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Anatomical changes within the medullary dorsal horn in chronic temporomandibular disorder pain

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ABSTRACT

Accumulated evidence from experimental animal models suggests that neuroplastic changes at the dorsal horn are critical for the maintenance of various chronic musculoskeletal pain conditions. However, to date, no study has specifically investigated whether neuroplastic changes also occur at this level in humans. Using brain imaging techniques, we sought to determine whether anatomical changes were present in the medullary dorsal horn (spinal trigeminal nucleus caudalis) in subjects with the chronic musculoskeletal pain. In twenty-two subjects with painful temporomandibular disorders (TMDs) and forty pain-free controls voxel based morphometry of T1-weighted anatomical images and diffusion tensor images were used to assess regional grey matter volume and microstructural changes within the brainstem and, in addition, the integrity of ascending pain pathways. Voxel based morphometry revealed significant regional grey matter volume decreases in the medullary dorsal horn, in conjunction with alterations in diffusivity properties, namely an increase in mean diffusivity, in TMD subjects. Volumetric and mean diffusivity changes also occurred in TMD subjects in regions of the descending pain modulation system, including the midbrain periaqueductal grey matter and nucleus raphe magnus. Finally, tractography revealed altered diffusivity properties, namely decreased fractional anisotropy, in the root entry zone of the trigeminal nerve, the spinal trigeminal tract and the ventral trigeminothalamic tracts of TMD subjects. These data reveal that chronic musculoskeletal pain in humans is associated with discrete alterations in the anatomy of the medullary dorsal horn, as well as its afferent and efferent projections. These neural changes may be critical for the maintenance of pathological pain.

Introduction

In many chronic pain conditions, rather than simply being a reflection of peripheral inputs or pathology, the perception of pain is thought to also dynamically reflect central neuronal alterations (Latremoliere and Woolf, 2009). In particular, it has been proposed that alterations at the first (primary) synapse, that is, between peripheral nociceptor afferents and lamina I spinal or medullary dorsal horn neurons, are critical for the maintenance of pain that was initially induced by low-level afferent input such as chronic inflammatory and musculoskeletal pain (Ikeda et al., 2006).

Both animal models and clinical conditions suggest that some forms of chronic pain are associated with alterations in neuronal activity linked to long-term structural changes in the brain (Henderson et al., 2013; Jensen et al., 2013; Sessle, 2000). In contrast, other forms may not necessarily be associated with central changes, although evidence is mixed (Moayedi et al., 2012; Younger et al., 2010). For example, we found in humans that chronic neuropathic pain is associated with thalamic and cortical structural brain changes, but musculoskeletal pain is not (Gustin et al., 2011). However, in animal models of chronic inflammatory/musculoskeletal pain, investigations of changes at the primary synapse demonstrate long-term glial up-regulation and central sensitisation (Miyagi et al., 2011; Tsuboi et al., 2011). Hence, chronic musculoskeletal pain may also be associated with structural changes, but at the level of the primary synapse, rather than higher brain regions.

To date, no study has explored the anatomy of spinal or medullary dorsal horn in humans with chronic pain. The lack of studies focussed on the spinal cord is not surprising given its extremely small size (Valsasina et al., 2012), but the brainstem can be explored using current high resolution magnetic resonance imaging (MRI) techniques. In the orofacial system, the medullary dorsal horn or spinal trigeminal nucleus caudalis (SpVc) lies within the caudal brainstem. We have previously used functional MRI to explore brainstem activation patterns during experimentally evoked acute muscle pain in healthy individuals. Indeed, we found that acute orofacial muscle pain evokes signal intensity increases within the ipsilateral SpVc (Nash et al., 2009).

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Given the superior spatial resolution of anatomical MRI, it is also possible to explore the anatomy of this brainstem region in humans with chronic orofacial pain conditions.

The aim of this investigation was to use anatomical MRI techniques to explore brainstem anatomy, particularly the SpVc, in individuals with painful temporomandibular disorders (TMDs), a set of chronic musculoskeletal conditions involving the masticatory muscles, the temporomandibular joints, or both (Bender, 2012). If chronic pain conditions such as TMD are maintained by central neuronal alterations, we hypothesise that this will manifest as observable anatomical changes, particularly within SpVc. Furthermore, it is likely that the degree of change will be reflected in the intensity of on-going pain. High resolution voxel-based morphometry (VBM) of T1-weighted anatomical and diffusion weighted images will be used to determine changes in regional brainstem anatomy. In addition, deterministic tractography will be used to assess the integrity of ascending orofacial pain pathways. We hypothesise that TMDs will be associated with altered SpVc anatomy and altered integrity of ascending pain pathways as a result of prolonged peripheral input (Stankewitz et al., 2013).

Materials and methods

Subjects

Twenty-two subjects with painful TMDs (four males, mean [±SEM] age: 46.5 ± 2.6) and 40 pain-free controls (seven males, age: 48.3 ± 2.1) were recruited. There was no significant difference in age (*t* test; $p > 0.05$) or gender distribution (χ^2 test, $p > 0.05$) between the two subject groups. All subjects were recruited from the Faculty of Dentistry, Westmead Hospital and University of Sydney. Individual TMD subject demographics are given in Table 1. TMD subjects were diagnosed using the research diagnostic criteria for TMD (Dworkin and LeResche, 1992) by a clinician (C.P.) in the research group. Inclusion criteria for TMD subjects included: a primary pain complaint of TMD, pain duration of ≥1 year and an average pain rating of ≥1. In addition, TMD subjects were excluded if they also had suspected trigeminal nerve damage/neuropathic pain symptoms or other chronic pain conditions. They were included from the study if they reported other non-chronic musculoskeletal pain complaints in other body regions (n = 6) or infrequent migraines (n = 2). Controls were screened by self-report for exclusion criteria including chronic pain (pain lasting for more than 3 months), current usage of analgesic medications or any neurological disorder. Subjects from either group were also excluded if they fulfilled standard MRI exclusion criteria such as pregnancy, metal implants and pacemakers. Informed written consent was obtained for all procedures according to the Declaration of Helsinki and the study was approved by local Institutional Human Research Ethics Committees. Subjects used in this study were also used in previous investigations (Gustin et al., 2011, 2012; Henderson et al., 2013; Wilcox et al., 2013).

Pain measures

On the day of the MRI scanning session, each TMD subject's pain was assessed by asking each subject to place a vertical pen stroke indicating their current pain intensity on a 10 cm horizontal visual analogue scale (VAS), with 0 cm indicating "no pain" and 10 cm indicating "worst pain imaginable"—"scan pain", the duration of their pain, the location as a distribution map of their ongoing pain onto a standard drawing of the face, and quality by the McGill Pain Questionnaire (Melzack, 1975). In addition, during the seven days prior to the MRI session, each TMD subject reported three times a day, with a vertical pen stroke, their pain intensity on a 10 cm horizontal visual analogue scale (VAS). These pain intensity scores were averaged to create a mean "dairy pain" intensity score.

MRI scans

All subjects lay supine on the bed of a 3 Tesla MRI scanner (Philips, Intera) with their head immobilised in a tight-fitting head coil. In each subject, three high-resolution 3D T1-weighted anatomical image sets covering the entire brain were collected (turbo field echo; echo time = 2.5 ms, repetition time 5600 ms, flip angle = 8°, voxel size 0.8 × 0.8 × 0.8 mm). The three acquisitions were acquired to improve signal-to-noise ratios. In addition four high-resolution diffusion tensor imaging (DTI) image sets covering the entire brain were collected using a single-shot multisection spin-echo echo-planar pulse sequence (repetition time = 8788 ms; flip angle = 90°, matrix size = 112 × 112, field of view = 224 × 224 mm, slice thickness = 2.5 mm, 55 axial slices). For each slice, diffusion gradients were applied along 32 independent orientations with $b = 1000$ s/mm^2 after the acquisition

Table 1
TMD subject characteristics: Arth: arthralgia; MFP: myofascial pain; TMJ: temporomandibular joint, VAS: visual analogue scale, PRN: *pro re nata* (as needed).

Subject	*Age (yrs)*	*Gen-der*	*Subgroup*	*Site*	*Scan pain*	*Diary pain*	*Pain duration (yrs)*	*Analgesic medication*
1	38	F	MFP	Right	4.7	1.8	6	Paracetamol
2	59	M	MFP & pain localised to the hard palate	Bilateral	6.0	5.9	20	Amitriptyline hydrochloride
3	59	F	MFP	Bilateral	7.6	7.9	30	Venlafaxine, co-codamol
4	53	F	Right TMJ Arth & bilateral MFP	Bilateral	3.1	2.5	3	None
5	47	M	MFP	Bilateral	2.7	2.9	20	Paracetamol (PRN)
6	42	F	Left TMJ Arth & bilateral MFP	Bilateral	0.5	1.9	3	None
7	70	F	Left TMJ Arth & bilateral MFP	Bilateral	4.6	1.0	11	Aspirin
8	62	F	MFP	Bilateral	3.9	5.4	5	Diazepam, paracetamol
9	45	F	Bilateral TMJ Arth & right MFP	Bilateral	1.1	1.5	2	None
10	37	M	Bilateral TMJ Arth & MFP	Bilateral	7.0	7.3	6	Botox
11	41	F	Right MFP	Right	0.5	1.4	4	None
12	50	F	MFP	Bilateral	2.7	4.3	46	Diclofenac
13	45	F	MFP	Bilateral	7.3	4.1	5.5	Oxycodone (PRN), paracetamol, co-codamol (PRN), doxylamine succinate, codeine
14	33	F	Right MFP	Right	5.5	5.9	15	Doxylamine succinate, codeine (PRN)
15	28	M	Right TMJ Arth & bilateral MFP	Bilateral	6.7	5.8	1.5	Ibuprofen (PRN)
16	25	F	Bilateral TMJ Arth & MFP	Bilateral	5.9	5.5	7	Amitriptyline hydrochloride, co-codamol, sumatriptan
17	67	F	MFP	Bilateral	1.1	3.1	5	None
18	50	F	Left TMJ Arth & bilateral MFP	Bilateral	2.0	2.7	3	None
19	38	F	MFP	Bilateral	5.5	2.2	4	Ibuprofen (PRN)
20	31	F	MFP	Bilateral	1.7	3.5	2	Ibuprofen, paracetamol
21	56	F	MFP	Right	3.6	1.1	18	None
22	46	F	MFP	Bilateral	3.3	3.3	12	Paracetamol
Mean	46.4				4.0	3.7	9.7	

of $b = 0$ s/mm^2 (b_0) images. Four acquisitions were acquired to improve signal-to-noise ratios. Anatomical and DTI image sets were visually inspected for artefacts (ghosting, distortion, and blurring and/or signal dropout). No anatomical image sets were excluded. DTI image sets from 6 TMD subjects and 20 controls were either excluded or not collected due to scanner error, time limitations or subject withdrawal. The resulting DTI data set included 16 of 22 TMD and 20 of 40 controls subjects (TMD: two males, mean age 47.3 ± 3.0 years; controls: two males, mean age 50.1 ± 3.3, no significant difference in age [t test; $p = 0.97$] or gender [χ^2 test, $p = 0.81$]).

MRI analysis

Brainstem VBM

Using SPM8 (Friston et al., 1994), the three T1-weighted images from each subject were coregistered and averaged. The mean images were segmented by tissue type and spatially normalised with a dedicated symmetrical brainstem template (spatial resolution of template 1 × 1 × 1 mm). In brief, using the SUIT toolbox (Diedrichsen, 2006), each image was cropped and the brainstem was masked before spatial normalisation. The subsequent non-linear spatial normalisation and re-slicing process produces brainstem "maps" of grey matter probabilities modulated by the volume changes due to the normalisation. Finally, the images were re-sliced into Montreal Neurological Institute (MNI) space and spatially smoothed using a Gaussian filter (3 mm full-width-half-maximum). Nine of the 22 TMD subjects rated their pain the same on the left and right sides, 5 rated their pain greater on the left side, 4 greater on the right and the remaining 4 TMD subjects had only right side pain. In order to assess anatomical changes ipsilateral and contralateral to the side of highest on-going pain, for the 8 subjects with right sided dominate pain, their images were reflected in the X plane ('flipped'). Significant differences in grey matter between TMD and control subjects were determined using a voxel-by-voxel analysis (false discovery rate corrected for multiple comparisons, $p < 0.05$). The effects of age and gender were excluded by adding them as nuisance variables. Clusters of significant difference were then overlaid onto the template T1-weighted image for visualisation. Furthermore, individual grey matter volumes (probability × volume) were extracted from clusters of significant difference and relations between grey matter volumes and pain characteristics, i.e. pain intensity and duration, were assessed using correlations (Pearson r, $p < 0.05$). In addition, to test for a possible confound of medication usage, the grey matter volumes were compared (2-sample t test) between TMD subjects using analgesic medication (n = 14) and those not on any medication (n = 8).

Brainstem DTI

Using SPM8 and custom software, the four diffusion tensor image sets were realigned based on the b0 images in each series and then averaged. Using diffusion-weighted images collected from 32 directions and b0 images, the diffusion tensor was calculated from the all images using a linear model. Once the elements of diffusion tensor were calculated, fractional anisotropy (FA) and mean diffusivity (MD) whole-brain maps were derived. These images were then spatially normalised and re-sliced using the same SUIT template used for the brainstem VBM analysis described above. This process resulted in brainstem maps of diffusion values, spatially normalised in MNI space with raw intensities preserved (non-modulated). The images were then smoothed (3 mm full-width-half-maximum) and reflected across the midline as previously described. Significant differences in diffusion values between TMD subjects and controls were determined using a voxel-by-voxel analysis. An initial threshold of $p < 0.005$ uncorrected for multiple comparisons was used for visualisation with subsequent small volume correction at $p < 0.001$ to account for multiple comparisons. The effects of age and gender were excluded by adding them as nuisance variables. Significant DTI differences were then overlaid onto the template T1-weighted image for visualisation. Furthermore, individual diffusion values were extracted from clusters of significant difference and relations between MD and FA and pain characteristics, i.e. pain intensity and duration, were assessed using correlations (Pearson r, $p < 0.05$). In addition, to test for a possible confound of medication usage, the MD values were compared (2-sample t test) between TMD subjects using analgesic medication (n = 10) and those who were not (n = 6) and those not on any medication (n = 8).

Deterministic tractography

Tractography was performed using MrDiffusion software (Dougherty et al., 2005), based on the diffusion tensor calculated as described above. Four fibre pathways were targeted: the root entry zone (REZ) of the trigeminal nerve, the spinal trigeminal tract, and the ipsilateral and contralateral ventral trigeminothalamic tracts. Four volumes of interest were specified anatomically, in accordance with Duvernoy's Atlas of the Human Brainstem and Cerebellum (Duvernoy, 1995), in each individual subject in their native space: the REZ of the trigeminal nerve, the caudal SpV nucleus, the medial lemniscus in the midbrain and the thalamus (Fig. 1). Fibres were tracked using a Runge–Kutta fourth order algorithm with a minimum FA value of 0.15 and a maximum turning angle of 60°. The REZ of the trigeminal nerve was defined as encompassing the trigeminal nerve within the pontine cistern, that is, from the point at which the nerve emerges from the pons to the point at which it exits the pontine cistern anteriorly. Fibres of the trigeminal nerve which descended to SpVc were classified as the spinal trigeminal tract. The ipsilateral ventral trigeminothalamic tract was defined as fibres that originated in the SpVc and passed through the ipsilateral medial lemniscus to the thalamus. The contralateral trigeminothalamic tract was defined as fibres that originated in the SpVc and passed through the contralateral medial lemniscus to the thalamus. The mean FA and MD diffusivity values for each of the tracts were calculated in each individual. For controls, left and right tract FA and MD values were compared using paired t-tests. These were not significantly different (all $p > 0.05$) and were subsequently combined for comparison with TMD subjects. Finally, significant differences between control and TMD tract FA and MD values were determined (2-sample t-test $p < 0.05$).

Results

Pain characteristics

Individual TMD subject characteristics are shown in Table 1. All TMD subjects reported myofascial muscle pain; of these eight subjects (36%) also reported TMJ athralgia. In four of the TMD subjects, pain was unilateral (right side) and the remaining subjects had pain bilaterally. Their average scan pain intensity was 4.0 ± 2.3 (moderate), the average diary pain was 3.7 ± 0.4 and their average duration of pain was 9.7 ± 1.9 years. TMD subjects most commonly described their pain as throbbing (70%), tender (60%) and shooting (40%).

Grey matter changes (VBM)

In TMD subjects, brainstem VBM analysis revealed significant regional grey matter volume changes in several regions (Fig. 2, Table 2). Compared with control subjects, significant reductions in grey matter volume occurred in the ipsilateral (to higher pain) SpV. This SpV volume decrease encompassed both the subnucleus interpolaris and caudalis (mean ± SEM grey matter probability × volume: controls 0.17 ± 0.009, TMD 0.13 ± 0.009). Significant reductions in grey matter volume also occurred in the region of the ipsilateral trigeminal principle sensory nucleus (Vp; controls 0.14 ± 0.009, TMD 0.097 ± 0.008) and in the rostral medullary raphe (controls 0.077 ± 0.008, TMD 0.044 ± 0.003). No brainstem region showed greater grey matter volume in TMD subjects compared with controls.

No regions of volume difference showed significant correlations between grey matter volume and scan pain intensity (SpV: r = 0.06,

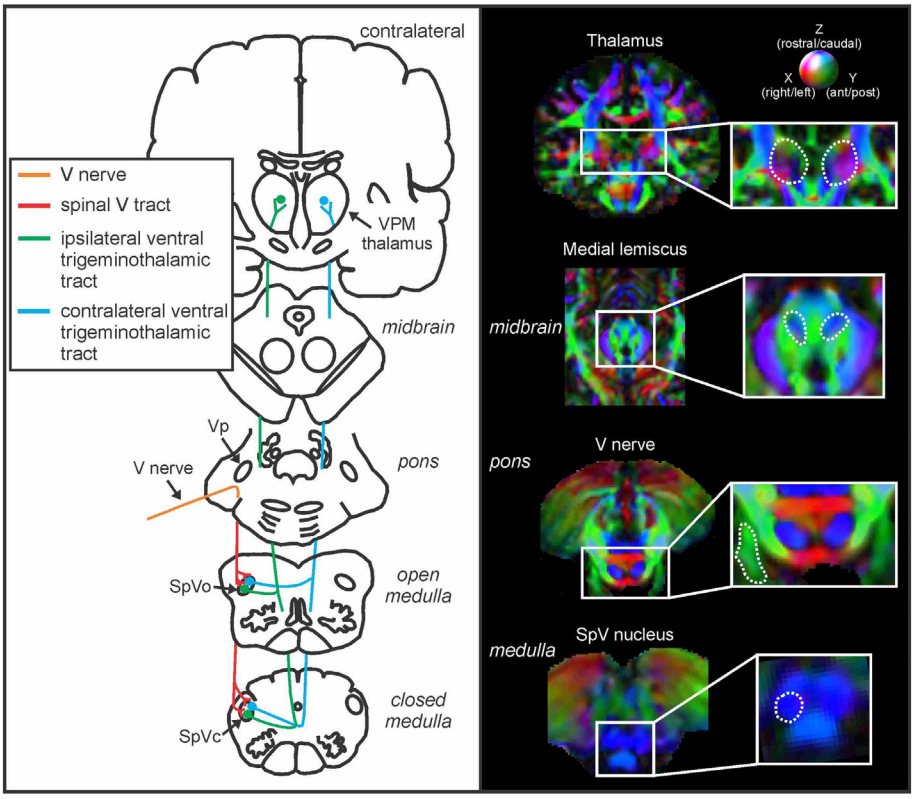

Fig. 1. *Left*: Diagram of the pathway of the trigeminal nerve, the spinal trigeminal tract, and the ipsilateral and contralateral trigeminothalamic tracts. *Right*: Volumes of interest (VOI) used to define the tracts of interest—the trigeminal root entry zone (REZ), the spinal trigeminal nucleus caudalis (SpVc), medial lemniscus, and thalamus. The dashed lines indicate the boundaries of each VOI overlaid on a vector RGB map (colour-coded for direction: green anterior–posterior, red medial–lateral, and blue inferior–superior) of an individual's brain.

$p = 0.80$; Vp: $r = -0.04$, $p = 0.87$; raphe: $r = -0.29$, $p = 0.18$), diary pain intensity (SpV: $r = 0.14$, $p = 0.52$; Vp: $r = 0.01$, $p = 0.98$; raphe: $r = -0.03$, $p = 0.91$), or pain duration (SpV: $r = -0.32$, $p = 0.15$; Vp: $r = -0.05$, $p = 0.82$; raphe: $r = 0.01$, $p = 0.98$). Furthermore, comparison of these clusters found no significant difference in grey matter volume between those TMD subjects who use analgesic medication and those who do not (mean ± SEM grey matter probability × volume: SpV: medication 0.14 ± 0.011, non-medication 0.11 ± 0.012, $p = 0.09$; Vp: medication 0.10 ± 0.009, non-medication 0.09 ± 0.015, $p = 0.53$; raphe: medication 0.042 ± 0.003, non-medication 0.048 ± 0.007, $p = 0.35$). Given that the majority of TMD subjects reported bilateral pain, in order to test for a possible specific laterality in grey matter changes, the SpV and Vp clusters were 'flipped' (reflected in the X plane) and grey matter volumes on the contralateral side were extracted. Although not revealed by the voxel-by-voxel analysis, this VOI analysis showed that TMD subjects also displayed significant grey matter volume reductions in the contralateral (to higher pain) SpV (mean ± SEM grey matter probability*volume: controls 0.15 ± 0.007, TMD 0.12 ± 0.009, $p = 0.0004$) and Vp (mean ± SEM grey matter probability*volume: controls 0.11 ± 0.006, TMD 0.07 ± 0.007, $p = 0.0005$).

Diffusion value changes (DTI)

In addition to changes in grey matter volume, DTI analysis revealed significant MD differences in a number of brainstem sites in TMD subjects (Fig. 3, Table 2). Compared with controls, MD was significantly increased in the region of the ipsilateral (to highest pain) SpV, specifically in SpVc and the caudalis–interpolaris transition zone (mean ± SEM [$\times 10^{-3}$] MD: controls 0.68 ± 0.04, TMD 0.85 ± 0.04), bilaterally in the region of the trigeminal nerve tract within the pons (ipsilateral: controls 0.67 ± 0.03, TMD 0.82 ± 0.04; contralateral; controls 0.81 ± 0.02, TMD 0.87 ± 0.02) and in the region of the midbrain periaqueductal grey matter (PAG; controls 0.83 ± 0.01, TMD 0.93 ± 0.02). No decreases in MD, and neither increases nor decreases in FA, were observed.

In the significant clusters, MD was not correlated with scan pain intensity (SpV: $r = 0.39$, $p = 0.13$; ipsilateral V nerve tract: $r = 0.38$, $p = 0.15$; contralateral V nerve tract: $r = 0.41$, $p = 0.11$, PAG: $r = 0.23$, $p = 0.39$), diary pain intensity (SpV: $r = 0.30$, $p = 0.25$; ipsilateral V nerve tract: $r = 0.47$, $p = 0.07$; contralateral V nerve tract: $r = 0.36$, $p = 0.17$, PAG: $r = 0.39$, $p = 0.13$), or pain duration (SpV: $r = -0.05$, $p = 0.84$; ipsilateral V nerve tract: $r = 0.31$, $p = 0.24$; contralateral V nerve tract: $r = 0.23$, $p = 0.38$, PAG: $r = 0.46$, $p = 0.06$). Furthermore, there was no significant difference in MD values between those TMD subjects who used analgesic medication and those who did not (mean ± SEM ($\times 10^{-3}$) MD: SpV: medication 0.86 ± 0.05, non-medication 0.83 ± 0.04, $p = 0.72$; ipsilateral V nerve tract: medication 0.81 ± 0.02, non-medication 0.83 ± 0.04, $p = 0.81$; contralateral V nerve tract: medication 0.89 ± 0.03, non-medication 0.83 ± 0.09, $p = 0.17$; PAG: medication 0.93 ± 0.07, non-medication 0. 92 ± 0.09, $p = 0.79$).

DTI and VBM overlap

Brainstem regions which displayed both grey matter volume and diffusion changes were identified. Within the medulla, grey matter volume decreased and MD increased in the region of the ipsilateral SpVc (MNI coordinates: $x = -5$, $y = -43$, $y = -63$ cluster size = 13 mm^3) (Fig. 4). There was however, no significant correlation between grey matter volume and MD within the SpVc ($r = 0.05$, $p = 0.86$). In addition to the SpVc, within the PAG, although MD was increased in TMD subjects, grey matter volume was not different compared to controls (mean ± SEM grey matter volume; controls: 0.52 ± 0.02; TMD: 0.51 ± 0.02, two-sample t(df) = 42, $p = 0.851$) and within

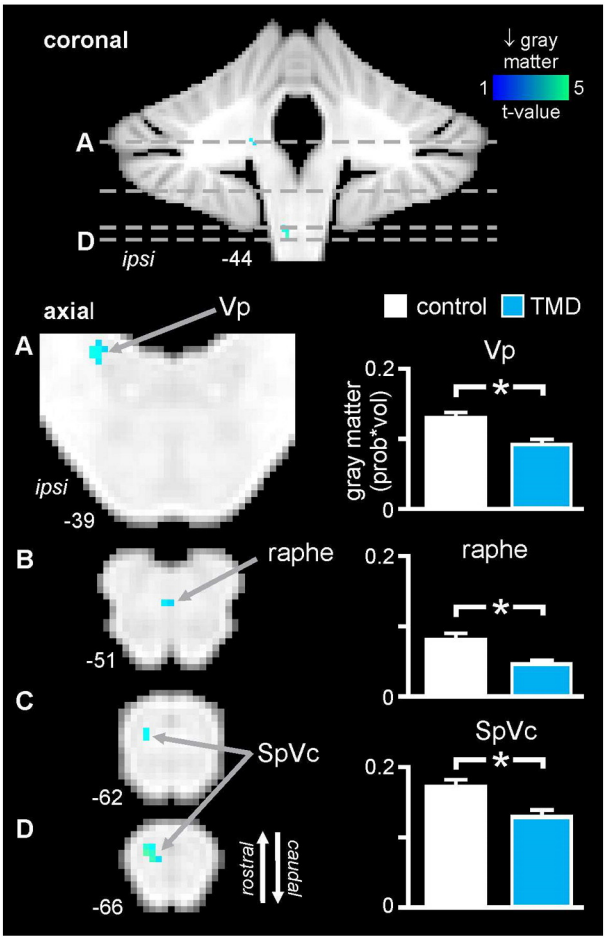

Fig. 2. Regional grey matter volume changes in TMD subjects compared with pain-free controls, overlaid onto coronal and axial brainstem template images. Significant grey matter volume decreases are represented by a t-statistic with a cool colour scale. Slice locations are indicated at the lower left of each slice in Montreal Neurological Institute space (y or z mm). Compared to controls, TMD subjects have lower grey matter volumes in the ipsilateral (to highest on-going pain) spinal trigeminal nucleus caudalis (SpVc), ipsilateral principle trigeminal sensory nucleus (Vp) and the rostral medullary raphe nuclei. Bar graphs of mean (±SEM) grey matter volumes calculated from the clusters of significant difference are shown to the right of the figure. * represents a significant difference at $p < 0.05$ (FDR corrected).

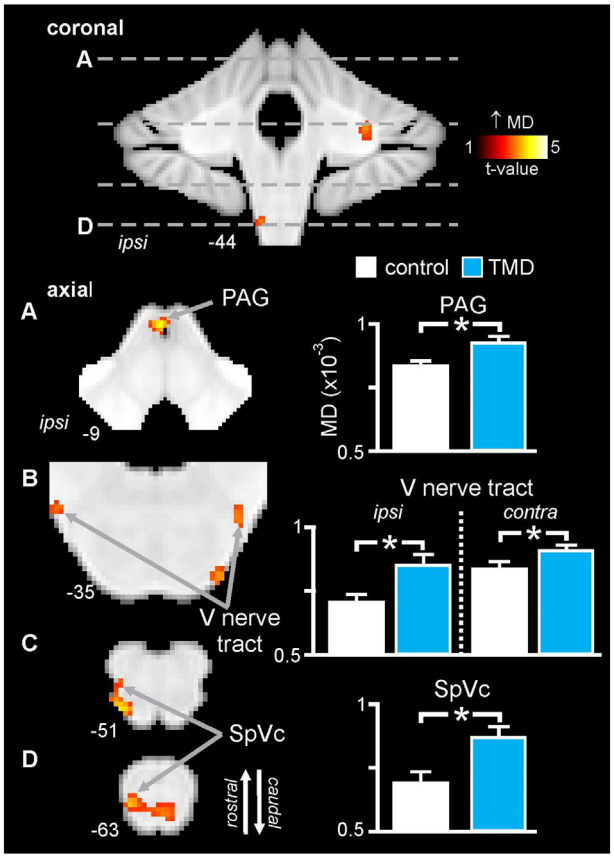

Fig. 3. Regional mean diffusivity (MD) changes in TMD subjects compared with pain-free controls, overlaid onto coronal and axial brainstem template images. Significant MD increases are represented by a t-statistic with a hot colour scale. Slice locations are indicated at the lower left of each slice in Montreal Neurological Institute space (y or z mm). Compared to controls, TMD subjects have higher MD in the ipsilateral (to highest on-going pain) spinal trigeminal nucleus caudalis (SpVc), bilateral trigeminal (V) nerve tract, and the midbrain periaqueductal grey matter (PAG). Bar graphs of mean (±SEM) mean diffusivity (MD [$\times 10^{-3}$] μm/ms) values calculated from the clusters of significant difference are shown to the right of the figure. * represents a significant difference at $p < 0.001$ small volume corrected.

the rostral medullary raphe, although grey matter was decreased in TMD subjects, MD values were not different compared to controls (mean ± SEM [$\times 10^{-3}$] MD; controls: 0.76 ± 0.01; TMD: 0.76 ± 0.01, two-sample t(df) = 34, $p = 0.918$).

Table 2
Montreal Neurological Institute (MNI) coordinates of significant grey matter volume and medial diffusivity differences between TMD and control subjects. SpV: spinal trigeminal nucleus, Vp: principle trigeminal nucleus, V: trigeminal, PAG: periaqueductal grey matter.

	MNI coordinates				
	x	*y*	*z*	Cluster size	Z-score
Grey matter volume: controls > TMD					
Ipsilateral SpV	−4	−46	−66	113	4.27
Raphe	−1	−39	−51	34	3.63
Ipsilateral Vp	−13	−43	−38	115	3.92
Mean diffusivity: TMD > controls					
Ipsilateral SpV	−6	−42	−64	79	3.36
V nerve tract					
Ipsilateral	−21	−33	−35	13	2.88
Contralateral	18	−30	−34	36	3.00
PAG	−1	−34	−8	98	3.73

Tractography

Tractography resulted in the identification of the REZ of the trigeminal nerve in all control and TMD subjects, the spinal trigeminal tract in 63% controls and 78% TMD subjects, the ipsilateral ventral trigeminothalamic tract in all control and TMD subjects and the contralateral ventral trigeminothalamic tract in 94% controls and 84% TMD subjects. An example of an individual subject's tracking is shown in Fig. 5.

Group analysis of the average FA and MD values in each ipsilateral (to pain) tract revealed significantly lower FA values in TMD subjects compared with controls in the REZ of the trigeminal nerve (mean ± SEM FA: controls 0.32 ± 0.02; TMD 0.27 ± 0.01), the spinal trigeminal tract (controls 0.54 ± 0.03; TMD 0.46 ± 0.02), the ipsilateral ventral trigeminothalamic tract (controls 0.58 ± 0.03; TMD 0.50 ± 0.01) and the contralateral ventral trigeminothalamic tract (controls 0.58 ± 0.03; TMD 0.50 ± 0.02). In addition, the REZ of the trigeminal nerve in TMD subjects displayed increased MD compared with controls (mean ± SEM [$\times 10^{-3}$] MD: controls 1.92 ± 0.14; TMD 2.40 ± 0.06) (Fig. 6).

Comparison of the contralateral (to pain) tracts in TMD subjects revealed the same significant differences in FA and MD. That is, TMD subjects had lower FA values in the REZ (mean ± SEM FA: TMD 0.27 ± 0.01), the spinal trigeminal tract (TMD 0.41 ± 0.02), the

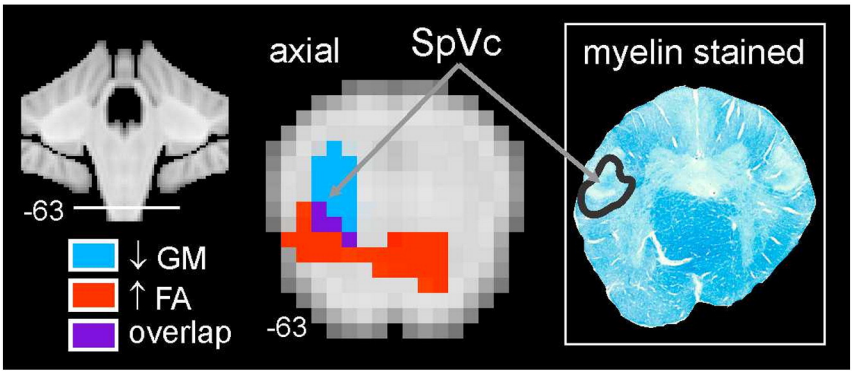

Fig. 4. Grey matter (GM) volume decreases (t-statistic cool colour scale) and medial diffusion (MD) increases (t-statistic warm colour scale) and their overlap (purple) in the spinal trigeminal nucleus caudalis (SpVc) of TMD subjects compared with pain-free controls. Clusters are overlaid onto the axial slice from the SUIT template. The slice location in MNI space is indicated at the bottom-left of the image. This overlap cluster (in purple) corresponds to the SpVc as shown on the myelin-stained axial section to the right.

ipsilateral ventral trigeminothalamic tract (TMD 0.51 ± 0.02) and the contralateral ventral trigeminothalamic tract (TMD 0.30 ± 0.01). In addition, the contralateral and REZ of the trigeminal nerve in TMD subjects displayed significantly increased MD compared with controls (mean ± SEM [$\times 10^{-3}$] MD: TMD 2.27 ± 0.01).

Discussion

This study reveals that chronic musculoskeletal pain is associated with anatomical changes at multiples levels of the ascending pain pathway. In subjects with TMD, decreased FA and increased MD occurred in

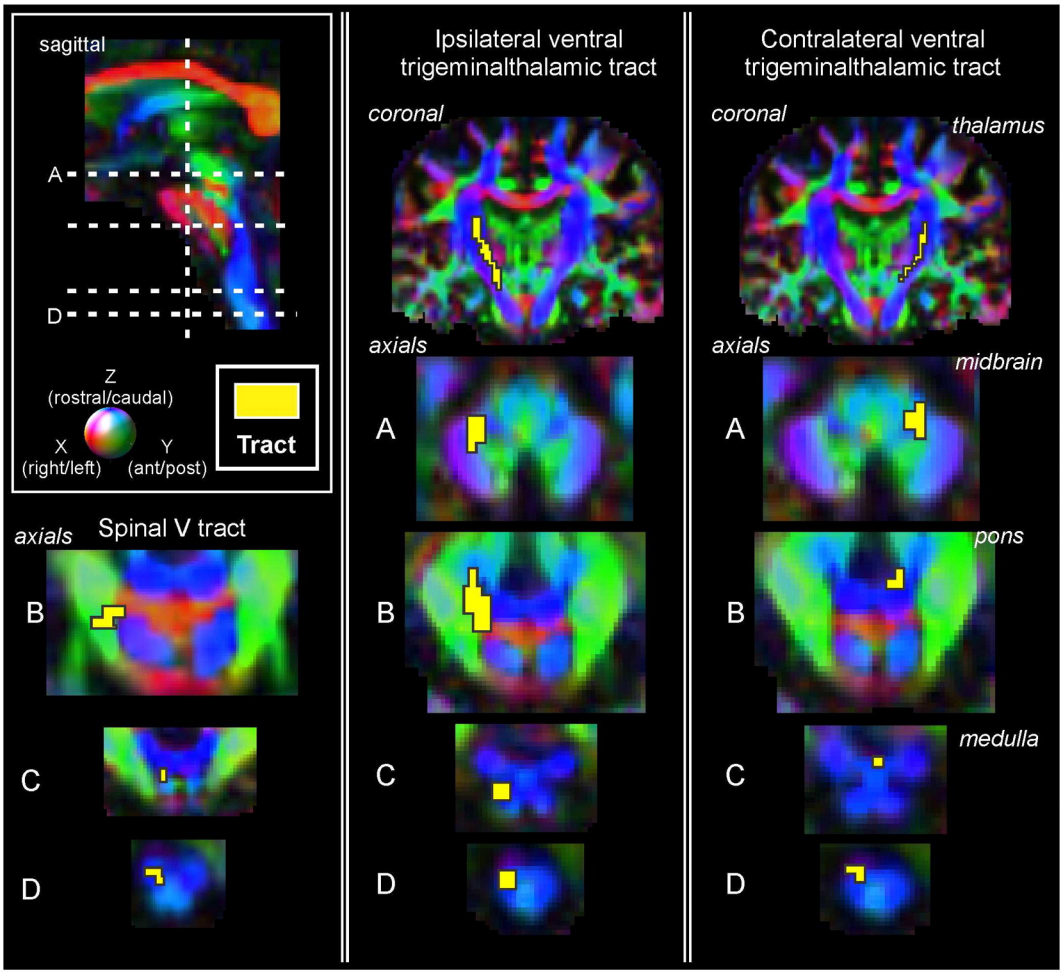

Fig. 5. Deterministic tractography in an individual subject. In each panel an isolated tract is visualised: (from left to right) the spinal trigeminal tract, the ipsilateral ventral trigeminothalamic tract and the contralateral ventral trigeminothalamic tract. The tracts are shown in yellow and are overlaid onto axial and coronal sections colour-coded for primary diffusion direction: green anterior–posterior, red medial–lateral, and blue inferior–superior. The levels of the axial and coronal sections are indicated as dashed lines on the sagittal image in the top-left box.

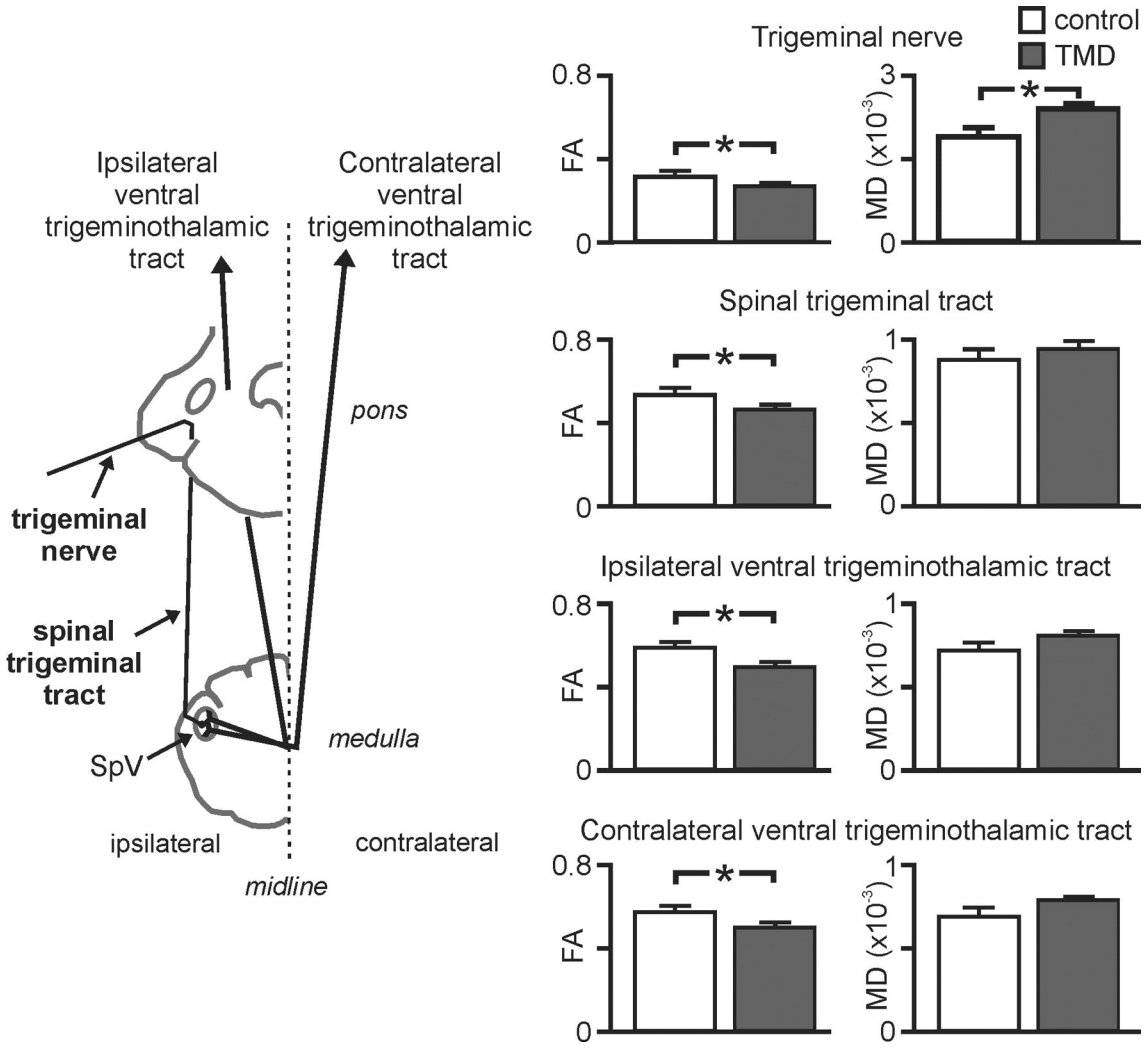

Fig. 6. *Left*: Diagram of the pathway of the trigeminal nerve, the spinal trigeminal tract, and the ipsilateral and contralateral trigeminothalamic tracts. *Right*: Bar graphs of mean (±SEM) fractional anisotropy (FA) and mean diffusivity (MD) in each ipsilateral (to pain) tract in TMD and control subjects. * represents a significant difference ($p > 0.05$).

the REZ of the trigeminal nerve, decreased grey matter paired with increased MD occurred in the region of the medullary dorsal horn, and decreased FA occurred in the ventral trigeminothalamic tracts.

Peripheral (trigeminal nerve) changes

Although the aetiology of TMD remains unresolved, there is evidence for the role of peripheral nociceptive afferent input, combined with hyperexcitability in the peripheral (i.e., the trigeminal nerve and ganglion) and central nervous systems (Maixner et al., 1995). Animal models of temporomandibular joint inflammation reveal upregulation of glial cells, increased neuron–glia communication, and enhanced neuronal excitability within the trigeminal ganglion (Takeda et al., 2005; Villa et al., 2010). Whilst our findings of increased MD (loss of barriers limiting water movement) coupled with decreased FA (loss of directional coherence/altered structural organisation) in the trigeminal nerve are usually interpreted as markers of neuronal degeneration, they may also reflect chronic inflammation (Assaf and Pasternak, 2008; Tievsky et al., 1999). The present findings of diffusivity changes may reflect long-term glial-induced microstructural changes and/or more general neuroplastic changes in response to increased peripheral nociceptive input. Whilst our current results are consistent with those of Moayedi et al. (2012) who also found lower FA in the trigeminal nerve in TMD and from DeSouza and colleagues (DeSouza et al., 2014) who found similar changes in trigeminal neuralgia patients, they do deviate from our previous findings of normal diffusivity within the trigeminal nerve in TMD (Wilcox et al., 2013). The reason for this discrepancy may be in the different methods used, and specifically the greater sensitivity of the current measurements, which likely gives a more accurate estimate of the tracts diffusion properties than our earlier approach (Dougherty et al., 2007).

Primary synapse (SpV) changes

As hypothesised we found a decrease in grey matter volume in the spinal trigeminal nucleus at the level of the subnucleus caudalis, with increased MD in the same region and extended into subnucleus interpolaris and the ventral trigeminothalamic tract. Whilst grey matter decreases may reflect neuronal loss, they may result from shrinkage or atrophy of glia (May and Gaser, 2006). In addition, we found increased MD coupled with no change in FA, possibly suggesting a decrease in axon/dendritic density but a preservation of directional coherence (Schwartz et al., 2005). Surprisingly, none of these changes were significantly correlated to individual pain intensity ratings, which may reflect the subjective nature of pain or that these changes underlie the presence of pain *per se*. Additionally, since we also found anatomical changes in pain modulatory regions and TMD is associated with altered endogenous analgesia, the lack of significant relationship between pain and SpVc anatomy may reflect interactions between these ascending and descending systems.

It is widely accepted that orofacial nociceptive input is processed in SpVc (Sessle, 2000), which exhibits anatomical similarity with spinal dorsal horn (Price et al., 1976). Additionally, the transition zone between subnucleus caudalis and interpolaris (SpVi/SpVc) is involved in orofacial pain processing, particularly that originating from muscle. Whilst SpVc is primarily concerned with sensory discrimination, SpVi/SpVc is involved in the integration of somatosensory and automatic functions and the engagement of descending pain modulation (Ren and Dubner, 2011). In animal models of orofacial pain, acute muscle inflammation produces up-regulation of microglia, enhanced SpVc and SpVi/SpVc neuronal excitability and prolonged neuronal plasticity and glial activity (Kiyomoto et al., 2013; Miyamoto et al., 2012). These data suggest that hyperexcitability of the central pain processing pathways, once established, may be critical for the maintenance of chronic pain (Guo et al., 2010). Our observed anatomical change within the SpV may reflect similar neural- and/or glial-plasticity, with the region serving as a locus for hyperexcitability within ascending pain pathways.

We also found a grey matter volume decrease in Vp, which is in direct contrast to an increase recently reported by Younger et al. (2010). The diverging findings may result from differences in pain durations of the patient populations (4.4 vs 9.7 years), potentially reflecting a speculative time-course of short term increases in grey matter volume followed by compensatory volume decreases over time. Although a classical view is that Vp is only involved in non-noxious orofacial somatosensory processing (Smith, 1973), Dessem et al. (2007) showed in rodents that nociceptive muscle afferents in the masseter nerve project to a discrete region of Vp. Furthermore, we demonstrated Vp activation during orofacial muscle (but not cutaneous) pain in humans (2009) and on-going Vp (and SpVc) blood flow increases in TMD subjects (Youssef et al., 2013). It is possible that the anatomical changes that occur in Vp of TMD subjects reflect hyperexcitability-related neuroplastic changes in a similar manner to those aforementioned in the SpVc.

Central ascending pathway changes

In addition to regional anatomical changes, we found significant diffusivity changes of the ascending pain pathways in TMD subjects, with decreased FA in the ipsilateral and contralateral ventral trigeminothalamic tracts. These bilateral tract changes are consistent with the majority of our TMD subjects reporting bilateral pain. Similarly, whilst the anatomical changes in SpVc and Vp were stronger ipsilaterally, these same regions were found to have similar, albeit smaller in magnitude, changes on the contralateral side. Considering that we found decreased FA coupled with no change in MD, one possible interpretation is that these changes reflect concomitant structural reorganisation in relation to the observed anatomical changes in trigeminal nuclei, i.e. Vp and SpV, rather than neuronal loss or degeneration which is usually accompanied by changes in MD.

Changes in brainstem descending pain modulation areas

In conjunction with alterations in the ascending pathways, evidence suggests that the descending pain modulatory pathway in TMD (and other musculoskeletal/inflammatory pain conditions) is also affected (Bragdon et al., 2002; Kashima et al., 1999; King et al., 2009, Linnman et al., 2012). Interestingly, the most significant area of MD difference in TMD subjects was located in the PAG, a region shown to display anatomical changes in other chronic pain conditions (DaSilva et al., 2007; Rocca et al., 2006; Seminowicz et al., 2010). The PAG did not display grey matter volume changes, suggesting that it may undergo subtle microstructural changes such as reduced dendritic fields or dendritic spine numbers whilst preserving neural numbers. It is known that neuropathic pain is associated with altered spine length and density within the pain processing regions (Tan et al., 2012) and it is possible that reduced dendritic spine numbers occur in TMD and result in reduced endogenous analgesic activity efficiency. Indeed we also found a grey matter volume decrease and no change in diffusivity in the region of the nucleus raphe magnus, the PAG recipient in the descending analgesic pathway (Stamford, 1995). Altered descending PAG input to the raphe magnus may result in altered neural numbers, indeed, it has been shown in experimental animals that raphe magnus inhibition can reduce various forms of chronic pain and reduces dorsal horn sensitisation (Vera-Portocarrero et al., 2006a,b). Anatomical changes within the PAG–raphe magnus–SpV pathway may help maintain the central sensitisation and on-going pain in TMD subjects.

Evidence of central neuronal plasticity in TMD

Several lines of evidence also suggest that neuroplastic changes at the primary afferent synapse contribute to neural hyperexcitability in TMD (Sessle, 2000). It is possible that an initiating pathology such as trauma or inflammation within the muscles of mastication or temporomandibular joint initiates local concomitant neural inflammation (i.e., glial up-regulation) and neuronal hyperexcitability within the trigeminal ganglion and SpV complex. Indeed, chronic lower back pain is associated with glial activation in rostral parts of the ascending pain system, i.e. the thalamus and somatosensory cortex (Loggia et al., 2015). These focal short term changes may result in more lasting changes in the anatomy and microstructure of the trigeminal nerve and SpV, which maintain neuronal hyperexcitability even after resolution of the initiating pathology (Guo et al., 2010). Such changes may also occur in areas relating to the descending modulation of pain, which may further contribute to the maintenance of central sensitisation by facilitating (even attenuated) peripheral nociceptive input. As a result of these localised changes, adaptive changes in secondary (and potentially higher) afferents may propagate this central sensitisation to higher cortical areas, ultimately resulting in the activation of cortical regions which generate the persistent perception of pain. Indeed, we have recently shown that TMD is associated with increased cerebral blood flow in pain-related cortical areas such as the anterior cingulate, dorsal lateral prefrontal and precuneus cortices (Youssef et al., 2013), a phenomenon consistent with greater sustained activation of these regions.

Limitations

There are a number of limitations of this study with respect to TMD subject properties and medication use. Although our inclusion criteria included a primary chronic pain complaint of TMD, two TMD subjects also reporting having infrequent migraines and six reported non-chronic musculoskeletal pain in other body regions. Although it is not known if migraine is associated with altered brainstem anatomy, interictally, migraineurs have altered spinal trigeminal nucleus processing (Stankewitz et al., 2011). However, given that only 2 TMD subjects reported migraine as an additional complaint, we are confident that our results reflect underlying changes associated with TMD and not migraine. In addition, it is known that opioid use can rapidly change brain anatomy, in particular in the reward circuitry (Younger et al., 2011). Although only 4 of the TMD subjects in this investigation were taking opioids, most were on some form of medications and it is possible that these could have altered brain anatomy. We did however find no significant difference in brainstem anatomy changes in TMD subjects on medication compared to those not on medications; although subject numbers were likely too low to adequately address this potentially confounding issue.

Conclusion

These data reveal that chronic musculoskeletal pain in humans is associated with discrete alterations in the anatomy of the medullary dorsal horn, as well as its afferent and efferent projections, and areas

associated with descending pain modulation. These neural changes may underlie the abnormal neuronal function thought to initiate or maintain chronic musculoskeletal pain which, if so, offers a valid therapeutic target.

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Conflict of interest

The authors declare no competing financial interests.

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