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Alterations in Endogenous Opioid Functional Measures in Chronic Back Pain

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The absence of consistent end organ abnormalities in many chronic pain syndromes has led to a search for maladaptive CNS mechanisms that may explain their clinical presentations and course. Here, we addressed the role of brain regional μ-opioid receptor-mediated neurotransmission, one of the best recognized mechanisms of pain regulation, in chronic back pain in human subjects. We compared μ-opioid receptor availability *in vivo* at baseline, during pain expectation, and with moderate levels of sustained pain in 16 patients with chronic nonspecific back pain (CNBP) and in 16 age- and gender-matched healthy control subjects, using the μ-opioid receptor-selective radioligand [^{11}C]carfentanil and positron emission tomography. We found that CNBP patients showed baseline increases in thalamic μ-opioid receptor availability, contrary to a previously studied sample of patients diagnosed with fibromyalgia. During both pain expectation and sustained pain challenges, CNBP patients showed regional reductions in the capacity to activate this neurotransmitter system compared with their control sample, further associated with clinical pain and affective state ratings. Our results demonstrate heterogeneity in endogenous opioid system functional measures across pain conditions, and alterations in both receptor availability and endogenous opioid function in CNBP that are relevant to the clinical presentation of these patients and the effects of opioid analgesics on μ-opioid receptors.

Introduction

Back pain is an exceedingly common condition affecting 70–85% of all people during their lifetimes (Andersson, 1999). Most patients recover from an episode of acute back pain, but in a sizable number of individuals it persists or frequently recurs, leading to chronicity. In the majority of these patients no pathoanatomical diagnosis can be established, and, likely due to poor understanding of the cause of the pain, the treatment options for chronic nonspecific back pain (CNBP) have remained unsatisfying (Kuijpers et al., 2011; Balagué et al., 2012). The lack of apparent end organ pain generators raises the question of whether the pathogenesis of CNBP involves hyperalgesia maintained by pain-related neuroplastic changes, otherwise primarily characterized at the level of primary sensory and dorsal horn neurons (Woolf and Salter, 2000). In patients with chronic back pain, the hypothesis of maladaptive CNS changes has received considerable support from recent imaging studies showing brain structural and functional alterations related to the individual pain characteristics (Apkarian et al., 2004; Baliki et al., 2011, 2012).

A large body of evidence demonstrates the central role of the brain μ-opioid receptor (MOR) system in pain regulation (Zubieta, 2008). The significance of brain MORs in the endogenous regulation of acute pain in humans has been highlighted by positron emission tomography (PET) studies demonstrating μ-opioid system activation during painful stimulation in multiple brain regions, including the periaqueductal gray, mid- and lateral thalamus, hypothalamus, nucleus accumbens, and amygdala, as well as in the insular, anterior cingulate, and prefrontal cortices (Zubieta et al., 2001, 2002; Bencherif et al., 2002). Consistent with its role in pain and stress suppression, pain-induced MOR activation was negatively correlated with the sensory and affective ratings of the pain experience, as well as with the negative affective state experienced during the challenge (Zubieta et al., 2001, 2002).

In patients with neuropathic pain, studies using the nonselective opioid receptor radioligand [^{11}C]diprenorphine have shown reductions in baseline opioid receptor availability when compared with healthy control (HC) subjects (Jones et al., 2004; Willoch et al., 2004; Maarrawi et al., 2007). Similar reductions, albeit with a different regional involvement, have been found in fibro-

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Table 1. Demographic data of HC subjects and patients with CNBP and FM

	CNBP		FM	
Demographic data	Patients ($N = 16$)	Control ($N = 16$)	Patients ($N = 19$)	Control ($N = 19$)
Age (years)				
Mean (SD)	38 (11)	36 (11)	45 (13)	43 (12)
Range	20–50	20–49	19–68	20–61
Sex (*N*, male/female)	8/8	8/8	0/19	0/19
Race/ethnicity [*N* (%)]				
African American	0	1 (6)	1 (5)	1 (5)
Caucasian	14 (88)	15 (94)	16 (84)	18 (95)
Hispanic	1 (6)	0	2 (11)	0
Native American	1 (6)	0	0	0
Pain duration (years)				
Median (IQR)	5.0 (3.8)		4.0 (6.5)	
Range	2–15		1–18	
Pain intensity (VAS)				
Mean (SD)	49 (20)		59 (17)	
Range	10–80		24–83	

Pain intensity was measured on a 0–100 visual analog scale (VAS). IQR, Interquartile range.

myalgia (FM) using [^{11}C]carfentanil (Harris et al., 2007). The reductions in opioid receptor availability in these chronic pain conditions have been interpreted as reflecting persistent endogenous opioid system activation and a downregulation of these receptor sites. Indeed, after successful treatment, recovery of opioid receptor availability has been shown in small samples of chronic pain patients in parallel with improvements in pain report (Jones et al., 1994, 1999).

Here, we examined whether CNBP is associated with dysfunctions in brain regional MOR neurotransmission, studied at baseline, during the expectation of pain, and during sustained, experimental muscle pain (Stohler and Kowalski, 1999). We hypothesized that alterations in brain MOR functional measures would be associated with clinical pain, affective state, and reductions in gray matter volume (Apkarian et al., 2004), as the latter may be related to deficits in pain regulatory mechanisms.

Materials and Methods

Subjects. We compared 16 CNBP patients [8 males and 8 females; mean (±SD) age, 38 ± 11 years] with 16 age- and gender-matched HC subjects (8 males and 8 females; mean age, 36 ± 11 years). For baseline receptor measure comparisons with the CNBP sample, we also examined data from a previously studied sample of 19 patients with FM (all females; mean age, 45 ± 13 years) and a separate age- and gender-matched HC group (all females; mean age, 43 ± 12 years; Table 1).

The study participants were as follows: right-handed nonsmokers, who did not use alcohol >10 units per week), did not perform physical exercise >1 h/d, and had no recreational drug use. The HC subjects had neither current somatic or psychiatric diseases nor a history of them, and were not taking any regular medications. All participants provided written informed consent before entering the study. The study protocol was in accordance with the Declaration of Helsinki, and was approved by the Institutional Review Board and the Radioactive Drug Research Committee.

The CNBP patients were recruited from a local pain clinic with the following main inclusion criteria: current average back or neck pain intensity between 3 and 8 on a 0–10 verbal rating scale (0 representing no pain, 10 representing the greatest pain intensity imaginable) with pain duration of at least 1 year; no current or past opioid use within the past year; and no history of psychiatric disease (except for mild depressive symptoms).

The CNBP patients were classified as having nonspecific back or neck pain (i.e., patients with specific diagnoses, nonspinal etiology, or radicular symptoms were not included in the study). The pain was localized in the low back in four patients, in the neck in four patients, and in larger areas in the back and neck in the remaining eight patients. The most intense pain was localized in the low back in 10 of 16 patients. The CNBP patients were using several different kinds of analgesic medications, with the most common being acetaminophen and nonsteroidal anti-inflammatory drugs (7 of 16 patients), muscle relaxants (3 of 16 patients), selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs; 3 of 16 patients), and gabapentin (3 of 16 patients).

The FM patients were part of a subject group that had previously participated in a PET study examining the effects of acupuncture on brain MOR availability (Harris et al., 2009). Seventeen of the 19 FM patients studied were part of our earlier study, for which baseline MOR nondisplaceable binding potential (BP_{ND}) data were available (Harris et al., 2007). The patients with FM met the American College of Rheumatology 1990 diagnostic criteria for FM for a minimum of 1 year (Wolfe et al., 1990), with a continued presence of pain on at least 50% of days. The analgesic medications used by the FM patients were SSRIs and SNRIs, which were used by a total of 10 of 19 patients. As in the CNBP sample, the FM patients had not been exposed to opioids for at least a year.

Pain expectation and sustained pain challenges. Performed only in the CNBP sample and its control group, the challenges consisted of a pain expectation condition (0.9% isotonic saline, administered 5–25 min after the start of the scan) and a painful condition (5% hypertonic saline, administered 45–65 min after the start of the scan). The participants were informed of these two conditions, but not of their order or laterality, allowing for the assessment of the effects of pain expectation. In the pain condition, a steady state of moderate muscle pain was maintained by the infusion of a small amount of 5% hypertonic saline into the relaxed left masseter muscle via a computer-controlled closed-loop system (Zhang et al., 1993; Stohler and Kowalski, 1999). This prolonged painful stimulation during the PET scan allows reliable determination of MOR BP_{ND} during the individual pain experience (Zubieta et al., 2001), while the feedback mechanism ensures that the pain experience is comparable across individual subjects and subject groups (Stohler and Kowalski, 1999). Moreover, because none of the volunteers had pain in the jaw or face area, choosing the masseter muscle for painful stimulation allowed for a better differentiation between experimental and clinical pain ratings, while at the same time introducing an increase in pain signal for the assessment of endogenous opioid system functional integrity.

The pain level was measured every 15 s by an electronic 0–100 visual analog scale (VAS), representing "no pain" to "the highest pain intensity imaginable." Here, a computer-controlled pump injected an average volume of 2.4 ± 1.0 ml into the masseter muscle, with a target of 40 VAS units; the actual average VAS rating in this study over the whole subject group was 31 ± 13 units. The isotonic saline solution was infused at the same rate as the hypertonic solution and was applied in the right masseter muscle, opposite to where pain was induced. In the data analysis, MOR activation during pain expectation and pain was defined as a reduction in MOR BP_{ND} during the experimental condition when compared with the corresponding time frame in the baseline PET scan. The pain challenge was completed by all HC subjects ($N = 16$) and 15 CNBP patients (i.e., one patient with CNBP did not complete the pain challenge).

At baseline and immediately after the isotonic and hypertonic saline infusions, the subjects completed the expanded form of the Positive and Negative Affect Schedule (PANAS; Watson and Clark, 1999) and the McGill Pain Questionnaire (MPQ; Melzack and Torgerson, 1971), which uses weighted word descriptors for the pain, and 0–100 VAS ratings of the pain intensity and unpleasantness. These measures, together with the average pain intensity ratings acquired every 15 s during the 20 min challenge, provided the measures of the individual pain experience during the challenges. Individual pain sensitivity was measured as the total volume of hypertonic saline solution (in milliliters) needed to keep the pain intensity in the target range.

Neuroimaging. The PET scans were acquired with a Siemens HR+ scanner in 3D mode with septa retracted and scatter correction [reconstructed full-width at half-maximum (FWHM) resolution, 5.5 mm in-plane and 5.0 mm axially]. Each participant was positioned comfortably in the PET scanner gantry, and an intravenous (antecubital) line was placed in the right arm. A light forehead restraint was placed to eliminate head movement during the scan. [^{11}C]carfentanil was synthesized at

high specific activity by the reaction of [^{11}C]methyliodide and a nonmethyl precursor, as described previously (Dannals et al., 1985; Jewett, 2001). An exposure of 15 ± 1.0 mCi (555 ± 37 MBq) was administered during the scan, with a mass of carfentanil injected of <0.05 μg/kg per scan. Fifty percent of the [^{11}C]carfentanil dose was administered as a bolus, and the remaining 50% as a continuous infusion for the remainder of the study. Twenty-eight frames of images were acquired over 90 min with an increasing duration (30 s up to 10 min). The HC subjects and CNBP patients underwent two 90 min PET scans with [^{11}C]carfentanil, one scan without any intervention for baseline MOR BP_{ND} assessment, and another scan for the measurement of MOR activation during pain expectation and experimental pain. Scan order was randomized and counterbalanced. The FM patients and the corresponding HC subjects participated only in the baseline PET scan.

PET images were reconstructed using iterative algorithms (brain mode; Fourier rebinning with ordered subsets-expectation maximization, four iterations, 16 subsets; no smoothing) into a 128 × 128 pixel matrix in a 28.8-cm-diameter field of view. Attenuation correction was performed through a 6 min transmission scan (^{68}Ge source) obtained before the PET study, and with iterative reconstruction of the blank/transmission data followed by segmentation of the attenuation image. Small head motions during emission scans were corrected by an automated computer algorithm for each subject before analysis, and the images were coregistered with the same software (Minoshima et al., 1993). After this, time points were decay corrected during reconstruction of the PET data.

Image data were transformed on a voxel-by-voxel basis into the following two sets of parametric maps: (1) a tracer transport measure (K_1 ratio); and (2) a receptor-related measure (i.e., BP_{ND}). A modified Logan graphical analysis (Logan et al., 1996) was used to calculate the tracer transport and BP_{ND}, obviating the need for arterial blood sampling. The occipital cortex, an area devoid of MORs, was used as the reference region. The slope of the Logan plot was used for the estimation of the BP_{ND}, a measure equal to $f_{ND}B_{max}/K_d$, where B_{max} represents the concentration of receptors and K_d their affinity for the radioligand, and the term f_{ND} refers to the concentration of free radiotracer in the extracellular fluid, which is considered to represent a constant and very small value.

Anatomical T1-weighted magnetic resonance imaging (MRI) data were acquired on a 3 T scanner (Signa LX; General Electric), using 3D inversion recovery-prepared fast spoiled gradient recalled acquisition (echo time = 1.9 ms; repetition time = 9.2 ms; inversion time = 500 ms; flip angle = 15°; bandwidth = 16 kHz; number of excitations = 1; 256 × 256 matrix; field of view = 25/26 cm; number of contiguous images = 154; isotropic voxel size = 1 mm).

MR, K_1, and BP_{ND} images were coregistered to each other and to the Montreal Neurological Institute (MNI) stereotactic atlas orientation using Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm/) and Matlab (MathWorks). MOR binding maps were normalized with the deformation field obtained from the normalization of the MR images to the MNI atlas orientation using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/). The accuracy of the coregistration and normalization algorithms was confirmed for each subject individually by comparing the transformed images to each other and to the MNI atlas template. Statistical parametric maps of group differences were generated with SPM8. No global normalization was applied to the data; therefore, the calculations presented are based on absolute $f_{ND}B_{max}/K_d$ estimates. Only regions with specific MOR BP_{ND} were included in the analyses (i.e., voxels with BP_{ND} values >0.1). To compensate for small residual anatomic variations across subjects and to improve signal-to-noise ratios, a 6 mm FWHM Gaussian filter was applied to each scan.

MRI data processing for voxel-based morphometry (VBM) analysis was performed using VBM8 toolbox, with default parameters for image processing. This included bias regularization, and tissue classification and registration using linear (affine) and nonlinear transformations within a unified model (Ashburner and Friston, 2005). High-dimensional spatial normalization was made to the DARTEL template (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra; Biomedical Image Analysis Group, Imperial College London, London, UK). The analysis was performed on the volume of gray matter, multiplied by the nonlinear, but not linear, components derived from the normalization matrix. This procedure preserves actual local gray matter volume, accounting for individual brain size (modulated gray matter volume). The realigned and normalized gray matter segments were smoothed with an 8 mm FWHM Gaussian kernel.

Data analysis. We examined the effects of CNBP and FM on baseline MOR BP_{ND} by applying a general linear model (GLM) on a voxel-by-voxel basis using SPM8. The PET data from the FM sample were processed and reanalyzed by the same person in parallel with the PET data from the CNBP sample, using the same assumptions and tools. Instead of simply comparing the new data from the CNBP sample to the previously published data on FM (Harris et al., 2007), this approach accounted for differences in data-processing streams and updated software that might potentially affect the results. The effects of CNBP on pain expectation and pain-induced MOR activation (defined as a reduction in the MOR BP_{ND} measure from baseline to nonpainful isotonic saline or painful hypertonic saline condition, respectively) were determined using a mixed-model ANOVA, with the diagnostic group (HC/CNBP) as the between-subject factor and the change in MOR BP_{ND} as the within-subject dependent variable. For all analyses, age was included as a nuisance covariate. For the pain challenges, we also included the average VAS pain ratings during the hypertonic saline infusion as a nuisance covariate to control for small differences between subjects. Significant effects were detected in the whole-brain voxel-by-voxel analysis using a statistical threshold that controls a type I error rate at $p < 0.05$ (false discovery rate corrected for multiple comparisons). These statistical thresholds were estimated using the Euler characteristic (Worsley et al., 1992), based on the number of voxels in the gray matter, image smoothness, and the extent of local changes (correction for cluster volume; Friston et al., 1991). The numerical values for MOR BP_{ND} were extracted from the image data by averaging the values of voxels contained in the area in which significant effects were obtained in the analyses.

We compared the gray matter of HC subjects and CNBP patients using a whole-brain VBM analysis with VBM8, covarying for age. Voxels with gray matter value <0.1 were excluded from the analysis. We used a height threshold of $p < 0.001$ (uncorrected) with an extent of 80 voxels (270 mm^3; corresponding to the expected number of voxels per cluster) across the whole brain for searching significant differences in brain gray matter volume, with a priori hypothesis of regional reduction of brain gray matter as demonstrated by earlier studies (Apkarian et al., 2004; Seminowicz et al., 2011; Ivo et al., 2013). Additionally, the clusters showing significant alterations in endogenous opioid function in the CNBP sample were used to perform a region of interest (ROI)-based VBM analysis. A multivariate GLM was used to evaluate the group effects on gray matter volume, where the diagnostic group was included as an independent variable, regional gray matter volume as a dependent variable, and age as a covariate.

Before performing statistical tests, the distribution of each variable was tested and a parametric or nonparametric test was selected for further analysis accordingly. SPSS version 19 (SPSS) was used for assessing group differences and planned correlations between MOR BP_{ND}, local gray matter volume and clinical measures. Statistical significance was set at $p < 0.05$.

Results

General characteristics of the patients and controls

Demographic data of the CNBP and FM patients and the corresponding HC subjects are shown in Table 1. There were no significant differences between the patients with CNBP and FM in terms of pain duration ($U_{(33)} = 150$, $Z = -0.03$, $p = 0.99$) or rating of current clinical pain intensity on a 0–100 VAS ($t_{(33)} = 1.5$, $p = 0.14$). Average age was slightly higher in the FM than the CNBP group, but this difference was not statistically significant ($t_{(33)} = 1.8$, $p = 0.08$).

Baseline MOR BP_{ND} in CNBP and controls

The CNBP patients showed significant increases in baseline thalamic MOR BP_{ND} compared with its control sample (Fig. 1), as follows: right thalamus, peak MNI coordinates (x, y, z) at (10, −12, 6): cluster size = 3070 mm^3, Z = 5.1, mean MOR BP_{ND} increase of 18%; left thalamus, peak MNI coordinates at (−9, −9, 4): cluster size = 1630 mm^3, Z = 4.1, mean MOR BP_{ND} increase of 23%. No significant clusters were found in the opposite contrast (HC > CNBP).

Regional MOR BP_{ND} in the CNBP group was negatively correlated with positive affect ratings, as measured with the PANAS, in both the right (r = −0.56, p = 0.03) and left thalamus (r = −0.66, p = 0.006; Fig. 2*A*,*B*). Negative affect (PANAS), duration of clinical pain, clinical pain ratings using VAS or MPQ total, and sensory and pain affect subscale scores were not significantly associated with changes in thalamic MOR BP_{ND} ($p > 0.4$).

To eliminate the possibility that the increases in MOR BP_{ND} in CNBP patients, not previously observed in other persistent pain conditions, could be due to technical factors such as changes in image-processing streams, previously acquired baseline data in FM patients was also analyzed against a matched control group (Harris et al., 2007). Contrary to the CNBP sample, patients with FM exhibited significantly lower MOR BP_{ND} than their matched HC sample in several brain areas. These included the thalamus bilaterally, with a peak in the right thalamus [peak MNI coordinates at (10, −15, 1): cluster size = 3510 mm^3, Z = 6.0, mean MOR BP_{ND} reduction of 22%], the nucleus accumbens bilaterally [right, (14, 16, −8): cluster size = 2390 mm^3, Z = 6.0, 19% reduction; left, peak MNI coordinates at (−10, 10, −11): cluster size = 1400 mm^3, Z = 4.8, 16% reduction], the left amygdala/left hippocampus [peak MNI coordinates at (−26, −9, −24): cluster size = 253 mm^3, Z = 4.8, 24% reduction], and the left insula [peak MNI coordinates at (−42, −3, 1): cluster size = 503 mm^3, Z = 4.4, 20% reduction; Fig. 3]. A small cluster was also found in the right amygdala, although it failed to reach significance after full correction for multiple comparisons [p = 0.09; peak MNI coordinates at (22, −6, −24): cluster size = 68 mm^3, Z = 4.4, 20% reduction]. The opposite contrast (HC < FM) did not show any significant effects.

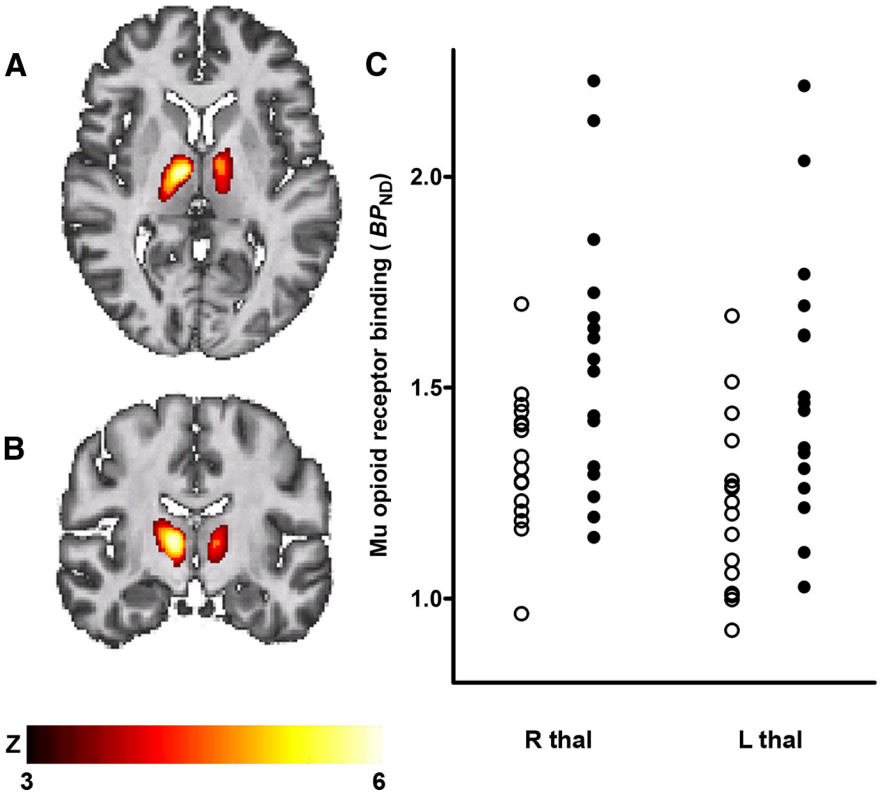

Figure 1. Increased thalamic MOR BP_{ND} in CNBP patients. Patients with CNBP demonstrated significant increases in MOR BP_{ND} in the right thalamus (R thal) and left thalamus (L thal). ***A***, ***B***, The thalamic clusters are shown in radiological convention in axial (Z = 5; ***A***) and coronal planes (Y = −14; ***B***). ***C***, Plots of average cluster MOR BP_{ND} values for HC subjects (empty circles, N = 16) and CNBP patients (filled circles, N = 16).

Function of MOR-mediated neurotransmission in CNBP

We also examined whether differences between the HC and CNBP groups would be observed for the capacity to activate endogenous opioid neurotransmission in response to pain expectation (pain is expected but not received) and experimental pain.

From the perspective of affective measures, there were no significant differences in affective state between HC and CNBP subjects, at baseline (PANAS positive: $F_{(1,29)}$ = 0.03, p = 0.87; PANAS negative: $F_{(1,29)}$ = 1.8, p = 0.19), during the expectation of pain (positive: $F_{(1,29)}$ = 1.8, p = 0.20; negative: $F_{(1,29)}$ = 2.0, p = 0.16), or during pain (positive: $F_{(1,29)}$ = 1.0, p = 0.32; negative: $F_{(1,29)}$ = 1.8, p = 0.19).

As would be expected with the use of the adaptive pain maintenance system used in the studies, no significant differences were observed between the HC subjects and CNBP patients in their average 0–100 VAS ratings acquired every 15 s during the experimental pain challenge (HC: 29 ± 8.4; CNBP: 33 ± 12; $t_{(27)}$ = −0.96, p = 0.35) or total MPQ score (HC: 23 ± 12; CNBP: 24 ± 11; $t_{(28)}$ = −0.22, p = 0.83), confirming a similar experiential state in both groups. The total amount of hypertonic saline needed to maintain the pain experience at target levels was, however, different: CNBP patients required significantly less hypertonic saline than HC subjects to maintain pain (average amount of hypertonic saline: 2.7 ± 0.84 ml for HC subjects; 1.8 ± 0.92 ml for CNBP patients; $t_{(25)}$ = 2.6, p = 0.02), consistent with the presence of generalized hyperalgesia in the patient group.

A mixed-model ANOVA on a voxel-by-voxel basis revealed significant group (HC/CNBP) × condition (pain expectation/pain) interactions. During the pain expectation condition, we detected a significant interaction in the left amygdala, whereby patients with CNBP demonstrated lower endogenous opioid system activation (ΔBP_{ND}) than HC subjects [peak MNI coordinates at (−20, −6, −17): cluster size = 560 mm^3, Z = 4.2; Fig. 4*A*,*B*]. During experimental sustained muscle pain, we also found a significant interaction in the same area, again with lower MOR activation in CNBP patients, compared with HC subjects [peak MNI coordinates at (−26, −13, −12): cluster size = 446 mm^3, Z = 4.6; Fig. 4*C*]. We did not detect significant group × condition interactions for the opposite contrasts (HC < CNBP).

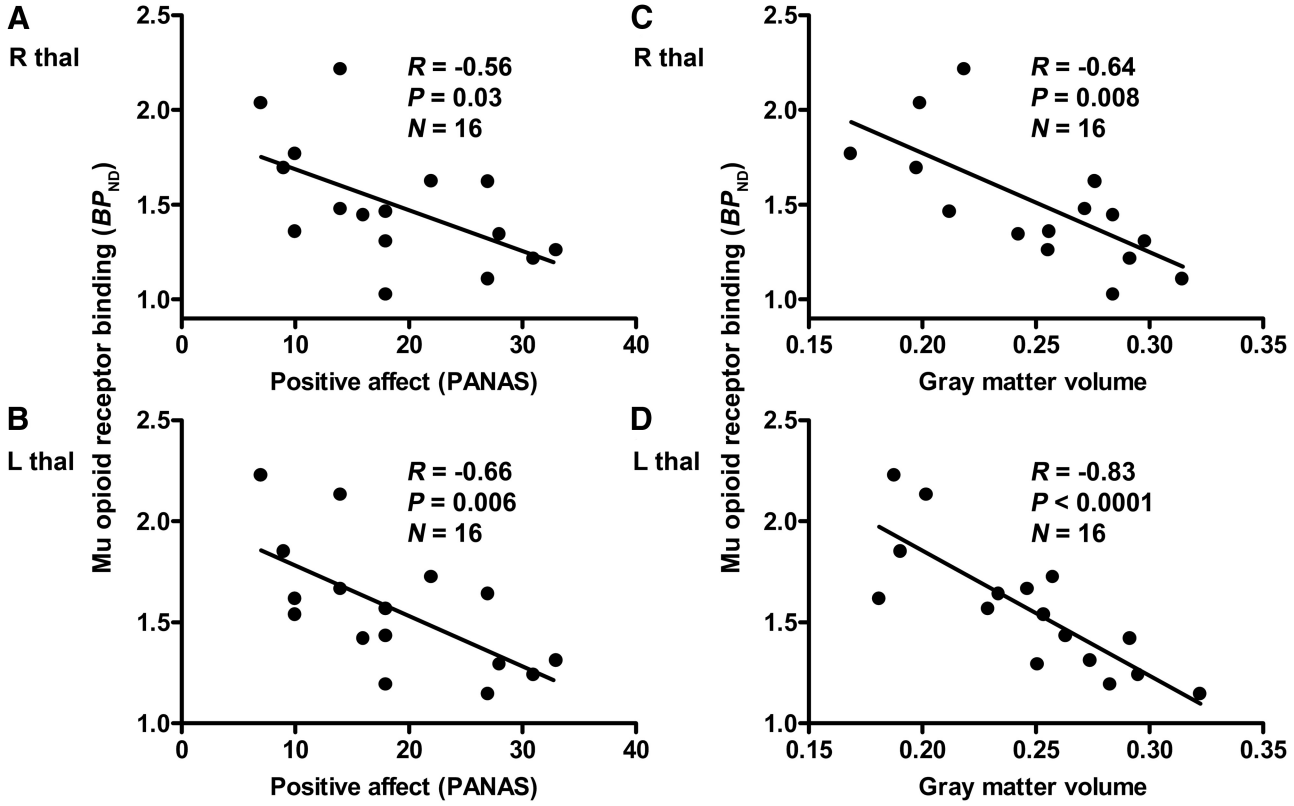

Figure 2. Associations between thalamic MOR BP_{ND} and positive affect and gray matter in CNBP patients. ***A***, ***B***, Significant negative correlations were found between MOR BP_{ND} and positive affect as measured by PANAS in the right thalamus (R thal; ***A***) and left thalamus (L thal; ***B***). ***C***, ***D***, The thalamic MOR BP_{ND} was also negatively correlated with gray matter volume in the right (***C***) and left thalamus (***D***).

Because the increased thalamic MOR BP_{ND} in CNBP at baseline may be related to a reduced endogenous opioid release in this region, we performed an additional mixed-model ANOVA that was spatially limited to the bilateral thalamic clusters showing increased MOR BP_{ND} at baseline. This analysis found reduced MOR activation in the CNBP group in the thalamus, bilaterally, during the pain challenge, but not in the pain expectation condition [right thalamus, peak MNI coordinates at (16, −24, 3): cluster size = 135 mm^3, $Z = 3.1$; left thalamus, peak MNI coordinates at (−9, −7, 0): cluster size = 672 mm^3, $Z = 3.4$]. No associations were found between the pain-induced MOR activation and baseline MOR BP_{ND} in these clusters, although a trend toward significant negative correlation was observed in the left thalamus (right thalamus: $p = 0.3$; left thalamus: $r = -0.33$, $p = 0.07$). Again, no significant group × condition interactions were found for the opposite contrasts (HC < CNBP).

We then examined the relationships between the magnitude of MOR activation during the two experimental conditions and ratings of experimental and clinical pain, as well as affective state. The activation of MOR neurotransmission in CNBP patients in the left amygdala during pain expectation was positively correlated with PANAS positive affect ratings during that condition ($r = 0.52$, $p = 0.046$). During the pain challenge, MOR system activation in the left amygdala was negatively correlated with back pain VAS intensity ($r = -0.59$, $p = 0.02$) and VAS unpleasantness scores ($r = -0.55$, $p = 0.04$), but not with back pain MPQ ratings or ratings of experimental pain ($p > 0.2$). Also, a positive correlation was observed between the magnitude of MOR activation and PANAS positive affect scores during the pain challenge ($r = 0.54$, $p = 0.04$). No relationships were detected between the MOR activation in the thalamic clusters and VAS or MPQ pain ratings ($p > 0.2$) or PANAS positive and negative affect scores ($p > 0.2$). These data then indicate the presence of a deficit in endogenous opioid neurotransmission in the left amygdala that is related to the clinical presentation of these patients from both emotional and pain perspectives, observed in both pain expectation and experimental pain challenges.

Voxel-based morphometry

An examination of gray matter volumes in HC and CNBP volunteers was conducted to determine whether the alterations in measures of endogenous opioid function (increases in MOR availability *in vivo* and reductions in MOR activation during pain expectation or experimental pain challenge) would be associated with reductions in gray matter volume, as the latter has been reported in CNBP samples (Apkarian et al., 2004). Conversely, for small regions such as the thalamus or the amygdala, potential increases in gray matter volume could explain the increases in MOR availability observed in CNBP as an artifact of measurement, due to lesser partial volume-averaging effects with surrounding structures with low MOR availability such as white matter or CSF.

Global brain gray matter volumes did not differ between HC subjects and CNBP patients (HC: 631 ± 75 ml; CNBP: 613 ± 69 ml; $F_{(1,29)} = 0.30$, $p = 0.59$). A VBM analysis showed significant regional reductions in gray matter volume in CNBP, compared with HC subjects: left inferior frontal gyrus, peak MNI coordinates at (−44, 21, 16): cluster size = 527 mm^3, Z = 4.5; medial aspect of the left superior frontal gyrus, peak MNI coordinates at

(−4, 33, 48): cluster size = 1220 mm^3, $Z = 4.0$; and lateral aspect of the left superior frontal gyrus, peak MNI coordinates at (−14, 18, 49): cluster size = 581 mm^3, $Z = 3.8$. In CNBP patients, gray matter volume in the left superior frontal gyrus (−4, 33, 48) was negatively correlated with PANAS negative affect scores ($r = -0.51$, $p = 0.04$). However, these prefrontal gray matter changes were not related to clinical pain ratings at the time of imaging ($p > 0.2$).

An ROI-based analysis was also used to compare gray matter volume in HC and CNBP samples for the regions where alterations in endogenous opioid mechanisms were observed. Significant gray matter loss was detected in the thalamus (right: HC, 0.29 ± 0.04; CNBP, 0.25 ± 0.04; $F_{(1,29)} = 6.6$, $p = 0.02$; left: HC, 0.27 ± 0.03; CNBP, 0.25 ± 0.04; $F_{(1,29)} = 4.8$, $p = 0.04$), but not in the left amygdala ($p > 0.7$). We found a significant negative correlation between gray matter volume and MOR BP_{ND}, but not MOR system activation during pain expectation or pain, in CNBP patients in both thalamic clusters (right: $r = -0.64$, $p = 0.008$; left: $r = -0.83$, $p < 0.0001$; Fig. 2*C*,*D*). The gray matter volume in these thalamic regions was also positively correlated with PANAS positive affect scores (right: $r = 0.58$, $p = 0.02$; left: $r = 0.66$, $p = 0.006$), but not with clinical pain ratings ($p > 0.2$).

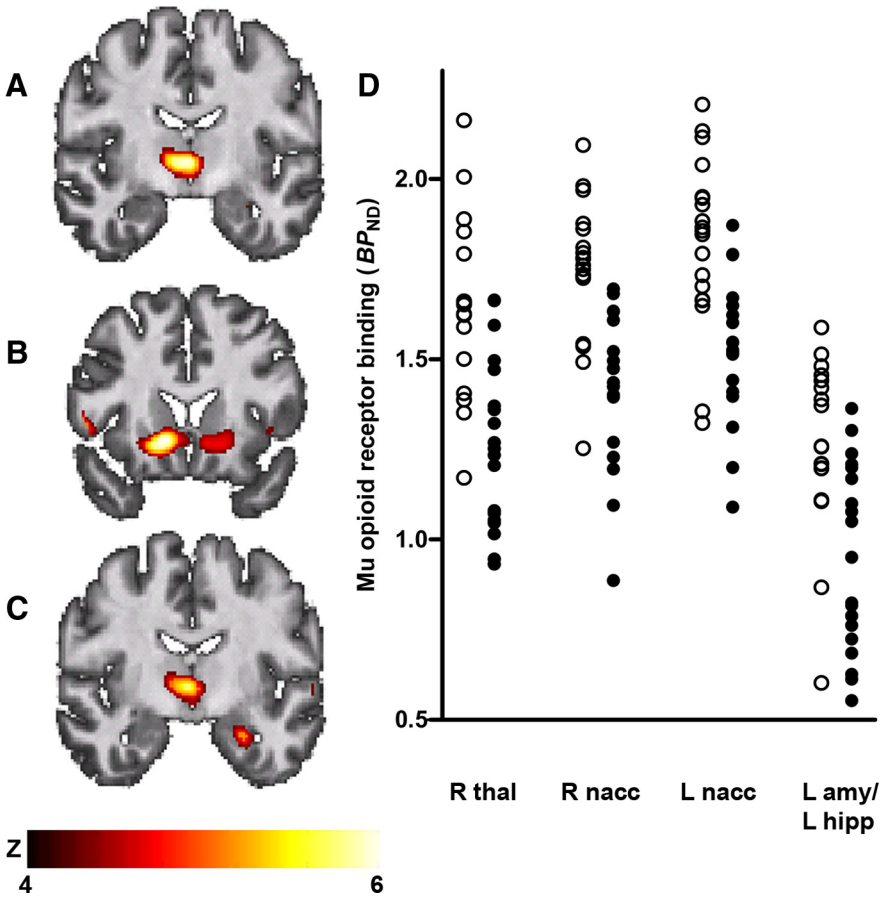

Figure 3. Reductions in brain regional MOR BP_{ND} in patients with FM. ***A–C***, Significant reductions in MOR BP_{ND}, compared with controls, were observed in the right thalamus (R thal; $Y = -11$; ***A***), the right nucleus accumbens (R nacc) and left nucleus accumbens (L nacc; $Y = 15$; ***B***), the left amygdala (L amy)/left hippocampus (L hipp; $Y = -9$; ***C***), and the left insula. ***D***, Plots of average cluster MOR BP_{ND} values for HC subjects (empty circles, $N = 19$) and FM patients (filled circles, $N = 19$).

Discussion

We report regionally specific alterations in measures of MOR-mediated neurotransmission in CNBP. At baseline, patients with CNBP showed increases in MOR BP_{ND} in the thalamus bilaterally when compared with HC subjects. These increases contrasted with reductions in regional MOR BP_{ND} in FM as well as reductions in overall opioid receptor availability reported in other forms of persistent pain (Jones et al., 2004; Willoch et al., 2004; Maarrawi et al., 2007). Baseline thalamic MOR BP_{ND} in CNBP was negatively associated with gray matter volume in the same regions, and also negatively with positive affect.

In a second set of analyses, we examined the activation of endogenous opioid neurotransmission in response to pain expectation and sustained pain. An ROI approach was adopted for the thalamus, in the areas where increases in receptor availability were detected. We observed lower levels of thalamic MOR system activation during the pain challenge in CNBP in comparison with the HC group, suggesting that the upregulatory changes observed for baseline MOR BP_{ND} are potentially secondary to a deficit in presynaptic endogenous opioid function in response to changes in pain signal. We also used a brain-wide, voxel-by-voxel comparison to compare the activation of the MOR system between the CNBP patients and the HC sample. During both experimental conditions, we found a significant group × condition interaction in the left amygdala, showing lower magnitudes of MOR system activation in the CNBP group with respect to the HC group. Consistent with the suppressive effect of MOR-mediated neurotransmission on pain but also affective regulation and stress responses, we showed that amygdala MOR activation during pain expectation was associated with the maintenance of positive affect during the challenge, while MOR activation in the same region during sustained pain was also negatively correlated with back pain ratings.

Significant alterations in MOR functional measures at baseline and during pain were found in the thalamus, a region with a critical role in conveying pain signals from the spinothalamic tract to higher-order cortical areas where pain is both represented and regulated. Reductions in blood flow to this region, as well as spontaneous neuronal hyperactivity have been reported in both patients and animal models of persistent neuropathic pain, suggesting reduced inhibitory neural activity (Guilbaud et al., 1990; Rinaldi et al., 1991; Iadarola et al., 1995; Paulson et al., 2002). In line with the hypothesis that reduced thalamic inhibition is present in persistent pain states, studies in patients with trigeminal neuropathy have found thalamic gray matter loss, dysregulated thalamocortical connectivity, and reduced concentrations of GABA, a major neurotransmitter mediating fast inhibition, in the thalamus (Gustin et al., 2011; Henderson et al., 2013). Although a considerable amount of severe chronic back pain may be neuropathic, at present it is not known whether these findings can be extended to CNBP (Schmidt et al., 2009). Nonetheless, our findings support the concept of reduced thalamic inhibition in chronic pain by showing that deficits in MOR activation may also lead to reduced inhibition of the pain signal and contribute to

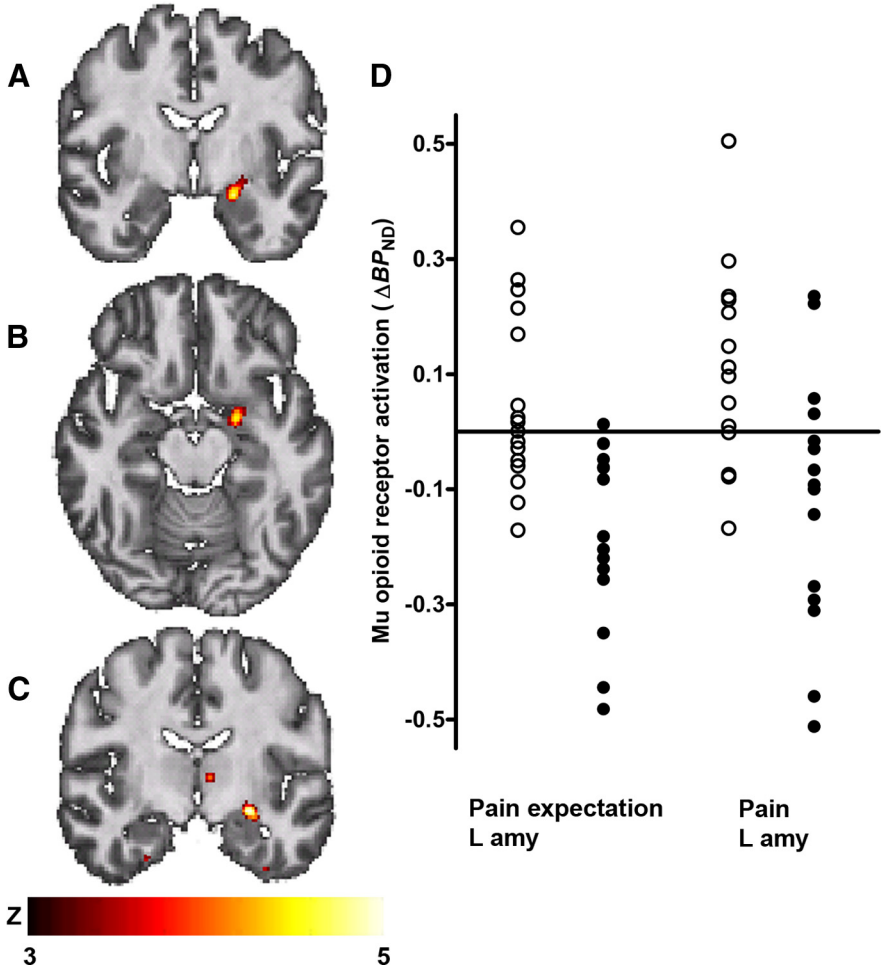

Figure 4. Differential effects of pain expectation and sustained experimental muscle pain on MOR BP_{ND} in HC subjects and CNBP patients. ***A–C***, Significant differences in MOR activation were observed between HC and CNBP samples in the left amygdala (L amy) during pain expectation, shown in coronal ($Y = -6$; ***A***) and axial planes ($Z = -15$; ***B***), and during sustained pain, shown in coronal plane ($Y = -12$; ***C***). ***D***, Plots of average MOR BP_{ND} changes for HC subjects (empty circles, $N = 16$) and CNBP patients (filled circles, $N = 15$).

enhanced pain, possibly by affecting pain-related thalamocortical circuitry (Carr and Bak, 1988; Brunton and Charpak, 1998; Baliki et al., 2011).

The amygdala, the region where reductions in endogenous opioid activity were observed during both pain expectation and experimental pain, has a central role in regulating emotional processes including acute and persistent pain (Neugebauer et al., 2004). Of the amygdala nuclei that participate in pain regulation, particularly the basolateral and lateral nuclei present high levels of MOR expression (Mansour et al., 1987), and these nuclei are also considered areas of interface and integration of multiple sensory inputs with emotional significance to the organism (Phelps and LeDoux, 2005). MOR receptor activation in this region has been shown to modulate descending inputs to the periaqueductal gray and rostral ventromedial medulla, reducing the magnitude of the pain signal (Helmstetter et al., 1998; McGaraughty and Heinricher, 2002). In healthy human subjects, the activation of endogenous MOR-mediated neurotransmission in the amygdala during sustained pain has been associated with reductions in pain intensity ratings (Zubieta et al., 2001). Here, we observe that deficits in the capacity to activate this neurotransmitter system during pain expectation and pain were associated with lower capacities to maintain positive affect, and with higher ratings of clinical pain intensity and its unpleasantness in CNBP patients. These findings are certainly consistent with the integrative and regulatory effects of the amygdala MORs on emotionally relevant sensory stimuli, including pain.

The regional deficits in MOR-mediated neurotransmission reported here are likely to participate in the amplification of the pain signal and in persisting back pain. These mechanisms may also lead to an increased sensitivity to experimental pain and to an increased risk of developing another chronic pain disorder, both features previously reported in CNBP and together implying dysfunctional pain control (Giesecke et al., 2004; Von Korff et al., 2005; O'Neill et al., 2007; Wiesinger et al., 2007). The hypothesis of dysfunctional endogenous opioid pain control in CNBP is supported by data showing low endogenous opioid concentrations in the CSF in chronic pain samples (Puig et al., 1982; Lipman et al., 1990), and an association between abnormal opioid system responses to naloxone, an opioid antagonist, and pain symptoms (Bruehl et al., 2004, 2010). At present, it is not possible to ascertain whether those deficits are secondary to the presence of persistent pain, or whether interindividual variations in the function of this neurotransmitter system represent a risk factor for the development of chronic back pain conditions, as has been suggested by other authors (Bruehl and Chung, 2006). Prospective studies after acute injury would be required to answer that particular question. Nevertheless, the data presented here demonstrate that endogenous opioid system markers do differ across persistent pain conditions and may represent a tool for the study of differences in the presentation and treatment response of varying forms of persistent pain. For example, reductions in the availability of opioid receptors in neuropathic pain and in FM have been suggested to underlie the poor response to opioid medications in those conditions (Jadad et al., 1992; Cherny et al., 1994; Dadabhoy and Clauw, 2006). In the case of CNBP, where mean increases in receptor availability were observed, however with substantial interindividual variability, opioid agonists are likely to induce varying levels of response, depending on receptor concentrations. The latter could contribute to a broader range of pain control efficacy by opioid medications, but also to individual variations in the physical dependence, rewarding, and tolerance effects of those drugs, which are also mediated by MORs (Sora et al., 1997).

The lower magnitudes of MOR-mediated neurotransmission observed in CNBP during both painful and nonpainful challenges may indicate the presence of reduced inhibition of nociceptive input, which in turn may lead to structural changes such as cortical reorganization (Flor et al., 1997) and gray matter loss in cortical and subcortical areas (Apkarian et al., 2004). Indeed, in the analyses of gray matter volume we were able to replicate the earlier finding of gray matter loss in the prefrontal cortex, which was correlated with negative affect (Apkarian et al., 2004; Ruscheweyh et al., 2011; Seminowicz et al., 2011; Ivo et al., 2013).

We also found significant gray matter reductions in the bilateral thalamus, in accordance with some (Apkarian et al., 2004; Ivo et al., 2013), but not all previous studies in chronic back pain patients (Schmidt-Wilcke et al., 2006). Thalamic gray matter loss was correlated with increases, interpreted as compensatory, in MOR availability in the same region, while the amygdala, where reductions in endogenous opioid system function were substantial and detected in brain-wide analyses, heavily innervates prefrontal cortical regions (Porrino et al., 1981). This suggests that alterations in MOR neurotransmission may be contributing not only to the sensory and affective presentation of CNBP patients, but also to the neurodegenerative effects of persistent pain on brain gray matter. The contribution of the gray matter changes to pain chronification is yet to be determined due to mixed evidence as to whether these alterations are reversible or, at least partly, irreversible (Grachev et al., 2000; Rodriguez-Raecke et al., 2009; Gustin et al., 2011; Seminowicz et al., 2011).

The present report identifies alterations in what is arguably the main central antinociceptive system, the endogenous opioids and MORs, in patients with CNBP. These alterations were related to the sensory and affective elements of the pain experience, but also to the emotional state, all of which are thought to impact disability and the clinical course of persistent pain conditions. Perhaps more important from a diagnostic and therapeutic perspective, the directionality of the effects observed at the receptor availability level differed from those previously reported in other persistent pain conditions. Future studies appear warranted to further delineate differences across persistent pain states, given the relevance of these findings to the effects of opioid drugs, and potentially the complications associated with their administration, such as abuse and dependence. The possibility that interindividual variations in endogenous opioid system function may predispose individuals to the development of persistent pain after acute injury would also require further exploration.

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