Thalamic activity and biochemical changes in individuals

with neuropathic pain following spinal cord injury

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Abstract

There is increasing evidence relating thalamic changes to the generation and/or maintenance of neuropathic pain. We have recently reported that neuropathic orofacial pain is associated with altered thalamic anatomy, biochemistry and activity, which may result in disturbed thalamocortical oscillatory circuits. Despite this evidence, it is possible that these thalamic changes are not responsible for the presence of pain per se, but result as a consequence of the injury. To clarify this subject, we compared brain activity and biochemistry in 12 people with below-level neuropathic pain after complete thoracic spinal cord injury to 11 people with similar injuries and no neuropathic pain and 21 age and gender matched healthy controls. Quantitative arterial spinal labelling was used to measure thalamic activity and magnetic resonance spectroscopy was used to determine changes in neuronal variability quantifying N-acetylaspartate and alterations in inhibitory function quantifying gamma amino butyric acid. This study revealed that the presence of neuropathic pain is associated with significant changes in thalamic biochemistry and neuronal activity. More specifically, the presence of neuropathic pain following spinal cord injury is associated with significant reductions in thalamic N-acetylaspartate, gamma amino butyric acid content and blood flow in the region of the thalamic reticular nucleus. Spinal cord injury on its own did not account for these changes. These findings support the hypothesis that neuropathic pain is associated with altered thalamic structure and function, which may disturb central processing and play a key role in the experience of neuropathic pain.

Introduction

Pain is a common and debilitating consequence of spinal cord injury (SCI) [52]. Although several types of pain can develop following SCI, neuropathic pain is one of the most intractable and the most prevalent, occurring in almost 50% of individuals following SCI and may be due to damage to the spinal cord or nerve roots [41]. Although the mechanisms responsible for generating SCI neuropathic pain remain unknown, some have suggested that activity within the spinal cord itself is important. For example, it has been hypothesized that SCI neuropathic pain results from on-going activity in intact residual spinothalamic tract pathways [50], or from an 'irritated focus' immediately above the injury [22]. Although such factors may help maintain neuropathic pain, they do not appear essential since complete sensory blockade or verified surgical removal of several spinal cord segments immediately above the injury, fail to relieve the pain in a considerable proportion of SCI subjects [4; 24]. These observations strongly suggest that neuropathic SCI pain may ultimately result from supraspinal alterations.

There is increasing evidence for a critical role of the thalamus in the generation and/or maintenance of neuropathic pain. For example, neuropathic pain is associated with altered thalamic anatomy [10; 12], altered thalamic biochemistry [12; 28] and decreased thalamic perfusion [12; 26; 27]. Furthermore, there is increasing evidence that neuropathic pain may be associated with thalamocortical dysrhythmia [21; 39; 48], which may result from altered inhibitory output from the thalamic reticular nucleus (TRN) [18; 31]. Consistent with the hypothesis, we have recently shown decreased thalamic gamma amino butyric acid (GABA) content and decreased perfusion in the region of the TRN in individuals with orofacial neuropathic pain [12].

Despite this evidence, it is possible that these thalamic changes (decreased GABA, perfusion, gray matter volume and neuronal viability) are not responsible for the presence of pain *per se*, but result as a consequence of deafferentation. Indeed, there is evidence that although thalamic ventroposterior lateral (VPL) neurons in spinally injured rats exhibit

increased burst firing, this dysrhythmia is linked to spinal injury and not to the presence of behaviours suggesting pain in animal models [9; 20]. Furthermore, we have previously reported that VPL thalamic anatomy was significantly altered in both SCI subjects with and without neuropathic pain, although a significantly greater change occurred in those with pain [10]. This raises the prospect that in neuropathic pain subjects, the injury itself may result in altered thalamic activity and biochemistry. Determining this distinction is important since future improvements in treatment are likely to hinge on our understanding of the neural mechanisms responsible for neuropathic pain.

To resolve this issue, we measured thalamic perfusion and biochemistry in both complete thoracic SCI individuals with and without neuropathic pain and in healthy controls. We hypothesised that individuals with SCI neuropathic pain would have reduced thalamic neuronal density (VP), GABA content and regional perfusion (TRN). Further, we hypothesised that in individuals without pain, both those with SCI and in healthy controls, no significant change in any of these parameters would occur.

Methods

Subjects

Twenty two subjects with established complete thoracic SCI (16 males; mean [±SEM] age 54±3 years) and 21 age and gender matched controls without pain or spinal cord injury (13 males; mean age 51±2 years) were recruited for the study. For the SCI subjects, the neurological level of injury was determined using the American Spinal Injury Association (ASIA) Impairment Scale (AIS) [23]. Each subject was examined by a clinician (P.W.) in the research group. The standardized AIS examination protocol was used to determine the most caudal level of the spinal cord with normal sensory and motor function on both sides of the body. An injury was termed complete when there was an absence of sensory and motor function in the lowest sacral segments. Twelve (8 males; mean age 57±4 years) of the 22

SCI subjects experienced persistent neuropathic pain. All 12 SCI subjects had below-level neuropathic pain as defined by the International Association for the Study of Pain SCI pain taxonomy [42]. These subjects experienced continuous burning pain with intermittent stabbing and electric shock-like sensations in the region of sensory loss at least three segments below the neurological level of injury (Figure 1). To assess the intensity of their pain, each subject completed a pain diary in which they indicated, with a vertical pencil stroke on a 10 cm horizontal line, the intensity of their pain (0cm = "no pain" to 10cm = "maximum imaginable pain") three times a day for the week prior to the scanning session. These pain diary values were averaged to provide an indication of each subject's chronic pain rating. Each subject also rated their ongoing pain intensity during the MRI scanning session (Table 1). The remaining 10 SCI subjects (8 males; mean age 50±4 years) did not have chronic neuropathic pain (Figure 2). The frequency of non-neuropathic pain did not differ between the two SCI groups. No significant differences in age (p=0.17) was found between SCI with pain and SCI without pain subjects. Both SCI groups were not significantly different in age compared to healthy controls (SCI with pain vs controls: p=0.18; SCI without pain vs controls: p=0.69). Informed written consent was obtained for all procedures and the study was approved by the institutional Human Research Ethics Committees.

MRI acquisition

Quantitative arterial spin labelling:

Subjects lay supine head first on the bed of a 3T MRI scanner (Philips, Intera) with their head immobilized in a tight-fitting eight-channel head coil. An isotropic 3D scan covering the whole brain was acquired (turbo field echo; echo time=2.5 ms; repetition time: 5600ms; flip angle: 8°; voxel size: 0.8x0.8x0.8mm). Whole brain cerebral blood flow (CBF) maps were then acquired using quantitative arterial spin labelling. The pulsed qASL version called QUASAR was used for this study and the sequence and subsequent CBF quantification has been described in details elsewhere [29; 30]. In short, the sequence is a

multi-slice, multiple time-points capable sequence based on pulsed qASL and a Look-Locker readout. In addition, the sequence acquires data with and without vascular crushers (gradients) (Velocity cutoff = 4cm/s) which allows the estimation of the arterial input function on a regional level. CBF is subsequently estimated by model free deconvolution, similar to what is done for gadolinium perfusion scans [29]. By adding a few dynamics at a lower flip angle, correct B1, T1 and M0 of tissue can be obtained which is subsequently used for CBF quantification [30]. General scan parameters were: repetition time (TR): 4000ms echo time (TE): 23ms, ΔTI: 300ms, TI1: 40ms, 13 inversion times (40-3640ms), 64×64 matrix, 7 slices, slice thickness: 6mm, 2mm gap, field of view (FOV): 240×240, flip-angle: 35/11.7°, SENSE: 2.5, 84 averages (48 with crusher: 4cm/s, 24 without crushers, 12 low flip angle, all implemented in a single sequence with a scan duration of 5min 52s.

Functional magnetic resonance imaging (fMRI)

In order to accurately place the 1H-MRS voxel in the somatosensory thalamus, we functionally defined the VPL thalamic nucleus in a group of 20 healthy subjects (14 males, mean [±SEM] age 43±3 years). A continuous series of 130 gradient echo, echo-planar fMRI image volumes using blood oxygen level dependent contrast were collected. Each image volume contained 43 axial slices covering the entire brain (voxel = 1.95 x 1.95 x 3.00 mm thick, repetition time = 3000ms; echo time = 40 ms). During each fMRI series, the right big toe pad was brushed innocuously using a plastic brush at approximately 2 strokes/second. Each of these stimulation paradigms were performed for a period of 10 fMRI volumes (30 seconds) following a baseline period of 10 fMRI volumes (30 seconds). This was repeated a further 5 times for a total of 6 stimulation and 7 baseline periods.

Magnetic resonance spectroscopy:

GABA-edited MEGA-PRESS spectra

Multi-planar (axial, sagittal, coronal) reformats were used for voxel placement. GABA-edited MEGA-PRESS spectra [6; 25] were acquired from a voxel (20x20x20 mm^3)

which was centred in each subject in the thalamus. We collected multiple pilot spectra to determine the minimum single voxel seize with reasonable signal-to- noise ratio. Since 11 of 12 subjects with pain had bilateral pain and the remaining subject had pain on the left side only, we collected MRS data from the right thalamus only. The MEGA-PRESS sequence parameters were as follows: TR: 2000ms, TE: 68ms, 1024 acquisition points, total acquisition time: 27min. 100 averages were acquired with the MEGA pulse centred at 1.9ppm ("ON spectra") and 100 averages with the pulse centred at 7.6ppm ("OFF spectra"). Automatic shimming (pencilbeam auto first order option) was performed resulting in line widths of <10Hz for all spectra.

Proton magnetic resonance spectra (1H-MRS)

Proton magnetic resonance spectra (1H-MRS) were acquired from a voxel (15x10x15mm^3) centred in the right ventral posterior lateral (VPL) thalamus. We collected multiple pilot spectra to determine the minimum single voxel seize with reasonable signal-to-noise ratio. The 1H-MRS sequence parameters were as follows: TR: 2000ms, TE: 29ms, 1024 acquisition points, spectral width of 2kHz, total acquisition time: 9min. Automatic shimming (pencilbeam auto second order option) was performed resulting in line widths of <10Hz for all spectra.

MRI analysis

Quantitative arterial spin labelling

QASL images were opened using custom software developed by Petersen and colleagues [29] and CBF maps created. In addition, from the CBF maps, anatomical (grey/white) image sets were created. These anatomical images were then co-registered to the T1-weighted anatomical image set collected at the same slice locations and the resulting parameters applied to the CBF maps. The T1-weighted anatomical images were then normalized to the standard MNI template and the normalization parameters applied to the

CBF maps. The resulting spatially-normalized CBF maps were then smoothed using a 6mm full-width-at-half-maximum Gaussian filter. One healthy subject had to be excluded due to excessive head movement. Significant differences in CBF within the thalamus between SCI subjects with neuropathic pain and SCI subjects without pain were determined using a random effects procedure (uncorrected $p < 0.005$, minimum cluster size 10 voxels, age and gender as nuisance variables, whole thalamus mask). CBF values from significant clusters were extracted from the SCI subjects with neuropathic pain, SCI subjects without pain and control subjects and compared using independent t tests ($p < 0.05$). Furthermore, in SCI subjects with neuropathic pain, a one-tailed Pearson correlation test was used to determine significant ($p < 0.05$) correlations between CBF values (thalamus cluster right) and thalamic biochemical ratios. Since these correlations were planned, we did not consider Bonferroni correction. Finally, in SCI subjects with neuropathic pain, correlations between CBF values and diary pain intensity, scan pain intensity and pain duration were determined (one-tailed Pearson correlation). To account for the cumulative α error due to multiple comparisons [three imaging modalities (QASL, NAA, GABA) correlated to three pain variables (scan pain, diary pain, pain duration), all of the results were corrected using the Bonferroni method ($p < 0.05/9$). The statistical analysis was performed with SPSS version 19.0 (IBM SPSS Statistics).

Functional magnetic resonance imaging (fMRI)

FMRI images were processed using SPM8 software. The images were motion corrected, global signal drifts removed using linear detrending, spatially normalized to the Montreal Neurological Institute (MNI) template and spatially smoothed using a 6mm full-width-at-half-maximum Gaussian filter. Using a repeated box car model convolved with hemodynamic delay function, significant increases in fMRI signal intensity related to brushing the big toe were determined (random effects, corrected $p < 0.05$, minimum cluster 10 voxels). For each subject, the percent changes in signal intensity were calculated in each significant cluster. These signal changes were then averaged (±SEM) across the group to

create a group time trend. For display purposes, the statistical maps were overlaid onto an individual subject's T1-weighted anatomical image.

<u>Magnetic resonance spectroscopy</u>

GABA-edited MEGA-PRESS spectra

For quantification of the GABA/creatine ratios the acquired spectra were analysed using the Java-based magnetic resonance user's interface (jMRUI 4.0, European Union project). First, the dominant water resonance was removed using the Hankel Lanczos Singular Valve Decomposition algorithm. The ''ON'' and ''OFF'' spectral subsets were summed producing single ''ON'' and ''OFF'' 68 ms sub-spectra for each spectra dataset. These 68 ms sub-spectra were then subtracted resulting in GABA-edited difference spectra to measure GABA concentration at 3.01 ppm. The GABA-edited difference spectra were then phased with respect to both the zero- and first-order phase. GABA was quantified using AMARES, a nonlinear least-square fitting algorithm operating in the time domain. Peak fitting for GABA was performed after manually defining the centre frequency and line width of the GABA peak, and modelling the GABA peak as a singlet. Furthermore, Lorentzian curves were used to obtain the peak amplitude for this resonance.

The ''OFF'' spectral subsets were summed producing single ''OFF'' 68 ms sub-spectra for each spectra dataset to measure Creatine (Cr) concentration at 3.02 ppm. The single ''OFF'' 68 ms sub-spectra was then phased with respect to both the zero- and first-order phase. Line broadening of 5 Hz was used to improve the display. Spectral fitting in AMARES was performed after manually defining the centre frequency and line width of the Cr peak, and modelling the Cr peak as a singlet. Furthermore, Gaussian curves were used to obtain the peak amplitude for this resonance.

Finally, ratios were calculated for GABA relative to Cr. Differences in metabolite ratios between SCI subjects with persistent neuropathic pain and SCI subjects without pain and healthy controls were determined using independent t tests ($p < 0.05$). Furthermore, in SCI subjects with neuropathic pain a one-tailed Pearson correlation (Bonferroni corrected)

was used to determine significant ($p < 0.05/9$) correlations between GABA/Cr ratios and the mean pain intensity scores for the week prior to scanning (diary pain), during scanning (scan pain) and pain duration. The statistical analysis was performed with SPSS version 19.0 (IBM SPSS Statistics). Two SCI subjects with pain had to be excluded due to excessive head movement.

Proton magnetic resonance spectra (1H-MRS)

MRS data were analysed in the time domain using the Java-based magnetic resonance user's interface (jMRUI 4.1, European Union project). First, the dominant water resonance was removed using the Hankel Lanczos Singular Valve Decomposition algorithm. All metabolite resonances were quantified using QUEST (containing a 29 ms TE metabolite basis set including N-acetyl aspartate (NAA), Cr, aspartate, glutamate, glutamine, myo-inositol (MI) and glycerolphosphocholine [5]. Ratios were calculated for NAA relative to Cr. Using SPSS version 19.0 (IBM SPSS Statistics), significant differences in metabolite ratios between SCI subjects with pain, SCI without pain and healthy controls were determined using independent t tests ($p < 0.05$). Furthermore, in SCI subjects a one-tailed Pearson correlation test was used to determine significant ($p < 0.05$) correlations between NAA/Cr ratios and GABA/Creatine ratios. Finally, in SCI subjects with neuropathic pain, a one-tailed Pearson correlation test (Bonferroni corrected) was used to determine significant ($p < 0.05/9$) correlations between NAA/Cr ratios and diary pain, scan pain and pain duration. One SCI subject with neuropathic pain, one SCI without pain and two healthy subjects had to be excluded due to excessive head movement.

Results

Individual and mean subject characteristics are shown in Table 1. On average, SCI subjects had on-going pain intensity (diary pain) of 5.2 ± 0.7, pain intensity during scanning

(scan pain) 3.6±0.8 and an average pain duration of 182.4±41.8 months. Eleven out of 12 SCI subjects had bilateral below-level neuropathic pain and one subject (SCI subject 7) had left sided below-level neuropathic pain.

Thalamic blood flow

Individuals with SCI neuropathic pain had significantly reduced CBF within the right thalamus compared to SCI subjects without pain (p=0.004) and healthy controls (p=0.01) (mean [±SEM] CBF ml/min/g: controls: 28.1±2.4; SCI without pain: 30.9±3.1; SCI with pain: 18.7±2.3). This CBF decrease was located on the lateral edge of the thalamus, including the region of the thalamic reticular nucleus (Figure 3), however, it is pertinent to know that other thalamic nuclei may also be encompassed. More specifically, it was located in the anterior dorsal aspect of the thalamic reticular nucleus, which in monkeys, receives collaterals from VP thalamic neurons [32]. In SCI subjects with neuropathic pain, thalamic reticular nucleus blood flow was not correlated to GABA/CR ratios (r=-0.28, p=0.22) and NAA/CR ratios (r=-0.02, p=0.49). Furthermore, thalamic reticular nucleus blood flow was not significantly correlated to diary pain (r=-0.24, p=0.23), scan pain (r=0.36, p=0.13) or pain duration (r=0.29, p=0.18).

Brain activation in response to brushing

Brushing of the right big toe in healthy controls evoked significant signal intensity increases in the contralateral VPL thalamus. Figure 3A shows the location of this thalamic activation overlaid onto an individual subject's T1-weighted anatomical image set. The mean (±SEM) signal intensity changes over time for this VPL cluster is shown in Figure 3B. It is clear that during each brushing period, signal intensity increased and returned to baseline in the intervening rest periods.

Thalamic GABA levels

The thalamic region from which GABA spectra were acquired as well as a typical spectrum are shown in Figures 4A and 4B. SCI subjects with neuropathic pain had significantly lower thalamic GABA levels compared with SCI subjects without pain (mean [±SEM] GABA/Cr ratio $x10^{-3}$: SCI subjects with pain: 1.7±0.2; SCI subjects without pain: 2.4±0.1; p<0.001) and controls (controls: 2.4±0.2; p<0.001) (Figure 4C). Individual GABA/Cr ratios are shown in Table 2. In SCI subjects with neuropathic pain, GABA/Cr ratios were not correlated to either diary pain (r=0.15, p=0.34), scan pain (r=0.07, p=0.42), or pain duration (r=-0.54, p=0.06).

Thalamic NAA levels

The thalamic region from which NAA spectra were acquired as well as a typical spectrum are shown in Figures 5A and 5B. SCI subjects with neuropathic pain had significantly lower thalamic NAA levels compared with SCI subjects without pain (mean [±SEM] NAA/Cr ratio: SCI subjects with pain: 1.19±0.03; SCI subjects without pain: 1.40±0.07; p=0.02) and controls (controls: 1.32±0.03; p=0.005) (Figure 5C). Individual NAA/Cr ratios are shown in Table 2. In SCI subjects with neuropathic pain, NAA/Cr ratios were positively correlated to GABA/CR ratios (r=0.59, p=0.04), that is the greater the reduction in thalamic NAA levels, the greater the reduction in thalamic GABA. Furthermore, in SCI subjects with neuropathic pain NAA/Cr ratios were not correlated to either scan pain (r=0.60, p=0.03), diary pain (r=0.31, p=0.18) or pain duration (r=-0.10, p=0.39) when Bonferroni corrected.

Discussion

This study demonstrates that the presence of neuropathic pain following SCI is associated with significant changes in thalamic biochemistry and on-going neuronal activity. More specifically, the presence of neuropathic pain following SCI and not SCI itself is

associated with significant reductions in thalamic NAA (a marker of neural viability) [35], GABA content and blood flow. However, it is pertinent to note that we do acknowledge that this reduction in blood-flow may also encompass other thalamic nuclei. These findings support the hypothesis that neuropathic pain is associated with altered thalamic structure and function that are likely to disturb central processing and play a key role in the experience of persistent pain. Specifically, it has been shown that neuropathic pain following SCI is associated with changes in thalamic neurons which subsequently may make these neurons hyperexcitable and as such they may act as a pain generator or amplifier [16; 51; 53].

A significant strength of this study is that it has been demonstrated that both chemical and functional thalamic alterations are exclusively associated with neuropathic pain and not the injury itself. Indeed, although all our SCI subjects had the same type and level of injuries (complete injury, thoracic level), those subjects that did not develop below-level neuropathic pain, displayed no changes in thalamic GABA, NAA concentration or blood flow. It is thought that NAA is localized almost exclusively in mature neurons and neuronal processes and is considered a putative marker of the number and viability of neurons [46]. Despite limited signal to noise ratios required us to obtain proton spectra from a region larger than the VP thalamus, we are confident that the NAA changes result largely from VP anatomical changes, since we have shown that SCI is associated with fractional anisotropy changes centred on the VP thalamus which are exacerbated in those with neuropathic pain [10]. Although a change in fractional anisotropy can result from neuronal loss, our finding that only SCI subjects with neuropathic pain had reduced NAA in the region of the VP thalamus, compared with SCI subjects without pain and controls, suggests that SCI itself does not result in a decrease in neuronal viability within the VP thalamus. Thalamic reorganization after peripheral deafferentation in the macaque is well known [33] and therefore the lack of change following SCI is surprising given the substantial deafferentation that has occurred and highlights the significance of decreased thalamic neural viability on the presence of neuropathic pain following SCI.

It is well known that VP thalamic output neurons that project to the primary somatosensory cortex also send collaterals to the thalamic reticular nucleus (TRN), which in turn sends inhibitory efferents back to the VP thalamus [31]. Consistent with a postulated role for these collaterals in modulating pain through inhibitory feedback mechanisms, we found that SCI pain is associated with decreased blood flow in the region of the TRN. More specifically, this blood flow reduction is located in the TRN region that responds to somatosensory stimulation and overlaps with the TRN blood flow decrease that occurs in individuals with orofacial neuropathic pain [12; 32]. Although human brain imaging investigations measuring evoked neuronal function (fMRI BOLD) have revealed that acute pain is associated with activation of a number of brain regions, including the thalamus, somatosensory, insular and cingulate cortices [7], chronic neuropathic pain does not appear to be associated with an increase in thalamic CBF [26]. Indeed, investigations measuring spontaneous neuronal function (positron emission tomography) have consistently reported on-going activity decreases in the thalamus [13; 15; 26]. Our data supports these previous findings and extends them by showing that the decrease in thalamic activity may be derived from reduced activity within the TRN, likely resulting from reduced on-going drive from the VP thalamic nucleus.

Immunohistochemical investigations have shown that the TRN contains almost exclusively GABAergic neurons [18; 31]. Consistent with these findings, we found that SCI subjects with neuropathic pain have reduced thalamic GABA content which is also consistent with our previous findings in orofacial neuropathic pain patients [12]. The decreased thalamic GABA could result from reduced presynaptic GABA production or increased GABA reuptake and catabolism. Consistent with our findings in humans, Ralston and colleagues demonstrated in the macaque that somatosensory pathway lesions reduce GABA-immunoreactive synapses within the VP thalamus [36]. Furthermore, Rausell and colleagues showed in the macaque that peripheral nerve lesions result in GABA receptor down-regulation within the VP thalamus and an increase in activity of calbindin cells preferentially innervated by central pain pathways [37]. Although pain behaviours were not

measured in these previous macaque studies, our data suggests that the GABAergic changes may underlie the presence of pain, since our SCI subjects without pain did not show any change in thalamic GABA content.

It has been hypothesised that the TRN alters brain function by regulating all sensory information, apart from olfaction, that passes through the thalamus on its way to the cerebral cortex [18; 31]. Indeed it was hypothesized by Crick nearly 30 years ago that the TRN may regulate internal attentional processes, i.e. the searchlight hypothesis [3]. Although not conclusive, thalamocortical and TRN neurons also appear to be critical for the production of thalamocortical rhythmic oscillations with evidence that the intrinsic and network properties of TRN neurons endow them with oscillatory properties [21]. For example, Steriade and colleagues [43] reported spindle-related rhythms in disconnected TRN neurons and abolition of spindle oscillations following TRN lesion. Additionally, reciprocal connections between excitatory relay neurons and local inhibitory neurons may be involved in generating thalamocortical oscillations [14; 45]. It has recently emerged that chronic neuropathic pain, including neuropathic pain following SCI, is associated with significantly altered thalamocortical rhythmic oscillations, characterized by higher spectral power in the 2-25Hz range and a shift of the dominant peak towards lower frequencies [2; 39; 48]. Furthermore, this altered rhythm is characterized by persistent theta and beta over-activations in pain-associated areas, including the primary and secondary somatosensory cortical regions [44].

Consistent with this, we have recently shown in orofacial neuropathic pain subjects, that reduced thalamic GABA content was negatively correlated to thalamocortical functional connectivity. That is, the greater the loss of thalamic GABA, the stronger the signal coupling between the VP thalamus and brain areas including the primary and secondary somatosensory cortices and the anterior insula [12]. This thalamocortical connectivity may take time to establish since it has been shown in an animal model of SCI pain that VP-SI connectivity initially decreases [40]. These data support the idea that chronic neuropathic pain is associated with altered thalamocortical activity [39; 47; 48] and raises the possibility that at least some forms of chronic neuropathic pain are not associated with overall

increases in activity in "acute pain-related" brain regions, but instead changes in connectivity strengths.

It has been hypothesized that altered thalamocortical rhythm associated with chronic pain results from altered thalamic firing patterns [17]. Studies in both humans [11; 19] and in an animal model of neuropathic SCI pain reveal that neuropathic pain is associated with increased frequency of burst firing in VP thalamic neurons [1; 9; 38; 49]. In addition, in an animal model of diabetic neuropathic pain it was demonstrated that neuropathic pain is associated with enhanced spontaneous activity of VPL neurons [8]. Although, this abnormal bursting is not definitively tied to the presence of neuropathic pain [34] it has been hypothesised that this altered activity is associated with thalamocortical dysrhythmia and the experience of pain [21]. Although we did not measure VP thalamic firing, our data are consistent with the idea that altered VP thalamic regulation by the TRN is associated with the presence of neuropathic pain.

Despite no correlations between pain intensity variables (both diary and scan pain have been calculated from the average of continuous burning pain and intermittent stabbing and electric shock-like sensations) and plastic changes were determined, it is pertinent to note that by separating both pains (continuous burning pain and intermittent stabbing and electric shock-like sensations), significant associations may have been emerged.

Although the thalamic changes were significantly related to the presence of pain, there was no significant correlation between the changes and pain intensity in the time just prior to or during scanning. This suggests that chronic pain is associated with thalamic changes that may underlie the development and maintenance of pain. However, these changes are not directly linked to the intensity of pain at any particular moment.

Further insights into the interplay between deafferentation, loss of inhibition, dysrhythmia and neuropathic pain are provided by the present study. Although no conclusions can be made regarding the nature of neuronal firing, there is certainly a reduction in GABA in the thalamus and a disruption to normal inhibitory function. As with other studies, the reduction in thalamic activity represented by a reduction in perfusion is

consistent but nevertheless difficult to interpret. These results suggest however that a reduction in thalamic inhibitory function, possibly mediated by altered function of the TRN, results in an increase in thalamic outflow to cortical structures involved in pain processing.

References:

[1] Bakkum BW, Henderson CN, Hong SP, Cramer GD. Preliminary morphological evidence that vertebral hypomobility induces synaptic plasticity in the spinal cord. J Manipulative Physiol Ther 2007;30(5):336-342.

[2] Boord P, Siddall PJ, Tran Y, Herbert D, Middleton J, Craig A. Electroencephalographic slowing and reduced reactivity in neuropathic pain following spinal cord injury. Spinal Cord 2007;46(2):118-123.

[3] Crick F. Function of the thalamic reticular complex: the searchlight hypothesis. Proc Natl Acad Sci U S A 1984;81(14):4586-4590.

[4] Davis L. Treatment of spinal cord injuries. American Medical Association Archives of Surgery 1954;69:488-495.

[5] Di Piero V, Ferracuti S, Sabatini U, Pantano P, Cruccu G, Lenzi GL. A Cerebral Blood Flow Study on Tonic Pain Activation in Man. Pain 1994;56:167-173.

[6] Edden RAE, Barker PB. Spatial effects in the detection of γ-aminobutyric acid: Improved sensitivity at high fields using inner volume saturation. Magnetic Resonance in Medicine 2007;58(6):1276-1282.

[7] Farrell MJ, Laird AR, Egan GF. Brain activity associated with painfully hot stimuli applied to the upper limb: a meta-analysis. Hum Brain Mapp 2005;25(1):129-139.

[8] Fischer TZ, Tan AM, Waxman SG. Thalamic neuron hyperexcitability and enlarged receptive fields in the STZ model of diabetic pain. Brain Research 2009;1268(0):154-161.

[9] Gerke MB, Duggan AW, Xu L, Siddall PJ. Thalamic neuronal activity in rats with mechanical allodynia following contusive spinal cord injury. Neuroscience 2003;117:715-722.

[10] Gustin SM, Wrigley PJ, Siddall PJ, Henderson LA. Brain anatomy changes associated with persistent neuropathic pain following spinal cord injury Cerebral Cortex 2010;20(6):1409-1419.

[11] Hains BC, Saab CY, Waxman SG. Alterations in Burst Firing of Thalamic VPL Neurons and Reversal by Nav1.3 Antisense After Spinal Cord Injury. Journal of Neurophysiology 2006;95(6):3343-3352.

[12] Henderson LA, Peck CC, Petersen ET, Rae CD, Youssef AM, Reeves JM, Wilcox SL, Akhter R, Murray GM, Gustin SM. Chronic Pain: Lost Inhibition? The Journal of Neuroscience 2013;33(17):7574-7582.

[13] Hsieh JC, Belfrage M, Stone-Elander S, Hansson P, Ingvar M. Central representation of chronic ongoing neuropathic pain studied by positron emission tomography. Pain 1995;63(2):225-236.

[14] Huguenard JR, McCormick DA. Thalamic synchrony and dynamic regulation of global forebrain oscillations. Trends in Neurosciences 2007;30(7):350-356.

[15] Iadarola MJ, Max MB, Berman KF, Byas-Smith MG, Coghill RC, Gracely RH, Bennett GJ. Unilateral decrease in thalamic activity observed with positron emission tomography in patients with chronic neuropathic pain. Pain 1995;63(1):55-64.

[16] Jeanmonod D, Magnin M, Morel A. Low-threshold calcium spike bursts in the human thalamus - common physiopathology for sensory, motor and limbic positive symptoms. Brain 1996;119(Part:2):2):363-375.

[17] Jeanmonod D, Magnin M, Morel A, Siegemund M. Surgical control of the human thalamocortical dysrhythmia: 1. central lateral thalamotomy in neurogenic pain. Thalamus & Related Systems 2001;1:71-79.

[18] Lam YW, Sherman SM. Functional organization of the thalamic input to the thalamic reticular nucleus. J Neurosci 2011;31(18):6791-6799.

[19] Lenz FA, Kwan HC, Dostrovsky JO, Tasker RR. Characteristics of the bursting pattern of action potentials that occurs in the thalamus of patients with central pain. Brain Research 1989;496:357-360.

[20] Liang L, Mendell LM. Bilateral transient changes in thalamic nucleus ventroposterior lateralis after thoracic hemisection in the rat. Journal of Neurophysiology 2013;110(4):942-951.

[21] Llinas RR, Ribary U, Jeanmonod D, Kronberg E, Mitra PP. Thalamocortical dysrhythmia: A neurological and neuropsychiatric syndrome characterized by magnetoencephalography. Proceedings of the National Academy of Sciences of USA 1999;96:15222-15227.

[22] Loubser PG, Donovan WH. Diagnostic spinal anaesthesia in chronic spinal cord injury pain. Paraplegia 1991;29:25-36.

[23] Marino RJ, Barros T, Biering-Sorensen F, Burns SP, Donovan WH, Graves DE, Haak M, Hudson LM, Priebe MM. International standards for neurological classification of spinal cord injury. J Spinal Cord Med 2003;26:S50–56.

[24] Melzack R, Loeser JD. Phantom body pain in paraplegics: evidence for a central "pattern generating mechanism" for pain. Pain 1978;4:195-210.

[25] Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. NMR in Biomedicine 1998;11(6):266-272.

[26] Moisset X, Bouhassira D. Brain imaging of neuropathic pain. Neuroimage 2007;37 Suppl 1:S80-88.

[27] Ness TJ, San Pedro EC, Richards JS, Kezar L, Liu HG, Mountz JM. A case of spinal cord injury-related pain with baseline rCBF brain SPECT imaging and beneficial response to gabapentin. Pain 1998;78:139-143.

[28] Pattany PM, Yezierski RP, Widerström-Noga EG, Bowen BC, Martinez-Arizala A, Garcia BR, Quencer RM. Proton magnetic resonance spectroscopy of the thalamus in patients with chronic neuropathic pain after spinal cord injury. American Journal of Neuroradiology 2002;23:901-905.

[29] Petersen ET, Lim T, Golay X. Model-free arterial spin labeling quantification approach for perfusion MRI. Magnetic Resonance in Medicine 2006;55(2):219-232.

[30] Petersen ET, Mouridsen K, Golay X. The QUASAR reproducibility study, Part II: Results from a multi-center Arterial Spin Labeling test–retest study. NeuroImage 2010;49(1):104-113.

[31] Pinault D. The thalamic reticular nucleus: structure, function and concept. Brain Res Brain Res Rev 2004;46(1):1-31.

[32] Pollin B, Rokyta R. Somatotopic organization of nucleus reticularis thalami in chronic awake cats and monkeys. Brain Res 1982;250(2):211-221.

[33] Pons T, Garraghty P, Ommaya A, Kaas J, Taub E, Mishkin M. Massive cortical reorganization after sensory deafferentation in adult macaques. Science 1991;252(5014):1857-1860.

[34] Radhakrishnan V, Tsoukatos J, Davis KD, Tasker RR, Lozano AM, Dostrovsky JO. A comparison of the burst activity of lateral thalamic neurons in chronic pain and non-pain patients. Pain 1999;80:567-575.

[35] Rae C. A Guide to the Metabolic Pathways and Function of Metabolites Observed in Human Brain 1H Magnetic Resonance Spectra. Neurochem Res 2013:1-36.

[36] Ralston HJ, Ohara PT, Meng XW, Wells J, Ralston DD. Transneuronal changes of the inhibitory circuitry in the macaque somatosensory thalamus following lesions of the dorsal column nuclei. The Journal of Comparative Neurology 1996;371(2):325-335.

[37] Rausell E, Cusick CG, Taub E, Jones EG. Chronic deafferentation in monkeys differentially affects nociceptive and nonnociceptive pathways distinguished by specific calcium-binding proteins and down-regulates gamma-aminobutyric acid type A receptors at thalamic levels. Proceedings of the National Academy of Sciences of USA 1992;89:2571-2575.

[38] Saghaei E, Abbaszadeh F, Naseri K, Ghorbanpoor S, Afhami M, Haeri A, Rahimi F, Jorjani M. Estradiol attenuates spinal cord injury-induced pain by suppressing microglial activation in thalamic VPL nuclei of rats. Neuroscience Research 2013;75(4):316-323.

[39] Sarnthein J, Stern J, Aufenberg C, Rousson V, Jeanmonod D. Increased EEG power and slowed dominant frequency in patients with neurogenic pain. Brain 2006;129(Pt 1):55-64.

[40] Seminowicz DA, Jiang L, Ji Y, Xu S, Gullapalli RP, Masri R. Thalamocortical Asynchrony in Conditions of Spinal Cord Injury Pain in Rats. The Journal of Neuroscience 2012;32(45):15843-15848.

[41] Siddall PJ, Taylor DA, McClelland JM, Rutkowski SB, Cousins MJ. Pain report and the relationship of pain to physical factors in the first 6 months following spinal cord injury. Pain 1999;81(1-2):187-197.

[42] Siddall PJ, Yezierski RP, Loeser JD. Taxonomy and epidemiology of spinal cord injury pain. In: Yezierski RP, Burchiel KJ, editors. Spinal cord injury pain: assessment, mechanisms, management. . Progr Pain Res Manag 2002;23:9-24.

[43] Steriade M, Deschenes M, Domich L, Mulle C. Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. J Neurophysiol 1985;54(6):1473-1497.

[44] Stern J, Jeanmonod D, Sarnthein J. Persistent EEG overactivation in the cortical pain matrix of neurogenic pain patients. Neuroimage 2006;31(2):721-731.

[45] Trageser JC, Burke KA, Masri R, Li Y, Sellers L, Keller A. State-Dependent Gating of Sensory Inputs by Zona Incerta. Journal of Neurophysiology 2006;96(3):1456-1463.

[46] Urenjak J, Williams SR, Gadian DG, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. J Neurosci 1993;13(3):981-989.

[47] Walton KD, Dubois M, Llinás RR. Abnormal thalamocortical activity in patients with Complex Regional Pain Syndrome (CRPS) Type I. PAIN 2010;150(1):41-51.

[48] Walton KD, Llinás RR. Central Pain as a Thalamocortical Dysrhythmia: A Thalamic Efference Disconnection? In: L Kruger, AR Light, editors. Translational Pain Research: From Mouse to Man. Boca Raton, FL: CRC Press, 2010.

[49] Wang G, Thompson SM. Maladaptive Homeostatic Plasticity in a Rodent Model of Central Pain Syndrome: Thalamic Hyperexcitability after Spinothalamic Tract Lesions. The Journal of Neuroscience 2008;28(46):11959-11969.

[50] Wasner G, Lee BB, Engel S, McLachlan E, Wasner G, Lee BB, Engel S, McLachlan E. Residual spinothalamic tract pathways predict development of central pain after spinal cord injury. Brain 2008;131(Pt 9):2387-2400.

[51] Waxman SG, Hains BC. Fire and phantoms after spinal cord injury: Na+ channels and central pain. Trends in Neurosciences 2006;29(4):207-215.

[52] Widerstrom-Noga EG, Felipe-Cuervo E, Broton JG, Duncan RC, Yezierski RP. Perceived difficulty in dealing with consequences of spinal cord injury. Archives of physical medicine and rehabilitation 1999;80(5):580-586.

[53] Yezierski R. Spinal cord injury pain: Spinal and supraspinal mechanisms. . J Rehabil Res Dev 2009;46(1):95-108.

FIGURE LEGENDS

Figure 1: Individual pain and injury diagrams for subjects with below-level spinal cord injury neuropathic pain. The light grey shading indicates each subject's on-going neuropathic pain distribution. The cross hatching indicates areas of cutaneous hypersensitivity. The fine dashed line illustrates the neurological level of injury and the coarse dashed line the sensory zone of partial preservation (ZPP). The details of each subject are contained in Table 1.

Figure 2: Individual pain and injury diagrams for subjects without neuropathic pain. None of these subjects had neuropathic pain or cutaneous hypersensitivity. The fine dashed line illustrates the neurological level of injury and the coarse dashed line the sensory zone of partial preservation (ZPP). The details of each subject are contained in Table 1.

Figure 3: A; Cerebral blood flow (CBF) decreases (cool color scale) in the right thalamus of subjects with spinal cord injury (SCI) and neuropathic pain compared with SCI subjects without pain overlaid onto an individual's axial and coronal T1-weighted anatomical images. The CBF decrease encompasses the region of the thalamic reticular nucleus (TRN). Functional blood flow increases (red color scale) in the ventroposterior lateral (VPL) thalamus during innocuously brushing the big toe in 20 healthy subjects. Slice locations in Montreal Neurological Institute space are indicated at the lower left of each image. **B;** Mean (±SEM) percentage changes in signal intensity in the VPL thalamus. The grey bars indicate the periods of innocuous brushing of the big toe. **C**; A plot of mean (±SEM) CBF in the TRN of SCI subjects with neuropathic pain, SCI subjects without pain and pain-free controls. **D**: Overlay of TRN CBF decrease in the right thalamus of SCI subjects with neuropathic pain and TRN CBF decrease in the contralateral (to pain) thalamus of subjects with painful trigeminal neuropathic pain (PTN) published previously (green shading) [12]. Note, the CBF TRN decreases in SCI subjects with neuropathic pain overlap CBF TRN decreases of subjects with PTN.

Figure 4: A; Axial slice showing location from which GABA-edited MEGA-PRESS spectroscopy was performed in the right thalamus of SCI subjects with SCI neuropathic pain, SCI subjects without pain and pain-free controls. Slice location in Montreal Neurological Institute space is indicated at the lower left of the image. **B;** Typical MEGA-PRESS spectrum obtained from the thalamus. Glx: glutamine; GABA: gamma-animobutyric acid; Cr: Creatine; ppm: parts per million; **C**: A plot of mean (±SEM) GABA/Cr ratios in the thalamus of SCI subjects with neuropathic pain, SCI subjects without neuropathic pain and pain-free controls.

Figure 5: A; Axial slice showing location from which proton spectroscopy was performed in the right thalamus of SCI subjects with neuropathic pain, SCI subjects without pain and pain-free controls. Slice location in Montreal Neurological Institute space is indicated at the lower left of the image. **B;** Typical proton spectrum obtained from the thalamus. PCr: Phosphocreatine; NAA: N-acetyl aspartate*;* Cr: Creatine; ppm: parts per million; **C**: A plot of mean (±SEM) NAA/Cr ratios in the thalamus of SCI subjects with neuropathic pain, SCI subjects without neuropathic pain and pain-free controls.

Table 1: Spinal cord injury subject characteristics: Table indicating subject characteristics where neurological level injury = the most caudal segment of the spinal cord with normal sensory and motor function on both sides of the body; AIS = American Spinal Injury Association impairment scale where A = no motor or sensory function is preserved in the sacral segments S4-S5; motor/sensory level = the most caudal segment of the spinal cord with normal motor/sensory function; sensory ZPP = zone of partial sensory preservation, which indicates the most caudal dermatome that remains partially innervated; VAS = visual analogue score; SR = sustained release.