly increased ISOs (<0.1 Hz) along the ascending pain pathway including at the level of the primary afferent synapse, thalamus and S1 contralateral to the ongoing pain (Alshelh et al., 2016). Curiously, the increases in infra-slow oscillation along the pain pathway occur at approximately the same frequency as calcium waves in astrocytes, that is, 0.03–0.06 Hz, and it is known that astrocytes can modulate synaptic activity by releasing gliotransmitters (Crunelli et al., 2002). In an animal model of CRPS, spinal astrocytes are chronically activated at 4 weeks after tibial fracture and contributed to the maintenance of hind paw allodynia and reduced weight bearing (Li et al., 2015). Additionally, a human postmortem study found that in an individual with long-standing CRPS, chronic astrocyte activation occurred in the spinal dorsal horn, most prominently at the level of the initiating injury (Del Valle, Schwartzman, & Alexander, 2009). Treatments inhibiting glial activation and its potential effects on neural activity may prove to be a promising avenue for an adequate treatment for CRPS.

Whilst we have shown altered resting rhythm in individuals with orofacial neuropathic pain, to date no one has reported if a similar phenomenon occurs in individuals with CRPS. The aim of this study was to use resting state functional magnetic resonance imaging (fMRI) to determine if, similar to orofacial neuropathic pain, CRPS is characterised by increased infra-slow oscillation (i.e., increased power) in the ascending pain pathway. While we were not able to image the spinal cord dorsal horn adequately for the purposes of the current study, we can focus on the thalamus and S1, and we hypothesise that CRPS subjects will display increased ISOs in these two regions contralateral to the pain. Furthermore, we hypothesise that resting functional connectivity between the thalamus and S1 will be significantly stronger in CRPS subjects compared with controls and that this strength will be correlated with the intensity of ongoing pain.

2 | MATERIALS AND METHODS

Fifteen subjects with upper-limb CRPS (11 females, mean [±*SEM*] age: 47.5 ± 3.2 years) and 30 age- and sex-matched pain-free healthy controls (20 females, mean [±*SEM*] age: 44.2 ± 2.6 years) were recruited for the study. Each CRPS subject was age- and sex- matched to 2 healthy control subjects. CRPS subjects were diagnosed according to the International Association for the Study of Pain 'Budapest' diagnostic criteria (Harden, Bruehl, Stanton-Hicks, & Wilson, 2007) and had ongoing pain for at least 3 months. They were eligible for the study if they reported pain in more than one body region, but their CRPS had to be their primary complaint and had to be in the upper limb. Handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Subjects were excluded if they did not satisfy standard MRI safety criteria and healthy control subjects were excluded if they suffered from any chronic pain. Informed written consent was obtained for all procedures, which were conducted under the approval by local Institutional Human Research Ethics Committees and consistent with the Declaration of Helsinki.

For the CRPS subjects, hyperalgesia was assessed via pinprick on the dorsal web space of the hand and allodynia with light brush strokes on the dorsum of the hands/forearms. Vasomotor signs of skin temperature asymmetry were assessed through touch, skin colour changes/asymmetry through visual observation, and sudomotor/oedema signs of sweating and oedema via touch and the use of a tape measure respectively. Visual observation of finger, hand and wrist movement was used to assess motor signs. Trophic changes to hair, nail and skin were visually assessed. CRPS subjects marked the intensity of their ongoing pain on a visual analogue scale (VAS) (0 = no pain to 10 = worst pain imaginable) three times a day for seven consecutive days during the week of the scanning session. The average of these 21 pain intensity ratings was taken as 'diary pain' intensity. Subjects also outlined the area of their ongoing pain on a standard drawing of the body and assessed their pain on the day of the scanning session on a 10 cm VAS, that is, 'scan pain' intensity.

In all 15 CRPS and in 19 of the control subjects several questionnaires assessing limb function and perception were also collected. These were the patient rated wrist/hand evaluation questionnaire which assesses pain in the wrist joint and functional difficulties of the wrist and hand (MacDermid, Turgeon, Richards, Beadle, & Roth, 1998), the foreignness of limb feelings questionnaire which assesses how one perceives their own limb (Galer & Jensen, 1999b), the Bath CRPS body perception disturbance scale which assesses self-perception of the affected limb (Lewis & McCabe, 2010), and the QuickDASH questionnaire which assesses CRPS symptoms and the individual's ability to perform related activities (Hudak, Amadio, & Bombardier, 1996). For all four of these questionnaires, higher scores indicate greater pain and/or disability.

2.1 | Tactile acuity

In addition to questionnaires assessing limb function, we measured tactile acuity with a two-point discrimination (TPD) tool immediately following the MRI scanning session in the same 15 CRPS and 19 control subjects. Subjects rested their hand in a supinated position and kept their eyes closed. A TPD wheel (Exacta™, CA) was applied to the surface of the distal pad of the index finger with distances of 0, 2, 3, 4 and 5 mm between the two points. Each of the five distances was

presented seven times in a pseudo-randomised order, resulting in a total of 35 trials for each hand. The subjects reported if they felt one point or two points touching the skin. When the subject could not feel two points at 5 mm spacing, then the distance was gradually increased until two points could be felt. The percentage of two-point perception was plotted against the distance between the points and fitted by a binary logistic regression (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.), resulting in a psychometric function of absolute threshold. From the binary logistic regression fit, the threshold was determined as the distance at which chance level (50%) of correct two-point perception was reached. Significant differences between the left and right hands in controls and between the painful and non(less)-painful hand in CRPS subjects were determined using paired *t* tests ($p < .05$, two-tailed).

2.2 | MRI scans

Each subject was positioned supine onto the MRI scanner bed and placed into a 3 Tesla MRI scanner (Achieva TX, Philips Medical Systems, The Netherlands), with their head in a 32-channel head coil to which padding was added to prevent head movement. With the subject relaxed and with their eyes closed, a series of 188 gradient echo echo-planar functional MRI image volumes using blood oxygen level dependent contrast were collected. The first 8 image volumes were not recorded but were included to allow for global signal stabilisation, leaving 180 fMRI image volumes collected over a period of 6 min. Each image volume contained 35 axial slices covering the entire brain (field of view = 240 × 240 mm, matrix size = 80 × 78, slice thickness = 4 mm, repetition time = 2,000 ms; echo time = 30 ms, flip angle = 90°, raw voxel size 3 × 3 × 4mm). In each subject, a high-resolution 3D T1-weighted anatomical image set, covering the entire brain, was collected (ultrafast gradient echo sequence [turbo field echo]; field of view = 250 × 250 mm, matrix size = 288 × 288, slice thickness = 0.87 mm, repetition time = 5,600 ms; echo time = 2.5 ms, flip angle = 8°, raw voxel size 0.87 × 0.87 × 0.87 mm).

2.3 | MRI analysis

Using SPM12 (Friston et al., 1995) and custom software, the fMRI image sets were realigned and movement parameters examined to ensure no subject displayed >1 mm volume-to-volume movement in the X, Y and Z planes and 0.05 rad in the pitch, roll and yaw directions. Cardiac (frequency band of 60–120 beats per minute +1 harmonic) and respiratory (frequency band of 8–25 breaths per minute +1 harmonic) noise was modelled and removed using the Dynamic Retrospective Filtering (DRIFTER) toolbox (Sarkka et al., 2012). Global signal drifts were then removed using the LMGS detrending method described by Macey and colleagues (Macey, Macey, Kumar, & Harper, 2004). Any fMRI signal pattern correlated with the movement parameters was removed, using a method similar to the nonlinear LMGS detrending method developed by Macey and colleagues (Macey et al., 2004). The fMRI images were then co-registered to each subject's T1-weighted anatomical image, the T1-weighted image spatially normalised to the Montreal Neurological Institute (MNI) template and the normalisation parameters applied to the fMRI images. This process resulted in the fMRI images being resliced into 2 × 2 × 2mm voxels. The fMRI images were then spatially smoothed using a 6 mm full-width at half maximum (FWHM) Gaussian filter. In 3 of the 15 CRPS subjects, pain was restricted to the left side of the body. Given the ascending pain pathways from the dorsal horn to the thalamus are primarily crossed, we reflected the T1 and resting state fMRI scans of these 3 CRPS subjects across the midline. As a consequence, for all 15 CRPS subjects in our analysis, the left side of the brain is contralateral to their ongoing pain.

2.3.1 | ISOs

Using the SPM Data Processing Assistant for Resting-State fMRI (DPARSF) toolbox (Chao-Gan & Yu-Feng, 2010), we calculated the sum of amplitudes of low-frequency fluctuations (ALFF) between 0.03 and 0.06 Hz for each voxel in control and CRPS subjects using the spatially smoothed fMRI image sets. We also divided ALFF values by power over the entire frequency range to obtain fractional ALFF (fALFF) values for each voxel. Both ALFF and fALFF have high test–retest reliability, particularly ALFF (Zuo et al., 2010). In an initial analysis we determined significant differences between control and CRPS subjects over the entire brain at a voxel-by-voxel level using a two-sample random effects procedure with age and sex as nuisance variables ($p < 0.05$, false discovery rate corrected for multiple comparisons). However, since our hypothesis was that CRPS subjects would display significant increases in 0.03–0.06 Hz power in the thalamus and S1 contralateral to the body area of the highest pain, we subsequently restricted our investigation to these brain regions by applying a mask (Figure 1a). Significant differences between groups were determined using a two-sample random effects procedure with age and sex as nuisance variables ($p < .05$, false discovery rate corrected for multiple comparisons). For each significant cluster, fast Fourier transforms were performed on the resting fMRI signal intensity. More specifically, resting fMRI signals were demeaned, power spectral density calculated using MATLAB's 'periodogram' function, and the total power calculated as area under the curve with the 'bandpower' function. Plots of power at each frequency between 0.001 and 0.025 Hz were calculated and plotted for control and CRPS subjects. Total 0.03–0.06 Hz power was also calculated for each subject and plotted in addition to mean ± *SEM* of control and CRPS groups. We also determined if there were any significant differences between controls and CRPS subjects at other frequency ranges by calculating ALFF power for three standard ISO frequency domains: slow 5:0.01–0.027 Hz, slow 3:0.073–0.198 Hz, and slow 2:0.198–0.25 Hz. Significant differences between controls and CRPS subjects within the contralateral thalamus and S1 were then determined using two-sample random effects procedures with age and sex as nuisance variables ($p < .05$, false discovery rate corrected for multiple comparisons).

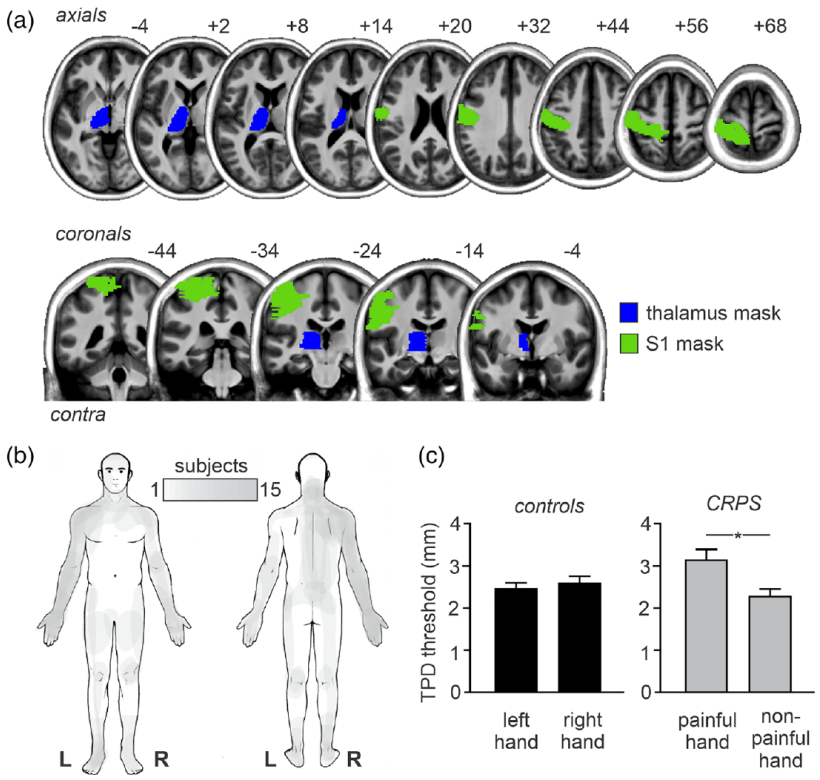


FIGURE 1 (a) Contralateral thalamus (blue) and primary somatosensory cortex (S1; green) masks used for restricted analysis overlaid onto a series of axial and coronal T1-weighted images. The locations of slices in Montreal Neurological Institute Space are indicated at the top right of each axial and coronal slice. (b) Maps of ongoing pain in 15 subjects with complex regional pain syndrome (CRPS). Note that all 15 subjects have pain in the upper limb. (c) Two-point discrimination thresholds (TPD) for the left and right hands in controls (black) and painful and non(less)-painful hand in subjects with CRPS. Note that controls show similar values for the left and right hand whereas in CRPS subjects, the painful hand shows significantly greater TPD, that is, reduced tactile acuity, than the nonpainful hand. $^{*}p < .05$

To determine the potential effects of head movement on infra-slow oscillation power, for each movement parameter (X, Y, Z, pitch, roll, yaw), in each subject, power spectra were calculated. The mean power between 0.03 and 0.06 Hz in control and CRPS groups was compared ($p < .05$, two-tailed, two-sample t test). The effects of pain variables were determined by assessing their relationships with 0.03–0.06 Hz power in significant clusters in CRPS subjects (Pearson's correlations, $p < .05$). Unfortunately, we were unable to test the effects of medication on infra-slow power as all but 2 CRPS subjects were taking some form of medication.

2.3.2 | Regional homogeneity analysis

To assess local signal covariation, we measured Kendall's coefficient of concordance (KCC), which evaluates the similarity of the time series within each voxel and its nearest neighbours. Using the DPARSF toolbox, the fMRI preprocessed images were band-pass filtered (0.03–0.06 Hz) and voxel-based graphs generated for each individual subject. For each voxel, the KCC was computed from the time course of that voxel and its 19 neighbouring voxels. The analysis was restricted to the contralateral (left) thalamus and S1 by applying a mask. After smoothing the KCC maps using a 6 mm FWHM Gaussian filter, significant differences between groups were determined using a two-sample random effects procedure with age and sex as nuisance variables ($p < .05$, false discovery rate corrected for multiple comparisons). We also extracted the KCC values from the thalamus cluster derived from the ALFF analysis and determined significant differences between controls and CRPS subjects using a two-sample t test ($p < .05$, two-tailed).

2.3.3 | Functional connectivity

ISO analysis revealed a cluster in the thalamus which displayed significant increases in ISO in CRPS subjects compared with controls. Using this cluster as a seed, we performed functional connectivity analyses to determine whether resting connectivity strengths between the thalamus and all other brain regions were significantly different in CRPS subjects compared with controls. For each subject, signal intensity changes over the 180 fMRI volumes were extracted from the thalamic seed and the relationship with ongoing signal intensities in each other voxel in the brain determined. The resulting thalamic connectivity maps were smoothed using a 6 mm FWHM Gaussian filter, and an initial analysis was performed in which we determined significant differences between control and CRPS subjects over the entire brain at a voxel-by-voxel level using a two-sample random effects procedure with age and sex as nuisance variables ($p < .05$, false discovery rate corrected for multiple comparisons). Since our hypothesis was that CRPS subjects would display significant differences in thalamo-S1 connectivity and that this relationship would be correlated to two-point discrimination ability, we subsequently restricted our investigation to the contralateral S1 by applying a mask. Significant differences

between groups were determined using a two-sample random effects procedure with age and sex as nuisance variables ($p < .05$, false discovery rate corrected for multiple comparisons). For each significant cluster, thalamic connectivity values were extracted from each individual subject and the mean ± *SEM* values plotted.

Finally, we determined if the degree of connectivity between the thalamus and the S1 was related to ongoing pain intensity, pain duration or individual two-point discrimination ability. Using the thalamic connectivity maps, voxel-by-voxel analyses were performed to determine significant linear relationships with either scan pain, diary pain or pain duration and the contralateral thalamus-S1 connectivity ($p < .05$, false discovery rate corrected for multiple comparisons). The analysis was restricted to the contralateral S1 by applying a mask. In addition, we determined if the degree of thalamo-S1 connectivity was correlated to two-point discrimination of the painful hand in CRPS subjects and the right hand in control subjects. Two-point discrimination measures were available in 12 CRPS and 19 control subjects. Using the thalamic connectivity maps, voxel-by-voxel analyses were performed to determine significant linear relationships with two-point discrimination ($p < .05$, false discovery rate corrected for multiple comparisons). For each significant cluster, thalamic connectivity values were extracted from each individual subject and plotted.

3 | RESULTS

The demographics and clinical characteristics of the 15 CRPS subjects are shown in Table 1 and the area of pain in all subjects is shown in Figure 1b. All 15 CRPS subjects reported ongoing pain in the upper limb and 7 also reported pain in the lower limb. Three CRPS subjects reported the highest ongoing pain in the left upper limb with the remaining reporting the highest pain in the right upper limb. Where subjects reported pain as bilateral in the upper limbs, the more painful side is termed the 'painful' side; the other as 'non(less)painful' from here on. The mean scan pain for CRPS subjects was 5.3 ± 0.5, mean diary pain was 4.6 ± 0.6 and the mean duration of pain was 4.7 ± 0.9 years. For the 3 CRPS subjects with pain on the left, their fMRI images were reflected across the midline so that the right side was ipsilateral to the highest ongoing upper limb pain.

3.1.1. | Questionnaires and two-point discrimination threshold

Three of the 15 CRPS subjects were excluded from TPD analysis due to ongoing pain preventing testing, resulting in 12 CRPS and 19 control subjects. As hypothesised, in CRPS patients, the painful hand had a significantly greater TPD threshold, that is, reduced tactile acuity, than the non(less)-painful hand (mean ± *SEM* TPD mm; painful: 3.16 ± 0.24, non(less): 2.30 ± 0.17; $p = .001$), whereas there was no significant difference between the left and right hands in controls (left: 2.47 ± 0.11, right: 2.60 ± 0.15; $p = .21$; Figure 1c). In addition to CRPS subjects having reduced tactile acuity in the painful hand, they also had significantly greater functional difficulties in their painful hand than controls as assessed by the Patient Rated Wrist/Hand Evaluation questionnaire (total mean ± *SEM* score: controls 3.7 ± 2.5, CRPS: 175.2 ± 21.7, $p < .0001$) and the QuickDASH questionnaire (disability/symptom mean ± *SEM* score: controls 1.9 ± 0.6, CRPS: 63.3 ± 4.8, $p < .0001$). As predicted CRPS subjects rated their limb as more foreign to themselves than controls as assessed by the foreignness of limb feelings questionnaire (total mean ± *SEM* score: controls 0.8 ± 0.8, CRPS: 48.3 ± 8.1, $p < .0001$) and had greater body perception disturbances than controls as assessed by the Bath CRPS Body perception disturbance scale (disability/symptom mean ± *SEM* score: controls 9.3 ± 1.5, CRPS: 22.5 ± 2.6, $p < .0001$). Finally, there were no significant relationships between TPD of the painful hand and either scan pain ($r = -.42$, $p = .17$), diary pain ($r = .26$, $p = .42$), or pain duration ($r = .11$, $p = .73$).

3.1.2. | Altered ISOs

Using a frequency range of 0.03–0.06 Hz, a voxel-by-voxel analysis of the entire brain revealed no significant group difference in either ALFF or fALFF values. However, for the ALFF values, when the statistical threshold was lowered to $p < .001$ uncorrected, a number of regions emerged. CRPS subjects had greater infra-slow oscillation power in the contralateral orbitofrontal cortex, insula, thalamus and secondary somatosensory cortex and in the ipsilateral anterior insula (Figure 2a, Table 2). A restricted analysis of ALFF in the contralateral thalamus and S1 regions revealed that at $p < .05$ FDR corrected, CRPS subjects had significantly increased infra-slow oscillation power in the contralateral thalamus (mean ± *SEM* 0.03–0.06 Hz ALFF power: *controls*: 1.01 ± 0.02, *CRPS*: 1.29 ± 0.05; Figure 2b, Table 2). This thalamus cluster overlapped with the region activated during innocuous brushing of the hand reported in our previous investigation (Wrigley et al., 2009), that is, in the region of the arm representation of the ventrocaudal (Vc) thalamus (Figure 2c). At no thalamus or S1 voxel was fALFF significantly different between controls and CRPS subjects. Extraction of power at each frequency in controls and CRPS subjects revealed that the frequency band showing significant power differences was remarkably restricted to between ~0.03 and 0.06 Hz (Figure 2c) and total power between 0.03 and 0.06 Hz was greater in CRPS compared with control subjects (mean ± *SEM* 0.03–0.06 Hz total power: *controls*: 11.7 ± 1.01, *CRPS*: 21.2 ± 3.3). The specificity of the power restriction to this particular band was confirmed by the findings that there were no significant ALFF power differences between control and CRPS subjects, in any thalamic region, in either slow 5 (0.01–0.027 Hz), slow 3 (0.073–0.198 Hz), or slow 2 (0.198–0.25 Hz) frequency bands.

In CRPS subjects, we found no significant relationship between thalamic 0.03 and 0.06 Hz power and scan pain ($r = -.07$, $p = .79$), diary pain ($r = -.11$, $p = .67$), or pain duration ($r = -.09$, $p = .74$). Furthermore, we found no significant relationship between thalamic 0.03 and 0.06 Hz power and Patient Rated Wrist/Hand Evaluation score ($r = -.21$, $p = .48$), QuickDASH score ($r = -0.19$, $p = .50$), Bath CRPS body perception disturbance scale ($r = -.46$, $p = .08$), or foreignness of limb feelings score ($r = -.50$, $p = .06$).

TABLE 1 Demographics and clinical characteristics of patients with CRPS

Subject	Age	Sex	EHI score	Pain duration (years)	CRPS affected region[1]	Medications[3]	Pain intensity (diary VAS)	Pain intensity (scan VAS)
1	49	M	100.0 (R)	7.0	**R UL**, R LL, L LL, face, abdomen	None	4.5	4.0
2	56	F	100.0 (R)	4.2	**L UL**, R UL, R LL, R and L chest	Duloxetine, gabapentin, oxycodone, Quetiapine, Tapentadol	8.1	7.8
3	56	F	60.0 (R)	0.9	**R UL**, R neck, R chest	Ashwagandha, budesonide, cannabis, codeine, Formoterol, oxycodone, Paracetamol, salbutamol	8.3	7.9
4	62	F	100.0 (R)	6.2	**L UL**, *R UL*[2]	Amitriptyline, Cannabidiol drops, codeine, levothyroxine, magnesium, Paracetamol, Topiramate, tramadol, valerian	5.8	4.3
5	58	F	−20.0 (A)	8.7	**R UL**, R LL, R face	Codeine, duloxetine, Linagliptin, meloxicam, metformin, Paracetamol	4.7	4.1
6	67	F	100.0 (R)	9.5	**R UL**, L UL, R LL, L LL	Amlodipine, gabapentin, ketamine in lipoderm cream, metformin, Metoprolol, pantoprazole, salbutamol	3.7	5.0
7	47	M	44.4 (R)	1.5	**R UL**	Amlodipine, atorvastatin, ibuprofen, Paracetamol, perindopril, Pregabalin	6.8	7.1
8	34	F	−23.1 (A)	5.3	**R UL**, R LL, R hip	Amitriptyline, buprenorphine patch	4.3	2.4
9	26	F	−40.0 (A)	1.3	**R UL**, L and R neck, Spine, L LL	None	5.4	6.8
10	46	F	80.0 (R)	3.9	**L UL**	Amitriptyline, Betahistine, duloxetine, naproxen, pantoprazole, Rizatriptan, Tapentadol, Valaciclovir,	0.6	5.6
11	24	F	70.0 (R)	2.6	**R UL**, L UL	Amitriptyline, gabapentin, levothyroxine	4.5	4.4
12	52	F	40.0 (A)	2.9	**R UL**, R torso	Ashwagandha, fish oil, ibuprofen, magnesium, mega B, melatonin, Paracetamol, Tapentadol, vitamin C, vitamin D	3.8	3.5
13	38	F	17.6 (A)	12.7	**R UL**, R neck, L LL	Duloxetine, gabapentin, naloxone, oxycodone, Palmitoylethanolamide (PEA)	0.0	5.9
14	52	M	−88.9 (L)	1.9	**R UL**, L and R neck, back	Cholecalciferol, ibuprofen, magnesium, oxycodone, Paracetamol, Pregabalin, tramadol, venlafaxine, Zopiclone	7.0	7.6
15	45	M	(L)	1.2	**R UL**		2.0	3.0

Note: **Bold** indicates the CRPS region with the most severe pain. *Italics* indicates remission of the CRPS region. Underline indicates medication taken in the last 24 hr of the day of testing.
Abbreviations: A, ambidextrous; L, left; LL, lower limb; R, right; *SEM*, standard error of mean; UL, upper limb.

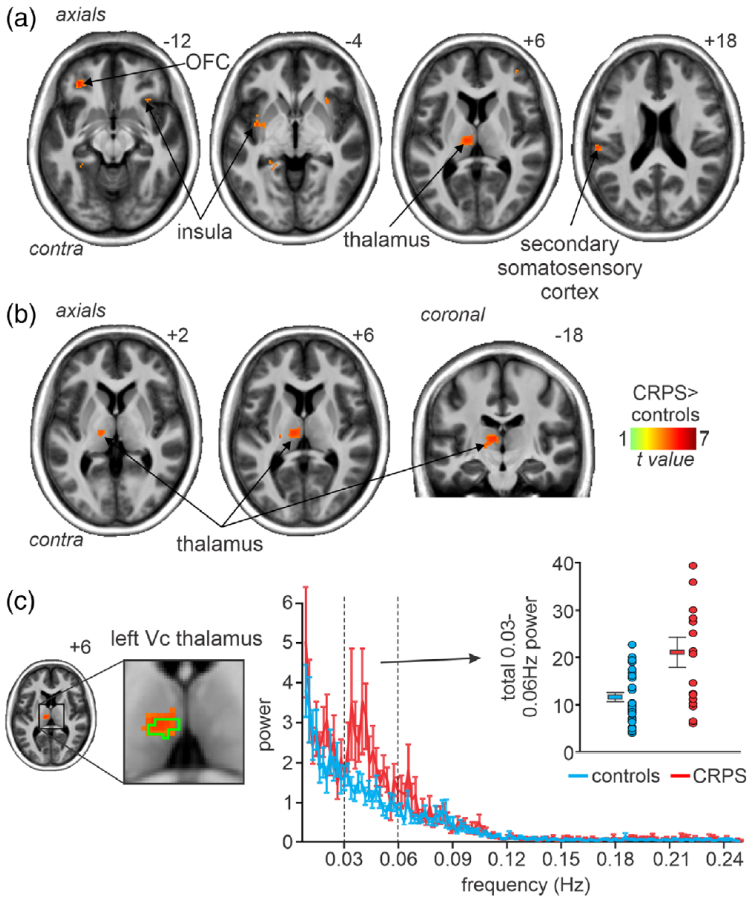


FIGURE 2 (a) Greater ($p < .001$, uncorrected) infra-slow oscillation power (0.03–0.06 Hz; hot colour scale) assessed over the entire brain in 15 subjects with complex regional pain syndrome (CRPS) compared with 30 matched controls overlaid onto a mean T1-weighted anatomical image set. The locations of slices in Montreal Neurological Institute Space are indicated at the top right of each axial slice. (b) Significantly greater ($p < .05$, FDR corrected) infra-slow oscillation power (0.03–0.06 Hz; hot colour scale) assessed in the contralateral (to the highest pain) thalamus and primary somatosensory cortex. Slices locations in Montreal Neurological Institute Space are indicated at the top right of each slice. Note that the significant difference is in the region of the ventrocaudal (Vc) thalamus. (c) Plots of mean ± *SEM* power at each frequency between 0.01 and 0.25 Hz in controls (blue) and CRPS subjects (red). It is clear that the frequency band in which power was significantly greater in CRPS subjects was that between ~0.03 and 0.06 Hz. The green outline on the image slice to the left indicates the region activated by innocuous brushing of the hand and hence in the Vc thalamus. To the right are plots of individual subject and mean ± *SEM* 0.03–0.06 Hz total power for the thalamus cluster

3.1.3. | Regional homogeneity

If increased ISO power results from increased synchronicity of astrocyte activation and recruitment of surrounding astrocytes and neurons, neighbouring voxels should display increased signal intensity synchronisation. We measured Kendall's coefficient of concordance (KCC) as an index of similarity of time series between voxels and found no significant differences between controls and CRPS subjects in the contralateral thalamus and S1. Furthermore, extraction of KCC values from the thalamic cluster derived from the ISOs analysis also revealed no significant difference in regional homogeneity in CRPS subjects compared with controls (mean ± *SEM* KCC *controls* 0.98 ± 0.01, *CRPS* 1.01 ± 0.01, $p = .08$; two-sample *t* test).

3.1.4. | Thalamic-somatosensory cortex connectivity

Using the thalamic cluster derived from the infra-slow oscillation analysis as a seed, we performed resting state functional connectivity between the contralateral thalamus and S1. A voxel-by-voxel analysis of the entire brain revealed greater thalamic connectivity in CRPS subjects compared with controls in a number of brain regions including the contralateral orbitofrontal cortex, ipsilateral insula, bilateral amygdala, bilateral posterior parietal, bilateral cingulate and bilateral S1 cortices (Figure 3a, Table 2). In no brain region was thalamic connectivity strength greater in controls compared with CRPS subjects. Restricted analysis of the contralateral S1 revealed two clusters in which CRPS subjects had significantly greater connectivity than controls. These clusters were in the locations that receive inputs from the arm (thalamic connectivity mean ± *SEM*: *controls* 0.10 ± 0.01, *CRPS* 0.19 ± 0.02) and the hand (*controls* 0.08 ± 0.02, *CRPS* 0.18 ± 0.03; Figure 3b,c, Table 2). In no voxel was thalamic connectivity strength greater in controls compared with CRPS subjects.

A voxel-by-voxel analysis of the contralateral S1 using scan pain intensity as a regressor resulted in a discrete region representing the arm where greater thalamic connectivity was associated with reduced pain ($r = -.83$; Figure 4a; Table 2). No significant correlation was found between thalamic connectivity and either diary pain or pain duration. In contrast, there was a significant positive relationship between thalamo-S1 connectivity strength and TPD threshold in all subjects in two regions of the contralateral S1, one in the region representing the hand and another in the region representing the arm (Figure 4b; Table 2). These positive relationships were similar in controls and CRPS subjects, that is, the lower the tactile acuity the

TABLE 2 Montreal neurological institute (MNI) coordinates, cluster size and t-score for regions of significant difference between control and CRPS subjects

Brain region	MNI co-ordinate			Cluster size	t-score
	x	y	z		
Infra-slow oscillation power (0.03–0.06 Hz)					
Wholebrain p < .001; CRPS > controls					
Contralateral orbitofrontal cortex	−32	38	−14	39	4.97
Contralateral insula	−36	−2	−2	122	4.23
Contralateral secondary somatosensory cortex	−58	−26	20	71	5.34
Contralateral thalamus	−8	−18	8	92	4.58
Ipsilateral insula	30	20	−4	26	3.80
Thalamus/S1 only p < .05 FDR; CRPS > controls					
Contralateral thalamus	−8	−18	8	128	4.58
Resting thalamic-S1 connectivity					
Wholebrain p < .001; CRPS > controls					
CRPS > controls					
Contralateral orbitofrontal cortex	−28	44	−8	53	4.64
Ipsilateral insula	34	18	0	50	4.21
Bilateral amygdala	−32	−4	−18	70	5.15
	32	−10	−14	42	4.08
Bilateral posterior parietal cortex	−54	−44	32	137	4.75
	58	−44	26	596	5.06
Cingulate cortex	−10	−46	44	1,737	5.29
Bilateral S1	38	−20	38	52	4.54
	−46	−26	42	25	3.65
S1 only p < .05 FDR; CRPS > controls					
Contralateral S1	−20	−42	52	189	4.79
	−46	−26	42	37	3.56
Increase thalamic-S1 conn-decrease scan pain					
Contralateral S1	−26	−34	72	10	5.71
	−34	−44	72	19	5.35
Increase thalamic-S1 conn-increase TPD					
Contralateral S1	−60	−12	42	64	4.42
	−36	−26	66	13	3.63

greater the connectivity strength in both the region of the arm (*controls* r = .48, *CRPS*, r = .77) and hand (*controls* r = .49, *CRPS*, r = .75).

4 | DISCUSSION

Consistent with our hypothesis, we found increased infra-slow oscillatory power in the contralateral thalamus of individuals with CRPS compared with controls. This thalamic increase overlapped with the region of the Vc thalamus and was restricted to the frequency band 0.03–0.06 Hz. Whilst we did not find a significant difference in infra-slow oscillatory power in the contralateral primary somatosensory cortex, we did find that resting thalamo-S1 connectivity strength was significantly greater in CRPS subjects compared with controls and was negatively correlated to scan pain intensity and tactile acuity. Finally, we found that regional homogeneity was not significantly different in the thalamus or S1 of CRPS subjects compared with controls.

We have previously shown that chronic orofacial neuropathic pain is associated with an increase in resting ISOs along the ascending pain pathway, including the Vc thalamus, thalamic reticular nucleus and S1 (Alshelh et al., 2016). In our previous study, we divided the thalamus contralateral to the side of pain into 240 regions of interest and found that the most common frequency band to display a significant infra-slow oscillation difference was between 0.03 and 0.06 Hz. Whilst we did not perform the same regions of interest analysis in the current study, extraction of power over the entire frequency band

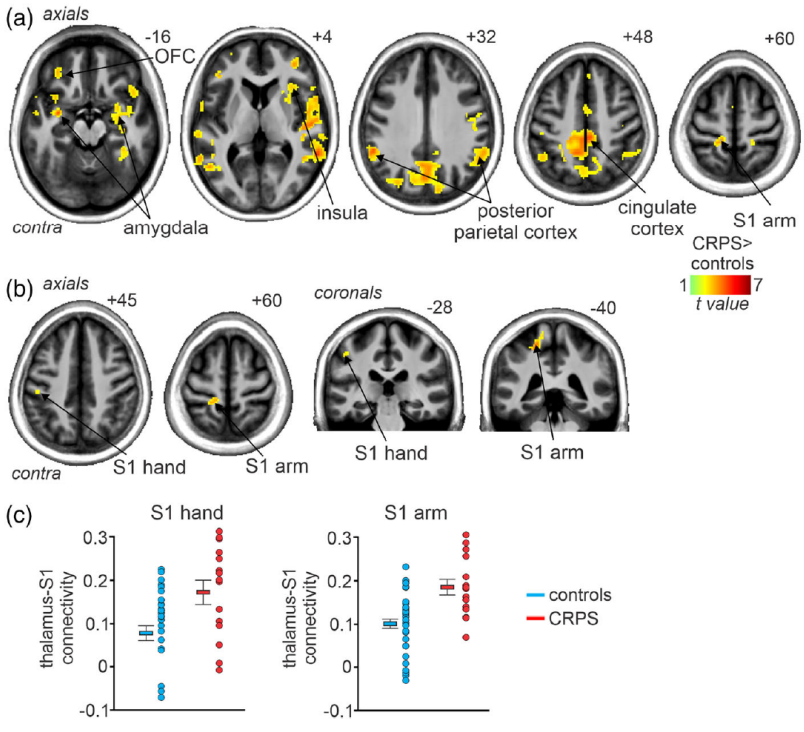
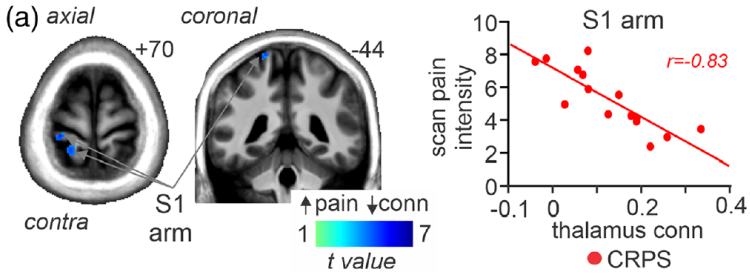


FIGURE 3 (a) Significantly greater ($p < .05$, FDR corrected; hot colour scale) resting functional connectivity between the thalamus and all other brain regions in 15 subjects with complex regional pain syndrome (CRPS) compared with 30 matched controls overlaid onto a mean T1-weighted anatomical image set. Slices locations in Montreal Neurological Institute Space are indicated at the top right of each axial slice. (b) Significantly greater ($p < .05$, FDR corrected; hot colour scale) resting functional connectivity between the contralateral (to highest pain) thalamus and primary somatosensory cortex (S1). Slices locations in Montreal Neurological Institute Space are indicated at the top right of each slice. Note that the significant greater connectivity in CRPS subjects includes the regions receiving inputs from the arm/trunk and hand. (c) Plots of individual subject and mean ± *SEM* thalamic connectivity in the S1 region representing the arm and the S1 region representing the hand



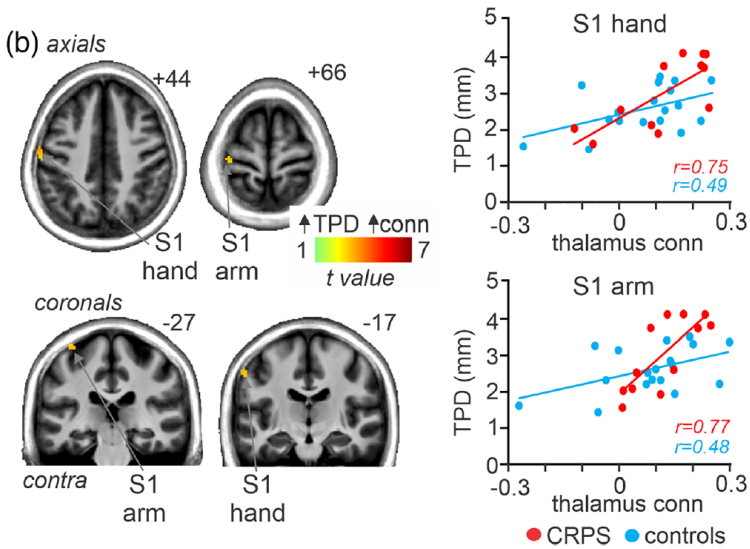


FIGURE 4 (a) Region of primary somatosensory cortex (S1) in which thalamic connectivity strength was significantly negatively (cool colour scale) correlated to scan pain intensity in 15 subjects with complex regional pain syndrome (CRPS). The locations of slices in Montreal Neurological Institute Space are indicated at the top right of each axial and coronal slice. To the right is a plot of individual subject connectivity strengths against scan pain intensity. (b) S1 regions in which connectivity strength was positively (hot colour scale) correlated to two-point discrimination (TPD; in mm) threshold in 12 CRPS and 19 control subjects. To the right are plots for individual subject connectivity strength values against TPD for two S1 clusters, one in the S1 region representing the hand and another in the S1 region representing the arm. Values for these S1 clusters are plotted for CRPS and control subject separately. conn, connectivity

(0.01–0.25 Hz) revealed that CRPS subjects also showed an increase in resting power that was restricted to the 0.03–0.06 Hz frequency band. Given that this frequency band is similar to that in which astrocyte calcium and gliotransmission release waves have been recorded (Scemes & Giaume, 2006) and given that there is preclinical and human postmortem evidence of chronic astrogliosis in individuals with CRPS and other neuropathic pain conditions (Del Valle et al., 2009; Li et al., 2015; Shi, Gelman, Lisinicchia, & Tang, 2012), we speculate that these oscillatory activity increases in CRPS result from chronic astrogliosis. It is known that in activated astrocytes, calcium waves can propagate to neighbouring astrocytes and this can result in neighbouring astrocytes responding with synchronous oscillatory gliotransmitter release (Cornell-Bell, Finkbeiner, Cooper, & Smith, 1990; Scemes & Giaume, 2006). Such oscillatory gliotransmitter release can elicit long-lasting NMDA-mediated currents in surrounding neurons and recent evidence shows that astrocytes can elicit prolonged neural firing increases (Crunelli et al., 2002; Deemyad, Lüthi, & Spruston, 2018). It has been shown that increased ISOs are coupled to high frequency power fluctuations in the cortex (Mantini, Perrucci, Del Gratta, Romani, & Corbetta, 2007; Vanhatalo et al., 2004) and it is possible that the increases in ISOs within the thalamus of CRPS subjects lead to altered higher-power activity in thalamocortical circuits. Altered thalamocortical loop dynamics is consistent with our finding of a significant increase in thalamo-S1 connectivity strength in CRPS subjects, which may contribute to the thalamocortical dysrhythmia observed in CRPS and other neuropathic pain conditions (Di Pietro et al., 2018; Jones, 2010; Sarnthein et al., 2006; Walton et al., 2010; Walton & Llinás, 2010).

In our previous study in chronic orofacial neuropathic pain, we found that within the ascending pain pathway, only the primary afferent synapse, that is, the spinal trigeminal nucleus, displayed a significant increase in regional homogeneity (Alshelh et al., 2016). An increase in regional homogeneity reflects an increase in local synchrony of ISOs which would occur if infra-slow frequency calcium waves propagate among neighbouring astrocytes. Whilst we were not able to explore the dorsal horn using fMRI in CRPS subjects in the current study, our finding that regional homogeneity was not different between CRPS and control subjects is consistent with the idea that chronic astrocyte activation at the primary afferent synapse is driving ISOs throughout the ascending pain pathway. Indeed, this idea is supported by an experimental animal investigation in which extracellular single-unit neuronal activity was recorded in naïve rats and in those with chronic neuropathic pain induced by sciatic chronic constriction injury (Iwata et al., 2011). Firstly, the investigation reported spontaneous oscillations in ventroposterior thalamus neural firing at approximately 0.03 Hz in animals with neuropathic pain but not in naïve animals. Secondly, they found that severing the connection between the dorsal horn and thalamus eliminated these increases in infra-slow neural activity oscillations, suggesting that they are being driven from the dorsal horn. We also recently found that in subjects with chronic orofacial neuropathic pain, administration of palmitoylethanolamide, a substance that can blunt astrocyte activation (Scuderi et al., 2012), significantly reduced ongoing pain intensity in 16 of 22 subjects (Alshelh et al., 2019). Whilst infra-slow oscillation power in the spinal trigeminal nucleus was reduced in all subjects, it was only those subjects that also displayed reductions in thalamic ISOs that experienced pain relief. Furthermore, only those that displayed pain relief also showed reductions in connectivity between the spinal trigeminal nucleus and the thalamus. These results add further weight to the hypothesis that chronic neuropathic pain is driven by chronic astrocyte activation at the level of the primary afferent synapse.

In addition to increased infra-slow oscillations, we found that connectivity strength between the thalamus and S1 was significantly correlated to pain and tactile acuity. Consistent with previous literature, in CRPS subjects, the painful hand displayed poor tactile acuity relative to the non(less)-painful hand and pain-free controls (Catley, O'Connell, Berryman, Ayhan, & Moseley, 2014; Lewis & Schweinhardt, 2012; Maihofner & DeCol, 2007; Pleger et al., 2006; Reiswich et al., 2012). We found, in both CRPS and control subjects, the strength of connectivity between the thalamus and S1 was positively correlated, that is, the poorer the tactile acuity the greater the connectivity strength. Similarly, despite no significant relationship between tactile acuity and ongoing pain intensity, scan pain scores were significantly negatively correlated to thalamo-S1 connectivity in the region of S1 that receives information from the upper limb.

The direction of these correlations appears at odds with what one might expect, that is, since thalamo-S1 connectivity is greater in CRPS subjects one might hypothesise that the greater the connectivity strength the greater the ongoing pain. Furthermore, it is surprising that greater thalamo-S1 connectivity strength is associated with higher TPD thresholds, that is, reduced tactile acuity in control and CRPS subjects. It is known that in healthy controls, increased tactile discrimination ability evoked by short-term co-activation protocols results in expanded S1 representations (Pleger et al., 2001; Pleger et al., 2003). CRPS patients have been shown to have decreased perceptual learning ability with a tactile stimulation training program (Maihofner & DeCol, 2007) as well as smaller functional S1 representation of the affected hand in S1, consistent with impaired two-point discrimination ability (Di Pietro et al., 2013). A recent investigation in pain-free controls reported that better tactile acuity was associated with stronger connectivity in somatosensory discriminatory networks, although this study did not specifically explore Vc-S1 connectivity strengths (Heba et al., 2017). Indeed, functional connectivity studies specifically targeting the Vc thalamus are rare and the interpretation of changes within this circuitry are difficult since Vc thalamus projections to S1 are complex. It is well-established from tract tracing studies that Vc projections to S1 involve collaterals to GABAergic neurons of the thalamic reticular nucleus, which in turn projects back to the Vc thalamus and itself receives input from S1 (Pinault, 2004). It is thought that this circuitry controls thalamocortical rhythm (Fuentealba & Steriade, 2005) and we recently reported that thalamic GABA levels are strongly correlated to thalamocortical rhythm in chronic orofacial pain patients, despite there being no difference in thalamic GABA levels between pain patients and controls, and interestingly the strong correlation only seems to exist in the pain state (Di Pietro et al., 2018). We have also shown that thalamic GABA content is

negatively correlated with Vc-S1 connectivity with no significant relationship in pain-free controls (Henderson et al., 2013). It is possible that the negative correlation between thalamo-S1 connectivity and pain and the positive relationship with tactile acuity result from a combination of excitatory ascending and inhibitory descending controls which may also be responsible for the thalamocortical dysrhythmia in CRPS (Walton et al., 2010). These relationships will only be fully appreciated with a more complete understanding of the significance of functional connectivity and the interaction between various nuclei that regulate thalamocortical loop dynamics.

There are several limitations needing discussion. Firstly, we recruited and investigated a limited number of CRPS subjects, although given the relatively rare nature of this disorder 15 is a considerable number and is larger than most of the previous neuroimaging studies exploring CRPS and brain function (Di Pietro et al., 2013). Whilst one could argue that a whole brain, voxel-by-voxel analysis was underpowered, our spatially restricted analysis based on our a priori hypothesis revealed significant difference at corrected p values using population based statistical tests, and thus our study was adequately powered. Secondly, our investigation was cross-sectional in nature and we did not assess individuals before they developed CRPS. Therefore, we cannot determine if any of the changes reported here occur throughout the development of CRPS or exist even before the initiating injury. A larger sample of subjects, recruited early in the course of the disorder and followed longitudinally, would allow us to explore changes that occur during the course of the condition. Thirdly, since we were interested in determining changes within the ascending pain pathway which is known to be lateralized, it was necessary to reflect the images of 3 CRPS subjects across the midline. Our results suggest that increased infra-slow oscillation patterns are consistent with the contralateral projection of these pain pathways, although there remains a potential issue of lateralized effects. Finally, we were unable to determine the effects of medication on our results since 13 of the 15 CRPS subjects were taking daily medication for pain relief. Whilst the use of medications almost certainly affects aspects of the changes reported in this study, it has been shown that pain medications such as gabapentin, which was used by many of the CRPS subjects in this study, reduces astrocyte calcium signalling (Tian et al., 2005), thus we may have underestimated the difference in infra-slow oscillation power between CRPS subjects and controls. In any case, further studies in which CRPS subjects are not taking medication are needed to confirm our results, though this is difficult on a practical and ethical level. Despite these limitations, the discrete changes in ISOs in the thalamus and the alteration in thalamo-S1 connectivity strengths are consistent with previous investigations in chronic pain and we are confident they are important in the maintenance of CRPS.

5 | CONCLUSIONS

Our findings show that the thalamic region that processes somatosensory information displays significantly increased ISOs in CRPS. Furthermore, CRPS is associated with increased thalamo-S1 connectivity which is correlated with reduced tactile discrimination of the painful hand. These changes may underlie the well-described alterations in thalamocortical loop dynamics, tactile discrimination and the constant perception of pain in individuals with CRPS.

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CONFLICT OF INTERESTS

The authors declare no potential conflict of interest

DATA AVAILABILITY STATEMENT

Research data are not shared due to human ethics requirements.

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