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The influence of pain-related fear levels on structural brain changes in pediatric complex regional pain syndrome

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THE INFLUENCE OF PAIN-RELATED FEAR LEVELS ON STRUCTURAL BRAIN CHANGES IN PEDIATRIC COMPLEX REGIONAL PAIN SYNDROME

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ABSTRACT

Complex Regional Pain Syndrome (CRPS) is a chronic neuropathic pain condition associated with significant alterations in the somatosensory and motor cortex brain regions. Cognitive-affective alterations have recently been recognized in patients suffering with CRPS, however, relatively little neuroimaging research has been done to examine these dimensions. Moreover, many children and adolescents suffer from CRPS, but very little is known about the impact of this condition on brain states in the pediatric population. The aim of this paper is to assess the structural brain differences between children with CRPS and healthy controls and to examine to what degree fear level influences such differences. This study is part of a larger investigation that integrates functional and structural brain differences to evaluate fear-related brain circuitry in patients with CRPS. Thirty-seven patients with CRPS were age and gender matched with 35 healthy controls. The two groups underwent structural magnetic resonance imaging (MRI) scans as well as completed the Fear of Pain Questionnaire, child report (FOPQ). To examine gray matter differences, voxel-based morphometry (VBM) and cortical thickness (CT) analysis was completed. Patients with CRPS in this study had an average age of 13.2 (SD=2.7) and were predominantly female (73%). Of the 35

patients who completed FOPQ, 49% reported clinically significant pain-related fear. Compared with healthy controls, CRPS patients had significantly less in gray matter (GM) volume in pain- and fear-related brain regions, including the dorsolateral prefrontal gyrus, motor and somatosensory cortex, anterior and posterior cingulate cortex, nucleus accumbens, putamen, amygdala, and hippocampus. Furthermore, gray matter decreases in regions such as anterior midcingulate cortex, nucleus accumbens, and putamen were associated with elevated pain-related fear in patients. Differences in gray matter volume in fear-circuitry areas could potentially be one mechanism by which abnormal fear learning and extinction develops in youth suffering with CRPS. Further research examining brain changes post-treatment is needed to determine if treatments that target improving pain and fear levels are associated with concomitant normalization of brain structures.

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ACC	Anterior Cingulate Cortex
AI	Anterior Insula
aMCC	anterior Midcingulate Cortex
Amy	Amygdala
BCH	Boston Children's Hospital
Cau	Caudate
CBP	Chronic Back Pain
CRPS	Complex Regional Pain Syndrome
CSF	Cerebral Spinal Fluid
Cu	Cuneus
dlPFC	dorsolateral Prefrontal Cortex
dmPFC	dorsomedial Prefrontal Cortex
dPCC	dorsal Posterior Cingulate Cortex
FAM	Fear-Avoidance Model
FDR	False Discovery Rate
FFG	Fusiform Gyrus
FM	Fibromyalgia
FMRIB	Functional MRI of the Brain
fMRI	functional Magnetic Resonance Imaging
FOPQ	Fear of Pain Questionnaire
FOV	Field of View

FSL	FMRIB Software Library
FSL-VBM	FMRIB Software Library-Voxel Based Morphometry
FWHM	Full-Width Half-Maximum
GM	Gray Matter
GMV	Gray Matter Volume
Hippo	Hippocampus
Hypo	Hypothalamus
ICV	Intracranial Volume
M1	Primary Motor Cortex
MFG	Middle Frontal Gyrus
mPFC	medial Prefrontal Cortex
MPRAGE	Magnetization-Prepared Rapid Acquisition Gradient Echo
MRI	Magnetic Resonance Imaging
MTG	Middle Temporal Gyrus
NAc	Nucleus Accumbens
NRS	Numerical Rating Scale
OFC	Orbitofrontal Cortex
optiBET	Optimized Brain Extraction Tool
pgACC	Pregenual Anterior Cingulate Cortex
p	calculated probability
PCC	Posterior Cingulate Cortex
PCu	Precuneus

PMA	Premotor Area
PCL	Paracentral Lobule
PMC	Premotor Cortex
PTG	Pars Triangularis Gyrus
Put	Putanem
r	Correlation coefficient
rACC	rostral Anterior Cingulate Cortex
ROI	Region of Interest
S1	Primary Somatosensory Cortex
SD	Standard Deviation
SFG	Superior Frontal Gyrus
SPSS	Statistical Package for the Social Sciences
TE	Time to Echo
Thal	Thalamus
TI	Time for Inversion
TIV	Total Intracranial Volume
TR	Repetition Time
VAS	Visual Analog Scale
VBM	Voxel-Based Morphometry
vmPFC	ventromedial Prefrontal Cortex
WM	White Matter

INTRODUCTION

Pain is a commonplace experience that is well known to almost every individual. Acute pain is usually caused by an inciting event, such as an injury or inflammation, and dissipates once the injury is healed or inflammation subsides. Chronic pain, on the other hand, endures even in the absence of inflammation or tissue injury. According to the National Institutes of Heath Pain Consortium, the public health burden of pain affects one-third of Americans and costs up to $635 billion each year (www.painconsortium.nih.gov).

Acute to Chronic Pain

Normally, the healing process occurs after an acute injury through inflammatory reaction, tissue restoration, and tissue remodeling, which lead to a resolution of the injury as well as pain sensation (Li et al., 2007). However, the transition from acute to chronic injury stems from an interruption in this process. Pathophysiology and histolopathological steps alter the afferent nociceptive information, leading to a reduced ability to inhibit pain impulses that normally produce an analgesic effect. Neuroplasticity of the central nervous system plays a key role in prolonging the peripheral lesion through continually sending signals to the spinal cord and eventually higher brain areas, remodeling and intensifying nociceptive transmission (Voscopoulos and Lema, 2010).

Although the transition to chronic pain involves an essential event of central sensitization that increases spinal cord inputs to the brain, many cognitive, affective, and

psychosocial factors play an important role in the development of chronic pain as well. In a study by Young Casey and colleagues (2008) who looked at the transition from acute to chronic pain and disability in neck and back pain, depressed mood and greater exposure to past traumatic life events were most predictive of chronic pain, whereas depressed mood and negative pain beliefs were most predictive of chronic disability (Young Casey et al., 2008). Chronic pain leads to cognitive and behavioral impairments that are oftentimes disproportional to the initial inciting event, causing increased anxiety, depression, and a reduced quality of life. Although pharmaceutical drugs are deemed highly effective in targeting acute pain, there does not seem to be a consistent pharmacological treatment for chronic pain. The reason is that such drugs aims to alleviate inflammation and local analgesia, which may relieve the initial injury but do little to affect pain in chronic conditions. Mansour and colleagues (2014) suggest that chronic pain is the consequence of plastic changes in cortical-limbic circuitry. While patients are in pain from their acute injury, aversive stimuli create salient memories and associations that translate into new learning of pain in other areas of life that is continually reinforced by their current pain (Mansour et al., 2014). Therefore, it is worthwhile to examine the structural and functional brain changes in patients with chronic pain in order to correlate such physical differences with clinical symptoms.

Brain Changes in Chronic Pain

In studies that have examined brain changes in chronic pain, involvement of both emotional circuitry and cortical reorganization is critical. A novel hypothesis proposed by

Mansour and colleagues (2014) suggests that chronic pain is a state of continuous learning. Acute pain perception involves activation of the anterior cingulate cortex (ACC) and insula as well as the limbic circuitry. The limbic circuitry includes a number of subcortical brain regions that are in constant communication with each other, driving emotion and behavior. The nucleus accumbens (NAc) plays a central role in the reward circuitry based on two neurotransmitters dopamine and serotonin, which promote desire and satiety, respectively (thebrain.mcgill.ca). The amygdala (Amyg) is the major region of emotions and arousal and is implicated as the center of negative affect (Simons et al., 2014). It is intricately connected to the hippocampus, the hub for memory. In the case of pain, the limbic system works together to promote fear learning. Although learning takes place in healthy patients, it is effectively extinguished over time with a concomitant resolution of pain. However, in development of chronic pain, the synaptic pathways enforced by learning interact with prefrontal cortex, specifically the medial and lateral areas, shifting nociceptive perception to an emotional suffering state. The lateral prefrontal cortex is involved in goal-directed and stimulus-driven attention (Asplund et al., 2010) while the medial prefrontal cortex plays a large role in memory and decision-making (Euston et al., 2012). The prefrontal cortex circuitry in turn influences cortical reorganization as a result of continual suffering and coping mechanism employed by the patient (Mansour et al., 2014). Over time, the persistent involvement of the limbic-cortical and prefrontal circuitry induces structural and functional brain changes in chronic pain patients.

As brain imaging methods advance, it has become possible to investigate potential morphometric brain region changes by comparing patients in chronic pain with healthy controls. Apkarian and colleagues (2004) observed that patients with chronic back pain (CBP) demonstrated impaired emotional decision-making that correlated with the magnitude of their back pain, suggesting that their pain extended to their ability to make decisions in other emotional tasks. In analyzing functional magnetic resonance imaging (fMRI) of these patients, they found that spontaneous pain engages the medial prefrontal cortex (mPFC) as well as the NAc. Interestingly, when comparing patients with healthy controls, deactivation of the NAc in response to a nociceptive cue was observed in patients, implying an abnormal pain processing mechanism in CBP patients (Apkarian et al., 2004).

In another study investigating structural and functional brain changes in fibromyalgia (FM) patients, Jensen and colleagues (2013) concluded that central plasticity is critical for the transition from acute to chronic pain. When compared to healthy controls, FM patients exhibited decreased cortical thickness, brain volume, and functional regional coherence in the rostral anterior cingulate cortex (rACC). The rACC plays a key role in descending inhibition of pain. The changes seen in FM patients may therefore indicate an abnormal dysfunction of descending pain modulation (Julien et al., 2005). Increased morphometric changes were associated with longer FM pain duration, indicating that long-term exposure to pain causes reduction in both activity and gray matter (GM) volumes in certain brain regions (Rodriguez-Raecke et al., 2009). In addition, brain changes in the mesolimbic system were associated with severity of

comorbid depression symptoms, which suggests that depression symptoms are associated with cerebral changes, independent of pain (Jensen et al., 2013).

Pediatric Chronic Pain

Chronic pain in children and adolescents has a prevalence rate of 11-38% (King et al., 2011). Chronic pain is especially impressionable on children because it influences cognitive and social development in a period that is particularly vulnerable to change. Moreover, the peak onset of pediatric chronic pain occurs in adolescents (King et al., 2011). Factors such as increased susceptibility to fear learning can result in pain persistence, treatment resistance, and increase risk of continuation of chronic pain condition into adulthood (Pattwell et al., 2012).

In a recent review by Goubert and Simons (2013), the determinant of chronicity of pain experience in pediatrics depends heavily on the cognitive styles of both the child and parents. Goubert and Simons suggested that the pain outcome is determined by the way an individual perceives and interprets pain. Primarily, a fearful temperament and pessimistic outlook are associated with poorer mental health and more long-term somatic pain (Goubert and Simons, 2013). Catastrophic thinking, specifically "focusing and exaggerating the threat value of painful stimuli and negatively evaluating one's own ability to deal with pain" (Sullivan et al., 2001), is predictive of persistence of pain and disability (Linton et al., 2000). Child catastrophizing may manifest in the way the child expresses his or her pain experience, which provokes anxious and protective behaviors of parents and may further encourage fear-avoidance beliefs and behavior of the child. This

cycle ultimately leads to maintenance of pain experience and increased functional disability (Goubert and Simons, 2013).

With the continuance of such fear-avoidant cognition and motivation, it follows that the presence of chronic pain will undoubtedly influence areas of the brain mediating pain perception and emotion circuitry. Based on the relatively high plasticity of pediatric brain (www.albertafamilywellness.org), the impact of chronic pain may have different magnitude and direction and may affect dissimilar brain regions than the corresponding pain conditions in adults. Unfortunately, there is a relative small amount of literature that has examined the brain changes in pediatric chronic pain disorders, furthering the need to do so in the current study.

Complex Regional Pain Syndrome (CRPS)

Complex Regional Pain Syndrome (CRPS) is a neuropathic pain condition that is characterized by burning and exaggerated painful sensation (hyperalgesia) and pain in response to non-painful stimulus (allodynia) (Birklein et al., 2000). Other autonomic symptoms may accompany the condition: sweating, edema, change in skin color, and temperature change. In addition, motor disturbances, such as weakness, tremor, and muscle spasms may be present (Veldman et al., 1993).

CRPS typically develop after an inciting injury and affects the corresponding part of the body. However, in some cases, the condition is developed idiopathically in less than 5% of patients (Veldman et al., 1993). Two types of CRPS are diagnosed. CRPS-1, formally known as reflex sympathetic dystrophy or Sudeck's atrophy, precipitates

without identifiable peripheral nerve damage. In CRPS-2, formally known as causalgia, neural damage can be identified (Stanton-Hicks et al., 1995).

Several studies investigated the incidence of CRPS and found that women ages 61-70 years are at most risk. Upper limb is more frequently affected than lower limb (de Mos et al., 2007; Sandroni et al., 2003). In studies that tested the efficacy of a range of treatment for CRPS, it was found that sympathetic suppression, radical scavenging, and acupuncture had limited results, whereas interventions involving exercise and calcium-regulating drugs proved most effective (Forouzanfar et al., 2002; Perez et al., 2001). Current treatment takes on a multidisciplinary approach that involves cognitive-behavioral therapy, such as relaxation training and readjusting catastrophizing cognition, as well as continuation of pharmaceutical drugs (Bruehl and Chung, 2006).

Brain Changes in CRPS

Several studies have assessed neural organization of patients with CRPS and possible treatment approaches aimed to specifically target cortical changes. In a study by Swart and colleagues (2009), cortical involvement of CRPS was found to include mislocalizations of tactile stimuli, changes in size and organization of the somatosensory map, changes in motor cortex representation, and body perception disturbances. Swart and colleagues concluded that the pain of patients with CRPS is a result of a mismatch between motor intention and incorrect sensory feedback of the affected limb. The chronic development of this pain condition is essentially a self-perpetuation of motor cortex reorganization that is caused by this incongruence of efferent and afferent inputs, and the

consequential disuse of the affected body part that leads to further reorganization of the cortex (Swart et al., 2008). Therefore, therapies that aim to resolve this mismatch, for example, mirror therapy (Ramachandran and Hirstein, 1998) or motor imagery programming, (Moseley, 2004) will eventually restore the disorganized body scheme (Harris, 1999).

Several other studies have taken a neuroimaging approach to examine structural brain changes in patients with CRPS that involve both gray matter (GM) and white matter (WM) changes as well as differences in functional connectivity. In a pioneering study, Geha and colleagues (2008) found GM atrophy in the right anterior insula (AI), right ventromedial prefrontal cortex (vmPFC), and right nucleus accumbens (NAc). Correlation studies associated right AI with duration of CRPS pain (Geha et al., 2008). Since the insula is activated in acute pain tasks (Apkarian et al., 2005) and its activation correlated with ratings of allodynia and hyperalgesia (Maihöfner et al., 2005), alteration in AI may point to abnormal autonomic function. The right AI is also implicated in emotional representation and correlates with subjective ratings of visceral awareness (Critchley et al., 2005), suggesting that atrophy in the right AI supports the clinical finding of negative emotional state of patients with CRPS. Atrophy in vmPFC correlates with duration and intensity of CRPS pain (Geha et al., 2008). Neurons in the vmPFC project to the hypothalamus and brainstem areas that link autonomic regulation with emotional responses (Ongür and Price, 2000), as well as the periaqueductal gray in modulating nociceptive inputs (An et al., 1998). Taken together, these findings suggest

that the alterations in GM density in the AI and vmPFC are involved in pain manifestation and autonomic irregularities in patients with CRPS.

Other studies have found contradictory results for the direction of brain changes or the differences in affected cortical regions. In their study, Pleger et al. (2014) concluded that CRPS affects brain structures in the prefrontal and motor cortex. More specifically, patients with CRPS showed an increase in GM density in the dorsomedial prefrontal cortex (dmPFC), as well as an increase in GM density in the primary motor cortex (M1) contralateral to the affected limb. Since the dmPFC is involved in coding emotional correlates of pain (Porro et al., 2003) and top-down modulation of central pain networks (Napadow et al., 2009), the increased GM density of dmPFC can be seen as a result of a response to the emotional process involved in modulating pain (Pleger et al., 2014). An increased GM density in M1 has been shown to correlate with individual extent of motor dysfunction (Maihöfner et al., 2007), therefore reflecting an amplified activation in motor response. In examining gray-white matter interaction between motor cortex and internal capsule, Pleger and colleagues (2014) found that an increase in GM density in M1 was associated with a decrease in WM density in the ipsilateral internal capsule. This finding possibly suggests a compensatory mechanism of the central motor system caused by motor dysfunction (Pleger et al., 2014).

A study by Barad and colleagues (2014) examined structural abnormalities specifically in pain-related regions. The analysis yielded the following findings: (1) decreased GM volume in the dorsal insula, left orbitofrontal cortex (OFC), and cingulate cortex, and (2) increased GM volume in the bilateral dorsal putamen and right

hypothalamus. Regions such as the cingulate and the OFC are found in the limbic system and are implicated in the general pain population (Ruscheweyh et al., 2011). Volumetric abnormalities of the limbic system also suggest dysregulated emotional processing of pain information in CRPS (Barad et al., 2014). The pregenual anterior cingulate cortex is involved with unpleasantness and suffering (Ploner et al., 2002), while the OFC exhibits an increase in activation during expectation of pain (Mohr et al., 2005). An increased GM volume in the putamen, an area involved in processing pain (Brooks et al., 2005), could be driven by a compensatory action against sustained nociceptive input (Barad et al., 2014). Increase in hypothalamus GM volume could be responsible for the irregular autonomic symptoms in CRPS (Lebel et al., 2008).

Pediatric Complex Regional Pain Syndrome

CRPS is also found in children and adults, albeit it is a rarer condition. Although it is well characterized in adults, pediatric CRPS is a relatively new diagnostic entity (Tan et al., 2008). There are several notable differences in the disease state between pediatrics and adults. The lower limb is more frequently affected (Low et al., 2007), the skin temperature of the limb is often cooler, and there are less pronounced neurological and sympathetic symptoms (Tan et al., 2008). In addition, significant trauma is much less frequently a precipitating event than in adults (Wilder et al., 1992). CRPS in pediatrics, as in the adult population, predominantly affects females (Low et al., 2007).

When examining the prognosis of pediatric CRPS, children have shown better response to noninvasive treatment (Murray et al., 2000), and psychosocial factors play a

greater role in the outcome of treatment (Wesdock, 1991). Several studies have reviewed an array of treatment approaches and compared them for efficacy. Kachko et al., (2008) found that early recognition and management are major factors in improving the outcome of CRPS. Early psychological evaluation and cognitive behavior treatment are crucial to preventing resistant CRPS (Kachko et al., 2008). In another study, Low et al., (2007) concluded that a combination of physiotherapy, psychological intervention, and medication, as well as an early diagnosis (average of 5 weeks), is key to a shorter time to symptom resolution (average 10 weeks). Interestingly, bone scans are also a useful diagnostic and prognostic tool. Diffuse hypoperfusion achieved symptom resolution in a shorter time (average 12.2 weeks) compared with patients with normal or hyperperfusion scans (average 16 and 28.4 weeks, respectively) (Low et al., 2007).

As previously mentioned, most children achieve full recovery either spontaneously or through therapeutic interventions within a year (Low et al., 2007). Because of the resiliency of the nervous systems of children and the modifiable nature of their brain changes (Becerra et a., 2014), pediatric patients with CRPS make excellent candidates for neuroimaging studies to investigate brain changes pre- and post-treatment and to examine morphometric and functional alterations as they correspond to treatment outcomes.

Erpelding and colleagues (2014) examined the differences in brain functionality and structure in pediatric patients with CRPS. A combination of a cross-sectional study and longitudinal study was conducted in order to assess disease effect (comparing patients and healthy controls) and treatment effect (comparing patients pre- and post-

treatment). When compared with healthy controls, patients with CRPS had significant GM differences in pain-related cortical areas including dorsolateral prefrontal cortex (dlPFC), motor (M1) and somatosensory cortex (S1), regions within anterior (ACC) and posterior cingulate cortex (PCC). Furthermore, patients exhibited reduced GM volumes in clusters of subcortical regions including caudate (Cau), putamen (Put), nucleus accumbens (NAc), anterior thalamus (Thal), amygdala (Amy), and anterior hippocampus (Hippo), providing possible markers for a disease state brain. When brain structure was assessed post-treatment, patients showed significant reversal of some structural alterations, including increased GM in the dlPFC, Put, Cau, and Thal, that correlated with behavioral improvements (Erpelding et al., 2014). These results suggest that such rapid brain changes may be indicative of improvement of the disease state.

Influence of Fear in Context of Pain

When assessing treatment outcome in patients with chronic pain, psychological factors, specifically pain-related fear and avoidance, play an important role in progress and persistence of pain-related disability in the adult population (Leeuw et al., 2007). Simons and Kaczynski (2012) also found this to be true in children and adolescents with chronic pain. In their study examining associations with treatment response in the context of intensive pain rehabilitation, pain-related fear was significantly associated with disability and depressive symptoms. Furthermore, high levels of pain-related fear at baseline predicted worse treatment response as measured by less reduction in functional disability and depression at discharge (Simons and Kaczynski, 2012).

A possible explanation that provides a link between pain-related fear and treatment response can be demonstrated in the fear-avoidance model (FAM) of chronic pain, modified for pediatric patients (Simons and Kaczynski, 2012). Patients who develop fear-of-pain tend to experience events such as hypervigilance and guarding behaviors that maintains their pain, leading to avoidance and eventually disuse, disability, and depression (Leeuw et al., 2007). On the other hand, if patients continue with physical activities, testing and correcting their pain expectations, pain symptoms are likely to resolve (Lohnberg, 2007).

Figure 1: Fear-avoidance model of chronic pain. Predictive components, pain catastrophizing and pain-related fear, have been shown to be predictive components of pain-related disability. Adapted from Simons et al., 2012.

A validated and reliable measure used to evaluate pain-related fear and avoidance in pediatric chronic pain patients is the Fear of Pain Questionnaire (FOPQ; Simons et al., 2011). The psychological measure is subdivided into two scores: Fear of Pain and Avoidance of Activities, and will be used in this study as the principal measurement to assess patient level of fear.

Pain-Related Fear Circuitry

Advances in neuroimaging technique have made it possible to examine brain structures and functional connectivity as they relate to pain and analgesia. Regions such as the somatosensory cortex, thalamus, insula, anterior cingulate cortex, and prefrontal cortex have been consistently implicated in the pain process, with recent discoveries of involvement of subcortical areas such as the hippocampus, basal ganglia, and amygdala (Simons et al., 2014). The amygdala is well known as the hub of negative affect, receiving pain stimuli and attaching emotional significance to sensory information (Paré et al., 2004). Within the amygdala, the lateral nucleus connects with the centromedial nucleus to control expression of conditioned fear responses. The prefrontal cortical areas, such as ventromedial prefrontal cortex (vmPFC), project to the lateral nucleus of the amygdala, demonstrating a cognitive component of memories and expectations for pain. The vmPFC along with the intercalated cells are also responsible for inhibition of fear expression through connections with the centromedial nucleus of the amygdala. The hippocampus projects to the basal nucleus of the amygdala and is involved in processing contextual information during fear conditioning (Simons et al., 2014). The lateral nucleus

of the amygdala also receives projections from the anterior cingulate cortex, which is involved in affective processing, selection of motor responses, and memory to predict and avoid pain (Devinsky et al., 1995).

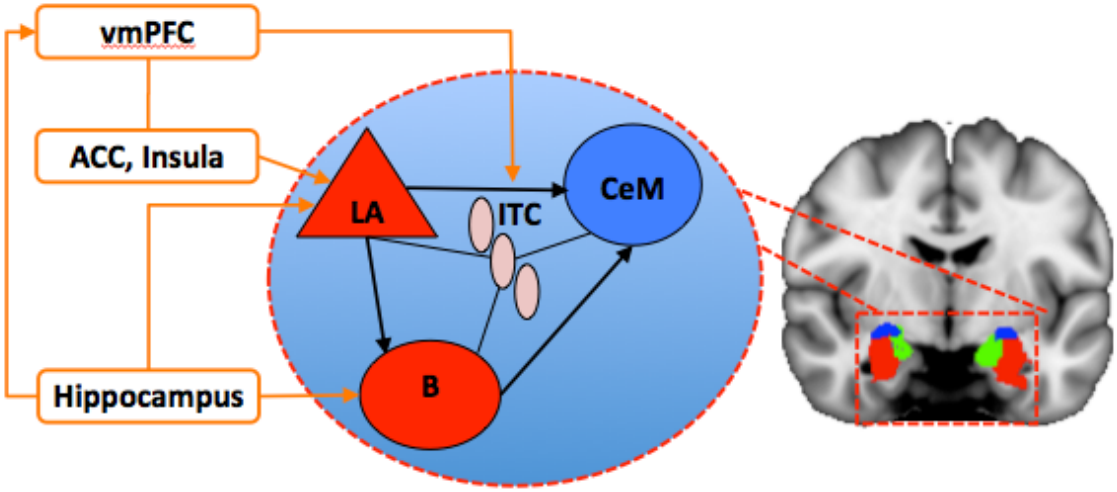

Figure 2: Neural pathways of fear learning. The lateral nucleus (LA) connects with the centromedial nucleus (CeM), controlling expression of conditioned fear responses. Projectsion exist from ventromedial prefrontal cortex (vmPFC), insula, anterior cingulate cortex (ACC), and hippocampus to LA. Additional projections exist between hippocampus to basal nucleus (B). Intercalated cells (ITC). Adapted from Simons et al., 2014

The Present Study

The present data and analysis are part of a larger investigation that integrates functional and structural brain differences to evaluate fear-related brain circuitry in patients with CRPS. Our patient population consists of patients with CRPS recruited primarily from the Pain Treatment Service and Pediatric Pain Rehabilitation Center at Boston Children's Hospital (Boston, MA). Our goal is to establish structural and functional differences in pediatric chronic pain patients compared with healthy controls and to evaluate the unique influence that pain-related fear plays on brain morphometry.

The specific focus of the current study aims to examine structural differences present in patient brains and to determine the degree to which fear level corresponds to such morphometric changes. In doing so, we hope to establish differences in brain structures as indicators of disease state that could provide insight for prognosis and treatment response.

Specific Aims

In this current study, we aim to:

(1) Examine structural brain differences between patients with CRPS and matched healthy controls.

(2) Investigate the influence of fear level by assessing structural brain differences in patients with high fear and non-elevated fear and by comparing them with healthy controls.

We hypothesize that the study will show:

(1) The existence of a significant difference in brain structures, especially in pain- and fear-related regions (e.g. dlPFC, M1, S1, Amy, Hippo) between patients and healthy controls.

(2) The importance of the high fear group in driving the differences in brain structures when compared with non-elevated fear patients and healthy control.

METHODS

Participants

A total of 37 patients with CRPS were recruited from the Chronic Pain Clinic in the Pain Treatment Service at Boston Children's Hospital (BCH) for this BCH Institutional Review Board approved study. Both the patient and a parent gave consented for this study. Parents were present during the study visits. Patients were included in the study if (1) they refrained from using analgesic medication more than 4 hours prior to the study session, (2) they experienced unilateral lower extremity CRPS (based on Budapest criteria; Harden et al., 2010), and (3) their pain intensity was greater than 5 on a 11-point numerical rating scale (NRS). They were excluded from the study if they had (1) claustrophobia, (2) significant medical problems (e.g., uncontrollable asthma and seizures, cardiac diseases, severe psychiatric disorders, and neurological disorders other than CRPS), (3) pregnancy, (4) medical implants, devices, or both and (5) weight more than 285 pounds which corresponded to the weight limit of the magnetic resonance imagining (MRI) table. Gender- and age-matched healthy controls (n=35) were recruited in the greater Boston area through advertisements. Each study session consisted of a neurological exam with a study physician, questionnaires, and an MRI scan.

Fear of Pain Measure

The Fear of Pain Questionnaire (FOPQ) (Simons et al., 2011) is a self-report inventory to assess pain-related fears. Each item is rated on a 5-point Likert-type scale

from 0 = “strongly disagree” to 4 = “strongly agree.” The FOPQ consists of 24 items with strong internal consistency (α =0 .92). The measure has two subscales: Fear of Pain (α = 0.89) and Avoidance of Activities (α = 0.86). Construct validity for this measure is supported with significant relations found for the FOPQ with child somatization, anxiety, and catastrophizing. Criterion-related validity is also supported with significant relations between higher FOPQ scores and greater functional disability and more frequent doctor visits in the previous three months. Stability of the FOPQ total scale score is adequate (α =0 .74) with decreases in FOPQ scores associated with concomitant decreases in functional disability (r = 0.45) at one-month follow-up, suggesting sensitivity to treatment response (Simons et al., 2011). High fear (FOPQ≥50) and non-elevated fear (FOPQ<50) groups used in this study were defined from tertiles in the validation sample (Simons et al., 2011).

Brain Imaging and Data Analysis

MRI Acquisition

Subjects underwent MRI on a 3T scanner (Siemens Medical Solutions, Erlangen, Germany) using a 12-channel head coil. For each participant, we collected a 3D T1-weighted anatomical scan using a magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence (128 slices; repetition time (TR) = 2100 ms; time to echo (TE) = 2.74 ms; time for inversion (TI) = 1100 ms; 256 × 256 matrix; field of view (FOV) = 200 mm; 1.33 × 1.0 × 1.0 mm voxels). Subjects were instructed to relax with their eyes open looking at a blank screen.

MRI Preprocessing and Data Analysis

Cortical Thickness

To analyze whether patients exhibited cortical GM alterations compared with healthy control subjects and by pain-related fear level, we performed cortical thickness analysis using FreeSurfer software (http://surfer.nmr.mgh.harvard.edu). MPRAGE preprocessing steps included (1) intensity normalization, (2) skull stripping, (3) Talairach transformation, (4) hemispheric separation, (5) tissue segmentation, (6) identification of white surface and pial surface, (7) cortical parcellation, and (8) registration to the average surface map. Finally, a 10-mm full-width half-maximum (FWHM) Gaussian smoothing kernel was applied. Cortical thickness analysis was performed at the whole brain level. Results were corrected for multiple comparisons based on Monte Carlo permutations with 5,000 iterations using the AlphaSim program (http://afni.nimh.nih.gov/afni/doc/manual/AlphaSim). With an image-wide threshold of $p < 0.01$ and a Bonferroni-corrected $p < 0.025$ (to correct for each hemisphere), AlphaSim simulations revealed that 76 contiguous vertices were required for clusters to be significant.

Subcortical Volume

To investigate subcortical GM volume differences by pain-related fear level among patients and in contrast to controls, we used two approaches: (1) whole structure subcortical volumes derived in the FreeSurfer processing stream (aseg stats) and (2)

voxel-based morphometry (VBM) using FSL-VBM (Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library-VBM) (Douaud et al., 2007) to examine differences within subcortical structures. For VBM, preprocessing steps included (1) brain extraction using an optimized brain extraction tool (optiBET) script (Lutkenhoff et al., 2014), (2) tissue-type segmentation into GM, white matter (WM), and cerebrospinal fluid (CSF), (3) nonlinear registration of GM partial volume maps to MNI152 standard space, (4) creation of a study-specific GM template, (5) nonlinear registration of GM images to study-specific GM template, and (6) modulation to correct for local expansion or contraction (i.e., by dividing them by the Jacobian of the warp field). Finally, the modulated registered GM images were smoothed with a FHWM kernel of 7.05 mm (i.e., sigma = 3). To control for variability in head size, the total intracranial volume (TIV) was extracted for each subject and entered as a variable of no interest for subcortical gray matter volume (GMV). To correct for multiple comparisons, the voxelwise threshold of significance was set at *p* values less than 0.05 when corrected for family-wise error (FEW) at the cluster level.

ROI Analysis

To increase power in our study, we conducted region-of-interest (ROI) analysis using a subcortical mask that contained the thalamus (Thal), caudate (Cau), putamen (Put), hippocampus (Hippo), nucleus accumbens (NAc), amygdala (Amy), and hypothalamus (Hypo). We also included the cingulate cortex, both anterior (ACC) and posterior (PCC). Selection of these regions was based on results from recent work

identifying these regions as having stronger synchronicity in activity with the amygdala at rest in pediatric CRPS (Simons et al., 2014). Masks for ROI analysis were created using the Harvard–Oxford Subcortical Structural Atlas in FSL (http://www.cma.mgh.harvard.edu/). When analyzing mean group differences between high fear patients, non-elevated fear patients, and healthy controls, FSL general linear model (GLM) F-test was conducted. If the F-test was significant, individual t-tests (i.e. high fear versus non-elevated fear, high fear versus healthy controls, and non-elevated fear versus healthy controls) were then used to determine the direction of the effect.

RESULTS

Participants

All 37 patients and 35 healthy controls were included for the cortical thickness and gray matter volume analysis. Within this sample, a subsample of 12 patients with CRPS had been previously used to investigate functional connectivity changes of specific brain regions (i.e., habenula, amygdala) (Erpelding et al., 2014; Simons, et al., 2014). Also, structural data from 23 patients and 21 controls had been previously reported (Erpelding et al., 2014). Of note, half of the patient brains had been flipped in the Erpelding et al. (2014), analysis to control for affected side in examining impact of disease state on brain structures, notably the somatosensory and motor cortices where laterality may act as a confounding variable. Given that the current study focuses on emotional circuitry and possible sidedness of such processing, brains were not flipped for this analysis.

Among the 37 patients with CRPS in the study, 73% were female with age range from 8-20 years (mean ± SD age 13.2 ± 2.7 years). This was commensurated with the matched healthy controls (77% female; mean ± SD age 13.4 ± 2.8 years). All patients with CRPS had a unilateral lower extremity affected limb (57% on the left side). Duration of pain ranged from 1-85 months (mean ± SD duration 13.2 ± 17.5 months). All study participants were right-handed.

Pain and Pain-Related Fear

Among the 37 patients, 89% of patients reported moderate (38%; VAS rating 4-6) to severe (51%; VAS rating 7-10) average pain levels (mean ± SD VAS 6.4 ± 2.1). Among the 35 patients who completed the FOPQ, 49% reported clinically significant pain-related fear (mean ± SD FOPQ 61 ± 7.3). To investigate the effect of fear levels on structural brain changes, we placed patients with FOPQ≥50 into a high fear group and patients with FOPQ<50 into a non-elevated fear group. Pain-related fear and pain were not statistically significantly associated with one another (r=0.13, $p=0.45$).

Patients Versus Healthy Controls

Cortical Thickness

There were several significant cortical differences between patients and controls with thinner cortices observed in each instance among patients. Compared with healthy controls, patients exhibited less cortical GM in pain-related brain regions such as the precentral gyrus (PreCG), postcentral gyrus (PoCG), paracentral lobule (PCL), superior temporal gyrus (STG), middle frontal gyrus (MFG), posterior cingulate cortex (PCC), fusiform gyrus (FFG), precuneus (PCu), and cuneus (Cu) (see **Table 1** and **Figure 2**). In addition, patients showed a decreased cortical thickness in the anterior midcingulate cortex (aMCC) compared with healthy controls.

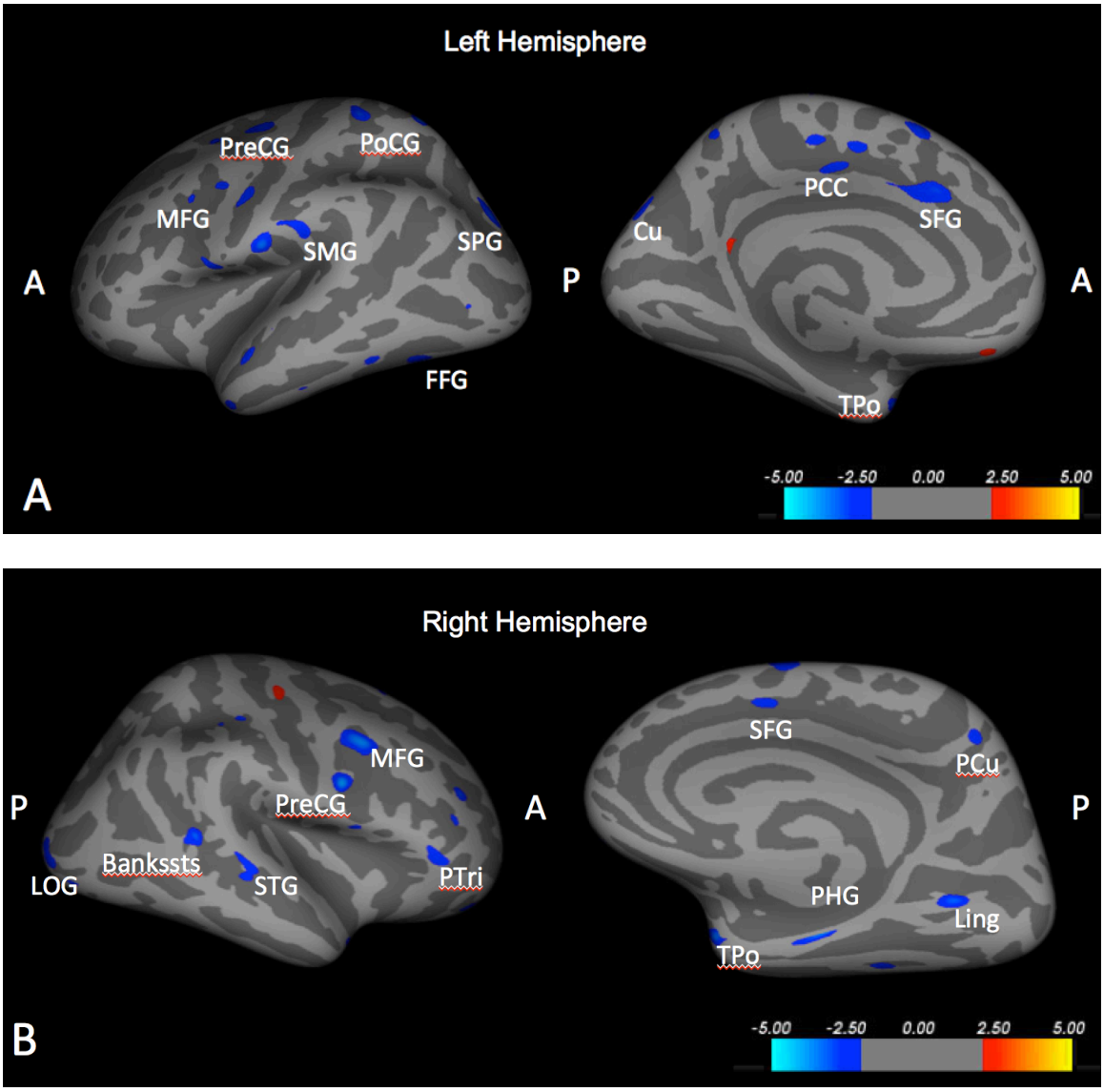

Figure 3: Significant cortical thickness differences between patients with CRPS and healthy controls. (A) *Left hemisphere*. Compared to healthy controls, patients exhibited less cortical GM in brain regions including the precentral gyrus (PreCG), postcentral gyrus (PoCG), middle frontal gyrus (MFG), superior middle gyrus (SMG), fusiform gyrus (FFG), superior parietal gyrus (SPG), superior frontal gyrus (SFG), posterior cingulate cortex (PCC), cuneus (Cu), and temporal pole (TPo). (B) *Right hemisphere*. Compared to healthy controls, patients exhibited less cortical GM in brain regions including the lateral occipital gyrus (LOG), banks of the superior temporal sulcus (Bankssts), precuneus (PCu), superior temporal gyrus (STG), pars triangularis (PTri), parahippocampal gyrus (PHG), and lingual (Ling). T-test of 2.50 corresponds to $p = 0.05$. Colors: blue to light blue = significant decrease compared with controls; red to yellow = significant increase compared with controls. N=37 (patients); N=35 (controls). CRPS = complex regional pain syndrome; GM = gray matter.

Table 1. Cortical and subcortical gray matter differences between patients and healthy controls. Patients show decreased GM cortical thickness in all regions.

Brain Region	Vertices/ Voxels	MNI Coordinates			*T*
		x	y	z	
Cortical Thickness: *Patients < Controls*					
Left Hemisphere					
Superior frontal gyrus	332	-13	17	34	2.95
Superior parietal cortex	290	-17	-55	59	3.72
Postcentral gyrus	240	-60	-17	17	3.40
Precentral gyrus	231	-26	-13	51	2.76
Postcentral gyrus	228	-24	-36	59	2.95
Supramarginal gyrus	217	-61	-29	20	2.41
Superior parietal cortex	212	-23	-83	27	3.03
Temporal pole	171	-34	13	-35	3.05
Precentral gyrus	165	-56	5	4	2.76
Superior frontal gyrus	161	-7	11	64	2.85
Precentral gyrus	161	-46	-8	30	2.70
Posterior cingulate cortex	160	-12	-16	38	2.66
Cuneus	133	-6	-82	31	2.48
Fusiform gyrus	131	-43	-63	-16	2.54
Superior frontal gyrus	125	-10	-12	50	2.59
Superior temporal gyrus	90	-50	-8	-17	2.35
Temporal pole	84	-44	2	-34	2.39
Paracentral lobule	83	-13	-28	48	2.33
Right Hemisphere					
Middle frontal gyrus	404	37	7	40	3.67
Supramarginal gyrus	372	45	-41	10	3.27
Superior frontal gyrus	340	8	13	63	2.64
Temporal pole	303	30	8	-34	3.42
Lateral occipital gyrus	252	29	-96	-1	2.52
Superior temporal gyrus	240	57	-25	-1	2.31
Precentral gyrus	222	50	6	27	3.90
Lingual gyrus	192	9	-63	5	3.22
Pars triangularis gyrus	179	51	34	-2	2.83
Precentral gyrus	157	37	-19	57	2.58
Parahippocampal gyrus	149	23	-24	-20	3.47
Precuneus	121	7	-50	54	2.73
Frontal pole	100	31	40	21	2.96
Superior frontal gyrus	93	13	10	42	2.62
Fusiform gyrus	79	38	-49	-19	2.40

T-test of 2.50 corresponds to $p = 0.05$. N=37 (patients); N=35 (controls). MNI = Monteal Neurological Institute.

Subcortical Volume

There were two significant clusters of subcortical areas that were different between patients and controls. Compared with healthy controls, patients exhibited reduced GM volume in a cluster that included the left nucleus accumbens (NAc) and putamen (Put) and in another cluster that included the left amygdala (Amy) and hippocampus (Hippo) (**Figure 4**). We did not find any subcortical regions with significant increased GM volume compared with healthy controls.

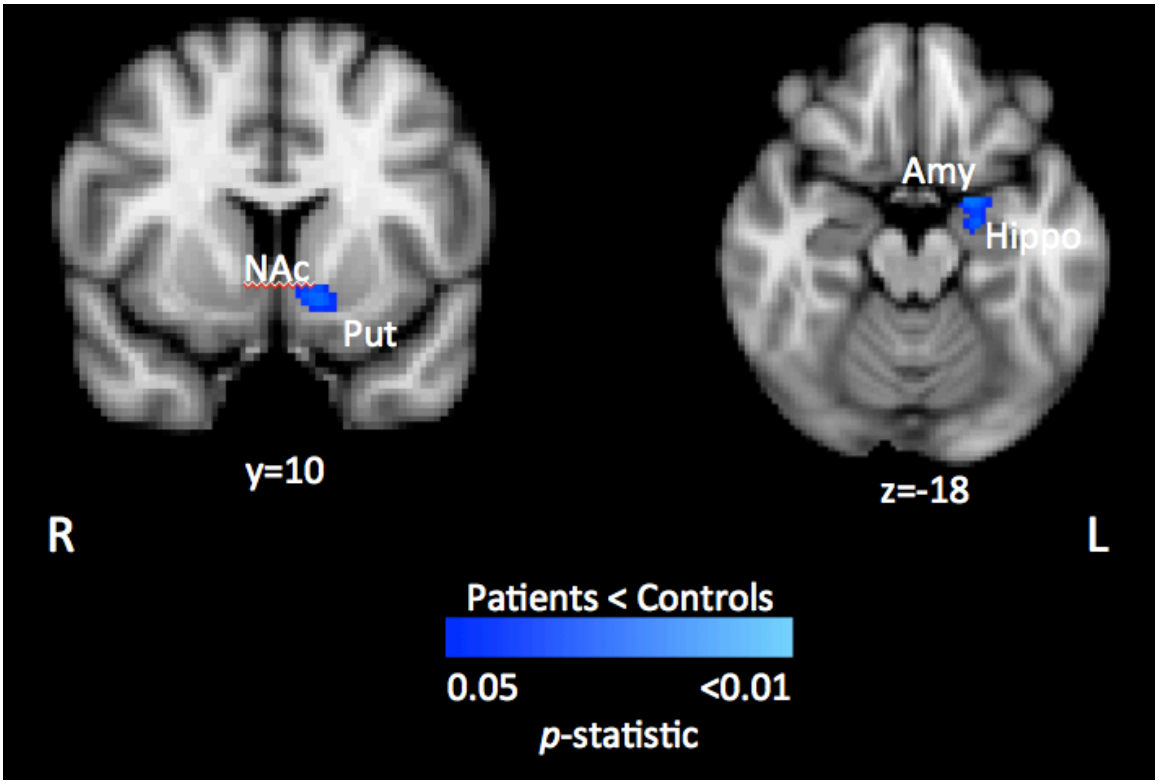

Figure 4: Significant subcortical volume differences between patients with CRPS and healthy controls. Compared with healthy controls, patients exhibited less subcortical GM volume in pain and fear-related brain regions including the left nucleus accumbens (NAc), putamen (Put), amygdala (Amy), and hippocampus (Hippo). Colors: blue to light blue = significant decrease compared with controls. N=37 (patients); N=35 (controls). CRPS = complex regional pain syndrome; GM = gray matter.

Influence of Pain-Related Fear Level

Cortical Thickness

There were several differences between high fear patients, non-elevated fear patients, and healthy controls. Compared with healthy controls, high fear patients had decreased cortical thickness in notable regions that were also found to be significantly different when comparing patients and healthy controls. These regions include middle frontal gyrus (MFG), superior temporal gyrus (STG), precentral gyrus (PreCG), superior frontal gyrus (SFG), superior parietal cortex (SPG), and cuneus (Cu). Unique brain areas that showed significant cortical thinning in high fear patients when compared to healthy controls include transverse temporal gyrus (TTP), anterior cingulate cortex (ACC), paracentral lobule (PCL), and pericalcarine cortex (PCAL). (see **Figure 5** and **Table 2**). In addition, ROI analysis revealed that compared with both non-elevated fear patients and healthy controls, high fear patients showed a thinner anterior midcingulate cortex (aMCC) and pregenual anterior cingulate cortex (pgACC) (**Figure 6**). High fear patients did not have any cortical regions with significant increased GM thickness compared with non-elevated fear patients.

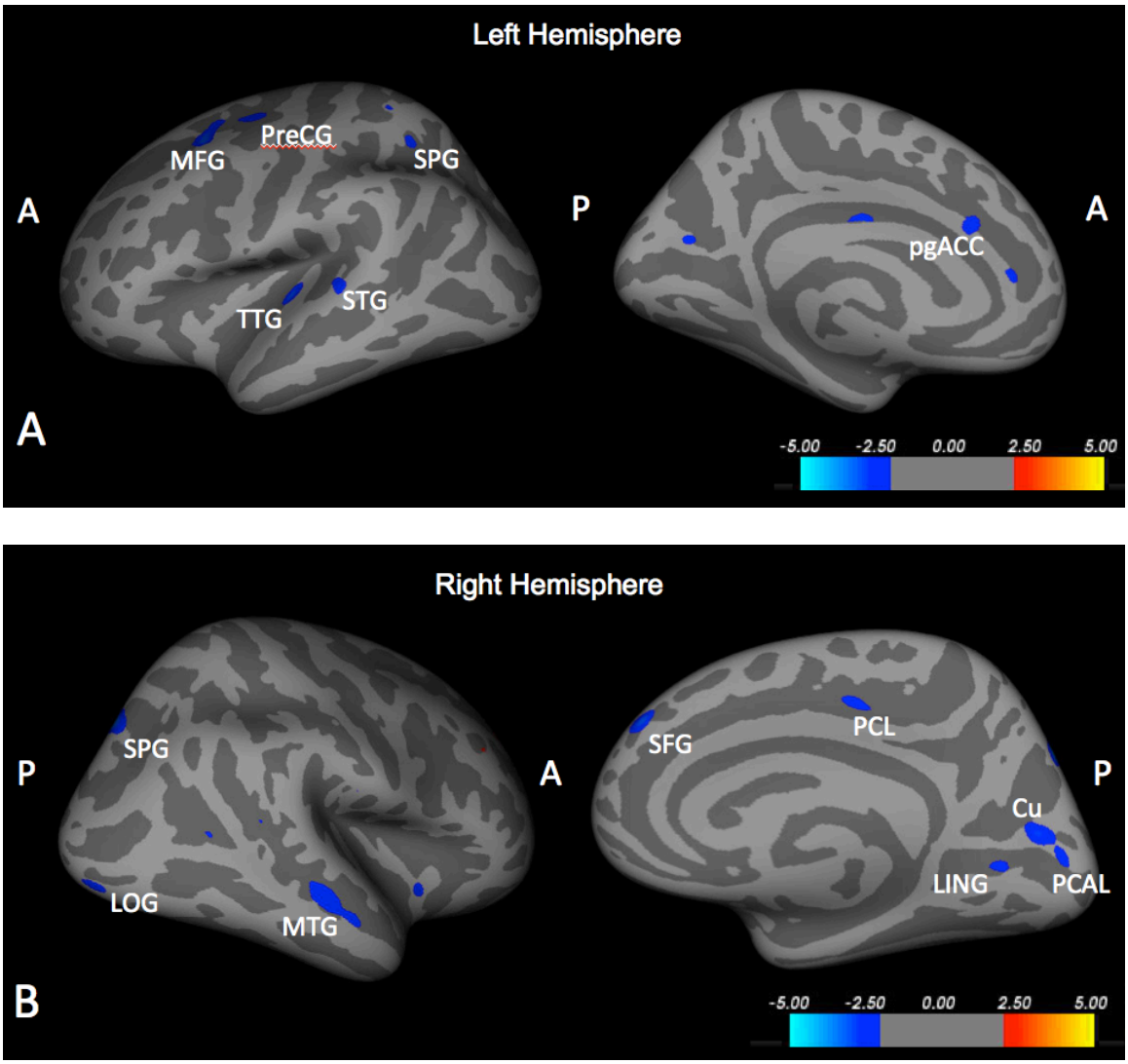

Figure 5: Significant cortical thickness differences between high fear patients and non-elevated fear patients. (A) *Left hemisphere*. Compared to non-elevated fear patients, high fear patients exhibited less cortical GM in brain regions including the middle frontal gyrus (MFG), precentral gyrus (PreCG), superior parietal gyrus (SPG), transverse temporal gyrus (TTP), superior temporal gyrus (STG), and pregenual anterior cingulate cortex (pgACC). (B) *Right hemisphere*. Compared to non-elevated fear patients, high fear patients exhibited less cortical GM in brain regions including lateral occipital gyrus (LOG), Cuneus (Cu), superior frontal gyrus (SFG), paracentral lobule (PCL), paracalcarine gyrus (PCAL), and lingual (LING). T test of 2.50 corresponds to $p = 0.05$. T-test of 2.50 corresponds to $p = 0.05$. Colors: blue to light blue = significant decrease compared with controls; red to yellow = significant increase compared with controls. N=37 (patients); N=35 (controls). CRPS = complex regional pain syndrome; GM = gray matter.

Table 2. Cortical thickness differences between high fear patients and non-elevated fear patients.

Brain Region	Vertices/ Voxels	MNI Coordinates			*T*
		x	y	z	
Cortical Thickness: *High < Non-elevated*					
Left Hemisphere					
Middle frontal gyrus	288	-30	8	54	3.11
Transverse temporal gyrus	168	-45	-21	2	3.07
Superior temporal gyrus	147	-64	-28	6	2.70
Precentral gyrus	143	-24	-10	47	2.67
Anterior cingulate cortex	89	-11	21	29	2.42
Superior parietal lobule	87	-30	-51	50	2.66
Right Hemisphere					
Middle temporal gyrus	468	49	-30	-10	2.70
Superior parietal cortex	330	20	-77	44	3.58
Cuneus	214	6	-68	16	2.92
Superior frontal gyrus	148	7	50	30	3.15
Paracentral lobule	99	15	-15	41	2.58
Lateral occipital gyrus	90	31	-93	-13	2.29
Lingual gyrus	88	21	-69	3	2.52
Pericalcarine cortex	79	8	-82	10	2.62

T-test of 2.50 corresponds to $p = 0.05$. N=37 (patients); N=35 (controls). MNI = Monteal Neurological Institute.

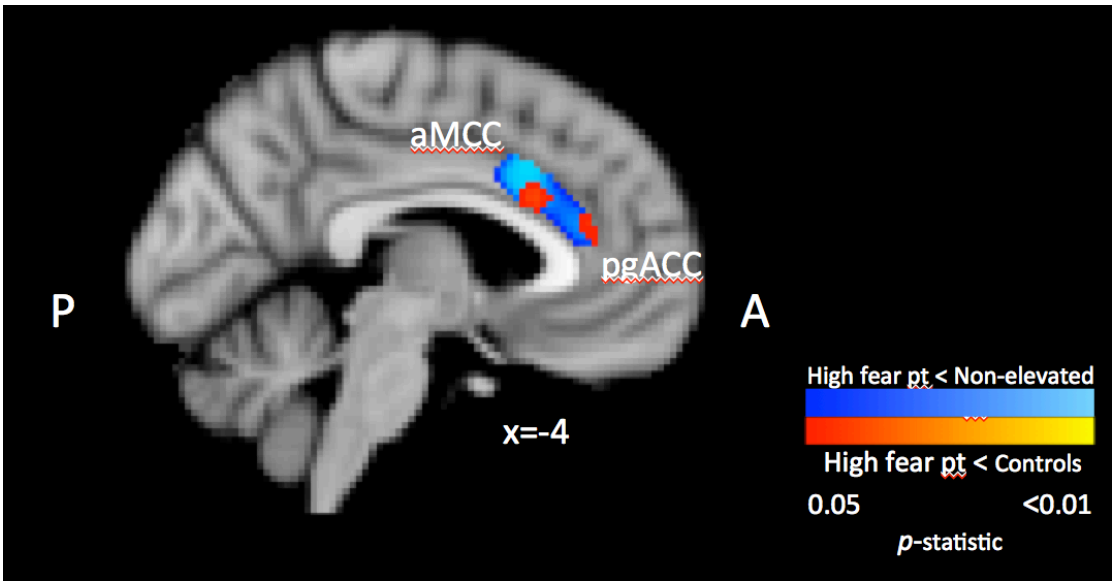

Figure 6: Significant VBM gray matter differences in high fear patients compared to non-elevated fear patients and healthy controls. Compared to both non-elevated fear patients and healthy controls, high fear patients exhibited less GM in a cluster consisting of the anterior midcingulate cortex (aMCC) and the pregenual anterior cingulate cortex (pgACC). Colors: blue to light blue = significant decrease compared with controls; red to yellow = significant increase compared with controls. N=37 (patients); N=35 (controls). CRPS = complex regional pain syndrome; GM = gray matter.

Subcortical Volume

There were no clusters that showed a significant difference between high fear patients and non-elevated fear patients in subcortical regions. However, we found one cluster containing the left nucleus accumbens (NAc) and the left putamen (Put) that exhibited a significant decrease in GM volume in high fear patients in comparison with healthy controls (**Figure 7**).

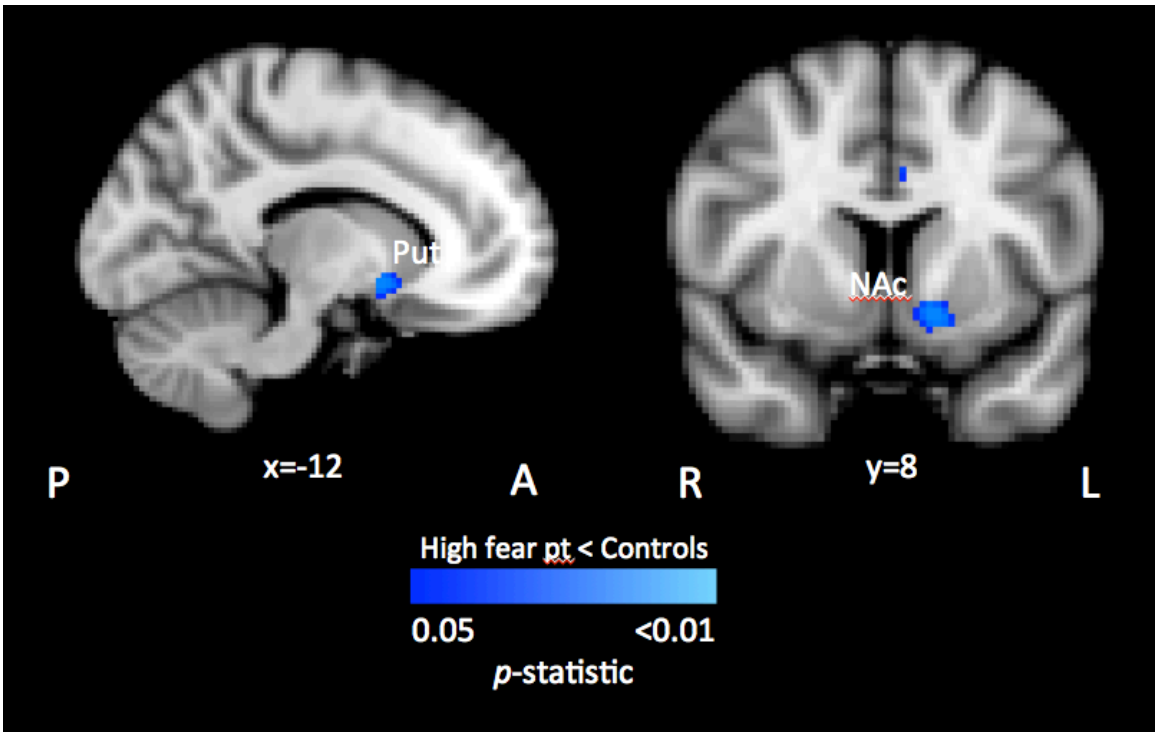

Figure 7: Significant subcortical volume differences between high fear patients and healthy controls. Compared with healthy controls, high fear patients exhibited less subcortical GM volume in a cluster consisting of the nucleus accumbens (NAc) and putamen (Put). Colors: blue to light blue = significant decrease compared with controls. N=37 (patients); N=35 (controls). GM = gray matter.

Correlation of FOPQ Scores with Cortical Thickness in Patients

Among patients, we examined the relation between pain-related fear level (FOPQ scores) and cortical thickness, controlling for pain level. Higher levels of pain-related fear were associated with cortical thinning in clusters found in the left middle frontal gyrus (MFG), right paracentral lobule (PCL), and posterior cingulate cortex (PCC). Cortical thickening was associated with higher levels of pain-related fear for clusters found in the right middle temporal gyrus (MTG) and right inferior parietal gyrus (IPG) (**Figure 8** and **Table 3**).

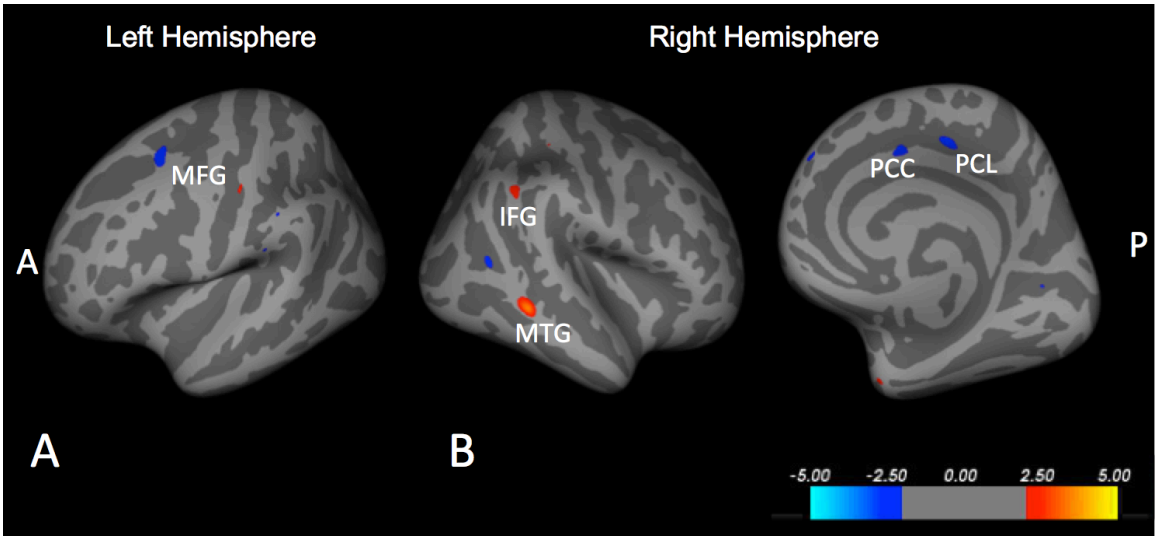

Figure 8: Within patients, significant correlation between cortical thickness and FOPQ scores, controlling for average pain level. (A) *Left hemisphere*. Higher FOPQ scores were correlated with less cortical GM in the middle frontal gyrus (MFG). (B) *Right hemisphere*. Higher FOPQ scores were associated with cortical thinning in the paracentral lobule (PCL) and posterior cingulate cortex (PCC). Higher FOPQ scores were related to cortical thickening in the middle temporal gyrus (MTG) and inferior parietal gyrus (IPG). T test of 2.50 corresponds to $p = 0.05$. Colors: blue to light blue = significant decrease compared with controls; red to yellow = significant increase compared with controls. N=37 (patients); N=35 (controls). FOPQ = fear of pain questionnaire; GM = gray matter.

Table 3. Correlation between cortical thickness and FOPQ scores in patients, controlling for average pain rating.

Brain Region	Vertices/ Voxels	MNI Coordinates			*T*
		x	y	z	
Cortical Thickness					
Left Hemisphere					
Middle frontal gyrus	138	-26	8	50	-2.92
Right Hemisphere					
Middle temporal gyrus	231	65	-42	-5	3.55
Inferior parietal gyrus	130	55	-52	37	2.22
Paracentral lobule	115	15	-16	41	-2.75
Posterior cingulate cortex	84	8	6	39	-2.34

T-test of 2.50 corresponds to $p = 0.05$. N=37 (patients); N=35 (controls). MNI = Monteal Neurological Institute.

DISCUSSION

In this study, we investigated the structural brain differences between patients with CRPS and matched controls and determined the degree to which fear level influences these differences. Compared with healthy controls, patients with CRPS had significant GM differences in pain-related cortical (PreCG, PoCG, PCL, MFG, PCC, PCu, and Cu) and subcortical regions (Put, NAc, Amy, and Hippo). Additionally, patients with CRPS showed significant GM differences in fear-related cortical areas (STG, PTG, frontal pole, and parahippocampal gyrus). Accordingly, our findings suggest that there are significant underlying morphometric differences between pediatric patients with CRPS and healthy controls. When examining the interplay of pain-related fear level on structural differences, high fear patients showed significantly decreased GM volume in the ACC compared to non-elevated fear patients and healthy controls. When comparing high fear patients with healthy controls, high fear patients exhibited a significant decrease in the left NAc and Put. The correlation of fear level with cortical structural changes in patients indicated that fear level was positively associated with GM differences in MTG and IPG and negatively associated with GM differences in MFG, PCL, and PCC. Taken together, children and adolescents with CRPS have altered brain structure compared to their healthy peers and pain-related fear levels appear to drive the differences for certain brain areas typically associated with fear learning circuitry.

Patient Versus Healthy Controls

The CRPS-related brain abnormalities involve brain areas that are important for somatosensory function, motor planning, pain modulation, and emotional processing. Compared with healthy controls, patients had cortical thinning in multiple regions, including pain-related areas such as PreCG, PoCG, PCL, MFG, PCC, PCu, and Cu. Consistent with previous studies on various chronic pain conditions such as chronic back pain, migraine, and fibromyalgia, patients with CRPS demonstrate brain alterations in regions located in the pain matrix that exacerbates pain (Borsook et al., 2010). Areas involved in motor planning found in PreCG, somatosensory function found in PoCG, and chronic pain processing (PCL, MFG, PCu, and Cu) have previously been shown to be altered in patients with CRPS (Barad et al., 2014; Juottonen et al., 2002; Maihöfner et al., 2007; Pleger et al., 2014), and may be associated with symptoms of high levels of spontaneous pain, allodynia, hyperalgesia, changes in motor control and limb protection and disuse (Bruehl and Chung 2006; Schilder et al., 2013).

A previous meta-analysis study by Smallwood and colleagues (2013) examining structural brain anomalies in patients with chronic pain revealed significant GM decreases in superior temporal gyrus (STG) and the putamen (Put). A possible role of the STG is monitoring of mismatches between predicted and actual sensation. Since STG receives input from the Put, the observed cortical thinning in STG and reduced volume in Put could be due to mismatch between pain expectation and perception, which leads to central fatigue (Smallwood et al., 2013).

Our results indicated a significant reduction in GM volume of subcortical regions in patients compared with healthy controls. ROI analysis showed a left-side specific difference in one cluster containing the NAc and Put and another cluster containing the Amy and Hippo. These are critical structures involved in the emotion-related limbic system, suggesting that patients have an abnormal emotional circuitry (Simons et al., 2014). The Put is part of the corpus striatum, which also includes the caudate nucleus and globus pallidus (http://healthline.com/human-body-maps). Recent evidence has implicated Put as playing a key role in operant conditioning and tracks how likely the conditioning stimuli lead to the correct response (Brovelli et al., 2011). The NAc is well known as being involved in the reward and motivation circuitry. An abnormal alteration of the NAc and Put may suggest impaired instrumental learning and decision-making in patients.

As we expected, key regions in the fear circuitry, such as the Amy and Hippo, show significant differences in patients when compared compared controls. A decrease in Amy GM volume may be a result of "emotional blunting," a condition seen in chronic pain patients in which inhibition from interneurons are constantly activated by consistent nociceptive input (Izquierdo et al., 2005). An intact hippocampus is needed to maintain conditioned learning as well as extinction. However, neuropathic injuries to the Hippo dramatically inhibit extinction, therefore prolonging fear learning (Mansour et al., 2014). Atrophy of Hippo GM volume may indicate a reduced ability in patients to properly undergo fear extinction and may extend fear-learning beyond specific cues and into associated context.

Influence of Pain-Related Fear Level

To our knowledge, this is the first imaging study to investigate the extent to which fear level influences brain structural differences in pediatric CPRS patients. When examining structural brain alterations within CRPS patient groups, we found significant correlation between fear levels as measured by FOPQ scores and cortical thinning in the middle frontal gyrus (MFG), paracentral lobule (PCL), and posterior cingulate cortex (PCC) and cortical thickening in the middle temporal gyrus (MTG) and inferior parietal gyrus (IPG). ROI analysis revealed a significant decrease in left Putnam (Put) and nucleus accumbens (NAc) volume in high fear patients compared with healthy controls, as well as a smaller anterior midcingulate cortex (aMCC) and pregenual anterior cingulate (pgACC) volume in high fear patients compared with both non-elevated fear patients and healthy controls.

The anatomical area of middle frontal gyrus (MFG) corresponds to the functional region of dorsolateral prefrontal cortex (dlPFC). Because the reduction of dlPFC thickness has been found in other types of pain conditions (Apkarian et al., 2004), this effect may not be unique to CRPS pathophysiology. The dlPFC is involved in cognition and executive functions in addition to pain modulation through top-down cognitive control. It is possible that GM atrophy may be a result of constant sensory input from regions such as the thalamus (Mesulam, 1998). In the context of pain, a thinning of the dlPFC seems to be more associated with sustained abnormal nociceptive input to the brain than reflecting a pre-existing defective pain inhibitory mechanism (Barad et al., 2013). In Erpelding and colleague's study, patients with CRPS had a significantly

decreased dlPFC when compared to healthy control. However, post-treatment analysis revealed a normalization of such alterations, suggesting that brain changes observed in dlPFC structure are likely related to presence of disease state and reversal of these changes reflect patients' improvement following treatment (Erpelding et al., 2014).

Results from our analysis showed that cortical thinning in dlPFC was related to increased pain-related fear in patients. In a study conducted by Wheelock and colleagues (2014) on threat-learning, the dlPFC is implicated in maintaining attentional resources in preparation for threat. The dlPFC showed stronger connectivity to other brain regions, including the amygdala and hippocampus, during predictable threats compared with unpredictable threats. Further findings correlating dlPFC connectivity with anxiety scores suggest that individuals with higher negative affect may require greater dlPFC connectivity to coordinate brain activity than individuals with low negative affect (Wheelock, 2014). Therefore, it is possible that the cortical thickness changes in dlPFC seen in high fear patients with chronic pain are a result of prolonged engagement of proactive cognitive processes in preparation to threat (i.e. pain) exposure.

ROI analysis showed significant reduction in aMCC volume in all three comparisons. Patients showed decreased aMCC volumes when compared with healthy controls. Furthermore, when analyzing within patients, high fear patients exhibited smaller aMCC volumes when compared to both healthy controls and non-elevated fear patients, suggesting that fear levels are associated with greater volume changes in the aMCC. The aMCC has a key role in cognitive aspects of movement generation (Hoffstaedter et al., 2014). It is possible that patients show atrophy in aMCC because of

increased motivation for limb protection and disuse, perpetuated by pain expectation of the affected limb. In a study by Vogt and colleagues, pain activity that was coupled with fear increased activation in the aMCC. aMCC has also been activated during anxiety related aversive conditional stimuli and pain anticipation (Buchel et al., 1998, Chua et al., 1999), suggesting that noxious activation in aMCC is associated with fear and anxiety that are critical to avoidance behaviors (Vogt et al., 2003). Taken together, alterations in the aMCC seem to be associated with increased pain-related fear and may be responsible for certain pain symptoms implicated in CRPS.

In a study conducted by Maihöfner and colleagues, patients with CRPS who had significant cortical reorganization were given treatment that consisted of physical therapy and anti-infammatory medication. After one year, there was a significant reduction in reported pain that correlated with the amount of cortical normalization (Maihöfner et al., 2004). These results demonstrate the relationship between clinical improvements and observed cortical normalization in patients with CRPS. Similarly, if brain alterations are assumed to be an indication of disease state, which are amplified by associated fear levels, it may be possible to normalize such brain structural changes by providing therapy that diminishes pain-related fear.

Limitations

There were several limitations in this study. We used two different methods of data analysis for cortical and subcortical regions, FreeSurfer and FSL-VBM, respectively. FreeSurfer uses a surface-based method to calculate vertices at each surface, which

"experience has shown results in a much better matching of homologous cortical regions than volumetric techniques" (www.surfer.nmr.mgh.harvard.edu). Although FreeSurfer was successful in extracting graphical representation of cortical changes, it was not possible to do so with subcortical volumes. On the other hand, FSL-VBM analyzes data by means of a volumetric-based method that investigate voxel-wise differences in local GM volume (www.fsl.fmrib.ox.ac.uk/fsk/fskwiki/FSLVBM). Therefore, the two analyses did in fact show differing results for areas in the cingulate cortex because they are two different methods of data analysis.

A larger sample size would have increased the power of our study. In addition, we were unable to obtain FOPQ scores from two patients, and therefore could not include them in our assessment of structural brain changes by fear level. However, by conducting ROI analysis and using the family-wise error rate (FWE-corrected P) correction *p*-value as a stringent threshold for significance, we believe we increased the power of our study to the best of our ability.

Another limitation extends to the nature of a cross-sectional design study. When examining changes in patients, it is impossible to attribute directionality of brain changes. It may be that these abnormal brain alterations were present in patients before the onset of the disease state, therefore predisposing these children to acquiring chronic pain condition. Conversely, brain abnormalities could be a result of disease symptoms and subsequent cognitive, affective, and somatosensory processing, and would thus be valid indicators of the patient's current clinical state. Thus it is important to conduct multi-modal imaging studies over time that can better inform the interpretation of the results.

Future Direction

A longitudinal study that assesses brain changes as well as psychological reports of chronic pain patients throughout their treatment sessions could correlate treatment progress with brain morphometry. Certain brain structure alterations may be able to identify characteristics of patients that predict likeliness of developing CRPS and examine risk factors of resistance to treatment.

In addition, a longitudinal study comparing MRI scans of patients before and after treatment would affirm better understanding of the causality of abnormal brain alterations in relation to the chronic pain condition. It would elucidate the plasticity of pediatric brain structures and possible normalization of brain morphometry and functionality after treatment. Furthermore, the study can examine patients who recovered and those who did not, and retrospectively assess each group's initial brain structures to establish risk factors for non-recovery. A combination of psychological and physical treatment therapy targeting diminishing patient fear level may show clinical improvements, which could be reflected in reversal of brain alterations.

Conclusion

In summary, we found that there are significant structural brain differences in pediatric patients with CRPS compared with healthy controls in particular regions that involve pain processing and emotional circuitry. Furthermore, differences in key regions such as the anterior midcingulate cortex, putamen, and nucleus accumbens are primarily

driven by high pain-related fear. In assessing our data, we believe that the use of psychology and physical therapy to incorporate components aimed at lowering fear levels should result in better treatment responses in patients who exhibit a high level of fear.

LIST OF JOURNAL ABBREVIATIONS

AnnuRev	Annual Review of Neuroscience
BJA	British Journal of Anaesthesia
E J Pain	European Journal of Pain
HBM	Human Brain Mapping
J Neubiorev	Journal of Neuroscience and Biobehavioral Reviews
J Neurosci	Journal of Neuroscience
J NiCl	Journal of Neuroimage Clinical
J Pain	Journal of Pain
PNAS	Proceedings of the National Academy of Sciences of the United States of America

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