Altered regional brain T2 relaxation times in individuals with chronic orofacial neuropathic pain

Z. Alshelh[a], F. Di Pietro[a], E.P. Mills[a], E.R. Vickers[a], C.C. Peck[b], G.M. Murray[b], L.A. Henderson[a],[*]

[a] Department of Anatomy and Histology, Sydney Medical School, University of Sydney, 2006, Australia
[b] Faculty of Dentistry, University of Sydney, 2006, Australia

ARTICLE INFO

ABSTRACT

The neural mechanisms underlying the development and maintenance of chronic pain following nerve injury remain unclear. There is growing evidence that chronic neuropathic pain is associated with altered thalamic firing patterns, thalamocortical dysrhythmia and altered infra-slow oscillations in ascending pain pathways. Preclinical and post-mortem human studies have revealed that neuropathic pain is associated with prolonged astrocyte activation in the dorsal horn and we have suggested that this may result in altered gliotransmission, which results in altered resting neural rhythm in the ascending pain pathway. Evidence of astrocyte activation above the level of the dorsal horn in living humans is lacking and direct measurement of astrocyte activation in living humans is not possible, however, there is evidence that regional alterations in T2 relaxation times are indicative of astrogliosis. The aim of this study was to use T2 relaxometry to explore regional brain anatomy of the ascending pain pathway in individuals with chronic orofacial neuropathic pain. We found that in individuals with trigeminal neuropathic pain, decreases in T2 relaxation times occurred in the region of the spinal trigeminal nucleus and primary somatosensory cortex, as well as in higher order processing regions such as the dorsolateral prefrontal, cingulate and hippocampal/parahippocampal cortices. We speculate that these regional changes in T2 relaxation times reflect prolonged astrocyte activation, which results in altered brain rhythm and ultimately the constant perception of pain. Blocking prolonged astrocyte activation may be effective in preventing and even reversing the development of chronic pain following neural injury.

1. Introduction

Chronic neuropathic pain is a complex disease resulting from actual or presumed damage to the somatosensory nervous system. The constant perception of pain that characterizes neuropathic pain is associated with increased thalamic bursting activity (Gerke et al., 2003; Iwata et al., 2011; Lenz et al., 1989), reduced thalamic blood flow (Hsieh et al., 1995; Iadarola et al., 1995; Moisset and Bouhassira, 2007; Youssef et al., 2014) and increased cortical power, often termed thalamocortical dysrhythmia (Di Pietro et al., 2018; Sarnthein et al., 2006; Walton and Llinás, 2010). The increase in cortical power is associated with an altered relationship with thalamic GABAergic content (Di Pietro et al., 2018) and we have proposed that the thalamocortical dysrhythmia that occurs in chronic neuropathic pain results from altered interactions between the ventrocaudal thalamus, thalamic reticular nucleus and the cerebral cortex (Henderson and Di Pietro, 2016).

In addition to cortical resting activity changes, as read with

electroencephalography (> 2 Hz) we have previously shown that chronic neuropathic pain is associated with increased infra-slow frequency (< 0.1 Hz) oscillatory activity throughout the ascending pain pathway, including the region of the primary afferent synapse, ventrocaudal thalamus, thalamic reticular nucleus and primary somatosensory cortex (Alshelh et al., 2016). Interestingly, these infra-slow oscillation increases occurred at approximately the same frequency range as reported calcium waves in astrocytes, i.e. 0.03–0.06 Hz (Crunelli et al., 2002). Astrocytes are known to modulate synaptic activity by the release of gliotransmitters and it is well-documented in preclinical animal models that neuropathic pain is associated with prolonged astrocyte activation in the dorsal horn/spinal trigeminal nucleus (Garrison et al., 1991; Okada-Ogawa et al., 2009). Given these data, we have speculated that following nerve injury, prolonged astrocyte activation results in increased infra-slow oscillations in the ascending pain pathway which in turn is associated with thalamocortical dysrhythmia and the constant perception of pain (Henderson and Di Pietro, 2016).

Whilst it is clear from preclinical animal models that neuropathic pain is associated with astrocyte activation in the dorsal horn and brainstem (Hsieh et al., 1995; Okada-Ogawa et al., 2009), robust evidence is lacking in humans. There is, however, evidence from a post-mortem study which revealed prolonged astrocyte activation, but no microglial activation, in the dorsal horn of individuals with neuropathic pain associated with HIV (Shi et al., 2012). Indirect measurements of glial activation using Positron Emission Tomography (PET) have also revealed chronic glial activation in humans with neuropathic pain. Banati and colleagues (Banati et al., 2001) reported increases in binding of [^{11}C](R)-PK11195 (a sensitive in vivo marker of glial cell activation) in the contralateral (to injury) thalamus of patients with phantom limb pain. Similarly, Loggia and colleagues recently used the same technique to show increased glial activation in the thalamus and primary somatosensory cortex, contralateral to pain, of individuals with chronic low back pain (Loggia et al., 2015). Whilst these brain imaging studies are important and suggest chronic glial activation in the thalamus and above, technical limitations mean that they cannot explore changes at the level of the brainstem or, more importantly, the primary afferent synapse.

One non-invasive technique that has been used to measure glial activation is T2 relaxometry (Jackson et al., 1994; Wagner et al., 2012), a magnetic resonance imaging technique that can characterise changes in the brainstem and above. There is immunohistochemical evidence that decreased T2 relaxation times are associated with increased glial activation (Schwarz et al., 1996) and in this investigation we hypothesize that chronic orofacial neuropathic pain is associated with significantly reduced T2 relaxation time in the ascending pain pathway, including the region of the spinal trigeminal nucleus, ventrocaudal (Vc) thalamus, thalamic reticular nucleus and the orofacial region of the primary somatosensory cortex.

2. Methods

2.1. Subjects

Thirty-seven subjects with chronic orofacial neuropathic pain (NP; 28 females, mean age 46.1 ± 2.5 years [± SEM]) and 40 healthy controls without ongoing pain (24 females, mean age 40.6 ± 2.7 [± SEM]) were recruited for this study. All NP subjects were diagnosed using the Liverpool criteria as having post-traumatic painful trigeminal neuropathy (Nurmikko and Eldridge, 2001). Written informed consent was obtained for all procedures and the study was approved by Institutional Human Research Ethics Committees, University of Sydney.

For each NP subject, the intensity of their on-going pain was recorded on a 10 cm horizontal visual analogue scale (VAS) with 0 indicating "*no pain*" and 10 indicating "*worst imaginable pain*", three times a day for the seven days prior to a magnetic resonance imaging (MRI) session (Carlsson, 1983). These pain intensity scores were averaged over the 7-day period to create a mean pain intensity score. On the day of the MRI, all subjects were experiencing pain and were asked to draw the distribution of their ongoing pain. Subjects were also asked to complete a McGill Pain Questionnaire (Melzack, 1975), the Beck Depression Inventory (BDI) (Beck et al., 1961) and the Pain Catastrophizing Questionnaire (PCS) (Sullivan et al., 1995). A subset of the control and NP subjects was used in a previous investigation (Alshelh et al., 2016).

2.2. MRI acquisition

All subjects lay supine on the bed of a 3 Tesla MRI scanner (Philips Achieva) with their head immobilized in a tight-fitting 32-channel SENSE head coil. With each subject relaxed and at rest, a high resolution T1-weighted anatomical image set covering the entire brain was collected (turbo field echo; field of view 250 × 250 mm; matrix size 288 × 288; slice thickness 0.87 mm; repetition time 5600 ms; echo time

2.5 ms; flip angle 8°; raw voxel size 0.87 × 0.87 × 0.87 mm). Following this, T2-weighted image sets covering the entire brain were collected (field of view 250 × 250 mm; matrix size 556 × 556; slice thickness 5 mm; repetition time 3000 ms; echo times 20, 40, 60, 80, 100 ms; raw voxel size 0.4 × 0.4 × 5 mm thick). Multiple echo times were used to create multiple image sets for subsequent calculation of T2-relaxation time maps.

2.3. MRI analysis

Using SPM12 (Friston et al., 1995) and custom software, the following equation was used to calculate T2 relaxation time brain maps: $T_2 = (TE_2 - TE_1)/ln(SI_1/SI_2)$ where TE_1 and TE_2 are the echo times for proton density and T2-weighted images, and SI_1 and SI_2 represent proton density and T2-weighted image signal intensities, respectively. A ceiling threshold of 500 ms was applied to eliminate cerebrospinal fluid. In each subject, T2 relaxation maps were co-registered to each individual's T1-weighted anatomical image set. The T1-weighted anatomical image was then spatially normalized to a template in Montreal Neurological Institute (MNI) space and the parameters applied to the T2 relaxation time maps. Finally, the T2 brain maps were spatially smoothed using an 8 mm full-width-at-half-maximum Gaussian filter. In those NP subjects with pain restricted to the left side of the face (n = 17), T2-weighted images were reflected across the midline so that T2 differences could be assessed ipsilateral and contralateral to the side of highest ongoing pain.

In addition to a wholebrain T2 relaxation analysis, we performed a brainstem-specific analysis. The T2 relaxation maps were cropped to include only the brainstem and cerebellum, and a mask of these regions was created using the Spatial Unbiased Infratentorial Template (SUIT) toolbox (Diedrichsen, 2006). These brainstem/cerebellum masks were then manually adjusted to accurately encompass the brainstem and cerebellum only. Using this mask, T2 relaxation brainstem images were spatially normalized to the SUIT template in MNI space and spatially smoothed using a 3 mm full-width-at-half-maximum Gaussian filter.

Significant differences in T2 relaxation time between controls and NP subjects were determined using a voxel-by-voxel analysis of the wholebrain and the brainstem only ($p < 0.05$, random effects, false discovery rate corrected for multiple comparisons, minimum cluster 10 voxels, age and gender as nuisance variables). A brain mask that excluded CSF as well as the brainstem was applied to the wholebrain analysis. For both the wholebrain and brainstem only analyses, mean (± SEM) T2 relaxation times of significantly different clusters were extracted and plotted. Significant correlations between T2 relaxation times and pain intensity, BDI and PCS scores were also determined ($p < 0.05$). In addition, to determine if medication use had a significant effect on T2 relaxation times, for each cluster, T2 relaxation times were compared between those NP subjects taking acute analgesic medication alone (n = 5) or those taking prophylactic analgesic medication (n = 11) with those not taking medication (n = 21) ($p < 0.05$, two-tailed, two-sample *t*-tests).

3. Results

3.1. Psychophysics

Individual NP subject characteristics are shown in Table 1, and overall pain distributions and pain descriptors in Fig. 1. In 34 of the NP patients, their on-going pain was unilateral (17 right, 17 left), and the remaining 3 NP subjects reported bilateral pain. In all NP subjects, pain was located in the 2nd and 3rd trigeminal nerve divisions, with 7 subjects also reporting pain in the 1st division. The mean (± SEM) pain intensity over the 7 days prior to the MRI scanning session was 3.8 ± 0.4 out of 10 and the mean duration of pain was 4.1 ± 0.9 years. Using the McGill Pain Questionnaire, NP subjects most frequently described their pain as "throbbing", "tender" and
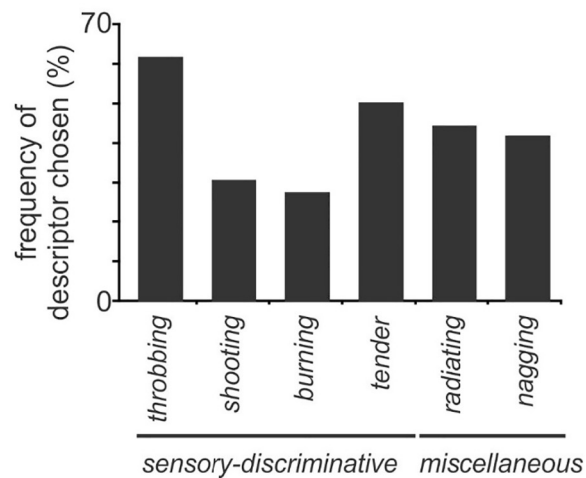
Table 1
NP subject characteristics.

| Subject	Age (years)	Gender	Pain duration (years)	Site	Pain Intensity (VAS)	Acute analgesic medication	Prophylactic medication
1	27	M	3.4	Right	0.5	–	Palmitoylethanolamide
2	21	F	12	Right	1.3	–	Palmitoylethanolamide
3	35	M	8	Right	1.4	–	Palmitoylethanolamide
4	45	M	14	Left	1.4	–	Palmitoylethanolamide
5	33	F	7	Left	2.4	–	–
6	53	F	2	Left	2.3	–	–
7	21	M	0.3	Left	1.9	–	Palmitoylethanolamide
8	64	F	17	Right	4.1	–	–
9	76	F	25	Left	5.7	–	–
10	67	F	2	Left	6.3	Diazepam	–
11	47	F	2.8	Left	4.1	–	–
12	40	F	1.5	Right	7.8	Ibuprofen	Pregabalin, carbamazepine
13	35	F	2	Left	4.9	–	–
14	46	F	0.6	Left	3.6	–	–
15	52	F	0.8	Right	2.7	–	–
16	58	M	1.8	Left	9.6	Ibuprofen	–
17	58	F	10	Right	5.1	–	–
18	42	F	2.8	Right	4.5	–	–
19	44	M	3.3	Bilateral	7.5	Acetylsalicylic acid, ibuprofen	Dextroamphetamine, metaprolol tartrate
20	47	F	0.4	Right	2	–	–
21	65	F	10	Left	6.1	Paracetamol	–
22	64	F	0.2	Right	1.5	–	–
23	24	F	2.6	Left	0	–	–
24	28	F	0.2	Bilateral	7.2	Ibuprofen, paracetamol, oxycodone hydrochloride	Pregabalin
25	58	F	0.5	Right	2.3	Ibuprofen, paracetamol	Pregabalin
26	44	F	2.3	Right	4	–	Desvenlafaxine
27	39	F	0.6	Left	3.1	Ibuprofen, paracetamol, codeine	
28	55	F	1	Right	0.8	–	–
29	41	M	0.5	Right	5.1	–	–
30	36	F	0.3	Left	0	–	–
31	20	F	0.5	Left	1.7	–	–
32	39	F	2.6	Left	5.6	–	–
33	74	F	0.4	Bilateral	4.2	–	–
34	53	M	0.6	Right	2.1	–	–
35	29	M	5.8	Left	4.8	Tramadol	–
36	73	F	3	Right	7.5	–	Efexor
37	53	F	2.3	Right	–	–	–
Mean ± SEM	46.1 ± 2.5		4.1 ± 0.9		3.8 ± 0.4		
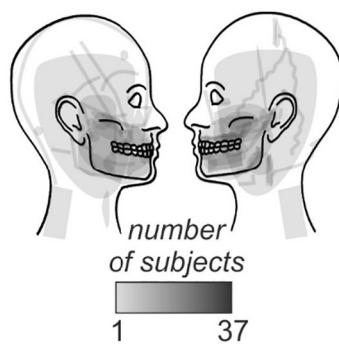
Fig. 1. Pain distribution and quality of pain in 37 subjects with chronic orofacial neuropathic pain.
A) Individual pain distribution patterns in all 37 chronic pain (NP) subjects. B) Frequency (percentage of subjects) of descriptors chosen from the McGill Pain Questionnaire to describe the quality of on-going pain, in NP subjects.
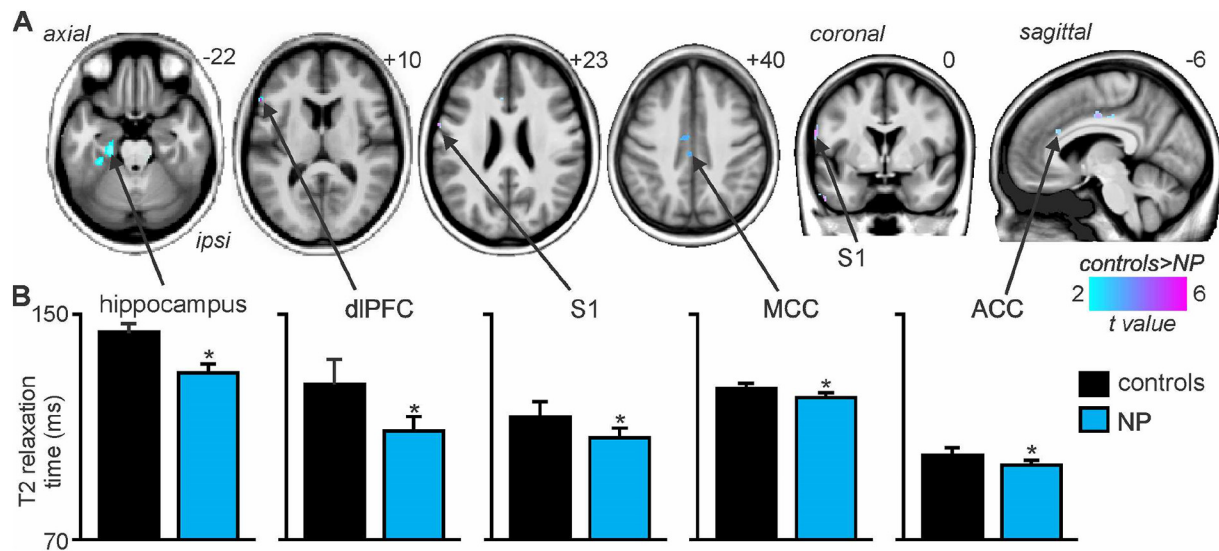
Fig. 2. Significant differences in T2 relaxation times in individuals with chronic neuropathic pain compared with controls.
A) Regions where chronic neuropathic pain (NP) subjects had significantly reduced T2 relaxation times compared with controls. Reductions are indicated by the cool colour scale and overlaid onto a mean T1-weighted anatomical image set derived from all 77 subjects. Note the decreases within the orofacial region of the primary somatosensory cortex (S1), mid-cingulate cortex (MCC), anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (dlPFC) and hippocampus. Slice locations in Montreal Neurological Institute space are indicated at the top right of each slice. B) Plots of mean ± SEM T2 relaxation times (ms) in control (black bars) and NP (blue bars) groups for significant clusters. *denotes significant differences derived from the voxel-by-voxel random-effects analysis ($p < 0.05$, false discovery rate corrected). ipsi: ipsilateral to side of highest on-going pain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

"radiating". There was no significant difference in age (t-test; $p > 0.05$) or gender composition (chi-squared test, $p > 0.05$) between the two subject groups. Compared with controls, NP subjects had higher BDI scores (mean ± SEM: controls: 5.0 ± 1.0, NP: 26.6 ± 2.0, $p < 0.001$) and PCS scores (controls: 12.2 ± 1.7, NP: 15.8 ± 1.5, $p < 0.001$).

3.2. T2 relaxation times

Wholebrain analysis revealed that NP subjects displayed significantly decreased T2 relaxation times relative to controls in a number of brain regions (Fig. 2, Table 2). These included the left (contralateral to highest pain) mid-cingulate cortex (mean ± SEM ms: controls: 101.2 ± 1.4, NP: 95.4 ± 1.1), left anterior cingulate cortex (controls: 96.8 ± 1.1, NP: 92.7 ± 1.0), left primary somatosensory cortex (controls: 107.5 ± 1.1, NP: 100.1 ± 1.3), left dorsolateral prefrontal cortex (controls: 122.4 ± 4.6, NP: 105.5 ± 3.2) and the left hippocampus/parahippocampus (controls: 145.2 ± 2.2, NP: 131.3 ± 1.9).

Brainstem-specific analysis also revealed regions in which T2

Table 2
Location, cluster size and significance level of T2 relaxation time decreases in individuals with chronic neuropathic pain compared with controls. Cluster peak locations are indicated in Montreal Neurological Institute (MNI) space.

Brain region	MNI co-ordinate			Cluster size	t-score
Whole brain T2 relaxation time					
Left mid cingulate cortex	6	−11	39	75	4.18
	−3	−24	38	55	4.31
Left medial prefrontal cortex	−6	24	24	10	3.88
Left primary somatosensory cortex	−61	0	21	40	4.40
Left dorsolateral prefrontal cortex	−56	24	9	18	4.11
Left hippocampus/ parahippocampus	20	−32	−17	313	4.42
Brainstem T2 relaxation time					
Right spinal trigeminal tract	9	30	−11	17	4.67
Right trigeminal nerve entry	11	−26	−37	54	5.03
Right spinal trigeminal nucleus	10	−42	−46	30	4.62

relaxation times were significantly lower in NP subjects than in controls in three discrete brainstem regions (Fig. 3, Table 2). Decreased T2 relaxation times in NP subjects occurred in the region encompassing the right spinal trigeminal nucleus (controls: 106.8 ± 1.1, NP: 101.2 ± 1.3), the right spinal trigeminal tract (controls: 118.2 ± 1.7, NP: 108.9 ± 2.1) and in the region of the right trigeminal nerve entry (controls: 107.3 ± 2.0, NP: 98.1 ± 1.8). In no brainstem region was there a significant increase in T2 relaxation time in NP subjects compared with controls.

In NP patients, there were no significant relationships between pain intensity or duration and T2 relaxation times in the left mid-cingulate cortex (intensity: $r = 0.08$, duration: $r = 0.08$), left anterior cingulate cortex (intensity: $r = 0.11$, duration: $r = 0.36$), left primary somatosensory cortex (intensity: $r = 0.05$, duration: $r = 0.22$), left dorsolateral prefrontal cortex (intensity: $r = 0.25$, duration: $r = 0.26$), left hippocampus/parahippocampus (intensity: $r = 0.23$, duration: $r = 0.26$), right trigeminal nerve entry (intensity: $r = 0.22$, duration: $r = 0.13$), right spinal trigeminal tract (intensity: $r = 0.11$, duration: $r = 0.20$), and right spinal trigeminal nucleus (intensity: $r = 0.09$, duration: $r = 0.14$, all $p > 0.05$). Furthermore, in NP subjects there were no significant linear relationships between PCS or BDI values and T2 relaxation times in the left mid-cingulate cortex (PCS: $r = 0.26$, BDI: $r = 0.31$), left anterior cingulate cortex (PCS: $r = 0.16$, BDI: $r = 0.06$), left primary somatosensory cortex (PCS: $r = 0.38$; BDI: $r = 0.22$), left dorsolateral prefrontal cortex (PCS: $r = 0.19$, BDI: $r = 0.28$), left hippocampus/parahippocampus (PCS: $r = 0.08$, BDI: $r = 0.01$), right trigeminal nerve entry (PCS: $r = 0.03$, BDI: $r = 0.26$), right spinal trigeminal tract (PCS: $r = 0.04$, BDI: $r = 0.24$), and right spinal trigeminal nucleus (PCS: $r = 0.07$, BDI: $r = 0.11$; all $p > 0.05$).

Finally, exploring the effects of medication use on T2 relaxation times in NP subjects revealed that in no cluster was T2 relaxation time significantly different in those taking either acute analgesic or prophylactic medications compared with those not taking medication: left mid-cingulate cortex (mean ± SEM ms: acute meds: 96.5 ± 3.4, prophylactic meds: 89.9 ± 1.4, no meds: 91.6 ± 1.1, acute v control $p = 0.08$; prophylactic vs controls $p = 0.36$), left anterior cingulate cortex (acute meds: 94.2 ± 0.6, prophylactic meds: 91.0 ± 1.3, no
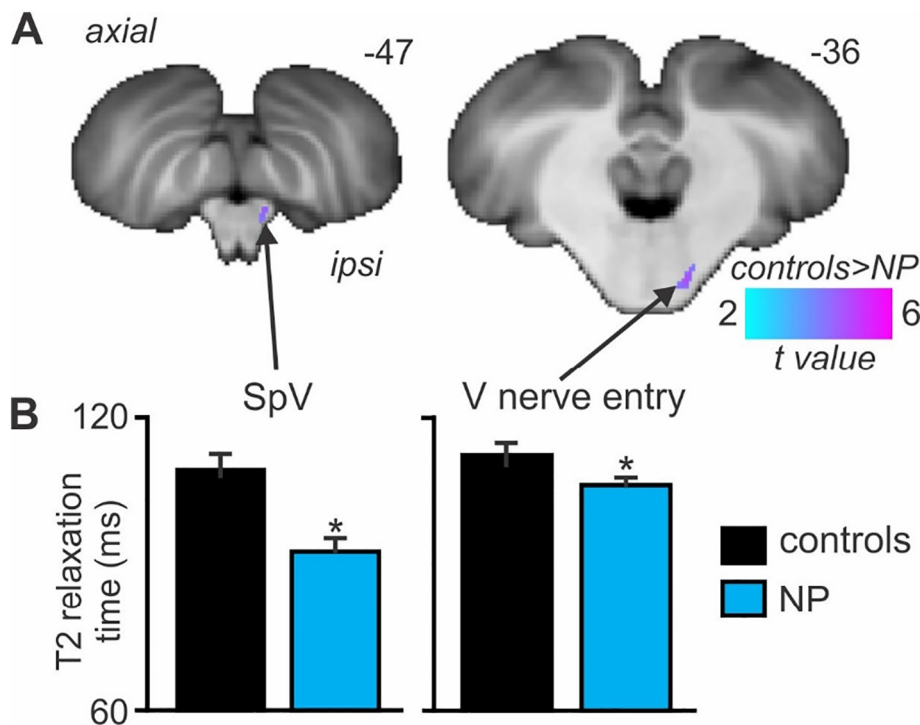
Fig. 3. Significant differences in T2 relaxation times in individuals with chronic neuropathic pain compared with controls, within the brainstem only.
A) Regions in which chronic neuropathic pain (NP) subjects had significantly reduced T2 relaxation times compared with controls. Reductions are indicated by the cool colour scale and overlaid onto a brainstem template image set. Note the decreases within the region where orofacial nociceptor afferents terminate – the spinal trigeminal nucleus (SpV), as well as the area where the trigeminal nerve (V) enters the pons. Slice locations in Montreal Neurological Institute space are indicated at the top right of each slice. B) Plots of mean ± SEM T2 relaxation times (ms) in control (black bars) and NP (blue bars) groups for significant clusters. *denotes significant differences derived from the voxel-by-voxel random-effects analysis (p < 0.05, false discovery rate corrected). ipsi: ipsilateral to side of highest on-going pain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

meds: 93.3 ± 1.6, p = 0.78, p = 0.36), left primary somatosensory cortex (acute meds: 99.8 ± 3.6, prophylactic meds: 103.0 ± 0.2.4, no meds: 98.5 ± 1.7, p = 0.73, p = 0.12), left dorsolateral prefrontal cortex (acute meds: 101.4 ± 8.5, prophylactic meds: 101.9 ± 5.4, no meds: 108.8 ± 4.4, p = 0.46, p = 0.34), left hippocampus/para-hippocampus (acute meds: 134.6 ± 6.5, prophylactic meds: 130.9 ± 2.8, no meds: 130.6 ± 2.8, p = 0.54, p = 0.95) and right spinal trigeminal nucleus (acute meds: 79.8 ± 5.8, prophylactic meds: 87.7 ± 5.4, no meds: 91.4 ± 3.4, p = 0.12, p = 0.53).

4. Discussion

The results of this investigation reveal significant decreases in T2 relaxation times in patients with chronic orofacial neuropathic pain at multiple points along the ascending pain pathway. Changes occurred in NP subjects in the region of the trigeminal nerve entry, the region of the ipsilateral (to pain) spinal trigeminal nucleus and in the contralateral orofacial representation of the primary somatosensory cortex. In addition, changes occurred in higher processing regions such as the cingulate and prefrontal cortices. These anatomical changes are consistent with pathological processes associated with astrocyte activation.

T2 relaxation times are influenced by the chemical characteristics of tissue. One factor that will significantly affect T2 relaxation time is the static dipolar fields created by neighbouring dipoles; in solids and polymers the static dipolar field is large. Thus, an increase in the proportion of solids and polymers within a region of the brain for example, would result in an increase in T2 relaxation rate, i.e. decrease in T2 relaxation time, in that region (Goldman and Shen, 1966; Solomon, 1955). Such an increase in solids/polymers within a specific brain region could result from numerous factors, including astrogliosis. In mild to moderate astrogliosis, there is an increase in glial fibrillary acidic protein, an intermediate filament composed of a variety of proteins, which would contribute to an increase in static dipolar field and a decrease in T2 relaxation time (Sofroniew, 2009; Sofroniew and Vinters, 2010; Stewart, 1993). Consistent with this, an experimental animal study that investigated changes following stroke reported decreased T2 relaxation times in areas of increased astrocyte and microglial activation and iron deposition (Justicia et al., 2008). Furthermore, a human

post mortem study of two individuals with multiple system atrophy reported reduced T2 relaxation times in the putamen, an area that also displayed pronounced reactive astrogliosis and microgliosis (Schwarz et al., 1996). Whilst there are other anatomical changes besides astrogliosis that could have contributed to the change in T2 relaxation times shown in this investigation, several lines of evidence suggest that astrogliosis is a likely contributing factor.

We found decreased T2 relaxation time in the region encompassing the ipsilateral (to pain) spinal trigeminal nucleus, the area in which orofacial nociceptor afferents terminate. It is known from both human post-mortem and experimental animal investigations that the presence of chronic pain following nerve injury is associated with prolonged astrocyte (and not microglial) activation at the level of the primary afferent synapse (Garrison et al., 1991; Okada-Ogawa et al., 2009; Shi et al., 2012). In a previous investigation, we found an increase in on-going infra-slow oscillatory activity in the region encompassing the spinal trigeminal nucleus in individuals with chronic orofacial neuropathic pain (Alshelh et al., 2016). Importantly, these increased infra-slow oscillations were at similar frequencies as calcium waves in activated astrocytes, which are associated with the release of gliotransmitters and the subsequent modulation of neural activity (Parri and Crunelli, 2001). We speculated that although chronic neuropathic pain is *not* associated with increased on-going activity in the ascending pain pathways, the modulation of this pathway by gliotransmitters results in altered activity patterns, thalamocortical dysrhythmia and the constant perception of pain (Alshelh et al., 2016; Henderson and Di Pietro, 2016). A decrease in spinal trigeminal nucleus T2 relaxation time is consistent with this hypothesis, although whether this change in relaxation time represents astrocyte activation remains debatable.

In our previous investigation, we also found altered infra-slow oscillation increases associated with chronic neuropathic pain in the region of the ventrocaudal thalamus, thalamic reticular nucleus and in the primary somatosensory cortex (Alshelh et al., 2016). In addition, others have reported increased [^{11}C](R)-PK11195 binding, a marker of glial activation, in the thalamus and primary somatosensory cortex in individuals with NP (Banati et al., 2001; Loggia et al., 2015). Whilst altered [^{11}C](R)-PK11195 binding may reflect changes in microglial or astrocyte activation, surprisingly we found no change in T2 relaxation

time in the thalamus in this investigation, despite showing changes in the spinal trigeminal nucleus and primary somatosensory cortex. Why no change in T2 relaxation rate occurred in the thalamus is unknown, but it might be the case that the thalamus is somehow more resistant to the development of astrogliosis than the primary afferent synapse or somatosensory cortex, or that recurrent circuits between the thalamus and cortex somehow reduces the potential for thalamic astrocyte activation following nerve injury. Indeed, in contrast to reports of prolonged astrocyte activation at the primary afferent synapse, it has been reported in the chronic constriction model of neuropathic pain that the ventroposterior thalamus displays microglial and not astrocyte activation at 7 and 14 days post injury, and that inhibiting microglial activation reduces thermal hyperalgesia (LeBlanc et al., 2011). This raises the possibility that increased infra-slow oscillations in the ventrocaudal thalamus in individuals with NP are driven by such changes at the primary afferent synapse. This is consistent with the finding of a preclinical study that shows infra-slow oscillatory activity in the ventroposterior thalamus was eliminated by severing the connection between the primary afferent synapse and the thalamus (Iwata et al., 2011).

Whilst the focus of this investigation was on changes in the ascending pain pathway, it is likely that other brain regions are also involved in the maintenance of neuropathic pain. We also found decreases in T2 relaxation time in the cingulate, dorsolateral prefrontal and hippocampal cortices in individuals with neuropathic pain. There is evidence from preclinical models of chronic pain of astrocyte activation in the anterior cingulate cortex and suggestions that these changes are associated with sleep disturbances (Kuzumaki et al., 2007; Yamashita et al., 2014). Whilst we did not measure sleep patterns in our chronic pain subjects it is possible that the altered T2 relaxation in the cingulate cortex may reflect changes in sleep patterns that occur in individuals with various forms of chronic pain (Pilowsky et al., 1985). Alternatively, there is growing evidence that the cingulate cortex codes the affective dimension of pain and thus cingulate astrocyte activation may reflect this aspect of the chronic pain condition, an idea that has support from preclinical investigations (Chen et al., 2012; Narita et al., 2006).

Indeed it is well established that neuropathic pain is often associated with psychological changes such as depression and pain catastrophizing (Bair et al., 2003; Keefe et al., 2004) and we found significant differences in pain catastrophizing scores and depression scores between the healthy controls and NP subjects. We did find significant changes in T2 relaxation times in the cingulate cortex, whose function is associated with depression (Fonseka et al., 2017; Guo et al., 2012). Although we found no correlation between T2 relaxation in any brain region and depressive scores, there is evidence of increased infra-slow oscillation power in the anterior cingulate cortex of individuals with treatment-resistant depression (Guo et al., 2012). Furthermore, these regions were also not correlated to pain intensity or duration of neuropathic pain. Whilst the lack of linear correlations does not exclude the possibility that the changes in these areas are related to pain and psychological measures, it may be that the underlying mechanisms responsible for T2 relaxation time alterations reflect anatomical changes that are simply not linearly coupled to changes in neural activity.

In contrast to the cingulate cortex, we know of no investigation exploring astrocyte changes in the dorsolateral prefrontal cortex or hippocampus in individuals with neuropathic pain or in preclinical models. Whilst the role of the dorsolateral prefrontal cortex in neuropathic pain remains unclear, there is evidence that its anatomy and function is altered in various pain conditions (Apkarian et al., 2004; Lorenz et al., 2003; Seminowicz and Moayedi, 2017). Furthermore, there is evidence for a role for the dorsolateral prefrontal cortex in descending pain modulation, which is altered in individuals with chronic pain (Bingel and Tracey, 2008; Youssef et al., 2016). We also found T2 relaxation time differences in the ventral aspect of the hippocampus, a region thought to control hypothalamopituitary adrenal

(HPA) axis function (Strange et al., 2014). Consistent with the idea that these T2 relaxation time changes represent astrogliosis, there are multiple investigations that report hippocampal volume increases in individuals with chronic pain, although there are some inconsistencies (Schweinhardt et al., 2008; Smallwood et al., 2013; Tu et al., 2010; Younger et al., 2010). Since chronic pain is also associated with the presence of stress-related disorders and HPA axis dysfunction (Griep et al., 1998; Korszun et al., 2002; Tennant and Hermann, 2002), it is possible that the changes in ventral hippocampal T2 relaxation times are related to changes in HPA axis function in our chronic pain subjects.

We found that neither acute analgesic nor prophylactic medication use had significant effect on T2 relaxation times in NP subjects, making it unlikely that medication itself has a significant effect on T2 relaxation times and the relationship between T2 relaxation times and pain and psychological measures. Whilst the effects of medication use on regional astrocyte activation and T2 relaxation rate needs to be explored in a longitudinal manner, our hypothesis that neuropathic pain results from chronic astrocyte activation is consistent with finding that neuropathic pain medications such as valproate, phenytoin and gabapentin, reduce astrocyte calcium signalling (Tian et al., 2005). This raises the propect that other medications with potential astrocytic modulatory functions, such as Clozapine, Riluzole, Chromipramine and Palmitoylethanolamide, may also be effective at treating neuropathic pain.

In addition to reduced T2 relaxation rates, we have previously shown in individuals with chronic orofacial pain, the SpV displays reduced gray matter density, reduced mean diffusivity, increased infra-slow oscillations and increased functional connectivity strengths with brainstem endogenous pain modulation circuitry (Alshelh et al., 2016; Mills et al., 2018; Wilcox et al., 2015). Despite altered functional connectivity, we did not observe anatomical or infra-slow oscillatory changes in brainstem pain modulating regions such as the periaqueductal gray, dorsolateral pons, subnucleus reticularis dorsalis or rostroventromedial medulla. Given this, we speculate that the anatomical changes and resting oscillation changes that occur in SpV reflect astrocyte activation that results from excitotoxic damage and is exacerbated by reduced endogenous analgesic circuitry action at the level of SpV. Future studies are needed to provide solid evidence that such a chain of events occurs.

Finally, there are several limitations that need to be noted. Firstly, as mentioned above, altered T2 relaxation times are not a direct index of astrocyte activation and further combined MRI and histochemical investigations are needed to determine precisely how astrocyte activation alters T2 relaxation times. Although PET studies may provide a more accurate measure of glial activation, at this stage they are not suited to measure changes at the primary afferent synapse in the brainstem or spinal cord. Secondly, it is difficult to accurately localize clusters to specific nuclei, particularly those in the brainstem, due to the relatively low spatial resolution and the fine compartmentalization of brainstem nuclei. Increasing MRI field strengths and greater accuracy of brainstem spatial normalization procedures will improve our confidence in brainstem-specific analysis in future investigations. Thirdly, whilst we found that medication use had no effect on T2 relaxation times, this finding should be interpreted with caution given the small number of subjects taking acute and preventive medications. Despite these limitations, the discrete localization of decreases in T2 relaxation times found in chronic orofacial neuropathic pain subjects in this investigation, together with existing human and pre-clinical evidence, makes us confident that astrocytes play a critical role in the development and the maintenance of neuropathic pain following nerve injury.

Acknowledgements

References

Alshelh, Z., Di Pietro, F., Youssef, A.M., Reeves, J.M., Macey, P.M., Vickers, E.R., Peck, C.C., Murray, G.M., Henderson, L.A., 2016. Chronic neuropathic pain: it's about the rhythm. J. Neurosci. 36, 1008–1018.

Apkarian, A.V., Sosa, Y., Sonty, S., Levy, R.M., Harden, R.N., Parrish, T.B., Gitelman, D.R., 2004. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. J. Neurosci. 24, 10410–10415.

Bair, M.J., Robinson, R.L., Katon, W., Kroenke, K., 2003. Depression and pain co-morbidity: a literature review. Arch. Intern. Med. 163, 2433–2445.

Banati, R.B., Cagnin, A., Brooks, D.J., Gunn, R.N., Myers, R., Jones, T., Birch, R., Anand, P., 2001. Long-term trans-synaptic glial responses in the human thalamus after per-ipheral nerve injury. Neuroreport 12, 3439–3442.

Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., Erbaugh, J., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561–571.

Bingel, U., Tracey, I., 2008. Imaging CNS modulation of pain in humans. Physiology (Bethesda) 23, 371–380.

Carlsson, A.M., 1983. Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. Pain 16, 87–101.

Chen, F.L., Dong, Y.L., Zhang, Z.J., Cao, D.L., Xu, J., Hui, J., Zhu, L., Gao, Y.J., 2012. Activation of astrocytes in the anterior cingulate cortex contributes to the affective component of pain in an inflammatory pain model. Brain Res. Bull. 87, 60–66.

Crunelli, V., Blethyn, K.L., Cope, D.W., Hughes, S.W., Parri, H.R., Turner, J.P., Toth, T.I., Williams, S.R., 2002. Novel neuronal and astrocytic mechanisms in thalamocortical loop dynamics. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 357, 1675–1693.

Diedrichsen, J., 2006. A spatially unbiased atlas template of the human cerebellum. NeuroImage 33, 127–138.

Fonseka, T.M., MacQueen, G.M., Kennedy, S.H., 2017. Neuroimaging biomarkers as predictors of treatment outcome in Major Depressive Disorder. J. Affect. Disord. 233, 21–35.

Friston, K.J., Holmes, A.P., Worsley, K.P., Proline, J.B., Frith, C.D., Frackowiak, R.S.J., 1995. Statistical parametric maps in functional imaging: a general imaging approach. Hum. Brain Mapp. 2, 189–210.

Garrison, C.J., Dougherty, P.M., Kajander, K.C., Carlton, S.M., 1991. Staining of glial fibrillary acidic protein (GFAP) in lumbar spinal cord increases following a sciatic nerve constriction injury. Brain Res. 565, 1–7.

Gerke, M.B., Duggan, A.W., Xu, L., Siddall, P.J., 2003. Thalamic neuronal activity in rats with mechanical allodynia following contusive spinal cord injury. Neuroscience 117, 715–722.

Goldman, M., Shen, L., 1966. Spin-spin relaxation in LaF3. Phys. Rev. 144, 321–331.

Griep, E.N., Boersma, J.W., Lentjes, E.G., Prins, A.P., van der Korst, J.K., de Kloet, E.R., 1998. Function of the hypothalamic-pituitary-adrenal axis in patients with fi-bromyalgia and low back pain. J. Rheumatol. 25, 1374–1381.

Guo, W.-b., Liu, F., Xue, Z.-m., Xu, X.-j., Wu, R.-r., Ma, C.-q., Wooderson, S.C., Tan, C.-l., Sun, X.-l., Chen, J.-d., Liu, Z.-n., Xiao, C.-q., Chen, H.-f., Zhao, J.-p., 2012. Alterations of the amplitude of low-frequency fluctuations in treatment-resistant and treatment-response depression: a resting-state fMRI study. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 37, 153–160.

Henderson, L.A., Di Pietro, F., 2016. How do neuroanatomical changes in individuals with chronic pain result in the constant perception of pain? Pain Manag. Nurs. 6, 147–159.

Hsieh, J.C., Belfrage, M., Stone-Elander, S., Hansson, P., Ingvar, M., 1995. Central re-presentation of chronic ongoing neuropathic pain studied by positron emission to-mography. Pain 63, 225–236.

Iadarola, M.J., Max, M.B., Berman, K.F., Byas-Smith, M.G., Coghill, R.C., Gracely, R.H., Bennett, G.J., 1995. Unilateral decrease in thalamic activity observed with positron emission tomography in patients with chronic neuropathic pain. Pain 63, 55–64.

Iwata, M., LeBlanc, B.W., Kadasi, L.M., Zerah, M.L., Cosgrove, R.G., Saab, C.Y., 2011. High-frequency stimulation in the ventral posterolateral thalamus reverses electro-physiologic changes and hyperalgesia in a rat model of peripheral neuropathic pain. Pain 152, 2505–2513.

Jackson, G.D., Grunewald, R.A., Connelly, A., Duncan, J.S., 1994. Quantitative MR re-laxometry study of effects of vigabatrin on the brains of patients with epilepsy. Epilepsy Res. 18, 127–137.

Justicia, C., Ramos-Cabrer, P., Hoehn, M., 2008. MRI detection of secondary damage after stroke: chronic iron accumulation in the thalamus of the rat brain. Stroke 39, 1541–1547.

Keefe, F.J., Rumble, M.E., Scipio, C.D., Giordano, L.A., Perri, L.M., 2004. Psychological aspects of persistent pain: current state of the science. J. Pain 5, 195–211.

Korszun, A., Young, E.A., Singer, K., Carlson, N.E., Brown, M.B., Crofford, L., 2002. Basal circadian cortisol secretion in women with temporomandibular disorders. J. Dent. Res. 81, 279–283.

Kuzumaki, N., Narita, M., Narita, M., Hareyama, N., Niikura, K., Nagumo, Y., Nozaki, H., Amano, T., Suzuki, T., 2007. Chronic pain-induced astrocyte activation in the cin-gulate cortex with no change in neural or glial differentiation from neural stem cells in mice. Neurosci. Lett. 415, 22–27.

LeBlanc, B.W., Zerah, M.L., Kadasi, L.M., Chai, N., Saab, C.Y., 2011. Minocycline injection in the ventral posterolateral thalamus reverses microglial reactivity and thermal hyperalgesia secondary to sciatic neuropathy. Neurosci. Lett. 498, 138–142.

Lenz, F.A., Kwan, H.C., Dostrovsky, J.O., Tasker, R.R., 1989. Characteristics of the bursting pattern of action potentials that occurs in the thalamus of patients with central pain. Brain Res. 496, 357–360.

Loggia, M.L., Chonde, D.B., Akeju, O., Arabasz, G., Catana, C., Edwards, R.R., Hill, E., Hsu, S., Izquierdo-Garcia, D., Ji, R.R., Riley, M., Wasan, A.D., Zurcher, N.R., Albrecht, D.S., Vangel, M.G., Rosen, B.R., Napadow, V., Hooker, J.M., 2015. Evidence for brain glial activation in chronic pain patients. Brain 138, 604–615.

Lorenz, J., Minoshima, S., Casey, K.L., 2003. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 126, 1079–1091.

Mills, E.P., Di Pietro, F., Alshelh, Z., Peck, C.C., Murray, G.M., Vickers, E.R., Henderson, L.A., 2018. Brainstem pain-control circuitry connectivity in chronic neuropathic pain. J. Neurosci. 38, 465–473.

Moisset, X., Bouhassira, D., 2007. Brain imaging of neuropathic pain. NeuroImage 37 (Suppl. 1), S80–88.

Melzack, R., 1975. The McGill Pain Questionnaire: major properties and scoring methods. Pain 1, 277–299.

Narita, M., Kuzumaki, N., Narita, M., Kaneko, C., Hareyama, N., Miyatake, M., Shindo, K., Miyoshi, K., Nakajima, M., Nagumo, Y., Sato, F., Wachi, H., Seyama, Y., Suzuki, T., 2006. Chronic pain-induced emotional dysfunction is associated with astrogliosis due to cortical delta-opioid receptor dysfunction. J. Neurochem. 97, 1369–1378.

Nurmikko, T.J., Eldridge, P.R., 2001. Trigeminal neuralgia–pathophysiology, diagnosis and current treatment. Br. J. Anaesth. 87, 117–132.

Okada-Ogawa, A., Suzuki, I., Sessle, B.J., Chiang, C.Y., Salter, M.W., Dostrovsky, J.O., Tsuboi, Y., Kondo, M., Kitagawa, J., Kobayashi, A., Noma, N., Imamura, Y., Iwata, K., 2009. Astroglia in medullary dorsal horn (trigeminal spinal subnucleus caudalis) are involved in trigeminal neuropathic pain mechanisms. J. Neurosci. 29, 11161–11171.

Parri, H.R., Crunelli, V., 2001. Pacemaker calcium oscillations in thalamic astrocytes in situ. Neuroreport 12, 3897–3900.

Di Pietro, F., Macey, P.M., Rae, C.D., Alshelh, Z., Macefield, V.G., Vickers, E.R., Henderson, L.A., 2018. The relationship between thalamic GABA content and resting cortical rhythm in neuropathic pain. In: Human Brain Mapping, (accepted).

Pilowsky, I., Crettenden, I., Townley, M., 1985. Sleep disturbance in pain clinic patients. Pain 23, 27–33.

Sarnthein, J., Stern, J., Aufenberg, C., Rousson, V., Jeanmonod, D., 2006. Increased EEG power and slowed dominant frequency in patients with neurogenic pain. Brain 129, 55–64.

Schwarz, J., Weis, S., Kraft, E., Tatsch, K., Bandmann, O., Mehraein, P., Vogl, T., Oertel, W.H., 1996. Signal changes on MRI and increases in reactive microgliosis, astro-gliosis, and iron in the putamen of two patients with multiple system atrophy. J. Neurol. Neurosurg. Psychiatry 60, 98–101.

Schweinhardt, P., Kuchinad, A., Pukall, C.F., Bushnell, M.C., 2008. Increased gray matter density in young women with chronic vulvar pain. Pain 140, 411–419.

Seminowicz, D.A., Moayedi, M., 2017. The dorsolateral prefrontal cortex in acute and chronic pain. J. Pain 18, 1027–1035.

Shi, Y., Gelman, B.B., Lisinicchia, J.G., Tang, S.J., 2012. Chronic-pain-associated astro-cytic reaction in the spinal cord dorsal horn of human immunodeficiency virus-in-fected patients. J. Neurosci. 32, 10833–10840.

Smallwood, R.F., Laird, A.R., Ramage, A.E., Parkinson, A.L., Lewis, J., Clauw, D.J., Williams, D.A., Schmidt-Wilcke, T., Farrell, M.J., Eickhoff, S.B., Robin, D.A., 2013. Structural brain anomalies and chronic pain: a quantitative meta-analysis of gray matter volume. J. Pain 14, 663–675.

Sofroniew, M.V., 2009. Molecular dissection of reactive astrogliosis and glial scar for-mation. Trends Neurosci. 32, 638–647.

Sofroniew, M.V., Vinters, H.V., 2010. Astrocytes: biology and pathology. Acta Neuropathol. 119, 7–35.

Solomon, I., 1955. Relaxation processes in a system of two spins. Phys. Rev. 99, 559–565.

Strange, B.A., Witter, M.P., Lein, E.S., Moser, E.I., 2014. Functional organization of the hippocampal longitudinal axis. Nat. Rev. Neurosci. 15, 655–669.

Sullivan, M.J.L., Bishop, S., Pivik, J., 1995. The Pain Catastrophizing Scale: development and validation. Psychol. Assess. 7, 432–524.

Stewart, M., 1993. Intermediate filament structure and assembly. Curr. Opin. Cell Biol. 5, 3–11.

Tennant, F., Hermann, L., 2002. Normalization of serum cortisol concentration with opioid treatment of severe chronic pain. Pain Med. 3, 132–134.

Tian, G.F., Azmi, H., Takano, T., Xu, Q., Peng, W., Lin, J., Oberheim, N., Lou, N., Wang, X., Zielke, H.R., Kang, J., Nedergaard, M., 2005. An astrocytic basis of epilepsy. Nat. Med. 11, 973–981.

Tu, C.H., Niddam, D.M., Chao, H.T., Chen, L.F., Chen, Y.S., Wu, Y.T., Yeh, T.C., Lirng, J.F., Hsieh, J.C., 2010. Brain morphological changes associated with cyclic menstrual pain. Pain 150, 462–468.

Wagner, D.C., Deten, A., Hartig, W., Boltze, J., Kranz, A., 2012. Changes in T2 relaxation time after stroke reflect clearing processes. NeuroImage 61, 780–785.

Wilcox, S.L., Gustin, S.M., Macey, P.M., Peck, C.C., Murray, G.M., Henderson, L.A., 2015. Anatomical changes at the level of the primary synapse in neuropathic pain: evidence from the spinal trigeminal nucleus. J. Neurosci. 35, 2508–2515.

Walton, K.D., Llinás, R.R., 2010. Central pain as a thalamocortical dysrhythmia: a tha-lamic efference disconnection? In: Kruger, L., Light, A.R. (Eds.), Translational Pain Research: From Mouse to Man. CRC Press, Boca Raton, FL.

Yamashita, A., Hamada, A., Suhara, Y., Kawabe, R., Yanase, M., Kuzumaki, N., Narita, M., Matsui, R., Okano, H., Narita, M., 2014. Astrocytic activation in the anterior cingu-late cortex is critical for sleep disorder under neuropathic pain. Synapse 68, 235–247.

Younger, J.W., Shen, Y.F., Goddard, G., Mackey, S.C., 2010. Chronic myofascial tem-poromandibular pain is associated with neural abnormalities in the trigeminal and limbic systems. Pain 149, 222–228.

Youssef, A.M., Gustin, S.M., Nash, P.G., Reeves, J.M., Petersen, E.T., Peck, C.C., Murray, G.M., Henderson, L.A., 2014. Differential brain activity in subjects with painful tri-geminal neuropathy and painful temporomandibular disorder. Pain 155, 467–475.

Youssef, A.M., Macefield, V.G., Henderson, L.A., 2016. Cortical influences on brainstem circuitry responsible for conditioned pain modulation in humans. Hum. Brain Mapp. 37, 2630–2644.