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## Research Report

## Abnormal gray matter aging in chronic pain patients

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## ARTICLE INFO

## Article history:

Accepted 18 March 2012

## Keywords:

Aging

Chronic pain

Cortical thickness analysis

Voxel-based morphometry

Temporomandibular disorder

MRI

## ABSTRACT

Widespread brain gray matter (GM) atrophy is a normal part of the aging process. However, recent studies indicate that age-related GM changes are not uniform across the brain and may vary according to health status. Therefore the aims of this study were to determine whether chronic pain in temporomandibular disorder (TMD) is associated with abnormal GM aging in focal cortical regions associated with nociceptive processes, and the degree to which the cumulative effects of pain contributes to age effects. We found that patients have accelerated whole brain GM atrophy, compared to pain-free controls. We also identified three aberrant patterns of GM aging in five focal brain regions: 1) in the thalamus, GM volume correlated with age in the TMD patients but not in the control group; 2) in the anterior mid- and pregenual cingulate cortex (aMCC/pgACC), the TMD patients showed age-related cortical thinning, whereas the controls had age-related cortical thickening; and 3) in the dorsal striatum and the premotor cortex (PMC). Interestingly, the controls but not the patients showed age-related GM reductions. Finally, a result of particular note is that after accounting for the effects of TMD duration, age remained as a significant predictor of GM in the PMC and dorsal striatum. Thus, abnormal GM aging in TMD may be due to the progressive impact of TMD-related factors in pain-related regions, as well as inherent

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Abbreviations: ACC, anterior cingulate cortex; aMCC, anterior mid-cingulate cortex; CTA, cortical thickness analysis; FDR, false-discovery rate; FWHM, full-width half-maximum; GM, gray matter; M1, primary motor cortex; MCC, mid-cingulate cortex; MRI, magnetic resonance imaging; pgACC, pregenual anterior cingulate cortex; PMC, premotor cortex; S1, primary somatosensory cortex; SMA, supplementary motor area; SPM, statistical parametric mapping; TMD, temporomandibular disorder; VBM, voxel-based morphometry

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doi:10.1016/j.brainres.2012.03.040

Please cite this article as: Moayedi, M., et al., Abnormal gray matter aging in chronic pain patients, Brain Res. (2012), doi:10.1016/j.brainres.2012.03.040

factors in motor regions, in patients with TMD. This study is the first to show that chronic pain is associated with abnormal GM aging in focal cortical regions associated with pain and motor processes.

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## 1. Introduction

There is no doubt that the brain undergoes structural changes due to normal conditions like aging. For example, normal aging is characterized by cortical gray matter (GM) atrophy (Bergfield et al., 2009; Blinkov and Glezer, 1968; Good et al., 2001; McGinnis et al., 2011; Morrison and Hof, 2007; Sowell et al., 2003), although hypertrophy has also been reported in some brain areas (Fjell et al., 2009; Salat et al., 2004). GM changes in the brain also occur with dysfunction, injury, or specific disease (May, 2011a). Chronic pain in particular is associated with gray matter (GM) abnormalities in brain regions related to nociceptive processing, pain modulation and limbic function, such as the anterior cingulate cortex (ACC), the mid-cingulate cortex (MCC), the insula, the prefrontal cortex (PFC), the thalamus, the primary somatosensory cortex (S1) and the secondary somatosensory cortex (Blankstein et al., 2010; Davis et al., 2008; Gerstner et al., 2011; Gustin et al., 2011; Holle et al., 2011; May, 2011b; Moayedi et al., 2011; Robinson et al., 2011; Seminowicz et al., 2011). Additionally, some studies of chronic pain populations have identified GM abnormalities (increases and decreases) in motor regions, such as the basal ganglia (May, 2011b; Robinson et al., 2011; Seminowicz et al., 2010; Wartolowska et al., 2011) and the primary motor cortex (M1) (DaSilva et al., 2007, 2008; Kim et al., 2008; Schmidt-Wilcke et al., 2010).

Chronic diseases such as pain may interact with normal aging processes. For example, accelerated age-related whole brain GM atrophy has been reported in fibromyalgia (Kuchinad et al., 2007) and chronic back pain (Apkarian et al., 2004). However, most aging studies in chronic pain have assessed global GM and little is known about the interaction between chronic pain and age in GM volume/thickness of specific brain areas.

MRI-detectable changes in GM are thought to be related to functional changes, as has been demonstrated by studies of learning (Draganski et al., 2006a) and training (Draganski et al., 2004). Furthermore, a study of repetitive noxious stimulation (Teutsch et al., 2008) found that 20 minutes of painful heat stimulation over eight consecutive days resulted in structural GM increases within the S1, secondary somatosensory cortex and MCC. These data suggest that prolonged nociceptive processes in chronic pain (i.e., the duration of pain) may drive GM abnormalities. While it is plausible that age-related changes specific to chronic pain are the product of cumulative pain exposure, this hypothesis has not been tested empirically.

Therefore, the aims of this study were to determine (1) whether chronic pain in temporomandibular disorder (TMD) is associated with abnormal GM aging in focal cortical regions associated with nociceptive processes, and (2) the degree to which the cumulative effects of pain contributes to age effects.

Prolonged nociceptive activity may disrupt or even reverse normal GM atrophy in nociceptive and motor regions, and increase rates of atrophy in pain modulatory regions. Therefore, we hypothesized that normal age-related GM changes would be (1) increased in brain regions implicated in pain perception (e.g., the thalamus, S1, secondary somatosensory cortex, the posterior insula and the MCC), and (2) suppressed in motor regions (e.g., M1, premotor cortex (PMC), supplementary motor area (SMA), basal ganglia) and regions implicated in pain modulation (e.g., ACC and anterior insula) of patients with chronic pain.

## 2. Results

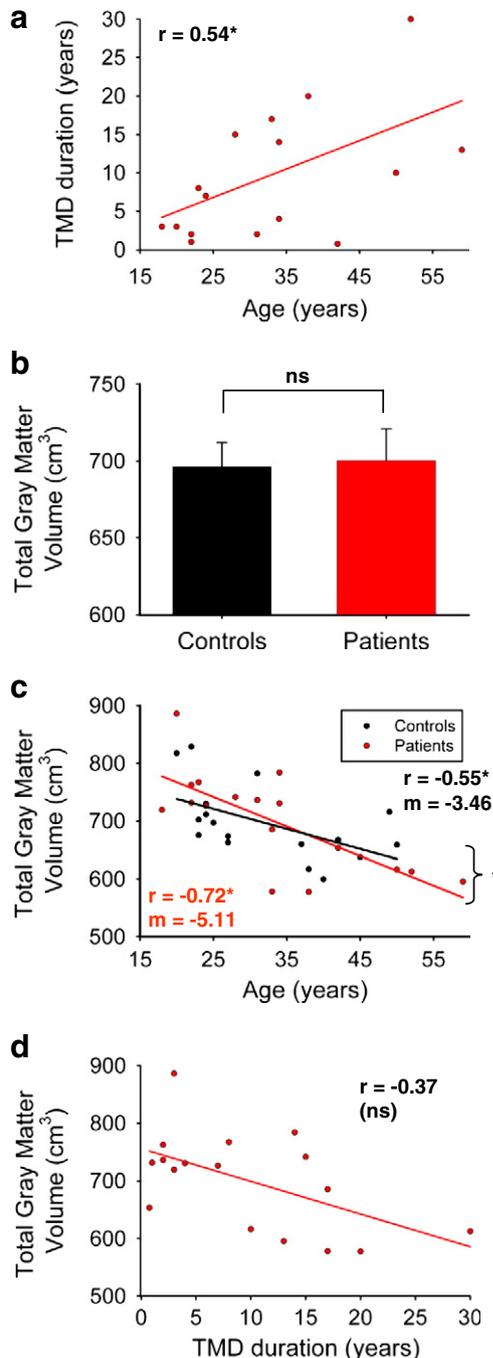
### 2.1. Patient characteristics

The mean age of subjects in the patient group (mean±SD: 33±12 years) was not significantly different than the control group (cortical thickness analysis (CTA) cohort: 33±9.8 years,  $p=0.94$ ; voxel-based morphometry (VBM) analysis cohort: 32±10.1 years,  $p=0.81$ ). The range of ages in the control group was 20 to 50 years old, and in the patient group was 18–59 years old. Patients reported having TMD for durations of 0.75–30 years (mean±SD: 9.8±8.3 years). Patient characteristic details are provided in Table 1, and

**Table 1 – Patient demographics.**

#	Age (years)	TMD duration (years)	Medications
1	22	2	
2	20	3	A*, cyc*
3	24	7	N, P
4	38	20	
5	42	0.75	F*
6	33	4	A*, F*
7	28	17	
8	34	14	A*, F*, Hy*
9	50	10	A, Ch, Di
10	59	13	
11	18	3	
12	34	15	
13	52	30	A
14	31	2	N
15	33	17	A, F
16	22	1	
17	23	8	

Abbreviations: A: Arthrotec (NSAID); cyc: cyclobenzapine; Ch: Champix; Di: Dixarit (Clonidine); F: Flexoril; Hy: Hydromorphone; N: Naproxen (NSAID); P: Prevacid; yrs: years. The asterisk (\*) denotes that subjects discontinued the use of the drug prior to our study.



**Fig. 1** – Total gray matter volume and correlations with age and duration. (a) TMD duration significantly correlated with and patients' age ( $r=0.54$ ,  $p=0.026$ ). (b) Total gray matter volume was not significantly different in the TMD group versus control group ( $p=0.88$ ). (c) Significant GM volume decreases with age for both controls ( $3.46\text{ cm}^3/\text{year}$ ) and TMD group ( $5.11\text{ cm}^3/\text{year}$ ) with a significant age-by-group interaction between the groups' slopes ( $p=0.0002$ ). (d) TMD duration is not significantly correlated to total gray matter volume ( $r=-0.37$ ,  $p=0.139$ ). Gray matter volumes were derived from the SPM segmentation pipeline and statistical analyses were performed in SPSS v.19.0 (see **Experimental procedures** for further detail). n.s. = not statistically significant ( $p>0.05$ ). Asterisks (\*) =  $p<0.05$ . m = slope (rate of GM change in  $\text{cm}^3/\text{year}$ ).

can be found elsewhere (Moayedi et al., 2011; Weissman-Fogel et al., 2011). Importantly, there was a significant correlation between patients' duration of TMD and their age ( $r=0.54$ ,  $p=0.026$ ; see Fig. 1a).

## 2.2. Global age effects

Both the control and TMD groups showed age-related whole brain GM atrophy, although there were no significant group differences in whole brain GM volume ( $p=0.88$ , see Fig. 1b). Specifically, there was a significant negative correlation between whole brain GM and age for the controls ( $r=-0.55$ ,  $p=0.024$ , slope ( $m$ )= $-3.46\text{ cm}^3/\text{year}$ ) and for the patients ( $r=-0.72$ ;  $p=0.001$ ,  $m=-5.11\text{ cm}^3/\text{year}$ ). However, there was an accelerated overall whole brain aging effect in the patients with a significant group interaction of the slopes of the GM/age curves (i.e., rate of change of GM with age) ( $p=0.0002$ ; see Fig. 1c). TMD duration was not significantly correlated to GM volume in the patient group ( $r=-0.37$ ,  $p=0.139$ , see Fig. 1d).

## 2.3. Focal age effects

A significant age-by-group interaction ( $p<0.05$ , corrected for multiple comparisons) was localized to two focal regions within the right cortex. In one region, on the border of the anterior MCC (aMCC) and the pregenual anterior cingulate cortex (pgACC) (BA32), patients had cortical thinning with age ( $r=-0.54$ ,  $m=-0.022\text{ mm}/\text{year}$ ), whereas controls had age-related cortical thickening ( $r=0.76$ ,  $m=0.033\text{ mm}/\text{year}$ ). In another region, the PMC, controls had age-related cortical thinning ( $r=-0.87$ ,  $m=-0.035\text{ mm}/\text{year}$ ), whereas patients did not have normal atrophy, but rather had a very modest age-related cortical thickening ( $r=0.11$ ,  $m=0.002\text{ mm}/\text{year}$ ) (see Table 2 and Fig. 2 for details).

Three subcortical clusters were identified that had a significant age-by-group interaction ( $p<0.05$ , FDR corrected; see Fig. 3): the left thalamus (extending to the ventral posterior lateral, ventral posterior medial, posterior and ventral lateral) and the right and left dorsal striata. Interestingly, Fig. 3 illustrates the strong positive correlation between GM volume with age in the TMD group ( $r=0.65$ ) in contrast to the weak correlation in the control group in the thalamus ( $r=-0.27$ ). Fig. 3 also illustrates the group differences in the dorsal striatum where the control group showed a clear age-related GM atrophy (left:  $r=-0.78$ ; right:  $r=-0.80$ ), in contrast to the age effect observed in the TMD group (left:  $r=0.42$ , right:  $r=0.33$ ) (see Table 2, Fig. 3).

## 2.4. The contribution of TMD duration to GM age effects

A schematic of the focal aging effects in control and TMD groups is shown in Fig. 4a (also see Section 3.2). The contribution of age and duration to the observed age-by-group interactions was examined because of the observed correlation between age and duration. We performed multiple regression analyses with age and duration as independent variables to parse out the relative contributions of these two factors. To describe our results, we used the standard annotation for partial correlations, e.g., age·duration, such that age is being correlated to the dependent variable while regressing out the

**Table 2 – Age-related group differences in cortical thickness and subcortical gray matter volume. Shown are the statistically significant ( $p < 0.05$ ) group differences in correlation coefficients of GM against age. Peak vertex/voxel Talairach coordinates (TAL) are reported.**

	Interaction	Region	#Vertices or #voxels <sup>a</sup>	Correlation (age vs. thickness)		TAL of peak			Peak t-score
				$r_{\text{patients}}$	$r_{\text{controls}}$	X	Y	Z	
Cortical (CTA)	C>P	R aMCC/pgACC	116	-0.54	0.76	14	30	20	4.42
	C<P	R PMC	109	0.11	-0.87	8	12	54	-5.18
Subcortical (VBM)	C<P	L dorsal striatum	1537	0.42	-0.78	-18	10	10	-4.99
		R dorsal striatum	4415	0.33	-0.80	11	-3	18	-4.65
		L thalamus	264	0.65	-0.27	-21	-23	14	-3.36

Abbreviations: C — controls; P — patients; aMCC — anterior mid-cingulate cortex; pgACC — pregenual anterior cingulate cortex; CTA — cortical thickness analysis; PMC — premotor cortex; VBM — voxel-based morphometry.  
<sup>a</sup> CTA results are provided as # vertices; VBM results are presented as # voxels.

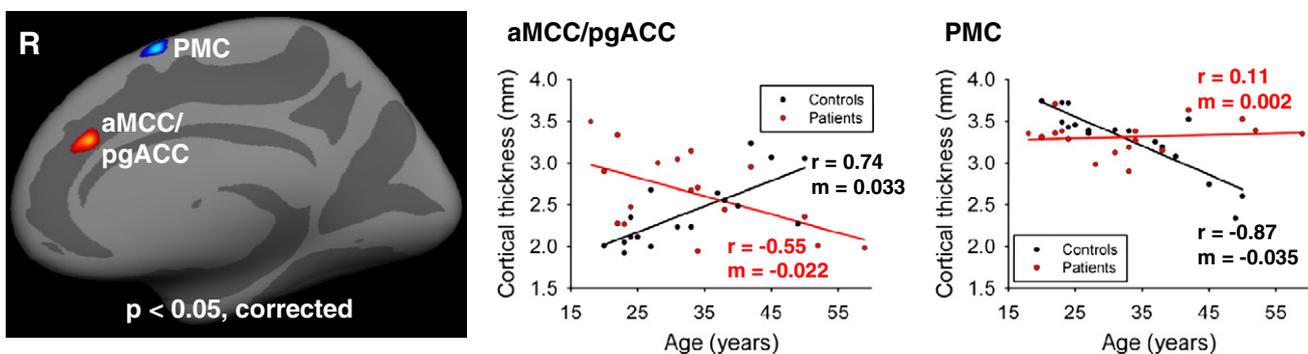
variance related to duration. The outcomes of these analyses are shown schematically in Fig. 4b. In the right thalamus, the partial correlation coefficient between age and GM volume was no longer significant when controlling for duration ( $r_{\text{age}} = 0.646$ ,  $p = 0.005$ ;  $r_{\text{age} \cdot \text{duration}} = 0.447$ ,  $p = 0.082$ ), whereas duration remained significant when age was included in the model ( $r_{\text{duration}} = 0.704$ ,  $p = 0.002$ ;  $r_{\text{duration} \cdot \text{age}} = 0.555$ ,  $p = 0.026$ ). In the aMCC/pgACC, the age-by-group interaction was driven by the shared variance between age and duration. When the age was regressed out of the correlation between duration and cortical thickness, the relationship remained insignificant ( $r_{\text{duration}} = -0.445$ ,  $p = 0.073$  to  $r_{\text{duration} \cdot \text{age}} = -0.222$ ,  $p = 0.409$ ). Further, when duration was regressed out of the correlation between age and cortical thickness in the aMCC/pgACC, the relationship was no longer significant ( $r_{\text{age}} = -0.535$ ,  $p = 0.027$  and  $r_{\text{age} \cdot \text{duration}} = -0.392$ ,  $p = 0.133$ ) (see Table 3).

In some cases, duration did not contribute to the observed age-by-group interaction. For instance, in the bilateral dorsal striatum, the partial correlations between age and GM in these regions did not show much change when duration was regressed out. Similarly, when age was regressed out of the correlation between duration and GM, the partial correlations were not significant (see Supplementary Table 1). In the case of the PMC, we found a suppressive effect (Cohen et al., 2003), i.e., when duration was included in the regression model, age became a better predictor of the

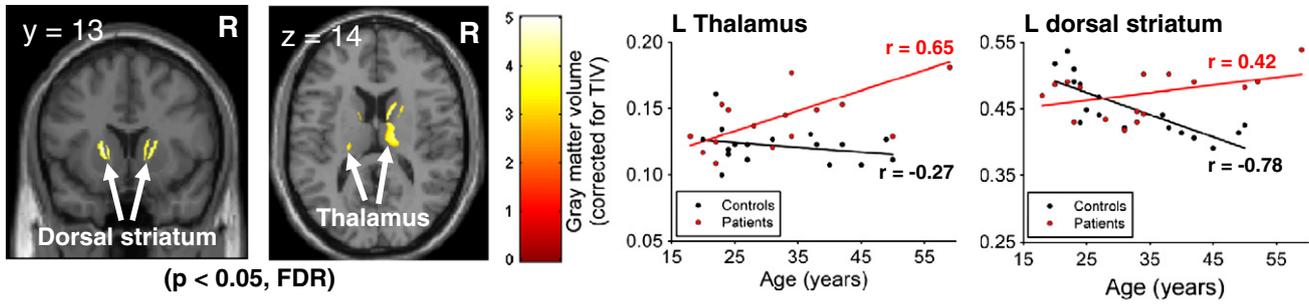
interaction as the correlation became more significant. That is, the partial correlation coefficient of age and thickness in the PMC, controlling for duration, was larger than the zero-order correlation (zero-order correlation: 0.11, partial correlation: 0.38). Similarly, when correlating thickness to TMD duration and controlling for the effect of age, the correlation coefficient for the PMC decreased from  $-0.36$  to  $-0.56$  (see Table 3).

### 3. Discussion

This study is the first to show that chronic pain is associated with abnormal GM aging in focal cortical regions associated with pain and motor processes. We found that patients with TMD have accelerated whole brain GM matter loss, compared to pain-free controls, but also identified three types of aberrant relationships between GM and age in five focal brain regions (see Fig. 4): (1) in the thalamus, TMD patients had age-related GM increases, whereas GM in controls was relatively sustained; (2) in the aMCC/pgACC, TMD patients had age-related cortical thinning, whereas the controls had age-related cortical thickening; and (3) in the dorsal striatum and PMC, the controls, but not the patients, had age-related GM decreases. Finally, after accounting for the effects of TMD duration, age remained as a significant predictor of PMC and



**Fig. 2 – Age-by-group interactions in cortical thickness. TMD patients had age-related thinning (0.022 mm/year) in the anterior mid-cingulate cortex/pregenual anterior cingulate cortex (aMCC/pgACC), whereas controls show age-related thickening (0.033 mm/year) in this region. In the premotor cortex (PMC), only the controls had age-related thinning (0.035 mm/year).**



**Fig. 3 – Group differences in age effects within subcortical GM volume.** Voxel-based morphometry revealed age-by-group interactions in the thalamus and dorsal striatum ( $p < 0.05$ , FDR). The graphs indicate gray matter volume (corrected for total intracranial volume versus age for each subject by group).

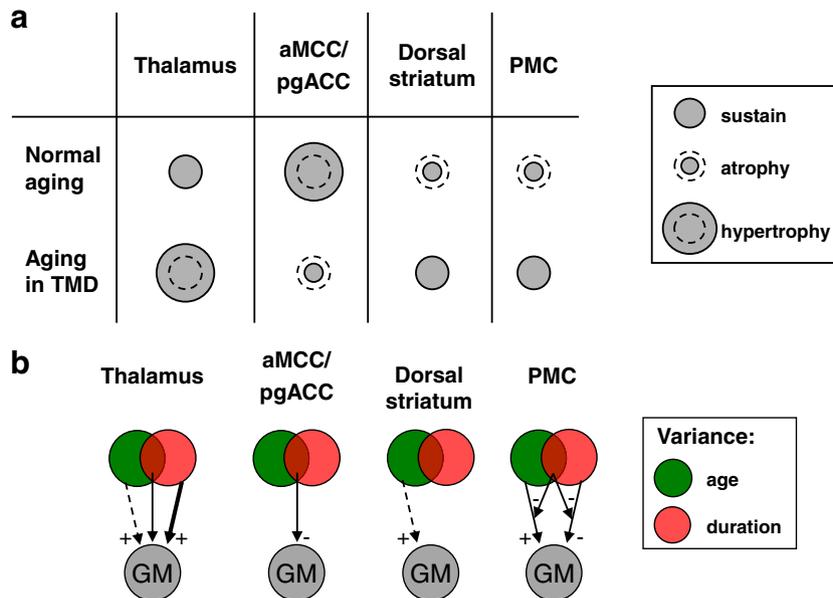
dorsal striatum GM. Abnormal GM aging in TMD may thus be due to the progressive impact of TMD-related factors in pain-related brain regions, as well as inherent factors in motor regions in patients with TMD.

**3.1. Whole brain GM atrophy**

Our finding of accelerated GM atrophy with age in TMD is consistent with previous studies of GM in fibromyalgia (Kuchinad et al., 2007) and chronic back pain (Apkarian et al., 2004). These previous studies attributed the increased rate of GM loss to excitotoxicity and inflammatory molecules. Specifically, they suggest that, because chronic pain is inherently harmful to the body and is associated with negative affect and increased stress, the inflammatory response is upregulated centrally, inducing cell death. These processes have been implicated in chronic age-related diseases (Mattson, 2003; Mattson and Chan, 2003), and therefore, it is plausible that they are implicated in age-related GM loss in chronic pain states.

**3.2. Age-related GM abnormalities**

The suppressive relationship identified in the PMC is of particular interest. The PMC is a region that is often activated in neuroimaging studies of experimental pain (Farrell et al., 2005; Lamm et al., 2011). In the current study, age and TMD duration uniquely and non-redundantly predicted variance in GM. When we included duration in the model, both variables (age and duration) better predicted the progression of cortical thickness over time. Furthermore, age and duration had differential effects on the PMC. Specifically, patients had sustained cortical thickness with age, whereas TMD duration was related to cortical thinning in the PMC. The PMC receives nociceptive input from the ventral caudal portion of the medial dorsal nucleus of the thalamus (Dum et al., 2009). Therefore, we would expect that a barrage of nociceptive input from the thalamus over an extended period of time could induce GM plasticity in the PMC, as we have observed in the thalamus, rather than the observed normalization. These paradoxical



**Fig. 4 – Summary diagrams of the contribution of age and duration to gray matter.** (a) Schematic diagram of GM regions with normal aging in healthy controls and abnormal aging in TMD. (b) Schematic diagram summarizing the relative contribution of age and TMD duration to the regions of GM with abnormal aging. The line thickness of each arrow depicts the relative contribution of age and duration and the plus (+) and minus (-) signs depict the direction of the relationship.

**Table 3 – Contributions of TMD duration to the age-gray matter relationships. Zero (0) order correlations and partial correlations are presented for age and TMD duration versus GM thickness/volume. The p-values in the age and duration columns represent the significance of the correlations.**

Region	Age				Duration			
	r	p	$r_{\text{age} \cdot \text{duration}}$	p	r	p	$r_{\text{duration} \cdot \text{age}}$	p
R PMC	0.109	0.677	0.381	0.145	–0.357	0.159	–0.496	0.051
R aMCC/pgACC	–0.535	0.027	–0.392	0.133	–0.445	0.073	–0.222	0.409
L dorsal striatum	0.417	0.096	0.416	0.109	0.129	0.639	–0.123	0.650
R dorsal striatum	0.325	0.204	0.327	0.216	0.092	0.742	–0.102	0.706
R thalamus	0.646	0.005	0.447	0.082	0.704	0.002	0.555	0.026

Abbreviations: aMCC — anterior mid-cingulate cortex; pgACC — pregenual anterior cingulate cortex; PMC — premotor cortex; S1 — primary somatosensory cortex.

findings warrant further study to better understand the relationship of the PMC and aging in the context of chronic pain.

Another key finding of this study is that GM volume in the dorsal striata is maintained (i.e., there is a loss of normal atrophy), which is unrelated to TMD duration. The basal ganglia have been implicated in the motor response to pain. Previous studies demonstrated that nociceptive neurons project directly to the globus pallidus and the putamen (Newman et al., 1996). Furthermore, the caudate nucleus of the rat and the cat has neurons that respond to noxious mechanical stimuli in the periphery and that are somatotopically organized (Chudler et al., 1993; Lidsky et al., 1979; Richards and Taylor, 1982; Schneider and Lidsky, 1981). Also, there is evidence that striatum is densely populated with opiate receptors in rats (Atweh and Kuhar, 1977) and humans (Blackburn et al., 1988). In line with these findings, stimulation of the caudate nucleus has been shown to produce analgesic effects in the monkey (Lineberry and Vierck, 1975). Stimulation of the striatum also modulates orofacial pain: stimulating dopaminergic neurons have been shown to modulate the nociceptive jaw-opening reflex in rat (Barcelo et al., 2012; Belforte and Pazo, 2005). In sum, pain–motor interactions are a hallmark of pain-related behaviors, the most obvious example being nocifensive behaviors. There is no doubt that the sensory and motor system interact, a concept supported by the many sensorimotor reflexes observed in animals and humans. However, the interactions of pain and motor function are not as clear. Furthermore, the effects of prolonged (or chronic) pain on motor output are equally ambiguous. It is therefore apparent that there is abnormal motor GM aging in TMD, independent of how long patients have had TMD.

The findings reported here are in contrast to previous findings that in some chronic pain conditions central GM changes are related to pathology where there exists a peripheral etiology, such as osteoarthritis. It has been reported that once the peripheral cause of the pain has been resolved, GM changes resolve (Gwilym et al., 2010; Rodriguez-Raecke et al., 2009; Seminowicz et al., 2011). However, there is evidence supporting both central and peripheral contribution to TMD pain (Maixner, 2008; Sarlani and Greenspan, 2005). Therefore the source of age-related variance may derive from multiple mechanisms in addition to (dis)use-dependent plasticity (see Section 3.3 and May, 2011a). Interestingly, recent studies have reported that development of some brain regions is tightly regulated by genes, rather

than the environment (Peper et al., 2007; Thompson et al., 2001). There is also evidence suggesting a genetic predisposition to functional chronic pain syndromes (Diatchenko et al., 2005, 2006a, 2006b; Slade et al., 2008). It is therefore feasible that there is a genetic contribution to the observed abnormal aging effects. These findings provide a genetic source of plasticity (Cannon et al., 2003; Toga et al., 2006) that may co-exist with other forms of plasticity.

### 3.3. Use-dependent plasticity

We have demonstrated that TMD duration added to the age effects in the thalamus and cingulate cortex. This form of plasticity is in line with the concept of use-dependent plasticity (May, 2011a) which comprises structural and functional changes in the brain in response to increased or decreased neuronal input (Garraghty and Muja, 1995; Schallert et al., 1997). For instance, studies in healthy human subjects have found that training (Draganski et al., 2004) and learning (Draganski et al., 2006a) can increase GM in the brain. Conversely, limb amputation, and other forms of sensory loss can induce reorganization of the cortical map that represents the affected limb (Merzenich et al., 1983, 1984) and GM loss (Draganski et al., 2006b; Taylor et al., 2009a). Of particular interest are studies that report reversible GM changes in nociceptive and antinociceptive regions of the brain in response to repeated noxious stimulation in healthy subjects (Bingel et al., 2008; Teutsch et al., 2008). Furthermore, patients with chronic pain show age-independent GM changes in both nociceptive and pain modulatory regions (e.g., Blankstein et al., 2010; Geha et al., 2008; Lutz et al., 2008; May, 2008; Rodriguez-Raecke et al., 2009; Schweinhardt et al., 2008; Younger et al., 2010). Similarly, we previously reported that the TMD cohort of the current study had GM thickening in the S1, ventrolateral and frontal polar cortices (Moayedi et al., 2011), and these group differences are not related to age (see Supplementary Materials and Supplementary Results). Studies have also demonstrated that some of the observed GM abnormalities are reversible (Gwilym et al., 2010; Rodriguez-Raecke et al., 2009; Seminowicz et al., 2011). Our findings of progressive thinning in the cingulate cortex and increasing thalamic GM are consistent with the concept of use-dependent plasticity. That is to say that increased input, activity or use will increase GM, and decreased use or activity is associated with decreased brain GM. Therefore,

the observed age-by-group interaction in the thalamus and aMCC/pgACC may, in part, be driven by prolonged nociceptive activity, which could oppose normal aging. These findings are consistent with our previous report that GM in the thalamus is positively correlated with TMD duration, and may be due to increased nociceptive activity, as previously discussed (see: Moayedi et al., 2011).

In the aMCC/pgACC, we found that the shared variance of both age and TMD duration contribute to progressive atrophy in the patients. This region receives orofacial nociceptive input from thalamic nuclei that are part of the spinothalamic and trigeminothalamic systems (Craig and Dostrovsky, 1997; Dum et al., 2009). The aMCC/pgACC is a complex, multimodal region that has been implicated in a number of functions (Beckmann et al., 2009; Yarkoni et al., 2011). For instance, this region has been identified as a node in the salience network, and has been implicated in aspects of salience (Davis, 2011; Davis et al., 2005; Downar et al., 2000, 2001; Seeley et al., 2007; Taylor et al., 2009b; Weissman-Fogel et al., 2010), and pain (Davis et al., 1995, 1997; Dostrovsky et al., 1995; Downar et al., 2003; Hutchison et al., 1999; Kwan et al., 2000; Lee et al., 2009; Mouraux et al., 2011; Wiech et al., 2010). The cingulate has also been implicated in the cognitive and affective processing of pain (Davis et al., 1997, 2000; Legrain et al., 2009; Rainville et al., 1997; Seminowicz and Davis, 2007a, 2007b; Wiech and Tracey, 2009; Wiech et al., 2008), and the MCC is involved in action selection and modulation of motor output in response to aversive stimuli (Shackman et al., 2011; Vogt, 2005; Vogt et al., 1993). Therefore, it is possible that the age-related thinning in the MCC is related to abnormalities in TMD with regard to cognitive and attentional processes related to prolonged TMD pain (Weissman-Fogel et al., 2011).

### 3.4. The cellular and molecular basis of GM changes

The cellular and molecular basis of MRI-detectable GM changes remains to be explained. However, several hypotheses for mechanisms of GM change have been postulated, such as neuronal and/or glial death (May, 2008), but recent evidence suggests that, to some extent, GM losses are likely related to density of small dendritic spines (Dumitriu et al., 2010; Metz et al., 2009), and the remodeling of neuronal processes (Lerch, 2011). Alternatively, reversible GM changes in chronic pain may be caused by neuroinflammation (DeLeo et al., 2004; Guo and Schluesener, 2007; Watkins et al., 1995), and induce MRI-detectable increases in GM. This mechanism could explain both abnormal age-related increases and maintenance of GM volume/thickness. For the observed GM losses, however, we cannot rule out that cell death is not occurring in age-related GM losses — healthy populations lose neurons as they age, and persons with neurodegenerative diseases suffer increased rates of atrophy related to cell death.

### 3.5. Caveats

Two issues that could not be controlled for in this study may have contributed to the observed age-related abnormalities. First, it was not possible to avoid inclusion of patients that were taking pain medications in the TMD group. Two recent studies have reported that NSAIDs have a protective effect

on GM volume, inhibiting age-related atrophy (Bendlin et al., 2010; Walther et al., 2011). Another study has reported that opiates have lasting effects on GM volume in the amygdala, MCC, PFC and the hypothalamus (Younger et al., 2011). As half of the patients in the current study were taking NSAIDs (see Table 1), it is possible that medication effects may contribute to findings of GM maintenance. However, only one patient was taking opiates to manage their pain. The second issue of consideration is that the TMD patient effects were potentially impacted by depression and anxiety that is sometimes seen in the patient group (Dworkin, 1994; Slade et al., 2007; Tenenbaum et al., 2001). Although we did not explicitly examine these co-factors, the patients did not self-report major depression or anxiety.

Another issue that must be acknowledged is that the current study is a cross-sectional analysis of age-related GM changes, not a longitudinal study. Therefore, our results were restricted to correlation analyses, and causal inferences need to be interpreted with caution.

### 3.6. Conclusion

In sum, our findings provide novel evidence that chronic pain patients have abnormal age-related gray matter changes in cognitive, motor and nociceptive brain regions. Our study highlights the importance of understanding the effects of age and TMD duration in structural studies of chronic pain, as progressive changes in GM may require differential therapeutic approaches.

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## 4. Experimental procedures

### 4.1. Subjects

Seventeen patients with non-traumatic TMD (mean age  $\pm$  SD:  $33 \pm 12$  years) and 17 pain-free, healthy subjects (mean age  $\pm$  SD:  $33 \pm 9.8$  years) with no prior history of chronic pain were recruited and provided informed written consent to procedures approved by the local research ethics boards. Structural MRI data in this cohort unrelated to age have been presented (Moayedi et al., 2011). All subjects were right-handed females. Dentists at the Mount Sinai Hospital Dental Clinic screened the patients for inclusion based on experimental research diagnostic criteria from the TMD research diagnostic criteria (Dworkin and Leresche, 1992). Patient demographics and specific screening criteria have been previously described elsewhere (Moayedi et al., 2011; Weissman-Fogel et al., 2011) and can be found in Table 1. All patients reported the number of years of TMD symptoms, herein described as TMD duration. Additional exclusion criteria (for all study participants) included: a history of serious diseases (metabolic, rheumatoid, and vascular), concurrent craniofacial pain disorders, or any contraindication to MRI scanning (e.g., claustrophobia, metal).

### 4.2. Imaging and analysis

Each study participant was placed in a 3-tesla GE MRI (Signa HDx) system fitted with an eight-channel phased array head

coil, lying supine with foam padding around their head to reduce movement. The 3D brain scan was acquired with a T1-weighted scan (IR-prep 3D-FSPGR with 128 axial slices,  $0.94 \times 0.94 \times 1.5 \text{ mm}^3$  voxels,  $256 \times 256$  matrix size, field of view =  $24 \times 24 \text{ cm}$ , one signal average, flip angle =  $20^\circ$ , inversion time = 300 ms, echo time = 5 ms, repetition time = 12 ms).

#### 4.2.1. Global age effects

To assess group differences in global age effects, we estimated whole brain GM volume from tissue segmentation performed in the SPM5 software package (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>) running under Matlab v.7.0.4 (Mathworks). We used a general linear model to test for age-by-group interactions for whole brain GM. Specifically, the model tested the main effects of group, age, and age-by-group interactions on whole brain GM volume. We also performed two correlation analyses: (1) whole brain GM volume versus TMD duration, and (2) TMD duration versus age. All statistical analyses were performed in SPSS v.19.0 ([www.spss.com](http://www.spss.com)).

#### 4.2.2. Cortical thickness analysis (CTA)

FreeSurfer software (<http://surfer.nmr.mgh.harvard.edu>) was used to assess cortical thickness. Detailed methods are described elsewhere (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 1999a, 1999b, 2001). Briefly, T1-weighted scans underwent intensity normalization, skull stripping and segmentation of each hemisphere based on tissue type (GM, white matter, cerebrospinal fluid). Within each hemisphere, the boundary between each tissue type was modeled as a surface so that the distance between each surface could be calculated at each point on the cortex. Each subject's homologous gyri and sulci were aligned to the standard average brain provided in FreeSurfer. A Gaussian spatial smoothing kernel of 6 mm full-width half-maximum (FWHM) was applied to compensate for topographical heterogeneity prior to statistical analysis. For this study, the distance between two points on the cortex (or two vertices) was 0.71 mm. Herein, CTA results are reported as the number of vertices. To restrict our search to regions hypothesized to be involved in TMD chronic pain, a single mask using FreeSurfer's cortical parcellations was constructed (Fischl et al., 2004) that included the prefrontal cortex, insula, M1, SMA, cingulate cortex, postcentral gyrus and sulcus (including S1 and the secondary somatosensory cortex) and the posterior parietal cortex. Age-by-group interactions in cortical thickness were tested at each vertex on the cortex within the cortical mask, using the "different offset, different slope" option. To correct CTA results for multiple comparisons, we used a Monte Carlo simulation with 1000 permutations with the AlphaSim software package (<http://afni.nimh.nih.gov/afni/doc/manual/AlphaSim>), as done previously (Blankstein et al., 2010; Moayedi et al., 2011; Taylor et al., 2009a). Thus, we calculated a mask-wise corrected threshold of  $p < 0.05$  from an uncorrected voxelwise  $p < 0.01$  and a cluster threshold of 178 contiguous vertices.

#### 4.2.3. Voxel-based analysis (subcortical gray matter volume)

A VBM analysis was used to measure subcortical structures. One additional control subject was recruited and consented

to research ethics board approved procedures to replace one subject subsequently removed from the subcortical analysis because of poor segmentation in the pre-processing pipeline.

Preprocessing of T1-weighted images and statistical analyses were performed in the Statistical Parametric Mapping version 5 (SPM5) software package (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>) running under Matlab (Mathworks). We used the VBM 5.1 toolbox (<http://dbm.neuro.uni-jena.de/vbm>) implemented in SPM 5 to perform VBM (for details see: Ashburner and Friston, 2000). Briefly, images were normalized to a standard template (International Consortium for Brain Mapping template), tissue types were then segmented using a Markov random field model, and underwent Jacobian modulation, followed by spatial smoothing of GM with a 10 mm FWHM Gaussian kernel. We set an absolute threshold mask of 0.10 to restrict the results to GM. All coordinates were converted from MNI space to Talairach space (Talairach and Tournoux, 1988) using the Lancaster transform (Lancaster et al., 2007) implemented in GingerALE v.2.0.4 (<http://www.brainmap.org/ale>).

A GM mask of subcortical regions was constructed using the WFU Pickatlas toolbox ([http://www.nitrc.org/projects/wfu\\_pickatlas](http://www.nitrc.org/projects/wfu_pickatlas)) and included the basal ganglia, amygdala and the thalamus. To do so, we selected the "Sub-lobar" label mask in the atlas, and restricted it to regions of GM, as defined by the "Gray Matter" label in the atlas. Age-by-group interactions in GM volume were tested for each voxel within the subcortical mask. All significant results are reported at a voxelwise false discovery rate (FDR) (Genovese et al., 2002) corrected  $p < 0.05$ , as implemented in SPM 5.

#### 4.2.4. Contribution of TMD duration to age-related GM abnormalities

We examined the degree to which any of the observed age effects are attributed to cumulative duration of chronic pain. To do this, we performed a forward model multiple linear regression. Age and TMD duration were entered as explanatory variables and each of the significant findings as dependent variables.

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## Disclosure and authors' contribution

We have no conflict of interest to report.

All authors have approved the final article.

- study design: MM, MBG, HCT, KDD
- data collection: MM, IWF, BVF, MBG
- analysis and interpretation of data: MM, TVS, APC, KDD
- writing of the report: MM, KDD

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## Funding

This study was supported by a Canadian Institute of Health Research (CIHR) grant and funds from the Canada Research Chair program [MOP 53304] (to KDD); CIHR studentship (to MM); Ontario Graduate Scholarship (to MM); CIHR Strategic Training programs: Pain: Molecules to Community (to MM

and IWF), and Cell Signals in Mucosal Inflammation and Pain [STP-53877] (to MM); University of Toronto Centre for the Study of Pain Clinician/Scientist Research fellowship (to IWF and TVS).

## Acknowledgments

We thank Mr. Eugen Hlasny and Mr. Keith Ta for expert technical assistance. The authors would like to thank Dr. Yair Lenga for patient screening.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at [doi:10.1016/j.brainres.2012.03.040](https://doi.org/10.1016/j.brainres.2012.03.040).

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