Published in final edited form as:

Pain. 2014 September ; 155(9): 1727–1742. doi:10.1016/j.pain.2014.05.023.

The Responsive Amygdala: Treatment-induced Alterations in Functional Connectivity in Pediatric Complex Regional Pain Syndrome

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Abstract

The amygdala is a key brain region with efferent and afferent neural connections that involve complex behaviors such as pain, reward, fear and anxiety. This study evaluated resting state functional connectivity of the amygdala with cortical and subcortical regions in a group of chronic pain patients (pediatric complex regional pain syndrome) with age-gender matched controls before and after intensive physical-biobehavioral pain treatment. Our main findings include (1) enhanced functional connectivity from the amygdala to multiple cortical, subcortical, and cerebellar regions in patients compared to controls, with differences predominantly in the left amygdala in the pre-treated condition (*disease state*); (2) dampened hyperconnectivity from the left amygdala to the motor cortex, parietal lobe, and cingulate cortex after intensive pain rehabilitation treatment within patients with nominal differences observed among healthy controls from Time 1 to Time 2 (*treatment effects*); (3) functional connectivity to several regions key to fear circuitry (prefrontal cortex, bilateral middle temporal lobe, bilateral cingulate, hippocampus) correlated with higher pain-related fear scores and (4) decreases in pain-related fear associated with decreased connectivity between the amygdala and the motor and somatosensory cortex, cingulate, and frontal areas. Our data suggest that there are rapid changes in amygdala connectivity following an aggressive treatment program in children with chronic pain and intrinsic amygdala functional connectivity activity serving as a potential indicator of treatment response.

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Financial Disclosures

Dr. Simons, Melissa Pielech, Dr. Erpelding, Dr. Linnman, Dr. Moulton, Dr. Sava, Dr. Lebel, Paul Serrano, Dr. Sethna, Dr. Berde, Dr. Becerra, and Dr. Borsook reported no biomedical financial interests or potential conflicts of interest.

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Keywords

Fear; Children; Chronic Pain; Treatment Response; fMRI; Brain; Neuropathic

Introduction

Chronic pain is a complex experience that involves several neural networks tied to sensation, motor activity, cognition, and emotion. Brain regions often associated with the pain experience include the primary and secondary somatosensory cortex (S1 and S2), spinal cord, thalamus, insula, anterior cingulate cortex, prefrontal cortex [3,60,78], midbrain areas including the periaqueductal gray [44] and cerebellum [52], and subcortical structures including the hippocampus, basal ganglia, and amygdala [9,48,65,72]. There is a growing interest in the cognitive and emotional aspects of pain [10] with the amygdala emerging as a key region of interest [72].

A role for the amygdala in pain processing has been derived from a number of preclinical and clinical investigations. Preclinical data has provided a number of insights into the role of the amygdala in pain including: (i) a relay station for afferent nociceptive information from the parabrachial nucleus [6,20]; (ii) negative affective states [33,54]; (iii) increased excitability and plasticity in the amygdala in chronic pain [27]; and (iv) lesions inhibiting the development of chronic pain [41]. Clinical data on the amygdala's role in pain have been derived mainly from neuroimaging. While a number of human studies have implicated the amygdala in acute pain [5,8,58,59] and emotional disorders, such as anxiety [18] and depression [69], alterations in amygdala connectivity in chronic pain has been of recent scientific interest [72]. Alterations in amygdala connectivity have been identified among adult migraine [23], adult fibromyalgia [31], and adult irritable bowel syndrome [37] patients. Pain-induced amygdala hyperconnectivity has also been found in pediatric patients with complex regional pain syndrome (CRPS) that persists after symptom resolution [42]. These findings consistently suggest alterations in emotional-arousal circuitry among patients with chronic pain syndromes.

The aim of the present study was to characterize and compare functional connectivity of the amygdala with the rest of the brain in pediatric CRPS and healthy controls. Our group has previously reported that pediatric CRPS is highly responsive to intensive physical-biobehavioral treatments [45]. Based on this finding and recent reports of resting state network connectivity changes that occur with chronification of pain [26] and treatment [53] functional connectivity changes in the amygdala before and after intensive physical-biobehavioral treatment compared with age- and gender-matched healthy controls was examined. Using a relatively rapid treatment paradigm to examine the role of the amygdala in CRPS, we hypothesized to find (i) significant differences in functional connectivity in CRPS vs. Controls (*Disease Effect*); and (ii) a normalization in functional connectivity in CRPS patients following their treatment (*Treatment Effect*). In addition, given that the amygdala is involved in fear [39,56], we hypothesized that the psychological measure of fear avoidance in pain [73] correlates with the synchronicity of activity across typical fear networks (amygdala-insula connection, amygdala-anterior cingulate connection [19]).

Methods and Materials

Subjects

Twelve CRPS patients between the ages of 10–18 years with unilateral CRPS of the lower extremity were recruited from an intensive interdisciplinary pediatric pain rehabilitation program (see Table 1). Twelve healthy control controls were individually age- and gender matched. Selection Criteria: (i) for patients, diagnosis of CRPS based on the Budapest Criteria [24] as determined by an experienced pain physician on the basis of neurological examination and comprehensive record reviews; (ii) no other neurological, severe medical or psychiatric problems (e.g., active suicidality); (iii) absence of magnetic implants of any type; (iv) no current pregnancy; (v) no history of claustrophobia; (vi) weight <285 lbs. (the limit of the MRI table); (vii) right handed.

Procedure

The study was approved by the Boston Children's Hospital institutional Review Board (IRB). Informed parental consent and participant's assent were obtained at study enrollment. Participants attended two study sessions, at admission and at discharge from the Pediatric Pain Rehabilitation Center (PPRC) for patients and at a matched time interval for controls. No new medications were prescribed during treatment (i.e., each patient remained on the same pharmacological treatment as when they entered into the program). During each study session, participants underwent a focused neurological exam and MRI scanning.

PPRC Interdisciplinary Treatment

Patients referred to the PPRC from the multidisciplinary outpatient pain clinic have persistent pain with significant impairment of mobility and daily function. Admission was based on 1) problem duration or acuity and 2) inability to progress in outpatient treatment. PPRC enrollment was dependent upon 1) family willingness to enroll in the intensive program and 2) insurance provider coverage. Patients with active suicidality or current eating disorder were not eligible. The rehabilitation program entails intensive daily physical, occupational, and psychological therapies eight hours a day, five days per week for a typical length of stay of three weeks (for details see [45]). Patients received 3–4 hours of physical and occupational therapy sessions each day. Psychological treatment entails daily individual and group-based cognitive behavioral therapy and families are actively incorporated into the program, with family therapy and parent education provided. Psychological therapy targets include: 1) teaching a self-management approach to pain, 2) addressing negative thinking and fears about pain, 3) engaging in valued activities and relationships in the presence of pain, and 4) reducing parental attention and protective responses to pain [45]. A physician and nurse evaluate patients daily to ensure continued appropriateness of treatment (e.g., continued medical stability) and to address acute and/or ongoing medical issues.

Pain and Psychological Assessment

CRPS patients reported average pain ratings at admission and at discharge. On an 11-point numerical rating scale, scores between 0–3 were considered mild, 4–6 moderate, and > 7 severe [80]. CRPS patients also completed a battery of psychological measures at admission

and discharge from the program, which included the Fear of Pain Questionnaire ((FOPQ); [73]). For the FOPQ, scores of > 40 are considered elevated and scores of > 51 clinically significant.

MRI acquisition and analysis

Data acquisition—Participants were scanned on a 3T Tim Trio (Siemens Medical, Erlangen, Germany) scanner using a 12-channel head coil. *Anatomical*: A 3D T1-weighted anatomical scan was acquired using a magnetization prepared rapid gradient echo (MPRAGE) sequence (128 sagittal slices; field of view = 256×256 mm; TR = 2100 ms; TE = 2.74 ms; TI = 1,100 ms; $1.33 \times 1 \times 1$ mm voxels). *Functional*: A resting-state functional (f)MRI scan was acquired using a T2*-weighted echo-planar pulse imaging (EPI) sequence (41 slices; TR = 2.5 ms; TE = 30 ms; 64×64 matrix; $3 \times 3 \times 3$ mm voxels). During the restingstate fMRI acquisition period, subjects were asked to remain awake with their eyes open and observed a blank screen. All scans were examined separately for excessive motion (> 3 mm).

Structural and functional MRI analysis—All preprocessing, first-level and second-level group analyses were performed using FMRIB Software Library (FSL) <http://www.fmrib.ox.ac.uk/fsl>.

Preprocessing steps: For each subject, the following preprocessing steps were taken: (i) MPRAGE and EPI images were skull-stripped using the brain extraction tool (BET) [75]; (ii) functional images were B_0 unwrapped using FSL FUGUE; (iii) motion correction using FMRIB's Linear Motion Correction (MCFLIRT); (iv) spatial smoothing at 5 mm full-width at half maximum (FWHM); (v) affine registration of the resting state fMRI dataset to the Montreal Neurological Institute (MNI)-152 2mm template brain using FMRIB's Linear Image Registration Tool [28,29]; and (vi) highpass temporal filtering (0.01 Hz). Low pass filtering was not included as patients with chronic pain have been observed to have oscillations beyond 0.1 Hz [49,55].

Amygdala time courses: Using the Juelich probabilistic brain atlas [2] and a probability threshold of 50%, masks for the left and right amygdala as separate regions of interest (ROI) were defined (see Figure 1S) as has been done in prior functional connectivity analyses of the amygdala [63]. The ROIs were converted from standard space to each subject's native functional space and then binarized. Due to the risk of signal dropout in this specific region of the brain, seeds were refined using a whole brain binarized mask to eliminate regions with low signal intensity voxels. Subsequently, individual time courses were extracted.

Amygdala functional connectivity analysis—For each subject, GLM seed-region analyses using FSL FEAT were performed with the right and left amygdala simultaneously entered with WM, CSF, 6 motion parameters (i.e., 3 rotational and 3 translational), large motion artifact confound matrix (created using FSL Motion Outliers for motion < 3 mm) and added as variables of no interest. Once individual GLM FEAT analyses were completed, unpaired mixed-effects group analyses between patients and controls at Time 1 and paired mixed-effects group analyses from Time 1 (i.e., PPRC admission) to Time 2 (i.e., PPRC

discharge) within patients and within controls were conducted for each seed region. The dependence between functional connectivity and fear of pain, after controlling for pain levels, was also examined within patients at Time 1 and Time 2. To analyze changes in the correlation of amygdala connectivity with pain related fear within patients from Time 1 to Time 2, a second level FEAT analysis of individual amygdala connectivity with FOPQ scores and pain level was performed. The analysis consisted of adding demeaned FOPQ scores and demeaned pain levels as explanatory variables to perform group comparisons of individual connectivity results with the amygdala across time points. Areas of significant positive results indicate a correlation of FOPQ scores with strength of connectivity with the amygdala, i.e., these brain areas have a reduced connectivity with the amygdala when the FOPQ scores were lower whereas negative results would suggest that higher FOPQ scores are associated weaker connectivity.

Gaussian mixture modeling—A seed based analysis, such as this one, is a massive univariate general linear model subject to excessive false positives if multiple comparison effects are not taken into consideration. If there are unmodeled signals or the model assumptions do not hold, the null distribution could be shifted and scaled [57]. In these cases, standard approaches to correct for multiple comparisons (e.g. Bonferroni, false-discovery-rate) are not applicable. Gaussian mixture modeling (GMM; [57]) deconstructs the overall statistical map into several optimized Gaussian distributions. This approach has been used previously for seed-based functional connectivity analyses [79]. Most statistical maps for these analyses indicated a non-zero mean and a standard deviation different from one, violating the assumption of normality and supporting the use of GMM for the current analysis. Thresholds were determined from a partition of the statistical map distribution using GMM conducted in MATLAB [1] to correct for multiple comparison effects as well as account for possible variations in the null distribution. Each voxel is associated with a vector of probabilities of association with each of the modeled Gaussian distributions. The map for the distribution associated with activation (or deactivation) was thresholded at a probability of $p > 0.5$ (meaning that the chances of a voxel belonging to that class was > 0.5); the corresponding z -value in the original z distribution was obtained from matching the center of the activation distribution and used as the threshold for determining activated voxels for each analysis (thus does not provide the standard $p < .05$ value).

Results

Participants

Six scans displayed excessive motion ($> 3\text{mm}$) resulting in the following final group numbers: Controls: Time 1 $n=10$, Time 2 $n=10$; Patients: Time 1 $n=11$, Time 2 $n=11$. For the covariate analysis, complete data on pain ratings and fear of pain symptoms were available for 10 CRPS patients.

Pain and Pain-related fear

Prior to treatment, 91% of patients reported moderate (73%) to severe (18%) average pain levels with 70% of patients reporting elevated or clinically significant pain-related fear (Figure 1). At the end of treatment, 55% reported moderate pain while the remaining

patients reported only mild pain. Mean average pain ratings significantly decreased from $5.65 \pm 0.54(\text{SE})$ to $4.07 \pm 0.67(\text{SE})$ ($t(10)=3.49$, $p<.01$). For pain-related fear, average fear of pain scores significantly decreased from $43.8 \pm 4.2(\text{SE})$ to $22.9 \pm 3.3(\text{SE})$ ($t(8)=3.58$, $p<.01$). Pain-related fear and pain were not statistically significantly associated with one another at admission ($r=.23$, $p=.53$) nor at discharge ($r=.48$, $p=.19$).

Resting state functional connectivity analyses

Results for group differences, paired analyses from Time 1 to Time 2, and fear of pain covariate analyses with clusters of activation greater than 2.0 cm^3 are described in the text below. The tables provide greater detail with all significant clusters (> 26 voxels) listed.

Disease effect

Group differences at Time 1—Analysis revealed greater connectivity of the left and right amygdala in patients compared to healthy controls in several cortical and subcortical areas, with weaker connectivity in patients compared to controls limited to a few brain regions (see Figure 2).

Left amygdala—Greater connectivity in patients compared to controls was observed for the prefrontal cortex (right frontal pole, right middle, bilateral superior), motor cortices (bilateral paracentral lobule, bilateral precentral gyrus, right supplementary motor area), parietal lobe (right angular, right inferior, left primary somatosensory, right superior), and bilateral middle cingulate (see Table 2A). Greater connectivity was also observed in patients compared to controls from the left amygdala to several subcortical and cerebellar structures including the basal ganglia (bilateral caudate, bilateral putamen, left nucleus accumbens), bilateral thalamus, and bilateral cerebellum (with the largest segment in Crus 2). Weaker connectivity in patients compared to controls was observed for the left precuneus and occipital lobe (right calcarine, right cuneus, right middle, bilateral superior) (see Table 2B).

Right amygdala—Greater connectivity in patients compared to controls was observed for the right parietal lobe (precuneus, supramarginal), right occipital lobe (calcarine, superior), temporal lobe (bilateral middle, right superior), and right cerebellum (lobes IV, V) (see Table 2C). There were no brain regions that showed weaker connectivity in patients compared to controls (Table 2D).

Treatment effects

Within group changes over from Time 1 to Time 2—GLM-based within group paired functional connectivity analysis revealed several significant decreases in connectivity among patients for the left amygdala and only a few significant differences between controls in the right and left amygdala from Time 1 to Time 2.

Patients—Decreased connectivity between the left amygdala and the prefrontal cortex (bilateral inferior, bilateral middle), motor cortex (bilateral paracentral lobule, bilateral precentral, right supplemental motor area), parietal lobe (right angular gyrus, left inferior gyrus, bilateral S1), bilateral cingulate (anterior, middle), bilateral anterior insula, and lobule IX of the cerebellum was observed from Time 1 to Time 2 (see Table 3A and Figure 3). No

significant decreases were observed for the right amygdala nor in bilateral increased connectivity.

Controls—Greater connectivity at Time 2 was limited to between the left amygdala and the right thalamus and bilateral cerebellum (with the largest segment in Crus 1) compared to Time 1 (see Appendix Table 3B, 3C, and 3D). No increases in connectivity of greater than 2 cm³ were observed for the right amygdala and no decreases in connectivity were observed bilaterally among controls.

Patient covariate analysis – Fear of Pain—The dependence between functional connectivity and fear of pain after controlling for pain levels was examined within patients to examine the unique association with fear of pain. At both Time 1 and Time 2, several regions key to fear circuitry were correlated with higher fear of pain scores.

Fear of Pain at Time 1: Higher fear of pain was associated with stronger connectivity between the left amygdala and the prefrontal cortex (bilateral frontal pole, bilateral superior), bilateral middle temporal lobe, bilateral anterior cingulate cortex, left anterior insula, left hippocampus, parahippocampal gyrus, brainstem, and cerebellum (see Figure 4 and Table 4A). No brain areas were correlated with lower pain-related fear scores. For the right amygdala, no areas exceeding 2 cm³ of positively or negatively correlated activity were observed (see Appendix Table 4B and 4C).

Fear of Pain at Time 2: Although patients reported a significant decrease in pain-related fear at the end of intensive pain rehabilitation program, some residual levels of fear remained and were correlated with several brain areas and the left amygdala. Higher levels of fear of pain were associated with stronger connectivity between the left amygdala and the prefrontal cortex (bilateral frontal pole, right medial, bilateral middle, superior), bilateral middle temporal lobe, bilateral cingulate (anterior, middle), right hippocampus, and right putamen (see Figure 4 and Table 5A). For the right amygdala, higher levels of pain-related fear were associated with stronger connectivity with the cerebellum (with the largest segment Lobule VIII; see Appendix Table 5B and 5C).

Change in Fear of Pain and Change in connectivity: The change in left amygdala resting state connectivity in the CRPS patients correlated with the change in fear of pain, indicating weakened connectivity between the left inferior frontal gyrus, left superior frontal gyrus, left supplementary motor, left anterior cingulate, left insula, left thalamus and the amygdala in patients who had decreases in pain-related fear after treatment (see Figure 5 and Table 6). No significant correlations between right amygdala changes and fear of pain changes were observed. Additionally, there were no significant correlations between left and right amygdala changes and changes in pain level.

Discussion

The amygdala is involved in fear [16], reward [76], and pain [54]. This study evaluated functional connectivity of the amygdala with cortical and subcortical regions in a group of pediatric patients with CRPS with age-gender matched controls before and after intensive

pain rehabilitative treatment. To our knowledge, this is the first study to investigate amygdala resting state functional connectivity in children with CRPS.

Intensive psychophysical treatment of CRPS

CRPS is a debilitating, painful condition characterized by continued pain, disproportionate to any inciting event, with sensory, vasomotor, sudomotor, and motor/trophic changes [24,25]. Although CRPS in children is often reversible [47], some patients require intensive psychophysical treatment [45,70,74]. Outcomes from our intensive day hospital program show significant declines in disability, distress, pain intensity, and improvements in motor function [45]. In the current study, children prior to and after this intensive treatment were evaluated. Pain and fear significantly decreased, which is consistent with prior results [45,74].

Disease state

Significant differences in functional connectivity in patients compared with healthy controls at Time 1 (i.e., pre-treatment) were observed. Of the areas showing enhanced connectivity for the left amygdala, alterations were shown in cognitive/emotional (prefrontal, cingulate cortex, basal ganglia), sensorimotor (thalamus, somatosensory cortex) and integrative processing (cerebellum, parietal lobe, thalamus). Some of these regions have been implicated in the phenotypic presentation of CRPS and likely attribute to the disease state such as motor changes [40,66], altered cognitive and affective processing (prefrontal and cingulate cortex; [22]), and altered sensory processing (somatosensory cortex and thalamus [7]). Although the cerebellum-amygdala functional connectivity has not been well defined in humans, the cerebellum is an important region for pain processing [52]. In animal tracing studies, cerebellar axons are reported to extend to the amygdala [17]. Recent work has implicated the cerebellum-amygdala connection in fear memory and expression [64,81] showing strengthened functional connectivity between the cerebellum and amygdala in adolescents with generalized anxiety disorder [62], suggesting that this enhanced connection may be driven by both pain itself and the emotional aspects of pain. This pattern of heightened connectivity has been found previously in adults with CRPS [7].

Regarding differences observed in the right amygdala, changes were present in areas involved with visuo-spatial imagery, episodic memory retrieval and self-processing operations (precuneus; [12]) and complex sensory integration (temporal lobe). Neglect of the affected limb is often a consequence of CRPS and is consistent with heightened connectivity with the parietal lobe in prior research [35,51]. Although less is known regarding the temporal lobe, stronger connections between the amygdala and temporal pole have also been observed in patients with migraine [23]. In a prior report on connectivity in recovered CRPS patients, alterations in pain-induced connectivity between the amygdala and a number of regions [43] was observed, including many of those noted in this study. Altogether alterations in amygdala connectivity observed in pediatric CRPS appear to reflect motor, sensoryintegrative, and cognitive-affective dimensions of the pain experience.

Treatment effects

Dampened functional connections were between the left amygdala and the prefrontal cortex (inferior, middle), motor cortex, parietal lobe, and bilateral cingulate cortex. Diminished connectivity observed in sensorimotor regions and the parietal lobe may reflect improved motor function associated with treatment. This corresponds with the intensive dose of physical and occupational therapy (3–4 hours daily) that patients receive and motor improvements that have been previously documented [45]. With regards to cognitive-affective regions (prefrontal cortex, cingulate), diminished connectivity may indicate psychological improvement in response to cognitive-behavioral treatment (CBT). CBT is an essential component of the PPRC and is intensive (2–3 hours daily) [45].

As described further below, decreases in amygdala connectivity associated with decreases in pain-related fear were found for several brain regions, suggesting that amygdala connectivity may serve as an indicator of psychological treatment response in pain patients. Recent research has also tied pain-evoked increases in prefrontal cortex activation in adults with fibromyalgia [30] and increases in gray matter in prefrontal, parietal, cingulate, sensorimotor, and hippocampal areas in adults with chronic pain [68] after cognitive-behavioral therapy. Thus, the intrinsic brain functional connectivity observed between the amygdala and other brain regions is likely altered by a physical and psychological treatment paradigm in children with CRPS.

Pediatric CRPS and pain-related fear

Beyond its emerging role in pain, the amygdala has a major role in fear processing [39,56]. Patients with CRPS avoid use of [71] and neglect [36] their affected limb(s), resulting in exacerbated symptoms, muscle atrophy, and ongoing pain-related disability. The brain network associated with fear extends beyond the amygdala and includes other brain areas such as the insula, hippocampus, striatum, thalamus, and anterior cingulate cortex, with selected frontal and motor-sensory cortices also implicated [67]. After controlling for pain levels, at both Time 1 and Time 2, several regions key of the fear circuitry were correlated with higher fear of pain scores, most notably from the left amygdala to the prefrontal cortex, bilateral middle temporal lobe, bilateral cingulate cortex, and hippocampus.

Decreases in functional connectivity between the motor cortex, cingulate, insula, frontal areas and the left amygdala were associated with less fear at the end of treatment (after controlling for changes in pain level), suggesting that several changes in intrinsic brain functional connectivity can be linked to pain-related fear symptom improvement and maps closely to the broad changes in connectivity that were observed in patients after treatment (see Figure 6). Although PPRC treatment does not include a specific exposure-based treatment paradigm, such a graded in-vivo exposure [14], the role of fear is explicitly discussed and the milieu focuses on progressively engaging in previously avoided activities. Notably, changes in pain did not correlate with changes in amygdala connectivity. It is possible that improvements in pain are associated with treatment response, but that when both pain and pain-related fear are accounted for the model, fear emerges as a stronger predictor, particularly of amygdala connectivity. Prior work supports the potential independence of pain level and persistent brain circuit abnormalities in chronic pain patients

wherein almost complete remission in subjective pain scores in a cohort of pediatric CRPS patients was associated with persistent brain abnormalities [38] and alterations in amygdala connectivity observed in response to painful cold stimuli [43].

Lateralization of connectivity changes

Lateralization of functional connectivity in the amygdala was observed, with the left hemisphere dominant across all contrasts and analyses. In a recent meta-analysis examining the role of the amygdala in pain processing, the left amygdala was most commonly activated in patients with clinical pain conditions (e.g., fibromyalgia), with few clinical pain studies showing only right amygdala activation [72]. This is in contrast to experimental pain studies where the right amygdala predominates in human and animal models [11,32]. Potential reasons for these findings may be the left amygdala's more prominent role in affective encoding [50], fear processes [4], or reflect gender differences in amygdala functional connectivity [34], given the preponderance of females in this sample. Despite the consistency of our findings, the sample was too small and homogenous for affected limb (75% left) to make firm conclusions on laterality effects.

Caveats

Several caveats must be considered. Data on a small number of patients is presented, but inclusion of matched controls and a within subject design to assess treatment effects allows conclusions to be drawn from a difficult to recruit and important group, children with CRPS. Although duration of pain differed substantially, all patients were enrolled in this intensive program due to non-response to standard treatment. Gender differences could not be examined because of the preponderance of females, which is consistent with the literature [15]. Although many patients were on medications, patients remained on the same regimen during treatment; thus, medication effects should be consistent across imaging sessions. Functional connectivity does not define the direction of change and can only be inferred based on our knowledge of anatomical afferent and efferent amygdala connections [61]. Lastly, early and late adolescence is a developmental stage known for potentially marked changes in hormone and brain function. All patients in the study were 10 years or older where the developmental shift in amygdala connectivity from positive to negative has been demonstrated to occur [21], but examination of developmental influences is warranted in future studies. Lastly, observed changes in amygdala connectivity may reflect greater neural plasticity in youth with CRPS, compared to adults with this difficult to treat condition. In order to test if youth are potentially more neurally 'plastic', future research should include both pediatric and adult CRPS patients.

Conclusions

CRPS patients report significantly higher levels of somatic symptoms and functional disability compared to patients with other chronic pain disorders [13,46]. Adults who were diagnosed with CRPS in childhood report lower quality of life compared to healthy adults [77]. Fear of pain is a major component of the behavioral consequences of the disease. By targeting treatments that affect specific components of the chronic pain state, direct and indirect benefits of treatment on specific brain pathways may prove to be a useful approach.

Functional connectivity measures of such changes in areas such as the amygdala can produce objective evaluations of the efficacy of such treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The work was supported by NIH Grants to DB (NINDS R01NS065051 and NINDS K24NS064050), LS (NICHD K23HD067202), and CB Sara Page Mayo Endowment for Pediatric Pain Research and Treatment.

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Summary

Amygdala connectivity is altered in children with chronic neuropathic pain and is responsive to intensive interdisciplinary treatment with associated decreased in pain-related fear.

Figure 1. Changes in pain-related fear from Time 1 to Time 2

There was a significant mean decrease in fear of pain from Time 1 (pre-treatment) to Time 2 (post-treatment). Each red line reflects individual scores. Means scores with standard error bars at Time 1 and Time 2 are depicted in black. Although several patients continued to report modest levels of pain-related fear, only one patient reported clinically elevated fear scores at the end of treatment.

Figure 2. Connectivity differences between patients and controls at Time 1 from the left and right amygdala to the rest of the brain

The left image represent significant correlated activity from left amygdala to the rest of the brain, contrasting patients and controls at Time 1. The image on the right represents synchronous activity from the right amygdala to the rest of the brain contrasting patients and controls at Time 1.

Key: SMA: Supplementary Motor Area; PrCG precentral gyrus; SFG: superior frontal gyrus; MFC: middle frontal cortex; CP: caudate-putamen; FrP: frontal pole; Thal: thalamus; MCC: middle cingulate cortex; STG: superior temporal gyrus; MTG: middle temporal gyrus; LING: Lingual Gyrus; Pcun: Precuneus; Cb: cerebellum; S1: primary somatosensory cortex; Ins: insula; MeFG: Medial Frontal Gyrus; NAc: nucleus accumbens.

Figure 3. Decreased connectivity within patients after treatment (Left Amygdala)

Weakened connections from the left amygdala to frontal, motor, and somatosensory cortices were observed at the end of treatment.

Key: MFG: middle frontal gyrus; Ins: insula; IFG: inferior frontal gyrus; S1: somatosensory cortex I; MC: motor cortex (paracentral lobule, precentral gyrus, supplemental motor area); PaOP: opercular cortex; ACC: anterior cingulate cortex; MCC: middle cingulate cortex.

Figure 4. Connectivity strength by levels of pain-related fear in patients (Left Amygdala)

Across time, areas associated with fear circuitry were consistently associated with higher pain-related fear scores.

Key: MTG: middle temporal gyrus; BS: brain stem; Hi: hippocampus; Ins: insula; FrP: frontal pole; ACC: anterior cingulate cortex; SFG: superior frontal gyrus; Cb: cerebellum; MeFG: medial frontal gyrus.

Figure 5. Correlation between patient connectivity changes and pain-related fear changes (Left amygdala)

Weakened connectivity between the left amygdala and frontal, motor, somatosensory, and cingulate cortex is associated with greater decrease in pain-related fear scores after treatment.

Key: FOP: frontal opercular cortex; SFG: superior frontal gyrus; MC: motor cortex (precentral gyrus, supplemental motor area); ACC: anterior cingulate cortex; MCC: middle cingulate cortex; MeFG: medial frontal gyrus; Ins: insula; Thal: thalamus.

Figure 6. Treatment Response from Time 1 to Time 2: Decrease in functional connectivity and pain-related fear

Paired analysis of amygdala connectivity changes within patients is depicted in green while connectivity changes that correlated with changes in pain-related fear are displayed in red. Many of the amygdala connectivity decreases were correlated with decreases in pain-related fear after treatment, suggesting that changes in intrinsic brain functional connectivity can be linked to symptom improvement. Key: MFG: middle frontal gyrus; Ins: insula; SMA: supplementary motor area; ACC: anterior cingulate cortex; MCC: middle cingulate cortex; FOP: frontal opercular cortex.

Table 1

Demographic and clinical characteristics of the CRPS patients.

Patient	Gender	Age	Etiology	Pain location	Pain Duration (mo)	Medication
1	F	17	Knee injury	R knee	2.5	AC
2	F	10	Twisted ankle	R foot	18.7	AD
3	M	11	Foot injury	L foot	8	AC, AD
4	F	15	Crush injury foot	L ankle	5	AD
5	M	15	No known injury	R knee	12	AC, AD
6	F	11	Knee injury	L knee	6.5	AC, AD
7	F	16	Fractured distal fibula	L ankle	48	AD
8	F	17	Foot and knee injury	L ankle	19	AD
9	F	14	Sprained ankle	L foot	2.5	AC
10	M	13	Post surgery	L ankle	6	AC
11	F	17	Twisted ankle	R foot	85	AD
12	F	13	Ankle sprain	L foot	13	AC
Mean (SEM)		14.1 (.72)				18.9 (7.0)

R=right, L=left. AC, anti-convulsivants; AD, anti-depressants.

Table 2A

Patients > Controls Time 1 (Negative Activation): Amygdala Left.

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Frontal						
Frontal Pole	R	2.68	38	40	8	4.69
	R	2.92	20	40	40	1.54
Middle Frontal	R	2.89	42	24	34	1.35
	R	3.46	34	22	44	3.19
	R	3.09	36	22	38	1.02
	R	2.99	34	12	36	1.48
Paracentral Lobule	R	2.72	40	2	56	0.86
	R	2.70	34	2	38	0.37
	R	2.71	10	-26	70	2.63
	R	2.67	10	-28	76	0.70
	L	2.71	0	-18	68	2.35
	L	2.85	-10	-28	66	1.88
Precentral	L	2.70	-2	-30	72	0.68
	R	3.67	48	4	44	7.26
	L	2.80	-44	-2	34	2.90
	L	2.67	-32	-2	64	1.60
Superior	R	3.22	6	32	46	5.57
	L	2.76	-22	-2	68	3.03
Supplementary Motor Area	R	2.69	20	16	48	1.86
	R	2.92	14	14	54	0.39
	R	3.06	26	2	64	7.85
	R	3.46	8	20	54	8.71
Parietal	R	2.65	8	18	46	0.41
	R	2.72	2	-8	70	1.47
	R	2.74	2	-12	70	0.43

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Angular	R	2.68	40	-64	46	3.59
Inferior	R	3.16	34	-46	52	1.58
	R	2.70	52	-46	52	2.68
Postcentral	L	2.85	-38	-36	42	3.67
Precuneus	L	2.90	-6	-62	58	1.27
Superior	R	2.80	44	-44	58	0.70
	R	3.09	32	-44	40	1.78
	R	2.80	40	-48	60	0.66
SupraMarginal	R	2.79	32	-38	42	0.62
Temporal						
Pole Middle	L	3.02	-40	16	-32	1.44
Middle	L	2.97	-58	-62	4	1.20
Planum Polare	R	2.82	50	2	-4	0.78
Cingulum						
Middle	R	2.69	6	12	30	2.05
	R	2.79	6	-16	36	1.29
	L	2.69	-8	-12	40	1.10
	L	2.78	-4	-14	36	1.05
Parahippocampus						
Parahippocampal	R	2.82	32	-6	-30	1.37
Sub-Cortical						
Caudate	R	2.69	18	14	4	3.69
	R	3.13	12	4	8	0.56
	R	3.46	14	0	12	2.94
Nucleus Accumbens	L	3.22	-6	6	-12	2.30
Pallidum	R	2.71	16	0	2	0.70
	R	2.87	14	-6	-4	0.67
Putamen	R	2.63	24	0	6	1.66
	L	2.63	-26	-4	10	5.62
Thalamus	R	3.10	10	-14	18	1.82

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Brainstem/Cerebellum	R	2.98	10	-20	14	1.53
	L	3.29	-10	0	8	2.87
	L	3.13	-10	-10	12	2.34
	L	2.96	-10	-10	2	0.82
	L	3.01	-14	-16	-2	0.43
	L	3.11	-16	-20	0	1.82
Cerebellum 4 5	R	2.77	30	-32	-30	1.42
	L	2.81	-16	-40	-26	4.62
	R	2.75	32	-44	-38	0.89
	R	2.72	10	-74	-20	6.90
	L	2.72	-34	-40	-32	0.66
	L	2.91	-28	-42	-34	1.15
Cerebellum 7b	L	3.05	-46	-44	-42	0.28
	L	2.65	-30	-56	-52	2.03
	L	3.28	-14	-64	-50	2.46
	R	2.74	12	-52	-54	7.20
	L	2.80	-10	-50	-36	1.46
	L	3.62	-42	-44	-38	2.78
Cerebellum Crus1	L	2.80	-36	-60	-32	3.98
	R	2.93	42	-46	-40	2.44
	L	2.81	-42	-66	-46	2.08
	L	3.01	-38	-74	-46	3.32
	L	2.84	-6	-76	-38	2.06
	L	2.76	-4	-78	-24	4.83
Cerebellum Crus2	L	3.12	-12	-56	-26	3.49
	L	2.87	-2	-72	-46	3.72
V						
VIIb						

Table 2B

Controls > Patients Time 1 (Positive Activation): Amygdala Left

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Frontal						
Olfactory	R	2.75	4	16	-2	0.26
Precentral	L	2.42	-36	-24	62	0.92
Frontal Pole	L	2.69	-18	64	12	0.44
	R	2.73	16	54	32	0.25
Superior	L	2.58	-26	42	42	0.74
Parietal						
Angular	R	2.77	42	-60	24	0.26
	L	2.59	-50	-64	32	0.34
Precuneus	R	3.10	20	-58	26	2.75
	L	2.67	-12	-66	30	0.22
Occipital						
Calcarine	R	2.77	10	-58	16	1.06
	R	3.08	26	-60	10	0.74
	R	2.69	12	-66	16	0.43
	R	3.40	18	-80	16	1.15
Cuneus	R	2.66	8	-80	28	0.63
	R	3.37	12	-90	30	3.10
Inferior	R	2.86	40	-72	-12	0.54
Lateral	R	2.46	36	-66	24	0.30
	R	3.19	54	-70	10	0.43
Middle	R	2.96	44	-80	20	1.95
	R	2.80	30	-88	24	1.02
	L	2.65	-22	-82	20	0.23
Rolandic Operculum	R	2.81	52	-24	20	0.34
Superior	R	2.63	30	-80	38	0.30
	R	2.87	24	-88	34	0.22

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Temporal	R	2.76	16	-92	20	0.50
	L	2.71	-22	-80	26	0.33
	L	3.22	-16	-88	34	1.17
Temporal						
Fusiform	R	2.69	26	-76	-10	0.26
	L	2.71	-34	-78	-16	0.90
Heschl	R	2.46	50	-18	8	0.22
Lingual	R	2.40	14	-48	-6	0.23
	R	3.21	20	-82	-6	0.72
Superior	R	3.13	54	-36	6	0.97
Temporal Pole	R	2.70	40	24	-22	0.53
Insula						
Posterior	R	2.81	42	-12	6	0.32
Sub-Cortical						
Caudate	R	3.35	14	22	-4	0.79
	L	2.81	-18	24	-2	0.58
Nucleus Accumbens	L	2.56	-6	16	-4	0.23
Brainstem/Cerebellum						
Cerebellum Crus1	L	2.57	-46	-68	-22	0.67

Table 2C

Patients > Controls Time 1 (Negative Activation): Amygdala Right

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Frontal						
Frontal Pole	L	2.45	-42	48	16	0.82
Middle	L	2.46	-32	18	36	0.55
Superior	L	2.54	-26	44	40	0.48
	L	2.35	-2	28	60	0.43
Supplementary Motor Area	R	2.65	6	-20	50	0.46
Parietal						
Precuneus	R	2.49	12	-54	8	2.15
	R	3.11	12	-56	30	0.49
	R	2.56	8	-56	32	0.31
	R	2.66	16	-58	24	1.10
SupraMarginal	R	2.44	60	-24	38	0.66
	R	2.49	60	-26	30	0.74
	R	2.76	50	-46	32	2.80
Occipital						
Calcarine	R	2.54	20	-80	14	3.92
Inferior	R	2.98	40	-70	-12	0.52
Rolandic Operculum	R	2.38	54	2	4	0.89
	R	2.33	44	-6	18	0.22
	R	2.37	40	-8	18	0.26
	L	2.34	-62	-6	12	0.37
Superior	R	2.74	26	-64	26	2.44
Temporal						
Fusiform	R	3.01	38	-76	-18	0.45
Heschl	R	2.60	50	-18	8	0.76
	R	2.45	38	-28	14	0.62
Lingual	R	3.32	14	-48	-8	0.55

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Middle	R	3.08	28	-54	-4	0.86
	R	2.33	10	-62	8	1.48
	R	2.36	48	-44	6	1.48
	R	2.30	64	-48	4	0.67
	L	2.94	-54	-22	0	0.58
Pole Superior	L	2.28	-64	-42	6	0.47
	R	2.88	40	22	-24	0.96
	R	2.29	40	14	-26	1.14
Superior	R	2.35	50	-6	-14	0.90
	R	2.64	56	-16	6	0.64
	R	2.89	56	-22	6	0.49
	R	2.31	40	-28	10	0.64
	R	2.80	52	-32	6	1.74
Cingulum						
Middle	R	2.53	8	-12	46	0.50
	L	2.31	-10	12	42	0.51
Posterior	R	2.30	12	-44	32	0.34
Insula						
Anterior	R	2.32	38	12	-6	0.54
Posterior	R	2.49	32	-20	22	0.65
Parahippocampus						
Parahippocampal	L	2.72	-20	-12	-30	0.55
Sub-Cortical						
Caudate	L	2.55	-10	16	14	1.71
Putamen	L	2.45	-20	16	-2	0.46
Thalamus	R	2.30	16	-28	6	0.36
Brainstem/Cerebellum						
Cerebellum 4 5	R	2.50	14	-42	-20	0.82
	R	3.19	10	-56	-4	1.88
Cerebellum 8	L	2.59	-36	-58	-46	0.79

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Cerebellum Crus1	L	2.31	-48	-68	-22	0.74
Vermis 4 5	R	2.69	6	-48	-6	0.52
	R	2.59	4	-52	-6	0.64

Table 2D

Controls > Patients at Time 1 (Positive Activation): Amygdala Right

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Frontal						
Frontal Pole	R	2.87	34	40	6	0.29
	L	3.07	-36	52	-4	0.46
Middle	R	3.09	36	12	36	0.58
	L	3.07	-46	14	36	0.25
Superior Orbital	R	3.11	20	42	38	0.26
	R	2.65	26	-6	68	0.32
Parietal						
Postcentral	R	2.99	38	-38	56	0.52
Occipital						
Inferior	L	2.72	-50	-68	-12	0.27
	L	2.51	-22	-98	-10	0.39
Cingulum						
Anterior	L	3.00	0	46	0	1.42
Posterior		2.84	-10	-18	38	0.22
Brainstem/Cerebellum						
Cerebellum 6	R	2.73	26	-66	-20	0.25
Cerebellum Crus1	L	2.58	-48	-48	-40	0.45
	L	3.05	-40	-60	-32	0.25

Table 3A

Within Patients, Time 1 > Time 2 (Positive Activation): Amygdala Left

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Frontal						
Inferior Orbital	R	3.00	36	34	-8	0.24
Inferior Triangular	R	3.16	46	40	8	0.75
	R	2.96	38	34	10	0.34
	R	2.92	54	22	16	0.47
	R	3.03	40	38	10	0.38
	L	2.92	-42	42	14	1.10
Middle	L	2.94	-46	12	40	2.59
	L	3.09	-32	8	48	3.08
	R	3.04	34	20	42	1.07
	R	2.9	28	18	50	0.73
	R	2.84	44	16	42	0.42
	R	3.27	40	12	46	2.16
	R	3.07	28	8	52	1.06
	R	2.99	32	4	56	0.68
Paracentral Lobule	R	2.85	10	-28	64	0.49
	R	2.91	6	-30	70	0.44
	L	3.01	-12	-22	70	1.15
	L	3.2	-4	-36	70	1.38
	L	3.3	-4	-38	62	1.46
Precentral	R	3.2	52	8	36	1.29
	R	3.43	20	-20	68	2.55
	R	3.34	30	-20	56	1.05
	R	3.02	28	-22	62	0.83
	R	2.85	20	-32	70	0.56
	L	3.15	-46	-6	38	0.38
	L	3.12	-50	-6	32	0.84

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
	L	3.11	-46	-6	42	0.42
	L	3.19	-40	-10	38	0.63
	L	2.84	-20	-20	62	2.00
	L	3.04	-20	-24	72	0.79
Superior	L	3.07	-22	36	42	0.70
Superior Medial	L	3.54	-2	34	38	4.31
Supplementary Motor Area	R	3.09	12	-8	66	0.73
	R	3.79	12	-10	58	20.08
Parietal						
Angular	R	3.03	26	-60	48	2.38
	R	2.86	38	-68	44	1.58
Inferior	L	3.25	-38	-38	44	3.74
	L	2.83	-34	-60	40	3.03
Postcentral	R	3.11	50	-24	54	1.33
	R	3.09	14	-36	72	0.56
	R	2.83	18	-36	60	1.18
	L	3.37	-44	-10	48	0.58
	L	3.64	-46	-12	42	0.36
	L	2.94	-50	-12	48	0.98
	L	3.56	-42	-16	44	1.04
Precuneus	L	2.86	0	-74	42	0.52
Superior	R	2.96	46	-44	56	1.85
SupraMarginal	R	3.17	52	-24	28	1.02
	R	2.92	52	-44	42	0.26
Occipital						
Rolandic Operculum	L	3.14	-46	-18	20	1.58
Temporal						
Pole Middle	L	3.12	-30	10	-34	0.36
Superior	R	3.04	60	-22	8	0.32
Cingulum						

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Anterior	R	2.95	2	40	0	1.51
	L	3.06	-4	12	26	1.18
	L	2.92	0	4	28	0.58
Middle	R	3.27	4	-4	30	0.49
	R	3.32	0	-10	30	0.30
	R	3.49	4	-14	34	0.58
	L	3.19	0	12	36	0.57
	L	3.85	-6	-18	36	2.43
	L	2.89	-6	-32	40	0.62
Post	L	3.09	0	-32	30	1.70
Insula						
Anterior	R	2.89	40	24	4	1.46
	L	2.93	-40	18	-4	1.89
Posterior	L	3.1	-36	-18	18	0.34
	L	3.56	-34	-30	22	1.23
Parahippocampus						
Parahippocampal	R	2.87	24	8	-34	0.24
Sub-Cortical						
Caudate	L	3.16	-8	2	6	0.67
Thalamus	L	3.2	-8	-12	16	0.32
Brainstem/Cerebellum						
Cerebellum 9	L	2.96	-12	-44	-42	2.26

Table 4A

Time 1 Stronger Connectivity, Higher levels of pain-related fear: Amygdala Left

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Frontal						
Inferior Orbital	L	2.34	-32	34	-10	0.23
Inferior Triangular	R	2.55	50	26	16	1.74
Frontal Pole	L	2.87	-32	56	14	0.98
	R	2.83	34	54	18	2.02
	R	2.86	30	48	38	2.23
	R	2.60	34	48	6	0.35
Superior	R	2.44	28	32	50	0.26
	R	2.80	14	68	18	0.30
	L	2.62	-24	66	12	0.23
	L	2.56	-24	58	18	0.43
	L	2.61	-26	52	0	0.25
	L	2.37	-20	50	20	0.33
	L	2.56	-16	44	48	3.24
Parietal						
Inferior	L	2.44	-54	-54	38	0.37
Precuneus	L	2.50	-8	-70	34	0.28
Occipital						
Middle	L	2.47	-44	-80	28	0.70
Superior	L	2.46	36	-70	16	0.22
Temporal						
Fusiform	R	2.42	22	-54	-14	0.78
	L	2.43	-26	-58	-8	0.22
	L	2.58	-30	-64	-6	0.41
Inferior	L	2.75	-42	-14	-24	0.54
	L	2.61	-40	-14	-28	0.25
Middle	R	2.50	58	-18	-12	0.97

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Cingulum	R	2.64	58	-24	-14	0.30
	L	2.55	-50	6	-28	0.50
	L	2.49	-44	-2	-18	0.62
	L	2.43	-54	-4	-18	0.49
Anterior	R	2.67	8	42	10	2.60
	L	2.41	-2	42	0	0.58
Posterior	L	2.71	-8	28	-4	0.91
	L	2.45	-2	-16	30	0.56
Insula	R	2.45	-2	-16	30	0.56
	L	2.45	-38	18	-6	0.34
Anterior	L	3.29	-30	16	-12	1.04
	L	2.54	-34	10	2	0.37
Parahippocampus						
Parahippocampal	L	2.88	-26	-2	-30	0.65
	L	2.89	-20	-4	-36	0.50
Sub-Cortical						
Hippocampus	L	3.22	-26	-10	-22	0.44
	L	2.45	-28	-16	-18	0.70
Putamen	R	2.48	34	4	2	1.11
Thalamus	R	2.63	18	-10	2	0.82
Brainstem / Cerebellum						
Brainstem	R	2.71	6	-20	-26	1.02
	R	2.34	6	-36	-22	0.63
Cerebellum 3	L	3.04	-6	-40	-20	1.18
Cerebellum 4,5	R	3.01	10	-54	-8	2.04
	L	2.49	-18	-38	-28	1.98
Cerebellum 6	R	2.38	10	-58	-20	2.37
Cerebellum 7b	L	2.39	-40	-58	-48	1.26

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Cerebellum 9	L	2.47	-10	-48	-54	0.40
Crus1	R	2.56	36	-78	-26	0.77
Crus2	L	3.03	-46	-62	-40	2.41
Vermis 3	R	2.57	6	-46	-20	0.77
Vermis 4,5	R	2.69	6	-50	-22	0.87

Table 5a

Time 2 Stronger Connectivity, Higher levels of pain-related fear: Amygdala Left

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Frontal						
Frontal Pole	R	2.81	34	52	-8	0.43
	R	2.99	24	50	-8	0.30
	R	2.65	34	56	14	0.51
	R	2.54	26	50	28	0.73
	R	2.65	26	58	16	0.61
	R	2.79	20	52	0	0.47
	R	2.51	22	50	14	0.24
	R	2.95	24	46	18	0.62
	L	3.61	-26	50	-8	0.57
	L	3.22	-18	66	4	1.12
	L	2.78	-14	56	36	1.01
	L	2.56	-20	50	18	0.38
	L	3.14	-10	58	10	1.14
Frontal Orbital	R	3.47	28	38	-8	0.82
	L	2.62	-22	14	-24	0.22
Inferior Triangular	L	2.78	-40	40	4	0.70
	L	2.51	-56	24	10	0.26
Medial	R	3.41	8	44	-14	0.87
	R	3.06	12	40	-2	0.94
	R	2.93	6	46	0	1.27
	L	2.80	-12	52	0	0.32
Middle	R	2.57	40	34	34	0.49
	R	3.20	34	14	36	0.60
	R	2.59	40	16	32	0.33
	L	2.86	-26	28	34	0.70
Precentral	R	2.49	12	-30	76	0.50

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Superior	L	3.05	-6	62	26	1.97
	L	2.75	2	52	32	0.26
	L	2.77	-8	46	18	0.37
	L	2.71	-8	40	36	1.35
Supplementary Motor Area	R	2.98	2	0	70	0.39
	L	2.53	-10	6	70	0.64
Parietal						
Angular	R	2.90	52	-50	32	1.58
Postcentral	R	2.56	40	-28	42	0.43
SupraMarginal	R	2.54	48	-42	36	0.22
Temporal						
Fusiform	R	2.77	38	-38	-12	0.52
Inferior	L	3.07	-46	-38	-14	0.51
Middle	R	2.74	60	-44	8	1.89
	R	2.57	60	-54	8	0.66
	R	2.60	62	-56	-2	0.48
	L	2.74	-60	-56	-4	1.38
	L	3.19	-60	-58	18	0.87
Pole	R	3.26	34	12	-34	0.74
	L	2.83	-34	16	-36	0.43
Cingulum						
Anterior	R	2.83	4	20	26	0.65
	L	2.79	-8	52	0	0.33
	L	2.53	-2	48	16	0.49
	L	2.78	0	42	-2	0.48
	L	2.57	2	42	14	0.38
	L	2.54	0	38	6	1.20
	L	2.84	-12	36	20	1.06
Middle	R	2.50	4	30	34	0.62
	R	2.59	12	22	36	1.06

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Insula						
Anterior	L	2.64	0	22	34	0.72
	R	2.82	46	6	-10	0.26
	R	2.77	46	6	6	0.70
Parahippocampus						
Parahippocampal	R	2.80	32	-40	-6	0.43
Sub-Cortical						
Caudate	R	3.32	6	8	-4	0.50
	L	2.78	-16	2	22	0.22
Hippocampus	R	3.71	30	-18	-14	2.18
Nucleus Accumbens	L	2.66	-12	12	-8	0.27
Pallidum	L	2.73	-26	-10	-4	0.24
Putamen	R	3.10	24	18	-2	0.66
	R	3.17	30	16	-2	0.70
	R	2.77	28	-16	12	0.54
	R	2.80	18	16	-6	0.42
Brainstem / Cerebellum						
Crus I	L	3.03	-46	-72	-36	0.66
	L	2.89	-42	-74	-24	0.24

Table 6

Decreases in connectivity, decreases in pain-related fear: Amygdala Left

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Frontal						
Inferior Operculum	R	3.10	38	16	8	1.02
	L	2.72	-50	14	2	0.54
Inferior	L	2.76	-38	30	24	0.40
	L	2.53	-54	30	8	0.56
	L	2.80	-40	20	16	0.36
Middle	L	2.70	-26	34	40	0.60
	L	2.81	-26	28	32	0.59
Middle Orbital	R	2.53	26	24	42	0.43
Precentral	L	2.60	-56	6	38	0.42
	L	2.89	-44	-6	40	1.31
Superior	R	2.52	4	44	38	0.50
	R	2.83	8	20	42	0.96
	L	2.52	-14	24	56	0.38
	L	2.88	-22	0	64	1.51
Superior Medial	L	3.19	-10	58	6	1.02
	L	2.52	-4	44	42	0.27
	L	2.83	-6	40	32	0.51
Superior Orbital	R	2.62	20	8	62	0.66
Supplementary Motor Area	L	2.54	0	4	68	0.36
	L	2.60	0	12	58	0.39
	L	2.54	-12	8	66	0.46
	L	2.95	-4	0	58	8.54
	L	2.51	0	0	70	0.26
Parietal						
Postcentral	L	2.50	-48	-8	48	0.24
SupraMarginal	R	2.67	64	-46	26	0.34

Brain Region	Lat.	Z-stat	Coordinates (mm)			Vol cm ³
			x	y	z	
Occipital						
Rolandic Operculum	L	2.77	-52	8	2	0.31
Cingulum						
Anterior	R	2.55	10	42	6	0.33
	R	2.70	4	22	26	0.43
	R	2.72	6	26	14	0.31
	R	2.52	6	48	-2	0.22
	L	2.63	-12	44	10	0.43
	L	3.00	-2	42	-2	0.72
	L	3.19	-2	36	20	3.58
	L	2.94	-2	12	26	0.98
Middle	R	2.70	4	28	32	1.03
	L	3.14	0	-8	32	0.78
Insula						
Insula Anterior	R	2.70	40	2	4	0.34
	L	2.90	-38	22	2	0.90
	L	2.94	-30	20	10	0.67
Parahippocampus						
Parahippocampal	L	2.94	-22	-8	-36	0.27
Sub-Cortical						
Pallidum	R	2.79	20	6	-2	0.28
	R	2.96	20	-2	6	0.29
	L	2.59	-14	6	2	0.28
Putamen	R	2.94	30	16	-2	0.67
Thalamus	R	2.53	18	-12	12	0.82
	L	2.82	-34	-4	6	0.85
	L	2.84	-10	-12	14	0.34
	L	2.70	-20	-20	12	0.26
	L	2.73	-14	-8	10	0.80