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The stress model of chronic pain: evidence from basal cortisol and hippocampal structure and function in humans

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Recent theories have suggested that chronic pain could be partly maintained by maladaptive physiological responses of the organism facing a recurrent stressor. The present study examined the associations between basal levels of cortisol collected over seven consecutive days, the hippocampal volumes and brain activation to thermal stimulations administered in 16 patients with chronic back pain and 18 healthy control subjects. Results showed that patients with chronic back pain have higher levels of cortisol than control subjects. In these patients, higher cortisol was associated with smaller hippocampal volume and stronger pain-evoked activity in the anterior parahippocampal gyrus, a region involved in anticipatory anxiety and associative learning. Importantly, path modelling—a statistical approach used to examine the empirical validity of propositions grounded on previous literature—revealed that the cortisol levels and phasic pain responses in the parahippocampal gyrus mediated a negative association between the hippocampal volume and the chronic pain intensity. These findings support a stress model of chronic pain suggesting that the sustained endocrine stress response observed in individuals with a smaller hippocampii induces changes in the function of the hippocampal complex that may contribute to the persistent pain states.

Keywords: chronic pain; stress; functional MRI; hippocampus; parahippocampal gyrus

Abbreviations: BDI = Beck Depression Inventory; BOLD = blood oxygen level-dependent

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Introduction

Chronic pain is a self-reinforcing pathological state in which plastic changes in the stress system may contribute to the patient's suffering and pain-related disability (Turk, 2002; Gatchel *et al*., 2007). When facing prolonged, uncertain and uncontrollable threat, the organism modifies its level of metabolic activity to adapt to environmental demands that may eventually lead to maladaptive responses inducing a series of stress-related pathophysiological strain (McEwen, 1998*b*). Such a state has been referred to as allostatic load and may contribute to the triggering, the amplification and/or the persistence of the pain state (Borsook *et al*., 2012). More specifically, chronic pain has been associated with a diminution of brain grey matter volume or changes in cortical thickness (Kuchinad *et al*., 2007; Baliki *et al*., 2011) and functional reorganization of pain-related brain networks, including those related to the hippocampal formation (Baliki *et al*., 2010, 2012; Schweinhardt and Bushnell, 2010; Maleki *et al*., 2012). In addition, patients with chronic pain often display a dysregulation of the hypothalamic–pituitary–adrenal axis, reflecting the major adaption imposed by the pain state that is also known to impact the hippocampal structure and functions (McEwen and Kalia, 2010). The proposed interrelationships between maladaptive stress, chronic pain and hippocampal functions are novel and may contribute to explaining the persistence of chronic pain states and individual differences in the intensity of clinical pain.

One of the potential consequences of allostatic load is an over-activation of the hypothalamic-pituitary-adrenal axis and the subsequent structural and functional changes in the hippocampal formation owing to its sensitivity to the deleterious effect of sustained high levels of glucocorticoids (Sapolsky, 1985; Sapolsky *et al*., 1990; de Kloet *et al*., 2005; Mirescu and Gould, 2006). These changes may be critical when adapting to chronic pain, as uncertainty about upcoming pain (Ploghaus *et al*., 2000), exacerbation of pain by anticipatory anxiety (Ploghaus *et al*., 2001) and negative emotions (Roy *et al*., 2009) were shown to recruit the hippocampal formation. Moreover, structural and functional abnormalities in the hippocampus have been reported in an animal model of chronic pain and have been shown to predict the behavioural manifestation of anxiety and reduced extinction of contextual aversive conditioning (Mutso *et al*., 2012). These findings lead to the proposition that persistent pain promotes the establishment of a rich network of aversive associations subserved by the reorganization of the hippocampus and other limbic structures (Apkarian *et al*., 2009, 2011).

The origins of these changes are unknown, and the impact of the maladaptive stress response on the pain-related response bolstering the anxiety and aversive learning processes in the hippocampal formation remains to be examined. Interestingly, recent studies have suggested that only a subset of individuals faced with prolonged pain or stress appears to evolve toward persistent chronic states (McEwen and Stellar, 1993; Baliki *et al*., 2012). For instance, it has been suggested that smaller hippocampal volumes may increase the vulnerability to stress (Lyons *et al*., 2001), anxiety (Karatsoreos and McEwen, 2011) and the development of post-traumatic stress disorder in individuals exposed to trauma (Gilbertson *et al*., 2002). These studies suggest that the hippocampal volumes may constitute a pre-existing condition influencing the degree of resilience displayed by the organisms facing a major stressor.

The overall portrait is that prolonged pain may constitute an allostatic load in individuals showing more stress vulnerability, inducing long-lasting plastic changes that in turn instigate a spiralling down of the patient's condition. To gain understanding of the relationship between these different factors, we conducted a multivariate study examining the associations between the basal levels of cortisol (the major glucocorticoid in humans), the structural volumetric morphology of the hippocampus and the functional brain activity to phasic thermal noxious stimuli in patients with chronic back pain and age– and sex-matched healthy control subjects. Consistent with the model of allostatic load in chronic pain (Borsook *et al*., 2012), we first posited that basal levels of cortisol measured over seven consecutive days would be higher in patients with chronic back pain. We then hypothesized that patients with chronic back pain displaying higher levels of basal cortisol would have smaller hippocampii and stronger pain-evoked activity measured using blood oxygen level-dependent (BOLD)–functional MRI in the anterior hippocampal formation. Lastly, we used path modelling to test various ways by which these different biomarkers could interact to reflect or predict the patient's current clinical pain state. Based on previous literature, two main models were tested. A 'neurotoxic model' conceptualizes chronic pain as the instigator of the maladaptive stress response that impacts the structures and the functions of the hippocampal formation (Sapolsky *et al*., 1990; de Kloet *et al*., 2005). In contrast, a 'vulnerability model' proposes the smaller hippocampal volume as a predisposition to develop a maladaptive stress response (Lyons *et al*., 2001; Gilbertson *et al*., 2002; Lupien *et al*., 2007) that is associated with hyper-reactivity to stressors that lead to an amplification of acute pain/stress responses in the hippocampal formation associated with the clinical pain experienced by the patient.

Materials and methods

Participants

Twenty-one patients with chronic back pain (11 females; 23–49 years of age; mean 36 years) and 21 healthy control participants (10 females; 21–53 years; mean 36 years) participated in this study. The patients with chronic back pain were recruited through local pain treatment centres and newspaper advertisements in Montreal. Patients experiencing symptoms of back pain for >6 months were invited to participate in the study after a medical evaluation. The mean pain duration of the patients with chronic back pain was 11 ± 10 years (Table 1). Healthy control subjects were matched with patients with chronic back pain based on age and sex. Two participants (one healthy and one with chronic back pain) were excluded because of abnormally high levels of cortisol and because of drugs consumed during the week when saliva samples were collected. The full sample included 20 patients with chronic back pain and 20 control subjects in which cortisol samples were collected. Furthermore, four patients with chronic back pain and two control subjects were excluded from the brain imaging analysis because of head movements

Table 1 Characteristics of the patients with chronic back pain

Patients	Sex	Age	Location of pain	Duration of pain (months)	Intensity of pain	Origin of the pain	BDI	Medications
CBP01	Male	27	Low back; neck; shoulder	27	2	Spontaneous	2	NSAID
CBP02	Female	23	Low back up to the neck	108	3	Spontaneous	7	SNRI
CBP03	Male	45	Low back; right leg	252	1	Spontaneous	11	None
CBP04	Female	49	Low back; neck	69	4	Spontaneous	4	None
CBP05	Female	44	Low back	24	4	Spontaneous	0	None
CBP06	Male	27	Low back up to the neck; right foot	60	8	Spontaneous	15	None
CBP07	Male	46	Low back; right knee	63	5	Spontaneous	17	SNRI
CBP08	Female	28	Low back up to the neck	54	6	Spontaneous	11	None
CBP09	Male	30	Low back	212	3	Spontaneous	14	SNRI
CBP10	Male	35	Low back; neck	24	1	Spontaneous	18	None
CBP11	Male	48	Low back	68	4	Spontaneous	4	None
CBP12	Female	23	Low back up to the neck	48	3	Spontaneous	2	None
CBP13	Male	48	Low back	45	6	Spontaneous	4	NSAID
CBP14	Male	28	Low back up to the neck	180	6	Spontaneous	1	None
CBP15	Female	48	Low back; shoulders	400	3	Spontaneous	18	None
CBP16	Male	32	Low back	160	3	Spontaneous	5	NSAID

NSAID = non-steroidal anti-inflammatory drug; SNRI = serotonin–norepinephrine reuptake inhibitor.

exceeding 3.5 mm or owing to technical problems. The final sample consisted of 16 patients with chronic back pain (six females) and 18 control subjects (eight females) for the phasic pain functional MRI and structural analysis.

Six patients with chronic back pain used muscular relaxant, NSAIDs (non-steroidal anti-inflammatory drugs) or SNRIs (serotonin–norepinephrine reuptake inhibitors), to control their pain, and none of them used opiates. Some patients also used other medication for thyroid ($n = 1$) and high arterial pressure ($n = 1$). Eight patients with chronic back pain were medication-free. Additional analysis revealed that ruling out the non-specific effect of medication by covariance (i.e. currently taking some versus not taking any medication) did not change the results of the cortisol analyses, suggesting that the medication did not contribute significantly to the findings of the present study.

All experimental procedures conformed to the standards set by the latest revision of the Declaration of Helsinki and were approved by the Research Ethics Board of our institution ('Comité mixte d'éthique de la recherche du Regroupement Neuroimagerie Québec; CMER-RNQ'). All participants gave written informed consent, acknowledging their right to withdraw from the experiment without prejudice, and received compensation of $100 for their travel expenses, time and commitment and an additional $100 after the week of salivary sampling.

Questionnaires assessing depressive mood and clinical pain intensity

The Beck Depression Inventory—second edition (BDI-II) (Beck *et al*., 1996) is a self-report questionnaire with 21 items that was used to assess the participants' depressive symptoms experienced during the last 2 weeks. In this study, the mean scores on the BDI-II were higher in the subjects with chronic back pain (8.3 ± 6.5) compared with the control subjects (2.8 ± 3.5; $P < 0.01$). Interindividual BDI-II scores were therefore included as a variable of no interest (covariance) to prevent potential confounds. Nevertheless, including this covariate did not influence the results of the current study, suggesting that higher levels of cortisol were not related to the depressive symptoms of the participants. The present pain index of the McGill Pain Questionnaire (Melzack, 1975) was used to assess the intensity of clinical pain prior to entering the scan.

Salivary cortisol

Basal salivary cortisol levels were measured throughout a full week. Participants were asked to fill 10 mm of pure saliva (i.e. passive drool) in a small plastic vial using a straw. They were instructed to collect five samples per day over seven consecutive days, starting the day after the brain scanning session. On each day, participants collected their samples at awakening, 30 min after awakening, at noon, in the afternoon and at bedtime. The mean time at which the samples were collected was similar between groups: awakening (healthy 8:20 am; chronic back pain 8:05 am), 30 min after awakening (healthy 8:55 am; chronic back pain 8:35 am), noon (healthy 12:40 pm; chronic back pain 1:00 pm), afternoon (healthy 4:40 pm; chronic back pain 5:05 pm) and before going to bed (healthy 11:30 pm; chronic back pain 11:25 pm). The mean diurnal curve was computed for each subject based on the 7 days sampled, and the individual basal cortisol level was computed using the area under the curve with respect to ground (Pruessner *et al*., 2003). The samples were stored at -20°C until the time of cortisol concentration determination. Analyses were performed at the Centre for Studies on Human Stress (Douglas Mental Health Institute, Montreal Site, Canada; www.humanstress.ca) using an Enzyme Immunoassay (EIA) kit from Salimetrics LLC.

Experimental pain procedure and material

The brain imaging session consisted of two runs of thermal pain applied to the lower leg of the participants and two separate scans during which the participant observed images displaying pain-evoking situations. Data on pain-evoking images will be presented in a separate report. Each functional scan consisted of eight noxious and eight innocuous (control) thermal stimulations applied in a pseudorandom order making the intensity of the stimulation unpredictable. Thermal

stimulations were administered with a computer-controlled thermal stimulator using an MRI compatible $3 \times 3\,cm^2$ contact probe (Medoc TSA-II; Medoc). Baseline temperature between successive stimuli was set to 38°C. Prior to the functional MRI experimentation, pain sensitivity was assessed in each participant by a magnitude-estimations procedure to determine the pain-eliciting temperature for each person (⩽50.5°C; aiming at 75/100 on the pain scale). The control innocuous stimuli (⩽46°C) were adjusted individually to produce a clearly perceptible, but non-painful, warm sensation that was included as a control. The order of the conditions was pseudorandomized to introduce some uncertainty regarding the intensity of the upcoming stimulus. The rate of temperature increase from baseline (38°C) was adjusted individually to reach the target temperature in 2 s, and the following plateau lasted 5 s, before temperature returned to baseline in 2 s.

At the beginning of each trial, a fixation cross appeared for 3, 4 or 5 s before the noxious or innocuous stimulation, which was then followed by a long interval (18–25 s) to prevent sensitization and allow subjects to rate each stimulus. Each thermal sensation was evaluated with visual analogue scales displayed using E-Prime (Psychology Software Tools Inc.; http://www.pstnet.com) on a screen located at the head-end of the scanner and viewed via a mirror. After each stimulus, a visual cue first prompted the participant to indicate whether the thermal stimulus was warm or painfully hot by using the index and middle finger keys of an MRI-compatible response box. If the stimulation was classified as painful, the participant was then asked to rate successively the intensity and the unpleasantness of the painful experience on two separate computerized visual analogue scales. The scales were presented over 12 s each and labelled with the verbal anchor 'no pain' or 'not unpleasant' at 0 (left extremity) and 'extreme pain' or 'extremely unpleasant' at 100 (right extremity). If the stimulation was classified as warm, the subjects were requested to rate on a visual analogue scale ranging from 0 (no sensation) to 100 (very warm) over 18 s. The ratings were produced by moving a cursor with the index and middle finger of the right hand and were recorded in E-prime.

The temperature of the noxious stimulations was adjusted individually [mean ± standard deviation (SD): healthy: 48.5° ± 1.2°; patients with chronic back pain: 48.1° ± 1.3°] to produce strong pain intensity (healthy: 75.0 ± 9.4; patients with chronic back pain: 77.5 ± 13.5) and pain unpleasantness (healthy: 72.7 ± 8.7; patients with chronic back pain: 75.8 ± 16.8). The innocuous stimulations (healthy: 43.2° ± 1.0°; patients with chronic back pain: 42.8° ± 0.7°) were rated as non-painful and slightly warm (healthy: 31.2 ± 16.1; patients with chronic back pain: 28.1 ± 20.6). Fig. 2A and B show that there were no significant group differences in the temperature and the subjective ratings of the pain and warm sensations (all *P*-values > 0.13). Importantly, controlling for the individually adjusted temperature of the noxious stimulation or the pain intensity ratings by covariance did not change the imaging results.

Functional magnetic resonance imaging acquisition and analyses

Imaging was performed on a 3.0 T whole-body scanner (Siemens TRIO), using a 12-channel head coil, at the Unité de Neuroimagerie Fonctionelle (UNF), Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal (CRIUGM) in Montréal, QC, Canada. BOLD signal was acquired using a standard T_2*-weighted gradient-echo EPI sequence (repetition time = 3000 ms, echo time = 30 ms; flip angle = 90°; field of view = 220 × 220 mm^2; matrix = 40 interleaved, axial slices per whole-brain volume at 3.4 mm thickness; in-plane resolution of 3.4 × 3.4 mm for isotropic voxels; 227 volumes). Structural images were acquired using a high-resolution T_1-weighted MPRAGE sequence (repetition time = 2300 ms; echo time = 2.99 ms; flip angle = 9°; field of view = 256 mm; matrix = 256 × 256; 1 × 1 × 1.2 mm voxels; 160 slices per whole-brain volume). All data preprocessing and analysis were done using SPM8 (Statistical Parametric Mapping, Version 8; Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) executed in Matlab 7.8. (Mathworks). Offline preprocessing of functional images included realignment of functional time series, co-registration of each subject's functional and anatomical data, spatial normalization to the Montreal Neurological Institute (MNI) space and spatial smoothing (8 mm full-width at half-maximum Gaussian kernel).

The analysis of functional MRI data was based on a model accounting for fixed and random effects. For the fixed effect, a general linear model estimated changes in brain regional responses for each subject. The paradigm was modelled as six events: $warm_{ramp\text{-}up}$ (2 s), warm (7 s), $warm_{rating}$, $pain_{ramp\text{-}up}$, pain and $pain_{ratings}$. The warm/pain events included the 5 s plateau and the 2 s ramp-down. For each trial type, a given item was modelled as a delta function representing its onset and duration. The ensuing vectors were convolved with the canonical haemodynamic response function, and used as regressors in the individual design matrix. Movement parameters estimated during realignment (translations in *x*, *y* and *z* directions and rotations around *x*-, *y*- and *z*-axes) and a constant were also included in the matrix for each scanning run as variables of no interest. High-pass filter was implemented using a cut-off period of 128 s to remove the low-frequency drifts from the time series. Serial correlations in functional MRI signal were estimated using an autoregressive (order 1) plus white noise model and a restricted maximum likelihood (ReML) algorithm.

Linear contrasts tested the main pain-related effect by subtracting warm-related brain activity from the pain-related brain activity (pain versus warm) to control for non-specific neural response to thermal sensory input. From this contrast, a statistical parametric map [SPM(T)] was generated. The summary statistics image was then further spatially smoothed (Gaussian kernel 6 mm full-width at half-maximum) and entered in a second-level random-effect analysis. One-sample *t*-tests were performed on the data of all subjects for each group. A two-sample *t*-test was used to compare the main effect of pain versus warm between groups. We assessed the relationship between brain activity during pain and basal cortisol by regressing the individual within-subjects contrasts images for pain versus warm against the area under the curve with respect to ground in each group. Group differences were further tested using the interaction term.

The resulting set of voxel values for each contrast constituted a map of the *t*-statistic [SPM(T)] that was thresholded at an uncorrected $P < 0.001$ for visual inspection. Statistical inferences were based on the family-wise error correction (FWE, $P \leqslant 0.05$) over a region of interest delineating the bilateral hippocampus and parahippocampal gyrus, consistent with the *a priori* hypothesis suggesting a key role for the hippocampal formation in the stress-related model of chronic pain.

Hippocampal volumes

The cortical reconstruction and the volumetric segmentation of the hippocampus were performed using Freesurfer v5.0, an automated and widely documented software package for brain structural analysis (http://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferWiki).

The procedure includes motion correction, the removal of the skull using watershed/surface deformation procedure, normalization in Talairach space and segmentation of the subcortical structures based on the existing atlas containing probablistic information on the location of structures, and each voxel in the normalized brain volume is assigned one of ~40 labels. Technical details and method of the automated segmentation are provided in Fischl *et al*. (2002). The volume of each hemispheric hippocampus was extracted for each subject. The age and sex of each participant were included as covariates in the between-group comparison and the within-group correlations with the area under the curve with respect to ground.

Path analyses

Path analysis is a statistical procedure used to examine the direct and mediating links between multiple variables to test a given theoretical model empirically. In addition, path analysis can be used to compare the fit between two or more models, to assess which accounts best for the data (Lleras, 2005). In the present study, path analyses were performed with Amos statistical software package (http://www-01.ibm.com/software/analytics/spss/products/statistics/amos/) to test two hypothesized models. The models included (i) the cortisol diurnal area under the curve with respect to ground; (ii) the hippocampal volumes; (iii) the mean parameter estimates in a small sphere of 3 mm of radius around the peak of pain responses in bilateral parahippocampal gyrus; and (iv) the current level of clinical pain (McGill Pain Questionnaire, present pain index) or the chronic pain duration. The goodness-of-fit of each model was assessed using the ratio between chi-square and degrees of freedom (χ^2/df) (Bollen and Long, 1993), the comparative fit index (Bentler, 1990), the root mean square error of approximation (Steiger, 1990) and the Akaike information criterion (Akaike, 1974). A reasonable fit of a specified model to the data is indicated when the χ^2/df ratio is <5, the comparative fit index is >0.95 and the root mean square error of approximation is <0.05 (Bollen and Long, 1993).

Results

Basal cortisol levels are higher in patients with chronic back pain compared with control subjects

Our study is based on the expectation that patients with chronic back pain ($n = 16$) would show higher levels of basal cortisol than matched healthy control subjects ($n = 18$). Fig. 1 illustrates the mean diurnal cortisol values of each group computed from five samples per day over seven consecutive days, starting the day after the brain scanning session. As expected, in both groups, cortisol peaked 30 min after awakening and diminished throughout rest of the day, getting to its lowest point at bedtime. A repeated measures ANCOVA, controlling for BDI scores, performed on the five measures (time) over the 7 days (day) and including sex and group as independent variables revealed significant effects of time [$F(2.7, 76.9) = 34.76$; $P < 0.001$] and higher levels of cortisol in patients with chronic back pain [$F(1, 31) = 7.5$; $P = 0.01$], but did not show any effect or interaction involving the day of sampling or the sex of subjects (P-values $\geqslant 0.19$). The results further showed that the covariate, BDI scores, was not related to time or group effects (P-values $\geqslant 0.31$). The absence of significant group by time interaction reveals that cortisol levels were reliably higher in patients with chronic back pain throughout the day ($P = 0.50$). Consistent with these results, the cortisol area under the curve with respect to ground was significantly higher in patients with chronic back pain (mean 132.71 ± 54.40; ranging from 42.01–255.62) than in control subjects [mean 96.48 ± 30.85; ranging from 49.59–149.74; $F(1,31) = 6.6$; $P = 0.02$]. Additional sex effects found on the full sample (20 patients with chronic back pain and 20 control subjects) are presented in the online Supplementary material.

Pain-related brain activations do not differ between groups

BOLD responses evoked by painful and non-painful thermal stimulation administered to the lower leg of the participant were compared to assess pain-related brain activations in patients with chronic back pain and healthy participants. As expected, the contrast between heat pain and warm stimuli revealed robust brain activity in several regions often referred to as the 'pain matrix', including the thalamus, the sensorimotor regions (SI and M1), the parietal operculum (SII), the insular cortex and the anterior region of the mid-cingulate cortex (Supplementary material) (Apkarian *et al*., 2005). A conjunction analysis across groups revealed the typical pattern of pain-related activation (Fig. 2C), and a direct contrast between patients with chronic back pain and the control subjects yielded no significant voxels at $P < 0.001$ uncorrected (not shown). This suggests similar pain-related brain activation across groups.

Patients with chronic back pain with smaller hippocampal volumes have higher basal levels of cortisol

Measures of hippocampus volume were first submitted to a repeated-measures ANOVA comparing the hemispheres and the groups. Fig. 3A illustrates that no group effect or interaction involving the group was observed ($P \geqslant 0.33$). The only significant results reflected a slightly bigger hippocampus in the right hemisphere [$F(2.7, 84.8) = 35.19$; $P < 0.001$; as in Pruessner *et al*. (2000)]. We further tested the partial correlation between basal cortisol levels and hippocampal volume in each group using age, sex and BDI-II scores as covariates. Fig. 3B illustrates that, even after controlling for depressive symptoms, cortisol area under the curve with respect to ground still negatively correlated with the mean hippocampal volumes in the patients with chronic back pain ($r = -0.55$; $P \leqslant 0.05$) but not in the control subjects ($r = 0.12$; $P = 0.86$). When performing a Fisher r-to-z-score transformation to compare correlation coefficients across groups (Cohen and Cohen, 1983), the results show that the negative correlation between cortisol and hippocampal volumes was significantly greater in patients with chronic back pain than in healthy individuals ($Z = -1.95$; $P \leqslant 0.05$). This revealed that individuals with smaller hippocampii are more likely to show a maladaptive stress response as measured by the higher basal cortisol levels when facing persistent pain.

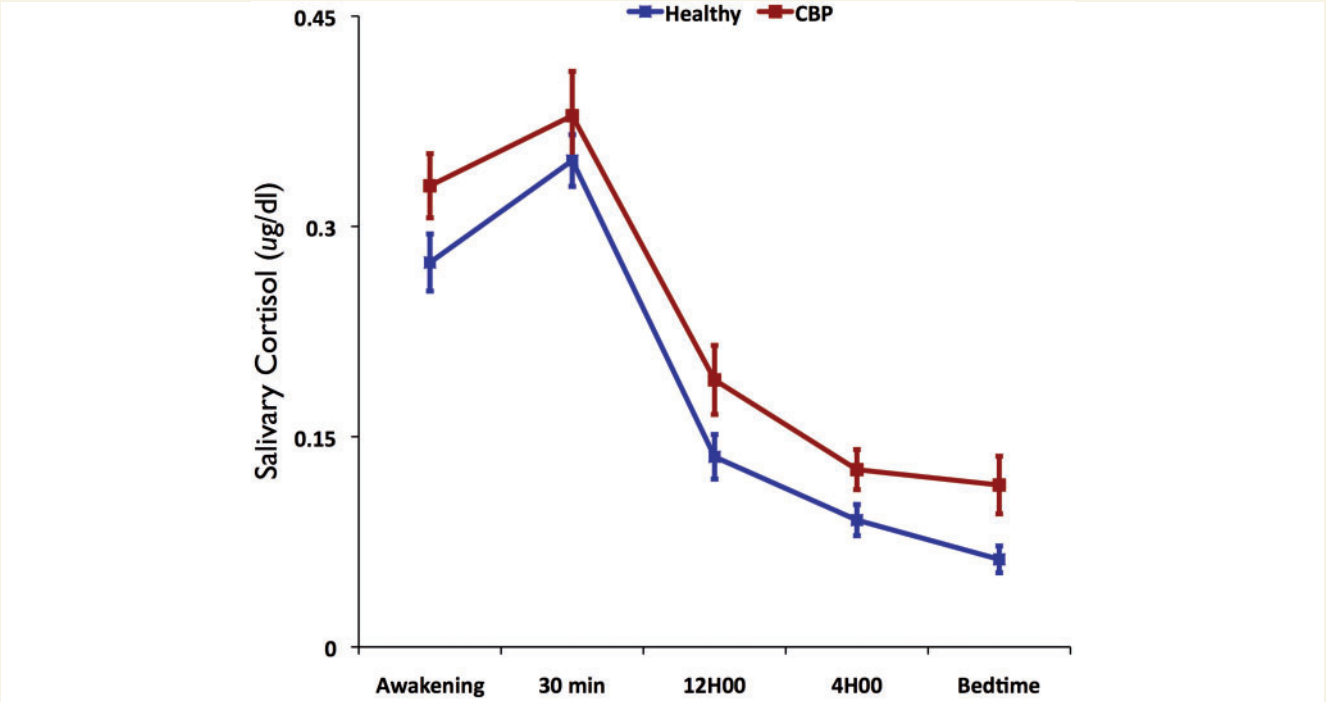

Figure 1 Diurnal basal glucocorticoid activity averaged over seven consecutive days. The patients with chronic back pain (CBP) had significantly increased stress hormone activity compared with the healthy control subjects ($P = 0.01$).

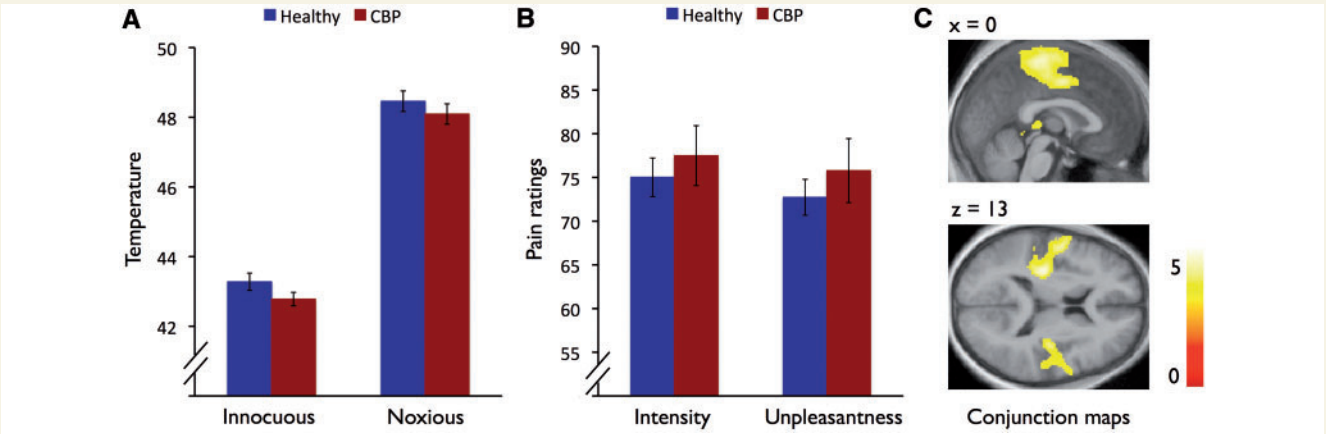

Figure 2 (**A**) Mean individually adjusted temperatures used to elicit comparable levels of warm sensations and strong pain in each group. (**B**) Mean pain intensity and unpleasantness ratings to the painful stimulations reported during the functional MRI session. (**C**) Conjunction map of the patients with chronic back pain (CBP) and the healthy participants to the pain versus warm contrast. Functional data are shown over the mean structural image of all participants ($P < 0.001$ uncorrected). In addition to the activation shown in the mid-cingulate cortex, supplementary motor area, insula and parietal operculum, significant responses were also found in SI/MI and the thalamus.

Basal levels of cortisol and chronic pain intensity are associated with a stronger pain response in the anterior hippocampal formation

We further examined the effect of basal cortisol on the phasic pain response (pain versus warm contrast). We used a region of interest that targeted the hippocampal formation (i.e. the hippocampus and the parahippocampal gyrus) because of its high affinity with cortisol and because of its involvement in pain processing and anxiety-related behaviour. Table 2 and Fig. 4A show that higher area under the curve with respect to ground in patients with chronic back pain correlated with stronger activation in the anterior parahippocampal gyrus (FWE-corrected $P \leqslant 0.05$), even after including the BDI-II scores as a variable of no interest. Using a less stringent threshold (uncorrected $P < 0.005$), we observed that this effect was bilateral and interacted with the group. This finding

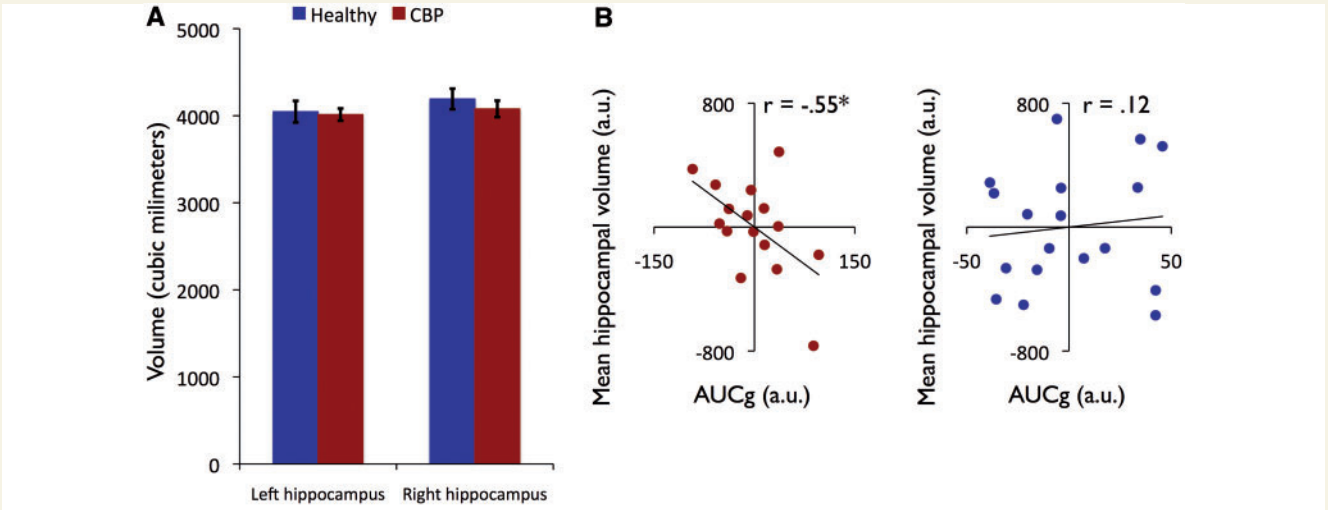

Figure 3 (**A**) Mean hippocampal volumes of the patients with chronic back pain (CBP) and control subjects. (**B**) In the patients with chronic back pain group, the residual of the mean hippocampal volume negatively correlated with the residual of the area under the curve with respect to ground (AUCg) of the basal levels of cortisol after removing the effects of the covariates sex, age and BDI-II scores ($P \leqslant 0.05$).

Table 2 Positive regression of pain versus warm over basal cortisol (area under the curve with respect to ground) within a region of interest delineating the hippocampal formation when controlling for BDI-II scores

Brain area	MNI coordinates			Local peak z-value	*P*
	x	*y*	*z*		
Parahippocampal gyrus	−36	−22	−28	3.80	<.001[a]
Parahippocampal gyrus	26	−18	−26	3.20	<.001
The between-group interaction term of pain versus warm over basal cortisol (area under the curve with respect to ground) when controlling for BDI-II scores					
Parahippocampal gyrus	−36	−26	−30	2.73	.003
Parahippocampal gyrus	24	−24	−28	2.88	.002
Positive regression of pain versus warm over chronic pain intensity (MPQ-PPI) within a region of interest delineating the hippocampal formation when controlling for BDI-II scores					
Hippocampus	36	−10	−26	3.99	<.001[a]
Hippocampus	−34	−12	−20	3.55	<.001[b]

a Significant when correcting for family-wise error (FWE).
b Marginally significant when correcting for family-wise error (FWE).

suggests a strong positive correlation of basal levels of cortisol with pain-related hippocampal activity in the patients with chronic back pain but not in healthy control subjects.

To test how a maladaptive stress response could impact the pain state of the patients, we further examined whether the phasic pain responses in a region of interest delineating the hippocampal complex (i.e. the hippocampus and the parahippocampal gyrus) was related to the chronic pain intensity reported by the patients on the McGill Pain Questionnaire immediately before the scan. As depicted in Fig. 4B and reported in Table 2, bilateral anterior hippocampal activity correlated with patients' scores on the McGill Pain Questionnaire (present pain index) (FWE-corrected $P \leqslant 0.05$), and this effect remained significant after controlling for BDI-II scores. This finding suggests that the response of the hippocampal formation to the painful/stressful experimental procedure partly reflects the current clinical pain condition.

Basal cortisol and pain responses in the parahippocampal gyrus mediate the relationship between the hippocampal volume and chronic pain intensity

Lastly, we evaluated two contrasting models (Fig. 5) proposed to explain the interactions between basal cortisol levels, hippocampal volumes, pain response in the hippocampal formation (i.e.

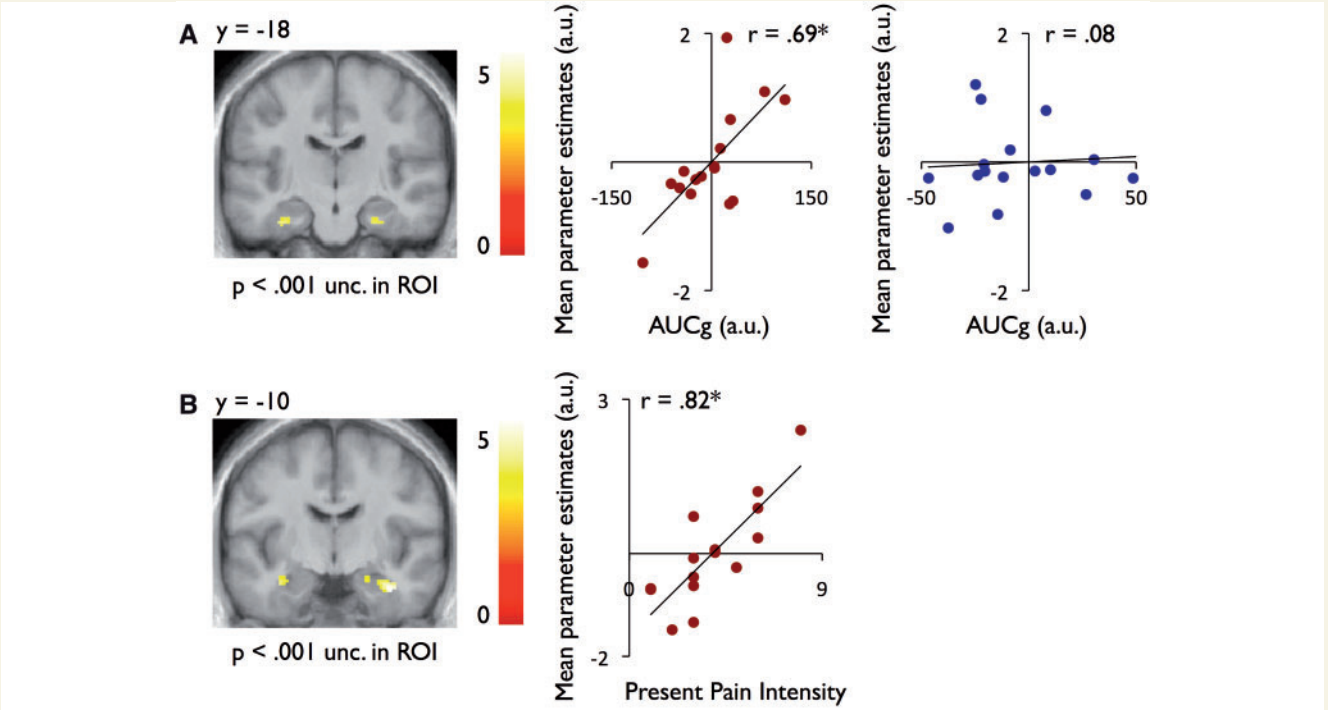

Figure 4 (**A**) The BOLD signal elicited by the (pain versus warm) painful stimulation in the anterior parahippocampal gyrus positively correlated with the area under the curve with respect to ground (AUCg) of the basal cortisol levels in chronic back pain even when controlling for BDI-II scores. Functional data are shown using a region of interest (ROI) delineating the hippocampal formation (shown at $P < 0.001$ uncorrected) over the mean structural image of all patients with chronic back pain. The red scatter plot represents the residual mean bilateral parahippocampal gyrus activity against the residual of the area under the curve with respect to ground of the basal cortisol in patients with chronic back pain. Consistent with the interaction reported in Table 2, such a relation was not observed in healthy individuals (blue scatter plot). (**B**) The BOLD signal elicited by the pain versus warm stimulations in the anterior hippocampus positively correlated with the McGill Pain Questionnaire (present pain index) even when controlling for BDI-II scores. Functional data are shown using a region of interest delineating the hippocampal formation (shown at $P < 0.001$ uncorrected) over the mean structural image of all patients with chronic back pain. The scatter plot represents the residual of the mean bilateral anterior hippocampus activity against the McGill Pain Questionnaire (present pain index) of all patients with chronic back pain.

parahippocampal gyrus and hippocampus) and chronic pain intensity. Model 1 was derived from the observation of a neurotoxic effect of cortisol (Sapolsky, 1985; Sapolsky *et al*., 1985, 1990) and proposes that chronic pain (its intensity or its duration) is the instigator of a maladaptive stress response leading to increased cortisol secretion that impacts on the hippocampus. Alternatively, Model 2 involves the notion of *a priori* vulnerability (Lyons *et al*., 2001; Gilbertson *et al*., 2002; Lupien, *et al*., 2007) and conceptualizes the smaller hippocampal volume as a predisposition to develop a maladaptive stress response when facing persistent pain, which may in turn alter the functional response of the parahippocampal gyrus to phasic pain.

The results suggest that Model 1 did not provide an optimal fit of the data and should not be accepted when including the chronic pain intensity [$\chi^2(3) = 12.25$, $P < 0.01$, $\chi^2/df = 4.08$, comparative fit index = 0.25, root mean square error of approximation = 0.45, Akaike information criterion = 26.25] or the chronic pain duration [$\chi^2(3) = 6.65$, $P = 0.08$, $\chi^2/df = 2.22$, comparative fit index = 0.38, root mean square error of approximation = 0.29, Akaike information criterion = 20.65] as the instigator of the stress response. In contrast, Model 2 clearly provided a better and acceptable fit of the data [$\chi^2(3) = 1.71$, $P = 0.63$; $\chi^2/df = 0.57$, comparative fit index = 1.0, root mean square error of approximation = 0.00, Akaike information criterion = 15.72] and was selected for further examination. Standardized regression weights obtained for all hypothesized paths of Model 2 are shown in Fig. 5B. To evaluate the mediation hypothesis, bootstrap analysis simulating 2000 samples were further performed to test whether or not the basal levels of cortisol and/or the pain response in the parahippocampal gyrus significantly mediated the relationship between hippocampal volume and chronic pain intensity. Fig. 5B shows that the relationship between the hippocampal volume and the pain response in the parahippocampal gyrus tended to be mediated by the basal cortisol levels [$b = -0.30$, $P = 0.057$; 95% confidence interval (CI): -0.55, -0.03], and that the relationship between the basal cortisol levels and the chronic pain intensity was mediated by pain response in the parahippocampal gyrus ($b = 0.34$, $P = 0.02$; 95% CI: 0.09, 0.60). The results further demonstrated that the indirect effect of basal cortisol levels and pain response in the parahippocampal gyrus mediated the relationship between the hippocampal volumes and the chronic pain intensity ($b = -0.16$, $P = 0.04$; 95% CI: -0.42, -0.02). Finally,

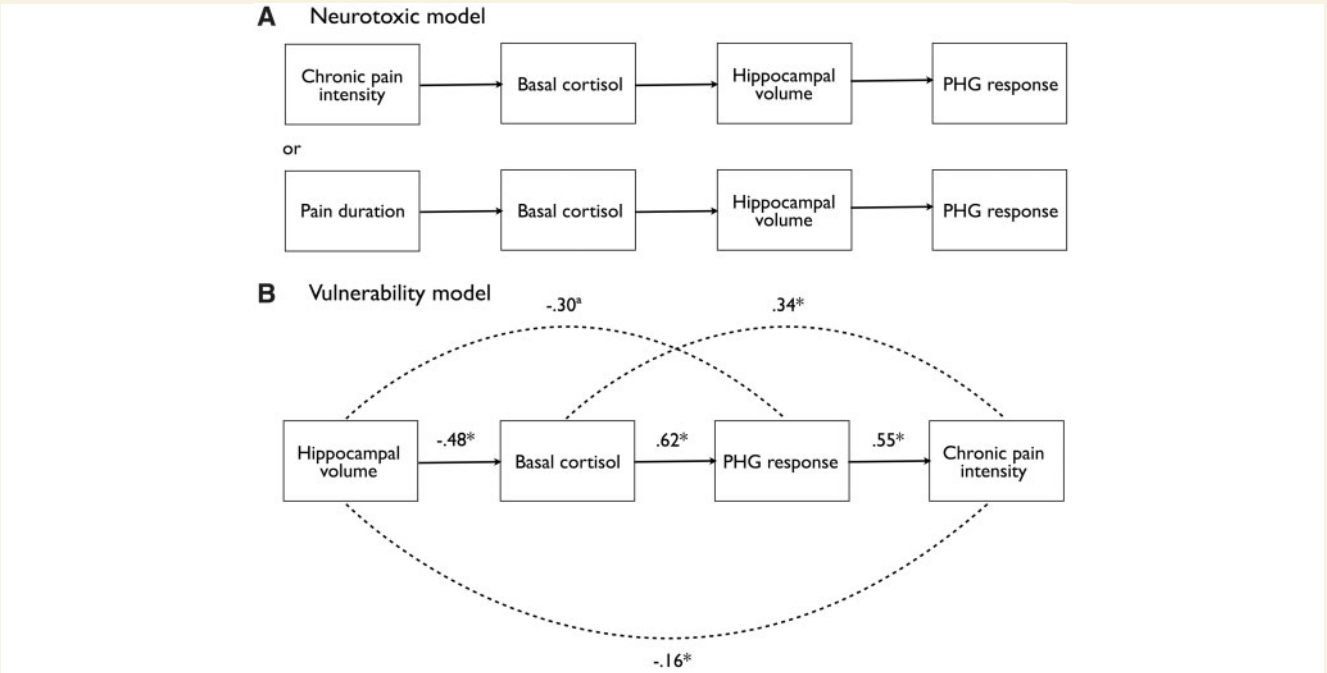

Figure 5 Two contrasting models proposed to explain the interactions between basal cortisol levels, hippocampal volumes, pain response in the hippocampal formation (i.e. parahippocampal gyrus and hippocampus) and chronic pain intensity were evaluated. (**A**) The first theoretical model conceptualizes the chronic pain intensity or pain duration as the instigator of the maladaptive stress response that impact the hippocampal formation and the parahippocampal gyrus (PHG) response to acute pain. (**B**) The second theoretical model conceptualizes the small hippocampal volumes as a risk factor for developing higher levels of stress hormones when exposed to recurrent or persistent pain, which in turn leads to enhanced parahippocampal gyrus responses and contributes to stronger ongoing/fluctuating levels of clinical pain. The results suggest that Model 1 should not be accepted while Model 2 significantly fitted the data. The direct (full lines) and indirect effects (dotted lines) are presented with maximum likelihood estimates (standardized estimates). $^{*}P < 0.05$ and [a]marginally significant, $P < 0.07$. All error terms are significant at $P < 0.001$.

path analysis results remained similar when controlling for BDI-II scores, suggesting that the observed relationships between the variables are not owing to depressive symptoms (the model including BDI-II scores is presented in the Supplementary material).

Discussion

The present study shows that patients with chronic back pain have higher basal cortisol levels than healthy individuals. Higher diurnal levels of cortisol were associated with smaller hippocampii and stronger phasic pain responses in the bilateral anterior parahippocampal gyrus. The phasic pain response of the anterior hippocampus was also stronger in patients reporting more intense clinical pain. Path analysis supported the vulnerability model in which a smaller hippocampal volume predicts higher levels of basal cortisol, which in turn amplifies pain-related parahippocampal gyrus activity and contributes to the chronic pain state. This suggests that chronic pain is associated with maladaptive stress responses possibly involving enhanced stress reactivity to acute pain and individual predisposition reflected in hippocampal structural differences. Interestingly, the healthy control subjects had comparable hippocampal volumes and did not show any correlation with the basal levels of cortisol, suggesting that it is the interaction between the persistence of pain and smaller hippocampii that predicted the maladaptive stress response.

Borsook *et al.* (2012) have recently proposed a theoretical model describing how the maladaptive stress response could contribute to developing and maintaining chronic pain. The authors argue that patients with migraine are facing repeated unpredictable stress that triggers a cascade of central and peripheral changes contributing to the allostatic load of the organism (Borsook *et al.*, 2012). According to this view, the pain episodes involve repetitive attacks to the organism resulting in a dysregulation of the normal adaptive response. Building on this model, our study demonstrates that patients with chronic back pain had elevated basal cortisol levels thought to reflect a maladaptive stress response induced by recurrent pain. Although this finding was expected, the literature on chronic pain and basal stress hormones has yielded conflicting results because different chronic pain conditions have been associated with hypocortisolism, hypercortisolism, or both (Crofford *et al.*, 1994; Catley *et al.*, 2000; Heim *et al.*, 2000; Peres *et al.*, 2001; Crofford, 2002; Bohmelt *et al.*, 2005; Chang *et al.*, 2009; McEwen and Kalia, 2010). However, such apparent discrepancy may be due to uncontrolled differential stress-related activity at the time of sampling and/or possible comorbid conditions such as depression (Wingenfeld *et al.*,

2010). In the present study, both groups showed week-long stable levels of cortisol, and depressive moods were generally below the clinical threshold and statistically controlled by covariance. Thus, we are confident that the observed difference in cortisol does reflect an abnormally high stress-related activity in chronic back pain.

One of the main findings of the current study is that the level of basal cortisol and the clinical pain intensity of patients with chronic back pain are associated with increased pain-related responses in the anterior hippocampal formation. The hippocampal formation is a complex structure where various functions are distributed in different locations. It has been proposed that the dorsal regions (posterior in primates) are involved in learning and memory while the ventral regions (anterior in primates) are involved in anxiety-related behaviour (including aversive associative learning) and the regulation of the neuroendocrine stress response (Moser and Moser, 1998; Kjelstrup *et al*., 2002; Bannerman *et al*., 2004; Fanselow and Dong, 2010). Previous studies have revealed that lesions to the ventral hippocampus or to the ventral parahippocampal gyrus reduce fear avoidance and neuroendocrine stress responses (Kjelstrup *et al*., 2002), suppress the behavioural stress response (Schulz-Klaus, 2009), block aversive learning (Schulz *et al*., 2004) and the expression of unconditioned fear (Schulz-Klaus *et al*., 2005). It has been shown that the glucose metabolic rate in the amygdala and the anterior hippocampus are both predictive of anxious temperament in non-human primates, but only the metabolic activity in the hippocampus seemed heritable (Oler *et al*., 2010). Interestingly, the anxiety model proposed by Gray and McNaughton (2003) lends further support to the critical role of the septohippocampal system in anxiety. The model states that the septohippocampal system acts as a comparator contrasting the upcoming sensory information to the predicted perceptual world. In the case of a mismatch, the organism shifts from an 'automatic' to a 'controlled mode' to evaluate the potential threat associated with this prediction error. Together, these studies indicate that the anterior hippocampal formation is involved with the neuroendocrine stress response and plays a critical role in anxiety-like behaviours that could become more prominent in patients with chronic pain.

Additionally, the involvement of the hippocampal formation in the processing of pain has been well documented. Human pain imaging studies have revealed that a mismatch between actual and expected thermal sensations increases brain activity in the hippocampus and the parahippocampal gyrus (Ploghaus *et al*., 2000) and that exacerbation of thermal pain by anxiety reflects increased brain activity in the anterior parahippocampal gyrus (Ploghaus *et al*., 2001). A more recent study demonstrated that the hippocampal formation and the periaqueductal grey are related to the anticipation of pain (Fairhurst *et al*., 2007). This is interesting because nocebo hyperalgesic effects and the blocking of opioid analgesia by negative expectancy have both been related to activity in the hippocampal formation (Kong *et al*., 2008; Bingel *et al*., 2011). These effects are consistent with an involvement of the hippocampus and related structures in the anticipation of pain and the regulation of associated responses.

Our results further indicate that the anterior hippocampus activity explained a significant part of the individual variability in the chronic pain intensity reported before the scan. Several studies have shown that chronic pain is associated with modifications in the functional response of the hippocampal formation to experimental pain. For instance, patients suffering from somatoform pain disorders show increased brain responses to thermal pain in the parahippocampal gyrus (Gundel *et al*., 2008), and experimental pain-related responses in the hippocampus of these patients has been linked with daily physical complaints (Gondo *et al*., 2012). Patients with migraine have also been shown to display stronger thermal pain responses in the anterior median temporal lobe (Moulton *et al*., 2011). Interestingly, patients with high compared with low frequency of migraine attacks also showed smaller hippocampal volumes (but not different from control subjects), stronger brain activity and reduced functional connectivity in response to thermal pain in the hippocampus (Maleki *et al*., 2012). These findings are consistent with our results suggesting that inter-individual differences in hippocampal volumes and/or functional responses may be related to the patients' clinical profiles. Finally, previous studies have also shown that chronic back pain changes the ventral striatum response to the offset of phasic painful stimulations (Baliki *et al*., 2010) and that the functional connectivity between the limbic system and the medial prefrontal cortex during spontaneous pain fluctuations predicts the transition from sub-acute to chronic pain (Baliki *et al*., 2012). These findings support the theory that sub-acute pain states modify the emotional and motivational responses to pain within limbic structures, which may in turn contribute to chronicity (Apkarian *et al*., 2009).

It has also been proposed that the unpredictable recurrence of pain episodes (Borsook *et al*., 2012) and/or the spontaneous fluctuations in chronic pain states (Apkarian *et al*., 2009, 2011) generate anxiety, fear and disability that modulate activity within limbic structures involved in learning and memory. Animal models and functional MRI studies in humans have established that contextual conditioning and extinction involves the hippocampus (Phillips and LeDoux, 1992) and that the consolidation of memory encoded in an emotional context recruits the amygdala and the locus ceruleus, which modulates hippocampal and parahippocampal activity (Cahill *et al*., 1996; McGaugh *et al*., 1996; Hamann *et al*., 1999; Dolcos *et al*., 2004; Phelps, 2004; Sterpenich *et al*., 2006). Stress hormones have been shown to promote memory consolidation (Beckner *et al*., 2006; Schwabe *et al*., 2008), and the modulation of this process by stress seems to be especially pronounced for emotional and arousing material (Cahill *et al*., 1994; Kuhlmann and Wolf, 2006; Payne *et al*., 2007). For instance, unpleasant emotions induced by affective pictures have been shown to increase the parahippocampal gyrus responses to painful shocks (Roy, *et al*., 2009), and enhanced memory for emotional events has been associated with elevated cortisol induced by a painful cold pressor test (Cahill *et al*., 2003). Importantly, repeated stress and encoding of emotional memories can lead to chronic anxiety (Roozendaal *et al*., 2009) and facilitate fear conditioning (Conrad *et al*., 1999). Our finding that higher levels of basal cortisol in patients with chronic back pain were associated with increased pain-related parahippocampal gyrus activity is thus compatible with the proposition that chronic pain changes hippocampal activity, fostering

the formation of rich contextual memory traces of painful events (Mutso *et al*., 2012).

One question arising is how pain instigates and maintains a maladaptive stress response in patients with chronic back pain. Prolonged activation of the stress system has important effects on the body and the brain (McEwen, 1998*a*). The hippocampus is highly sensitive to the effects of prolonged exposure to stress hormones (Sapolsky, 1985; Sapolsky *et al*., 1985, 1990). For instance, several studies have shown that stress and glucocorticoids inhibit cell neurogenesis in the hippocampus (for a review see Mirescu and Gould, 2006). Using transgenetic animal models, it has been shown that hippocampal neurogenesis is necessary for the expression of the behavioural, endocrine and neuroregulatory aspects of stress responses (Snyder *et al*., 2011) and that impairments in hippocampal neurogenesis increase anxious behaviours (Ageta *et al*., 2008; Bergami *et al*., 2008; Revest *et al*., 2009) and learned helplessness behaviours (Ho and Wang, 2010). Hence, the impact of the stress is such that the hippocampal formation would no longer regulate the hypothalamic–pituitary–adrenal response (Jankord and Herman, 2008); thereby inducing a vicious cycle resulting in allostatic load. A recent study using the spared nerved injury model of neuropathic pain in mice revealed reduced neurogenesis and altered short-term synaptic plasticity in the hippocampal complex (Mutso *et al*., 2012). As expected, these abnormalities gave rise to anxiety-like behaviours and impaired the extinction of contextual fear conditioning. These findings are consistent with our results showing that abnormal stress responses in patients with chronic back pain is associated with altered brain processing of acute painful stimuli within the anterior region of the hippocampal formation.

Path analyses were finally used to evaluate and compare the empirical validity of two contrasting models of stress in chronic pain. Our data were in accordance with a model positing that smaller volume of the hippocampus may constitute a factor of vulnerability for developing a maladaptive stress response (Lyons *et al*., 2001; Gilbertson *et al*., 2002; Lupien, *et al*., 2007) when facing a sustained or recurrent physical stressor. Our results, however, did not support the model presuming that chronic pain intensity generates the maladaptive stress response modifying the structures and the functions of the hippocampal formation. This suggests that individuals with smaller hippocampal volumes may have increased risk of developing persistent pain. A recent study reported that bilateral hippocampal volumes are reduced in patients with chronic back pain and complex regional pain syndrome, but not in patients with osteoarthritis, compared with healthy individuals (Mutso *et al*., 2012). Although our results did not show a significant group difference in hippocampal volumes, it does not disprove this model because in all of these cross-sectional studies, some pain-free control subjects with small hippocampal volumes may be considered at risk of developing a maladaptive stress response if confronted with sustained or recurrent pain.

In the current study, the medication effects could have modulated the levels of cortisol, the hippocampal volumes, and functional responses in the hippocampal formation. However, ruling out a non-specific impact of medication by covariance did not change the results of the cortisol analyses, and a recent longitudinal study elegantly demonstrated that medication in chronic back pain was not associated with structural or functional changes in the evolution of the disease over 1 year following a subacute pain state (Baliki *et al*., 2012). Nevertheless, we cannot entirely rule out the possibility that some drugs may affect the adaptation response of the hypothalamic–pituitary–adrenal axis and alter the pain- and stress-related changes in the structure and function of the brain.

In conclusion, results from the present study are consistent with a model in which reduced hippocampal volumes may be a predisposition to the maladaptive stress response and a general state of allostatic load when facing prolonged pain. In turn, this state may further contribute to chronic pain intensity through a more general enhancement of parahippocampal responses to stressors, as illustrated here by the stronger responses to phasic experimental pain. This provides a strong support to the recently proposed stress model of chronic pain, which highlights the importance of the maladaptive stress responses in the transition from acute to chronic pain (Borsook *et al*., 2012). Increased recognition of the important role of dysregulated stress responses in the transition towards, and the maintenance of, chronic pain might have important implications for its prevention and management. Indeed, interventions aiming at stopping or reversing chronic pain-related allostatic load could prove to be as important as treating the source of nociception itself (Luine *et al*., 1994; Rodriguez-Raecke *et al*., 2009; Seminowicz *et al*., 2011). Cultivating mental states aimed at down-regulating the impact of stress (Davidson and McEwen, 2012) and further implementing clinical interventions promoting anxiety and stress reduction may be essential to prevent and relieve chronic pain.

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Supplementary material

Supplementary material is available at *Brain* online.

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