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EJN European Journal of Neuroscience

FENS Federation of European Neuroscience Societies

European Journal of Neuroscience, Vol. 43, pp. 997–1005, 2016 doi:10.1111/ejn.13156

CLINICAL AND TRANSLATIONAL NEUROSCIENCE

Grey matter changes of the pain matrix in patients with burning mouth syndrome

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Keywords: burning mouth syndrome, dysgeusia, pain, voxel-based morphometry

Edited by Michel Barrot
Received 5 October 2015, revised 8 December 2015, accepted 18 December 2015

Abstract

Burning mouth syndrome (BMS) is characterized by a burning sensation in the mouth, usually in the absence of clinical and laboratory findings. Latest findings indicate that BMS could result from neuropathic trigeminal conditions. While many investigations have focused on the periphery, very few have examined possible central dysfunctions. To highlight changes of the central system of subjects with BMS, we analysed the grey matter concentration in 12 subjects using voxel-based morphometry. Data were compared with a control group (Ct). To better understand the brain mechanisms underlying BMS, the grey matter concentration of patients was also compared with those of dysgeusic patients (Dys). Dysgeusia is another oral dysfunction condition, characterized by a distorted sense of taste and accompanied by a reduced taste function. We found that a major part of the 'pain matrix' presented modifications of the grey matter concentration in subjects with BMS. Six regions out of eight were affected [anterior and posterior cingulate gyrus, lobules of the cerebellum, insula/frontal operculum, inferior temporal area, primary motor cortex, dorsolateral prefrontal cortex (DLPFC)]. In the anterior cingulate gyrus, the lobules of the cerebellum, the inferior temporal lobe and the DLPFC, pain intensity correlated with grey matter concentration. Dys also presented changes in grey matter concentration but in different areas of the brain. Our results suggest that a deficiency in the control of pain could in part be a cause of BMS and that BMS and dysgeusia conditions are not linked to similar structural changes in the brain.

Introduction

Burning mouth syndrome (BMS) is characterized by a burning sensation in the oral cavity, which appears without stimulation and for which no medical cause has been found. The burning sensation most often appears on the tongue, but can also involve the hard palate, the lips and the alveolar ridges, whereas the buccal mucosa and the floor of the mouth are less frequently affected (Ducasse *et al.*, 2013; Gurvits & Tan, 2013). These symptoms subside somewhat in the morning, and intensify during the day with the peak of pain in the late evening (Grushka *et al.*, 2002). The effects of BMS can be life-altering; it generally induces permanent stress increased by sleep disturbances which can turn into irritability, alterations of eating habits and depression. BMS lasts for at least 4–6 months and women are seven times more likely to be affected than men, with an onset during or after menopause. Two-thirds of the patients experience a spontaneous remission within 6 or 7 years, but in one-third of the patients, the condition is permanent (Grushka *et al.*, 2002; Chi *et al.*, 2008; Ducasse *et al.*, 2013; Gurvits & Tan, 2013).

The aetiology and pathophysiology of BMS are not yet well elucidated. Lesions of the oral cavity are usually not the reason for the burning sensation. Latest findings suggest a peripheral neuropathic condition of the trigeminal and/or taste nerves (Forssell *et al.*, 2002; Lauria *et al.*, 2005; Ducasse *et al.*, 2013). A hypofunction of the chorda tympani nerve has been found, by measuring the metallic taste recognition threshold evoked by electrical stimuli on the anterior two-thirds of the tongue (Eliav *et al.*, 2007). A biopsy of the lateral aspect of the anterior two-thirds of the tongue in 12 patients revealed a significantly lower density of the epithelial small fibres in taste buds, as compared with control subjects (Lauria *et al.*, 2005). While mainly the periphery of the trigeminal/taste oral functions have been investigated, very few studies have looked at possible central dysfunctions.

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In this study we investigated the change of grey matter concentration (GMC) in subjects with BMS, using voxel-based morphometry (VBM). This morphometric technique aims to detect differences in the regional concentration of grey matter at a local scale, without taking into account global shape differences (Ashburner & Friston, 2000). What the GMC represents in terms of cellular structure is still poorly understood. Cytoarchitectonically, measures would be necessary to correlate an increase in GMC with an actual increase in neuronal cell packing and a better wiring of neurons, resulting in improved function of the area. As initially proposed by Ashburner & Friston (2000), we will use the terminology 'grey matter concentration' and not 'grey matter density' to avoid confusion. Indeed, we present an increase and decrease of GMC, but no inference regarding a higher or lower density or neurons is suggested. In fact, histopathological data did not show a correlation between grey matter probability (data before normalization and smoothing steps leading to GMC) and neuronal density (Eriksson *et al.*, 2009). VBM provides information on structural changes of grey matter between two groups of individuals, and we propose to highlight here areas of the brain that could be linked to BMS. Therefore, we discuss our results in terms of morphological modification of functional areas that could at least partly explain BMS.

We explored the whole brain structure and did not focus on regions of interests as we were looking for various areas involved in either taste, trigeminal (hot, cold, prickling sensations) or somesthesic functions. To delineate the brain structural changes of BMS subjects specifically, we also analysed the brain structure of a closely related taste dysfunction which often accompanies BMS, namely dysgeusia. This condition is characterized by a distorted sense of taste, or an ongoing bad taste in the mouth, even in the absence of a gustatory stimulus (Fark & Hummel, 2013). Because dysgeusia mostly involves taste function and does not involve a burning sensation, the comparison between BMS patients, dysgeusia patients and controls was thought to permit a better definition of the function of central nervous structures involved in BMS.

Materials and methods

Subjects

The data for 42 subjects who participated in a previous study were used in this retrospective analysis. Participants were included in the study following diagnosis at the University Hospital Carl Gustav Carus, TU Dresden. Subjects provided written consent prior to participation. The study was conducted according to the *Declaration of Helsinki* and was approved by the Ethics Committee of the Technical University of Dresden Medical School (EK159042014). Three groups were studied, one with idiopathic BMS (BMS, $n = 12$, seven women, 35–72 years, 59.4 ± 12.1 years). The second group consisted of subjects with dysgeusia (Dys, $n = 17$, 11 women, 42–73 years, 58.4 ± 8.1 years). The control group comprised 13 healthy subjects (Ct, $n = 13$, 10 women, 50–73 years, 59 ± 3.4 years). Dys and BMS subjects may share some symptoms but patients who reported a daily burning sensation for more than 3 months were categorized as BMS. Participants received two questionnaires regarding their general health and BMS symptoms, respectively. In Dys and BMS groups, the symptoms of all the subjects started 3–24 months prior to the study, and three BMS subjects and one Dys subject had their symptoms for more than 2 years.

Gustatory assessment was performed with the 'Taste Strips' test (Landis *et al.*, 2009). A 2-cm² area on the right or the left side of the tongue was stimulated using paper strips previously impregnated with a tasty solution with one taste (sweet, sour, bitter or salty) in four different concentrations each. The 32 conditions (side of the tongue, taste, concentration) were presented in a pseudo-randomized order. In the BMS group, the 'Taste Strips' assessment results ranged from 8 to 23, with five hypogeusic patients (≤ 16) and seven normogeusic patients (> 16). In Dys, the results ranged from 9 to 24 with 14 patients being hypogeusic ($13 < x \leq 16$) and three patients being normogeusic; one patient did not perform the test. Regarding the three normogeusic patients and the patient who missed the taste test, all perceived an ongoing metallic or spicy sensation. Among the controls, assessment scores ranged from 17 to 27; all the participants were normogeusic.

Ratings of the pain consisted of three unanchored and unmarked scales, one each for the pain sensations in the morning, afternoon and evening. All BMS subjects, six Dys and no Ct subjects presented an oral pain sensation. BMS subjects reported that the most intense pain occurred in the evening. Pain sensation was significantly higher in BMS subjects than in Dys (mean ± $CI_{95\%}$ in Dys: 18.8 ± 0.3%; BMS: 40.7 ± 0.2%; Wilcoxon test with continuity correction $W = 145.5$, $P = 0.013$). The pain for Dys was an intense discomfort but was not described as a burning sensation.

Olfactory assessment was performed using the 'Sniffin' Sticks' odour identification test (Burghart Messtechnik, Wedel, Germany). The test consists of 16 common odours which participants had to identify by choosing between four odour descriptors (Hummel *et al.*, 1997). A few subjects presented with hyposmia (score < 12): three BMS, two Dys and none Ct.

Magnetic resonance imaging (MRI)

Structural MRIs were acquired from all participants using a 1.5-T MR scanner (Magnetom Sonata; Siemens Medical, Erlangen, Germany). A high-resolution T1-weighted sequence of the brain (3D IR/GR sequence: TR = 2180 ms, TE = 3.93 ms) was acquired with a 0.72 mm × 0.72 mm × 1 mm voxel size.

VBM analysis

For preprocessing, VBM analysis was performed by means of SPM8 software (Statistical Parametric Mapping; Welcome Department of Cognitive Neurology, London, UK) implemented in MATLAB R2013a (MathWorks Inc., Natick, MA, USA). We applied VBM implemented in the DARTEL Tools provided by Ashburner (2010, March 15) with default parameters. Origin was manually corrected. Images were then preprocessed, which included a very light regularization (0.0001) and a bias correction (60-mm cutoff), and tissue was classified (as detailed below) and registered using linear (12-parameter affine) and non-linear transformations (Warping & MRF), within a unified model. For the segmentation, we used the New Segment option of SPM8, into different tissue types (grey matter, white matter, cerebrospinal fluid, bone, air) with standard parameters (standard tissue probability maps; warping with ICBM space template – European Brains). Resulting images were controlled visually for correct segmentation. Inter-subject alignment was made with the DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra) toolbox, using default values. As DARTEL typically provides outputs in a slightly smaller space than MNI space, the resulting data were normalized to MNI space. Normalization was made with a 10-mm Gaussian full width. Data were also smoothed at half of the maximum smoothing, which preserved grey matter densities.

For statistical analysis with SPM8, we ran a full factorial design on pre-processed VBM images with a factor *group* (BMS subjects, Dys

subjects, Ct subjects) and *age* as a covariate. The covariate was centred on the overall mean and no interaction was set up, in order to remove the possible effects of age from the analysis. A proportional global normalization, based on the whole brain volume, an absolute masking with threshold of 0.2, as well as an explicit mask of grey matter (grey matter template of SPM8) were used to remove artefacts outside of the brain. We compared the GMC in all three groups. The threshold was set to an uncorrected P-value < 0.001 with a cluster threshold of $k = 100$. Each cluster of each contrast was then set as a region of interest (ROI), and the GMC values were extracted. Correlation between the GMC and the pain rated by the 42 subjects during the anamnesis was evaluated with the FDR-corrected Pearson correlation of the Hmisc package (Harrell & Dupont, 2012) implemented in R (significant results were determined for corrected $P < 0.05$ and tendencies for $P < 0.10$). When similar clusters were found in two contrasts, analysis of variance (ANOVA) and Tukey's *post-hoc* test were computed to determine the differences between the three groups (significance was set to corrected P-values < 0.05).

Results

Comparison between BMS and Ct

Eight clusters presented a highest GMC in BMS compared with Ct (Table 1, Fig. 1). The largest cluster was located in the right inferior temporal area ($k = 1702$). Contralateral to this, we observed a small cluster in the left hemisphere of the inferior temporal area. The second largest cluster was located in the piriform cortex ($k = 1385$). Next, a cluster covered parts of the insula and the frontal operculum. Finally, other areas were the primary motor area, the paracentral lobule, and two clusters in the right dorsolateral pre-frontal cortex [DLPFC: (34 12 42) and (50 13 37)]. The right inferior temporal gyrus, the paracentral gyrus and the two clusters located in the DLPFC showed a positive correlation with pain (i.e. the higher the pain the denser the area, Table 1).

The contrast Ct vs. BMS showed areas that had a reduced GMC in BMS compared with Ct (Table 1, Fig. 1). The left anterior and posterior cingulate gyrus (CG) and two clusters in lobules VI and VIIa crus I of the cerebellum had a lower GMC. The anterior CG and lobule VIIa crus I presented a negative correlation with pain (i.e. the higher the pain the more sparse the area, Table 1). The two other areas (lobule VI of the cerebellum and the posterior CG) presented a strong tendency for being sparser when the perceived pain was higher ($r_{40} = -0.49$, $P = 0.05$; $r_{40} = -0.37$, $P = 0.06$).

Comparison between Dys and Ct

The contrast Dys vs. Ct showed areas with a higher GMC in Dys compared with Ct. The three clusters were localized in the middle temporal gyrus, right and left, as well as in the primary somatosensory area. None of these regions presented a correlation of GMC with pain.

The contrast Ct vs. Dys revealed areas with a lower GMC in dysgeusic subjects compared with Controls (Table 1, Fig. 2). The four resulting clusters were located in the medial orbitofrontal cortex (OFC) which overlapped with the subgenual area, the right anterior CG and the middle CG as well as the pre-supplementary motor area (pre-SMA). The cluster found in the anterior cingulate gyrus was located in the right hemisphere whereas in the contrast Ct > BMS the cluster in the anterior cingulate gyrus was located in the left hemisphere. None of these clusters presented a significant correlation between GMC and pain.

Comparison between BMS and Dys

The contrast BMS vs. Dys showed areas with a higher GMC in BMS subjects compared with dysgeusic subjects (Table 1, Fig. 3). The six resulting clusters were located as follows: one in the piriform cortex, two in the left insula [one more anterior – which overlapped with the frontal operculum – and one more posterior, defined as Brodmann Area (BA) 13 – which overlapped with the secondary somatosensory area (SII)], the posterior OFC (post-OFC) and the subgenual area. All these regions were already highlighted in previous contrasts comparing BMS or Dys with Ct. Indeed, the exact clusters located in the piriform cortex, the insula/frontal operculum and the DLPFC ([50 13 37] in BMS > CT and [52 14 39] in BMS > Dys) were already identified in the contrast BMS > Ct. For these three areas, BMS subjects presented a higher GMC (piriform cortex: mean = 84.78 ± 6.07, insula/frontal operculum: 68.88 ± 5.52, DLPFC: 70.92 ± 7.13) than both Ct (piriform cortex: mean = 75.63 ± 5.46, insula/frontal operculum: 61.63 ± 2.57, DLPFC: 62.37 ± 5.00) and Dys (piriform cortex: mean = 76.12 ± 4.27, insula/frontal operculum: 62.62 ± 5.09, DLPFC: 61.82 ± 5.85; $P < 0.003$). The only region that was not previously identified was the more caudal part of the insula (BA 13). Similarly, the subgenual area appeared located in the same cluster highlighted by the contrast Ct > Dys. Thus, Dys subjects had a lower GMC in the subgenual area as compared with the two other groups (Dys: mean = 94.71 ± 6.88, Ct: 102.75 ± 8.34, BMS: 104.32 ± 7.20; $P < 0.02$). Only the GMC of the DLPFC positively correlated with pain (i.e. the higher the pain, the denser the area).

The last contrast showed areas with a reduced GMC in BMS compared with Ct (Table 1, Fig. 3). Clusters were located in the left middle temporal gyrus, the right inferior temporal gyrus, the posterior CG and the cerebellum. More precisely, three clusters were located in the cerebellum, one in the right lobule VI [14 –61 –27], one in the left lobule VI [–18 –73 –13] and one in the left lobule VIIa crus I [−27 –79 –18]. The left lobule VI and VIIa crus I, as well as the cluster located in the posterior CG were previously identified with the contrast Ct > BMS. Therefore, for these three clusters, BMS subjects had a lower GMC (lobule VI: mean = 62.71 ± 5.25, lobule VIIa crus I: 100.42 ± 7.18, posterior CG: 110.94 ± 5.52) than both Ct (lobule VI: mean = 70.35 ± 5.25, lobule VIIa crus I: 108.21 ± 3.64, posterior CG: mean = 120.06 ± 4.99) and Dys subjects (lobule VI: mean = 64.46 ± 5.81, lobule VIIa crus I: 105.77 ± 3.68, posterior CG: 117.77 ± 6.45; $P < 0.02$). The cluster in the middle temporal gyrus had also been found in the contrast Dys > C. Thus, Dys subjects had a higher GMC in the middle temporal gyrus compared with both other groups (Dys: mean = 45.38 ± 5.03, Ct: 39.66 ± 2.54, BMS: 40.58 ± 3.45; $P < 0.008$). The GMC of the posterior cingulate gyrus ($r_{40} = -0.38$, $P = 0.05$) and the lobule VIIa crus I of the cerebellum ($r_{40} = -0.36$, $P = 0.06$) had a strong tendency to negatively correlate with pain (i.e. the higher the pain the more sparse the area, Table 1).

Discussion

Our first objective was to identify whether BMS would induce a modification of the GMC. To the best of our knowledge, this is the first time that changes in GMC have been identified in several brain areas of BMS patients. The brain matrix fits very closely to a model of 'pain matrix' highlighted by a meta-analysis of positron emission tomography (PET) and functional MRI (fMRI) data (Peyron *et al.*,

TABLE 1. Contrasts of grey matter concentration (GMC) between BMS, Dys and Ct

		Contrast values			MNI coordinates			Correlation with pain	
Type of contrast	Area	*k*	*t*	*P*	*x*	*y*	*z*	*r*	*P* (FDR)
BMS > Ct	Piriform cortex L	1385	5.02	0.00001	−34	19	−24	0.26	0.15
	DLPFC R	398	4.03	0.00013	34	12	42	0.43	0.04*
	Inferior temporal area R	1702	3.97	0.00016	52	−42	−26	0.43	0.04*
	Inferior temporal area L	115	3.88	0.00020	−50	−46	−12	0.18	0.32
	Insula/frontal operculum L	194	3.82	0.00024	−45	17	−8	0.31	0.12
	Primary motor area L	546	3.69	0.00035	−19	−31	55	0.29	0.13
	DLPFC R	118	3.65	0.00039	50	13	37	0.43	0.04*
	Para-central lobule R	256	3.53	0.00055	4	−43	76	0.42	0.04*
Ct > BMS	Cerebellum lobule VI L	309	4.00	0.00014	−14	−76	−13	−0.49	0.05†
	Posterior cingulate gyrus L	706	3.85	0.00022	−15	−42	33	−0.37	0.06†
	Cerebellum lobule VIIa crus I L	713	3.81	0.00025	−28	−79	−19	−0.49	0.03*
	Anterior cingulate gyrus L	244	3.59	0.00047	−12	31	30	−0.39	0.04*
Dys > Ct	Middle temporal gyrus L	1538	4.03	0.00013	−67	−7	−11	−0.02	0.92
	Primary somatosensory area R	355	3.85	0.00022	52	−38	47	0.18	0.32
	Middle temporal gyrus R	320	3.59	0.00047	66	−13	−27	−0.13	0.45
Ct > Dys	Medial orbitofrontal cortex/subgenual area L	3661	4.33	0.00005	−12	30	−23	−0.24	0.18
	Anterior cingulate gyrus R	848	3.99	0.00014	9	39	26	−0.27	0.13
	Pre-SMA R	1260	3.97	0.00015	6	20	46	−0.12	0.47
	Middle cingulate gyrus R	221	3.56	0.00051	18	16	30	−0.30	0.12
BMS > Dys	Insula/frontal operculum L	981	4.97	0.00001	−45	17	−7	0.29	0.12
	Piriform cortex L	1860	4.88	0.00001	−33	19	−24	0.23	0.21
	DLPFC R	549	4.41	0.00004	52	14	39	0.37	0.04*
	Insula (BA 13)/SII L	860	3.85	0.00022	−37	8	14	0.28	0.13
	Posterior OFC L	837	3.72	0.00032	−10	9	−16	0.32	0.09
	Subgenual area L	437	3.51	0.00058	−5	26	−19	−0.14	0.42
Dys > BMS	Cerebellum lobule VI R	1379	4.17	0.00009	14	−61	−27	−0.19	0.31
	Middle temporal gyrus L	724	4.08	0.00011	−63	−9	−11	−0.09	0.61
	Posterior cingulate gyrus L	799	3.98	0.00015	−17	−43	32	−0.38	0.05†
	Inferior temporal area R	1364	3.97	0.00015	63	−12	−30	−0.16	0.37
	Cerebellum lobule VI L	308	3.80	0.00025	−18	−73	−13	−0.29	0.12
	Cerebellum lobule VIIa crus I L	116	3.56	0.00050	−27	−79	−18	−0.36	0.06†

Contrasts of GMC between burning mouth syndrome patients (BMS), dysgeusic patients (Dys) and control subjects (Ct). The direction of the difference of GMC is represented by the symbols < and >. The contrast outputs are the number of voxels per clusters (*k*), the t-value (*t*) and the *P*-value (*P*). Coordinates of the areas are given in MNI space. The last two columns give the correlation of the ROI, drawn from the cluster highlighted in the contrast. *r* is the correlation coefficients and *P* the *P*-value, degrees of freedom are 40 for all correlations. Significant FDR-corrected *P*-values are lower than 0.05 (*) and tendencies are lower than 0.1 (†). DLPFC, dorsolateral pre-frontal cortex; pre-SMA, pre-supplementary motor area; BA 13, Brodmann area 13; SII, secondary somatosensory area; R, right; L, left.

2000; Legrain *et al.*, 2011). This pain matrix was also highlighted by May (2008, 2011) with VBM studies of chronic pain (Fig. 4). Secondly, some of the areas highlighted correlated with the pain intensity rated by subjects. Thirdly, to characterize BMS more precisely we looked at subjects with dysgeusia only, grouping together a variety of taste dysfunctions but no burning mouth sensation. We highlighted that Dys presented also a change in GMC of several regions, although to a lesser degree. The areas affected in both conditions were different.

Pain matrix

As defined by Peyron *et al.* (2000), the 'pain matrix' is composed of insula/secondary somatosensory area (SII), anterior CG, thalamus, primary somatosensory area (SI), DLPFC, posterior parietal cortex, striatum, cerebellum (vermis and hemispheres), periaqueductal grey and SMA, in decreasing order of consistency. These areas appeared activated in pain conditions as compared with non-pain conditions in 30 PET and fMRI studies achieved between 1990 and 2000. The pain was either induced in normal volunteers or directly recorded in patients with neuropathic pain and compared with a non-painful condition under analgesia. We identified four out of these 10 areas which presented GMC changes in BMS patients compared with Ct. These areas were anterior CG, insula, DLPFC and cerebellum.

May (2008, 2011) identified a pain matrix similar to that identified by Peyron *et al.* (2000) in two meta-analyses of 13 and 30 VBM studies on different chronic pain, published between 2003 and 2008 and between 2004 and 2010, respectively. All areas presented a reduced GMC: the cingulate gyrus, the insular cortex, the temporal lobe, the frontal cortex, the thalamus/basal ganglia, the motor cortex, the brainstem and the DLPFC, in decreasing order of consistency. In the contrast between BMS and Ct we highlighted a decrease of GMC in the anterior and posterior CG and in the lobules of the cerebellum. However, the contrast also highlighted an increase of GMC in the insular cortex, the temporal lobe, the motor cortex and the DLPFC. Finally, we highlighted a GMC change in five of eight areas (anterior and posterior CG, insular/frontal operculum, inferior temporal gyrus, primary motor area and DLPFC) identified with VBM as a matrix involved in the processing of chronic nociceptive inputs.

Overall the areas highlighted fit very closely to the pain matrix previously identified. Regarding the difference in the direction of GMC changes (increase or decrease), in comparison with the consistent decrease identified by May (2008, 2011), it appears difficult to determine the underlying cellular mechanisms. As detailed in the Introduction, histopathological data did not show a correlation between grey matter probability (data before normalization and smoothing steps leading to GMC measures) and neuronal density

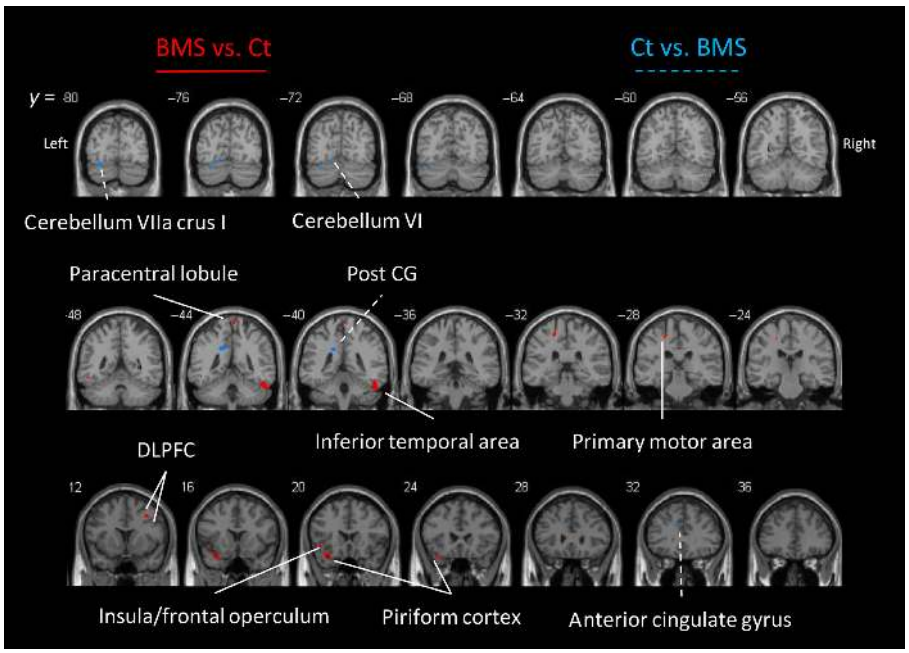

FIG. 1. Areas identified in contrasts between BMS and Ct. GMC differences between burning mouth syndrome patients (BMS) and controls (Ct), presented on coronal slices of the brain. Areas with higher GMC in BMS as compared with Ct are shown by a straight line, and areas with lower GMC in BMS as compared with Ct are shown by a dotted line. Contrasts were considered significant for uncorrected P-values < 0.001. The minimum number of voxels is $k = 100$. Post CG, posterior cingulate gyrus; DLPFC, dorsolateral pre-frontal cortex.

(Eriksson *et al.*, 2009). Thus, it is not possible to conclude regarding an increase of neurons when GMC increases.

Function of areas identified and implication of GMC changes in BMS

We found an increase GMC in the insula (insula/frontal operculum) and a decrease in the anterior CG, in BMS compared with Ct. May (2008) highlighted a decrease of GMC of the insula usually associated with a decrease of GMC in the anterior CG, in multiple chronic pain conditions (phantom pain, chronic back pain, migraine and fibromyalgia). May suggests that both regions operate as multi-integrator structures that participate in the anticipation of pain, as well as the experience of pain, and not directly in the perception of pain. The anterior CG may be identifed as an antinociceptive system. As the CG presented a decrease of GMC in 19 out of 26 studies, and is thus the area the most consistently associated with chronic pain, a decrease of GMC in anterior CG, more especially, appears as a biomarker of chronic pain. It has been shown to be highly activated through repeated heat painful stimulations, while other areas would decrease in activity under the control of anterior CG, and it correlated with a reduced pain perception (Bingel *et al.*, 2007). The changes of GMC observed in our study in these two areas may then reveal a dysfunction of this antinociceptive system in BMS patients.

A large part of the inferior temporal gyrus presented a higher GMC in BMS compared with Ct. The temporal lobe is a memory and learning area. In the context of pain, this area is activated when subjects expect to receive a painful heat shock (Brown *et al.*, 2008). It is interesting to note that in many pain studies using VBM, the superior temporal lobe, and the inferior temporal lobe, presented a decrease of GMC (Rocca *et al.*, 2006; Schmidt-Wilcke *et al.*, 2006, 2007, 2010; Rodriguez-Raecke *et al.*, 2009; Seminowicz *et al.*, 2010). However, the possible function of the area in pain was not discussed.

In addition, areas less discussed but often reported to be activated during the processing of pain are motor-related areas such as cerebellum, striatum and SMA (Peyron *et al.*, 2000). We highlighted a decrease of GMC in lobule VIIa crus I and lobule VI of the cerebellum. Helmchen *et al.* (2003) found that noxious heat stimuli (48.5 °C) activated more specifically the deep cerebellar nuclei and more specifically both areas we identified. They suggested that these areas were involved in nocifensive behaviour, i.e. withdrawal of part of the body from a burning object. In BMS, the impossibility of withdrawing from the burning sensation may have induced modification in the cerebellum.

Another motor-related areas, the primary motor cortex (pre-central gyrus) presented an increase of GMC. Patients usually report that burning is relieved during eating (Gurvits & Tan, 2013). One patient of our clinic (male, 62 years old) reported a decrease of the burning pain sensation whenever he was moving his tongue. Therefore, movements of the tongue could be a response to the burning sensation, which would result in a reduction of pain and may explain the increase of GMC in the primary motor area. Interestingly, electrical stimulation of this area decreases chronic pain in patients with central pain (Tsubokawa *et al.*, 1991, 1993) and burning oral sensation (Meyerson *et al.*, 1993).

Finally, we highlighted an increase of GMC in the DLPFC region. Prefrontal cortices, such as DLPFC, process attentional and memory components of noxious stimulation (Peyron *et al.*, 2000). However, in other chronic pains the DLPFC showed an increase of GMC (Apkarian *et al.*, 2004). BMS is often associated with depression and the DLPFC is probably a key area in depression, together with the hippocampus and neighbouring areas. As we did not measure depression, we could not include this variable in the analysis to assess the hypothesis that the decrease in DLPFC is linked to depression. However, we did not find any volume change in hippocampus and para-hippocampal fields, which is consistently observed in cases of depression (Sapolsky, 2001). Currently, depres-

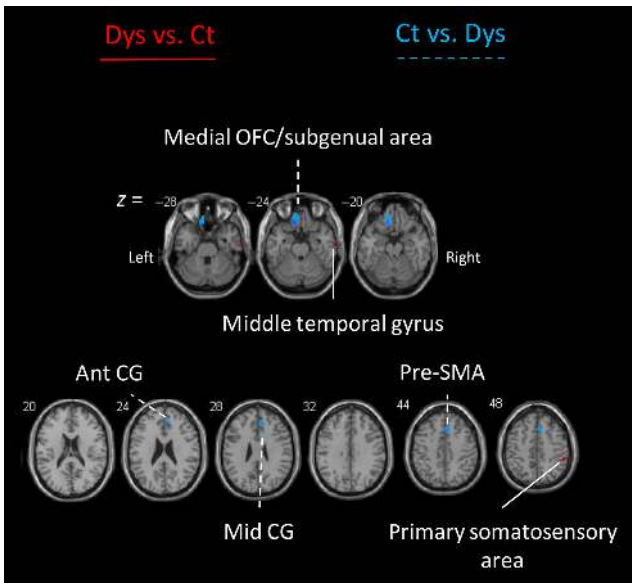

FIG. 2. Areas identified in contrasts between Dys and Ct. GMC differences between dysgeusic patients (Dys) and controls (Ct), presented on horizontal slices of the brain. Areas with a higher GMC in Dys as compared with Ct are shown by a straight line, and areas with a lower GMC in Dys as compared with Ct are shown by a dotted line. Contrasts were considered significant for uncorrected P-values < 0.001. The minimum number of voxels is k = 100. Medial OFC, medial orbitofrontal cortex; CG, cingulate gyrus; pre-SMA, pre-supplementary motor area.

sion in BMS is mostly considered as a result of the syndrome rather than its cause (Grushka *et al.*, 2002). Indeed, it was shown that BMS patients were more stressed and more depressed than a matched control group; however, initiation of the symptoms did not correlate with stressful life events (Eli *et al.*, 1994). Obviously, further studies are needed to better understand the role of the DLPFC in pain regulation and/or depression.

Correlation with pain intensity

Note that DLPFC, anterior CG, cerebellum, right inferior temporal area and para-central lobule presented a correlation of GMC with pain intensity rated by the patients. Although the pain measurements were rather weak, as it was provided by the general anamnesis performed when patients were diagnosed in the ENT department, some significant corrected correlations appeared. Interestingly, all these areas participate in pain modulation. Mainly, the coupling between anterior CG and DLPFC forms a descending pathway that may modulate nociceptive activity (Rainville, 2002; Lorenz *et al.*, 2003).

Changes in GMC are the cause or the consequence of BMS?

Two explanations for BMS prevail, a peripheral neuropathy or a central pain. Lauria *et al.* (2005) identified that BMS patients presented a reduced number of small trigeminal fibres located in taste buds. This result is supported by psychophysical study: Ito *et al.* (2002) showed that the threshold of pain perception on the tongue was higher in BMS subjects compared with a control group, whereas the threshold to different noxious stimulation applied on the finger was similar. As detection thresholds represent receptors' recruitment and/or fibre excitability (Willis, 1996), this result suggests a peripheral disorder of the trigeminal fibres of the tongue. The findings of Rodriguez-Raecke *et al.* (2009) also support the peripheral neuropathy theory. They showed, in hip osteoarthritis patients, that after replacement of the hip, patients were pain-free, and brain areas partly recover. Therefore, they suggested that a grey matter decrease is the consequence and not the cause of the pain. However, hip osteoarthritis may substantially differ from BMS. Indeed, hip osteoarthritis generally comes from inflammation or

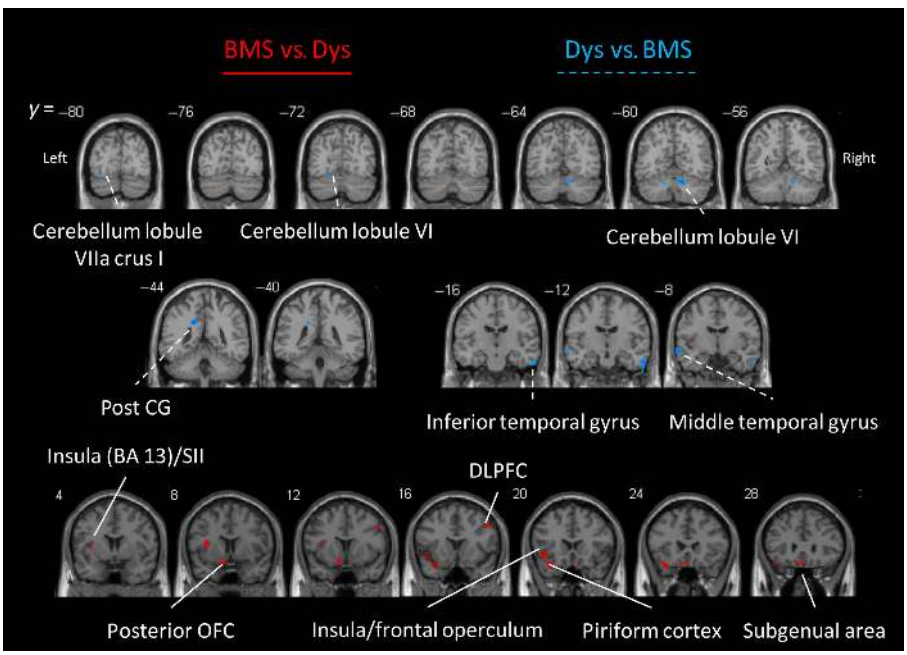

FIG. 3. Areas identified in contrasts between BMS and Dys. GMC differences between burning mouth syndrome patients (BMS) and dysgeusic patients (Dys), presented on coronal slices of the brain. Areas with a higher GMC in BMS as compared with Dys are shown by a straight line, and areas with a lower GMC BMS as compared with Dys are shown by a dotted line. Contrasts are significant for uncorrected P-values < 0.001. The minimum number of voxels is k = 100. BA 13, Brodmann area 13; SII, secondary somatosensory area; OFC, orbitofrontal cortex; CG, cingulate gyrus.

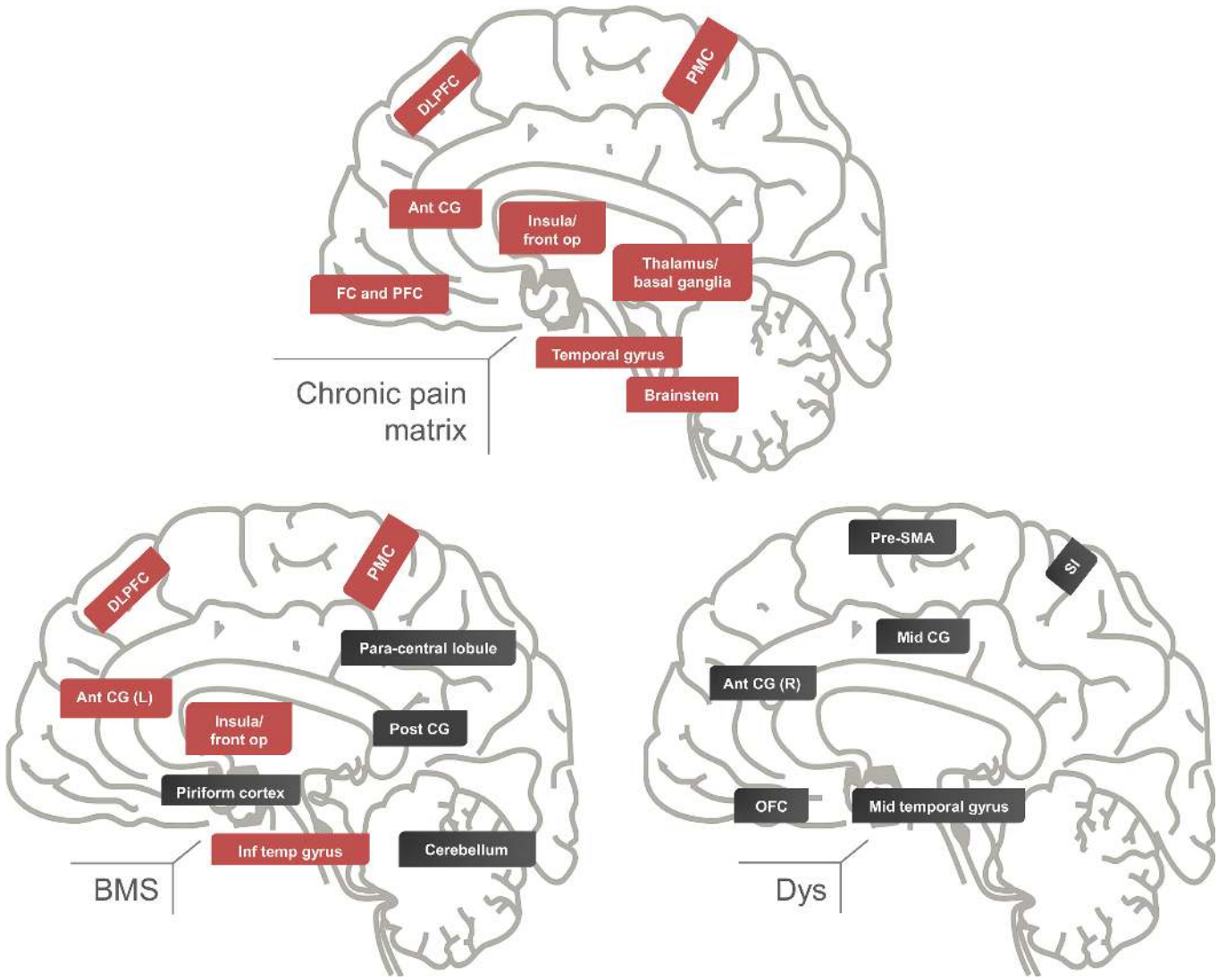

FIG. 4. Areas overlapping between the contrasts. Summary of areas identified as part of the 'chronic pain matrix' as identified by May (2011), areas with differences in grey matter concentration (GMC) in burning mouth syndrome (BMS) patients as compared with control (Ct), and areas with differences of GMC in dysgeusic patients (Dys) as compared with Ct. CG, cingulate gyrus; DLPFC, dorsolateral pre-frontal cortex; FC, frontal cortex; front. op., frontal operculum; OFC, orbitofrontal cortex; PFC, pre-frontal cortex; PMC, primary motor cortex; pre-SMA, pre-supplementary motor area.

injury to the hip joint, as well as from inherited factors. In comparison, BMS is identified, to date, as idiopathic. No evidence for a factor that may trigger small fibre neuropathy has been shown. One way to determine whether changes in grey matter are the consequence of BMS would be to measure GMC in early BMS and to show that, even if pain is perceived, no changes in GMC is observed.

If the pain does not come from the periphery, it may be driven by the brain itself. Central pain conditions, such as irritable bowel syndrome and fibromyalgia syndrome, are chronic idiopathic pain conditions resulting from abnormalities in pain processing, rather than from damage or inflammation of peripheral structures (Giesecke *et al.*, 2004). Previous studies suggest that BMS would fit with the concept of a 'central pain' condition (Gao *et al.*, 2000; Albuquerque *et al.*, 2006; Jääskeläinen, 2012). Ito *et al.* (2002) also highlighted that the pain sensation induced by nociceptive heat, cold and mechanical stimulation lasted much longer in BMS patients compared with a control group. Moreover, the recovery from pain was delayed and the return to baseline was not achieved, even 10 min after the stimulation, especially for heat stimulation. Our results showed a decrease of GMC in areas normally acting as antinociceptive areas (anterior and posterior CG, cerebellum and inferior temporal gyrus), which supports the hypothesis of a central pain. The defect in the control of pain induced by these areas may have led to sensitization to pain.

However, the fact that no factors have been found to explain BMS does not mean they do not exist. Therefore, GMC changes may be the result of the chronification process.

Dysgeusia patients

Dysgeusia is characterized by a lower taste sensitiviy and/or a taste distortion and/or a phantom taste (e.g. everything tastes salty or perception of a constant bitter taste). BMS and dysgeusia are often poorly discriminated, as hypogeusia sometimes accompanies BMS and conversely dysgeusic subjects may report pain. In our study, dysgeusic subjects who reported pain did not present a chronic and

acute pain and did not describe it as a burning sensation, therefore they were not included in the BMS group. Interestingly the brain areas identified were different from the BMS group, suggesting that BMS condition is different from Dys condition. Dys subjects presented fewer modifications in GMC as compared with BMS: primary somatosensory area, middle temporal gyrus, medial OFC/subgenual area, pre-SMA, middle CG and ant CG. Ant Cg was localized in the right hemisphere, on the contrary to BMS which presented a change of GMC in the left ant CG. Finally, the areas presenting a change of GMC in Dys did not correlate with pain when examined across all subjects.

In contrast to BMS, the Dys group presented an increase of GMC in the primary somatosensory area, notably involved in the processing of trigeminal inputs from the mouth. This result is coherent with a distorted or phantom taste that overstimulates the tongue and potentially trigeminal system. Dys had a reduced GMC in the medial OFC which overlapped with the subgenual area, in the anterior CG, in the mid CG and the pre-SMA, compared with Ct. Dys presented an increased GMC in the mid temporal gyrus on both sides and in the primary sensory area. Note that the complete medial OFC presented a lower GMC in Dys vs. Ct. Indeed, this area has been shown to encode expected value of a reinforcer (reward and punishment) in different sensory modalities (Kim *et al.*, 2010; Metereau & Dreher, 2015). As the anticipation is rather low with dysgeusia, because for example 'everything tastes salty' or because phantom tastes appear unexpectedly, we suggest that a decrease in GMC could result from the inability to anticipate food taste. As previously mentioned, the cingulate gyrus has an important function in pain regulation but it also encodes general emotional aspects of perception linked to taste and olfaction (Herwig *et al.*, 2007). The pre-SMA, usually associated with motor areas, is also involved in processing or the maintenance of relevant sensory information (Picard & Strick, 2001) and may be closely connected with the anterior CG as shown in primates (Wang *et al.*, 2001). Overall, Dys presented changes of GMC in the trigeminal region (increase of GMC in primary somatosensory area), but not in taste regions such as insula/frontal operculum and amygdala. It also showed grey matter changes in areas involved in emotion, motor anticipation and somesthesia.

Conclusions

The large modification of grey matter of the pain matrix in BMS subjects suggests a deficiency in the control of pain. This finding suggests a central pain condition. However, to understand is the central pain condition is the cause of the BMS or the result of a chronification process still need further investigations. Some studies indeed showed that behavioural cognitive therapy could help to reduce the pain perception even in patients with resistant BMS (Bergdahl *et al.*, 1995; Komiyama *et al.*, 2013). Therefore, two axis of research should be further investigated to better understand brain mechanisms underlying BMS. First, the identification of neuronal organization in areas with an increased or decreased GMC in the context of BMS and other chronic pain syndromes would be of huge interest. Seconldy, fMRI study with heat stimulation in BMS together with VBM analysis would probably help to explain the meaning of any increase or decrease in GMC. The measure of pain in the present study was rather weak as the focus was mainly on taste and trigeminal (sensations as hot, cold, spicy) areas. Therefore, studies focusing on the different components of the burning pain would be of interest. The group of patients assessed here was rather small due to the difficulty in recruiting patients with BMS; therefore, more studies on the topic would help in assessing the hypothesis expressed. Our study presents also a comparison with Dys. Although BMS and Dys appear to be closely related conditions, they present different central grey matter changes. BMS presents modification of GMC mostly in pain regions, while Dys shows changes of GMC in areas associated with emotions, motor anticipation and somesthesia. Therefore, BMS and Dys are driven by different brain mechanisms, which do not support the theory of a similar aetiology.

Conflict of interests

The authors declare that they have no conflict of interest.

Abbreviations

BA, Brodmann area; BMS, burning mouth syndrome; CG, cingulate gyrus; DLPFC, dorsolateral pre-frontal cortex; FDR, false discovery rate; fMRI, functional magnetic resonance imaging; GMC, grey matter concentration; IR/GR, inversion recovery/gradient recalled; OFC, orbitofrontal cortex; ROI, region of interest; SMA, superior motor area; TE, echo time; TR, repetition time; VBM, voxel-based morphometry.

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