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ORIGINAL ARTICLE

Dissociable Roles of Cerebral μ-Opioid and Type 2 Dopamine Receptors in Vicarious Pain: A Combined PET–fMRI Study

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Abstract

Neuroimaging studies have shown that seeing others in pain activates brain regions that are involved in first-hand pain, suggesting that shared neuromolecular pathways support processing of first-hand and vicarious pain. We tested whether the dopamine and opioid neurotransmitter systems involved in nociceptive processing also contribute to vicarious pain experience. We used in vivo positron emission tomography to quantify type 2 dopamine and μ-opioid receptor (D_2R and MOR, respectively) availabilities in brains of 35 subjects. During functional magnetic resonance imaging, the subjects watched short movie clips depicting persons in painful and painless situations. Painful scenes activated pain-responsive brain regions including anterior insulae, thalamus and secondary somatosensory cortices, as well as posterior superior temporal sulci. MOR availability correlated negatively with the haemodynamic responses during painful scenes in anterior and posterior insulae, thalamus, secondary and primary somatosensory cortices, primary motor cortex, and superior temporal sulci. MOR availability correlated positively with orbitofrontal haemodynamic responses during painful scenes. D_2R availability was not correlated with the haemodynamic responses in any brain region. These results suggest that the opioid system contributes to neural processing of vicarious pain, and that interindividual differences in opioidergic system could explain why some individuals react more strongly than others to seeing pain.

Key words: carfentanil, empathy, neurotransmitters, observed pain, raclopride

Introduction

Capacity for vicarious experiences is a fundamental aspect of human social behavior. For example, seeing others in pain often triggers in the observer strong unpleasant sensations resembling first-hand pain. Neuroimaging studies have established that some of the brain circuits involved in nociceptive processing are also engaged during vicarious pain (Singer et al. 2004, 2006; Jackson et al. 2005, 2006; Lamm et al. 2011) (but see also Krishnan et al. 2016). Both experiences are typically associated with activation of brain regions that are associated with the affective component of pain, namely anterior cingulate cortex (ACC) and anterior insulae (Rainville et al. 1997; Price 2000; Lamm et al. 2011), and sometimes also somatosensory cortices associated with the sensory dimension of pain (Singer et al. 2004). Moreover, haemodynamic activity in anterior insulae and ACC correlate with the observer's empathic concerns (Singer et al. 2004; Saarela et al. 2007), whereas activity in the somatosensory regions may relate to vicarious simulation of the intensity of observed pain (Bufalari et al. 2007). Such vicarious simulation of others' emotional and bodily states presumably mimics the negative emotional experience associated with pain perception, which may promote understanding others' painful feelings and facilitate helping behavior (Hein et al. 2010).

The similarities of haemodynamic activity during first-hand and vicarious pain experiences suggest that their neurochemical bases might also be similar. Endogenous opioid system and especially the μ-opioid receptor (MOR) is intimately involved in modulation of emotions (Nummenmaa and Tuominen 2017) and pain (Heinricher and Fields 2013). Human positron emission tomography (PET) studies have shown that noxious stimuli activate the MOR system, most consistently in ventral striatum, thalamus, and amygdala (Zubieta et al. 2001, 2002, 2003; Bencherif et al. 2002; Smith et al. 2006; Scott et al. 2007, 2008; Wager et al. 2007). Furthermore, the magnitude of MOR activation in thalamus and dorsal ACC (dACC) correlates with negative emotional experiences associated with pain (Zubieta et al. 2001), suggesting that differences in opioidergic neurotransmission in these regions may explain interindividual variation in the experience of pain. The endogenous opioid system could also affect how humans respond to seeing others in pain. Opioid antagonist naltrexone increases self-reported pain ratings and unpleasant experiences when seeing others in pain (Rutgen, Seidel, Silani, et al. 2015). Similarly, placebo analgesia that is supported by the opioid system (Pecina and Zubieta 2015) reduces the negative emotional experience of the observers, and this reduction is also reflected as attenuated brain responses related to pain's negative affect (Rutgen, Seidel, Riecansky, et al. 2015; Rutgen, Seidel, Silani, et al. 2015).

In addition to the opioid system, the endogenous dopamine system and particularly the type 2 dopamine receptor (D_2R) is also involved in nociceptive processing. In rats, pharmacological facilitation of the striatal D_2R system suppresses, and its blockade increases, pain behavior (Lin et al. 1981; Magnusson and Fisher 2000; Taylor et al. 2003). In humans, PET studies have revealed enhanced dopaminergic processing in dorsal striatum during pain (Scott et al. 2006, 2007, 2008; Wood et al. 2007). Dopamine release in striatum correlates with both sensory and affective components of pain (Scott et al. 2006; Martikainen et al. 2015), and striatal D_2R availability correlates negatively with pain sensitivity (Hagelberg et al. 2002; Pertovaara et al. 2004; Martikainen et al. 2005; Scott et al. 2006). Despite its well-established role in nociceptive processing, the role of the D_2R system in vicarious pain remains unexplored.

In sum, several lines of evidence suggest functional similarities between brain mechanisms underlying first-hand and vicarious pain. Even though both MOR and D_2R are involved in first-hand pain, it remains unresolved whether they also support vicarious pain. Here we tested this hypothesis using multimodal neuroimaging. We used PET with radioligands selective for MOR ([^{11}C]carfentanil) and D_2R ([^{11}C]raclopride) to estimate neuroreceptor availability in vivo. Subsequently, the subjects underwent a functional magnetic resonance imaging (fMRI) study, during which they watched videos of humans experiencing varying levels of pain. We found that seeing others in pain activates several nociceptive brain regions, including secondary somatosensory cortices (S2), thalamus and anterior insulae, as well as prefrontal cortices (PFC) and superior temporal sulci (STS). Critically, baseline cerebral MOR availability was negatively correlated with haemodynamic responses to others' pain in sensorimotor regions, anterior insulae, and STS. Positive correlations were found in the orbitofrontal cortex (OFC). In contrast, we found no connection between D_2R receptor availability and responses to others' pain. Our data suggest that MORs, but not D_2Rs, contribute significantly to vicarious pain.

Materials and Methods

Participants

The study protocol was approved by the ethics board of the hospital district of Southwest Finland, and the study was conducted in accordance with the Declaration of Helsinki. We studied altogether 36 women (mean ± SD age: 44 ± 10 years, range: 19–58). One subject was removed from the sample because her MRI revealed a previously nondiagnosed neurological disease. Exclusion criteria were lack of compliance, alcohol consumption exceeding 8 weekly doses, substance abuse determined by interview and blood tests, a history of or current psychiatric or neurological disease, current medication affecting the central nervous system, as well as standard PET and MRI exclusion criteria. Each subject participated in 3 imaging sessions. The 2 PET scans were separated, on average, by 4 days, while the PET and MRI scans were separated, on average, by 3 weeks. Subjects signed ethics-committee-approved informed consent forms, and they were compensated for their time and travel costs.

PET Imaging and Analysis

Figure 1 shows an overview of the experimental design and data analysis. PET data were acquired with the GE Healthcare Discovery TM 690 PET/CT scanner in Turku PET Center. Radiotracer production has been described previously (Karlsson et al. 2015). After a bolus of intravenous radioligand injection (251 ± 10 MBq of [^{11}C]carfentanil and 251 ± 24 MBq of [^{11}C]raclopride), radioactivity in the brain was measured with the PET camera for 51 min with increasing frame length (3 × 1 min, 4 × 3 min, 6 × 6 min) using in-plane resolution of 3.75 mm. The [^{11}C]carfentanil and [^{11}C]raclopride PET scans were performed on separate days. The subjects were lying in a supine position throughout the studies. Data were corrected for dead-time, decay and measured photon-attenuation, and dynamic PET scans were reconstructed with vendor-provided standard MRAC and MRP methods (Alenius and Ruotsalainen 1997).

Anatomical MR images (1 mm^3) were acquired with Philips Gyroscan Intera 1.5 T scanner using T1-weighted sequences. PET images were realigned frame-to-frame and coregistered with the anatomical and functional MR images (see below). Subject-specific regional time–activity curves (TACs) were then calculated for each region of interest (ROI; see below). Medial occipital cortex and cerebellum were used as reference regions in [^{11}C]carfentanil and [^{11}C]raclopride analyses, respectively. To ensure that the ROIs would not contain nondisplaceable

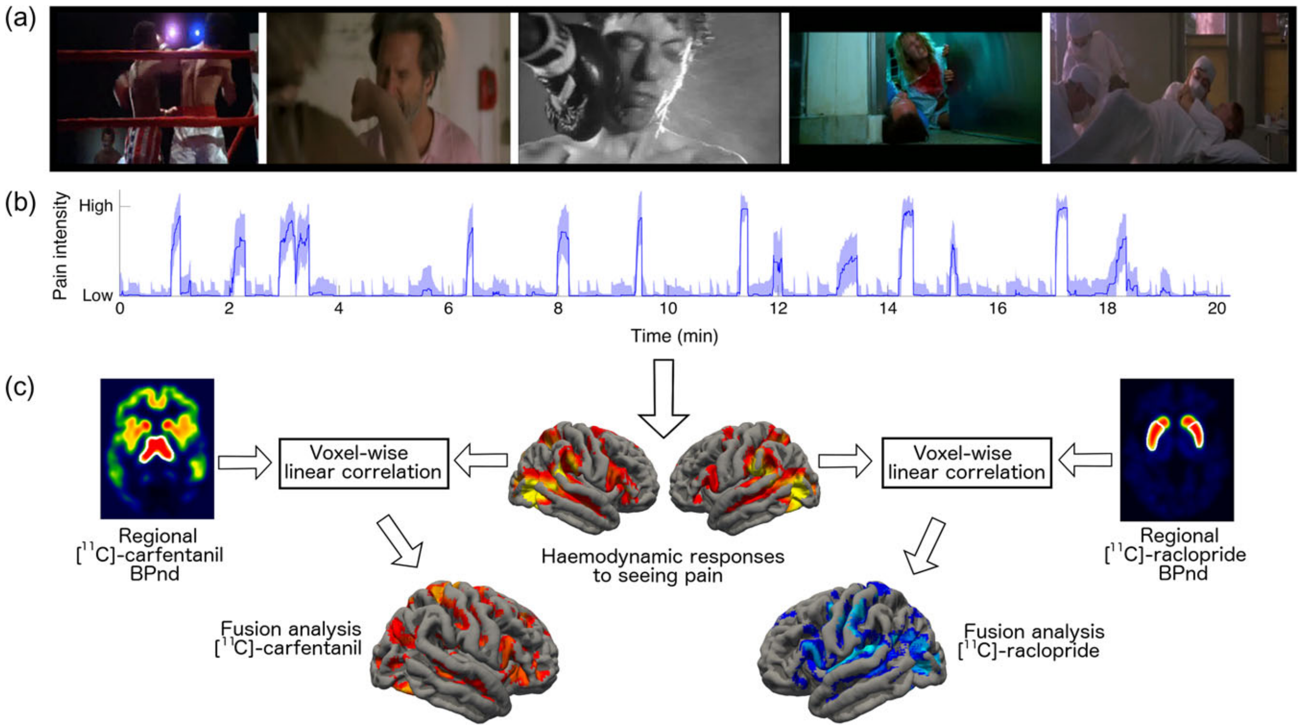


Figure 1. Experimental design and overview of the PET–fMRI fusion analysis. (*a*) Subjects watched 102 short movie clips depicting humans in painful and painless situations. (*b*) Dynamic pain ratings (means ± SEM) for the stimulus array were obtained in a separate condition. (*c*) Pain ratings were first used to predict subjectwise BOLD responses to seeing others in pain in the general linear model. Regional [^{11}C]carfentanil and [^{11}C]raclopride binding potentials were then used to predict the voxelwise regressor coefficients between pain ratings and BOLD to evaluate the contribution of MOR and D_2R on vicarious pain.

binding, voxels whose signal did not exceed mean reference tissue signal intensity were also excluded from the ROIs.

Simplified reference tissue model (SRTM; Lammertsma and Hume 1996) was used to model the tracer kinetics. Tracer binding was expressed in terms of BP_{ND}, which is the ratio of specific to nondisplaceable binding. ROI-level modeling was performed using an in-house implementation of SRTM. Voxel-level fitting was done using the basis-functions implementation of SRTM (Gunn et al. 1997); the parameter bounds for θ_3 (θ_3^{min}(carfe) = 0.06/min, θ_3^{max}(carfe) = 0.6/min; θ_3^{min}(raclo) = 0.082/min, θ_3^{max}(raclo) = 0.6/min) were chosen so that averaging over voxel-level BP_{ND}-estimates within a ROI would produce the same result as first calculating a ROI-specific TAC and then fitting the model to that.

ROI Selection

Tracer binding was quantified in 13 anatomical ROIs involved in nociceptive and socioemotional processing (Singer et al. 2004; Lahnakoski et al. 2012; Karlsson et al. 2015): amygdala, caudate, dACC, rostral ACC, thalamus, anterior insula, posterior insula, posterior STS, putamen, nucleus accumbens, precentral gyrus, postcentral gyrus, and OFC. The ROIs are visualized on top of tracer-specific mean binding potential maps in Figure 2. Specific [^{11}C]raclopride binding is low in many of these regions; we nevertheless included them in the analysis for the sake of consistency. The ROIs were derived separately for each subject using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/); such ROIs yield consistent estimates with those delineated manually (Johansson et al. 2016). Posterior STS was delineated manually because FreeSurfer does not segment it, and because corresponding anatomical ROI does not exist in atlases.

fMRI Data Acquisition and Analysis

Experimental Design and Stimuli

The experimental design has been previously described in detail (Lahnakoski et al. 2012) and is summarized in Figure 1. In brief, the stimuli consisted of 102 video clips (mean duration 12 s; to shorten the experiment we dropped 35 videos from the original design) extracted from mainstream Hollywood movies. The videos contained humans involved in painful and painless situations, as well as filler scenes without humans (scenery, inanimate objects, etc.). The clips were presented without breaks in a fixed order, and total duration of the experiment was 21 min. During the fMRI scan, the participants were asked not to move and watch the videos attentively as they would be watching a movie or TV.

Dynamic ratings for the intensity of vicarious pain seen in the videos were obtained in a separate condition from 17 participants (10 females) not participating in the neuroimaging study. Pearson correlation coefficient between mean male and female ratings was 0.96, and consequently ratings from both sexes were used in this study. While viewing each video clip, the participants used a mouse to move a small cursor at the right side of the screen up and down to indicate how much pain (from "not at all" to "highest imaginable pain") the character in the clip was experiencing. Ratings were sampled at 5 Hz, averaged across subjects, downsampled to one TR and finally convolved with the canonical HRF to provide regressors for the general linear model (GLM) analysis. The online rating tool is freely available at https://version.aalto.fi/gitlab/eglerean/dynamicannotations.

To control for low-level sensory confounds, we computed moment-to-moment mean luminosity and sound intensity from the video and audio tracks in 200 ms time windows using root mean square of the raw luminosity and intensity values

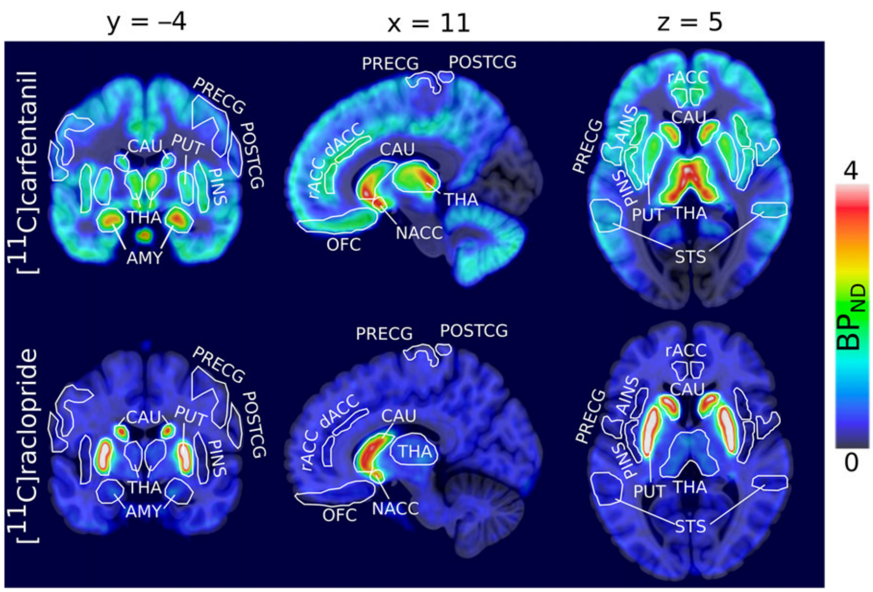


Figure 2. The regions of interest (ROIs) used in the study overlaid on study-specific mean binding potential maps of [^{11}C]carfentanil and [^{11}C]raclopride. AMY, amygdala; CAU, caudate; dACC, dorsal anterior cingulate cortex; rACC, rostral anterior cingulate cortex; THA, thalamus; AINS, anterior insula; PINS, posterior insula; STS, posterior superior temporal sulcus; PUT, putamen; NACC, nucleus accumbens; PRECG, precentral gyrus; POSTCG, postcentral gyrus; and OFC, orbitofrontal cortex. The ROIs are shown in MNI space for visualization purposes, in actual analyses the ROIs were obtained separately for each subject using FreeSurfer.

for each time window. These time series were convolved with HRF and further downsampled to one TR, orthogonalized with respect to the vicarious pain regressor, and finally included in the model as nuisance covariates (see below).

Image Acquisition and Analysis

Whole-brain functional data were acquired with T2*-weighted echo-planar imaging sequence, sensitive to the blood-oxygen-level-dependent (BOLD) signal contrast (TR = 3300 ms, TE = 50 ms, 90° flip angle, 192 mm FOV, 64 × 64 reconstruction matrix, 62.5 kHz bandwidth, 4.0 mm slice thickness, 33 interleaved slices acquired in ascending order without gaps). Altogether 390 functional volumes were acquired. Anatomical images (1 mm^3 resolution) were acquired using a T1-weighted sequence (TR 25 ms, TE 4.6 ms, flip angle 30°, 280 mm FOV, 256 × 256 reconstruction matrix).

Functional data were preprocessed with FSL using the FEAT pipeline: slice-time correction, motion correction, 2 steps coregistration to MNI 152 2-mm template, and 8-mm spatial smoothing using Gaussian kernel. Low-frequency drifts in data were estimated and removed using a 240-s-long Savitzky–Golay filter (Cukur et al. 2013). To control for head motion confounds, motion parameters were regressed out (Friston et al. 1996).

GLM was fitted to the data using SPM12 (version 6225; http://www.fil.ion.ucl.ac.uk/spm/). The design matrix consisted of 3 regressors: moment-to-moment ratings of pain in the videos, as well as their moment-to-moment brightness, and loudness of the audio track. Subjectwise contrast images were generated for main effect of vicarious pain intensity. The contrast images were then subjected to a second level analysis to reveal brain regions processing vicarious pain at the population level. As it has been recently argued that typical parametric statistical inference methods may produce inflated false-positive rates in neuroimaging (Eklund et al. 2016), we used nonparametric inference as implemented in SnPM13 toolbox (http://warwick.ac.uk/snpm).

PET–fMRI Fusion Analysis

To test for the contribution of MOR and D_2R on vicarious pain, the voxel-wise BOLD responses were modeled with ROI-wise [^{11}C]carfentanil and [^{11}C]raclopride binding potentials in each ROI separately using linear regression analysis (Fig. 1). We also investigated whether global MOR and D_2R availabilities, calculated as the within-subject mean binding potentials for both tracers (i.e., averaged across the ROIs shown in Fig. 2), predict haemodynamic responses during vicarious pain. In all analyses, 10 000 permutations were used to estimate the null distribution, primary threshold was set to $P = 0.05$, and only the clusters surviving FWE-correction ($P < 0.05$) are reported. In a complementary methodological approach, we also extracted subjectwise BOLD responses to seeing others in pain in the 13 ROIs described above. Subsequently, MOR and D_2R availabilities in these ROIs were correlated with the regional BOLD responses to characterize the regional interactions between MOR, D_2R, and BOLD responses while seeing others in pain. This enabled visualizing in which regions binding potential estimates best predicted the BOLD responses.

Results

Main Effect for Vicarious Pain

We first modeled the BOLD data with the vicarious pain intensity regressor to reveal brain regions activated when seeing others in pain. This analysis (Fig. 3) replicated our prior results using the same protocol (Lahnakoski et al. 2012), revealing bilateral activation clusters in regions including anterior insulae and S2 that are related to first-hand and vicarious experience of pain (Singer et al. 2004, 2006; Jackson et al. 2005, 2006; Hein et al. 2010; Lamm et al. 2011; Morelli et al. 2014). Additional clusters were observed in primary motor cortices, as well as PFC and STS that are linked to empathy and intention representation in general (Nummenmaa and Calder 2009; Morishima et al. 2012; Rameson et al. 2012). The unthresholded *t*-map is available at http://neurovault.org/collections/BHAGGQLK/.

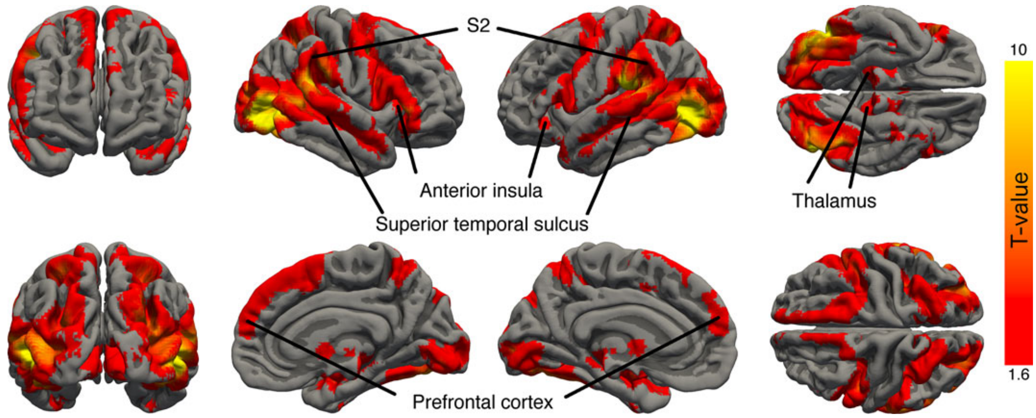


Figure 3. Brain regions whose responses were linearly dependent on the intensity of pain seen in the movies. The data are thresholded at $P < 0.05$, FWE corrected at cluster level. Colourbar indicates the t-statistic range. The results are shown on fsaverage pial surface from FreeSurfer.

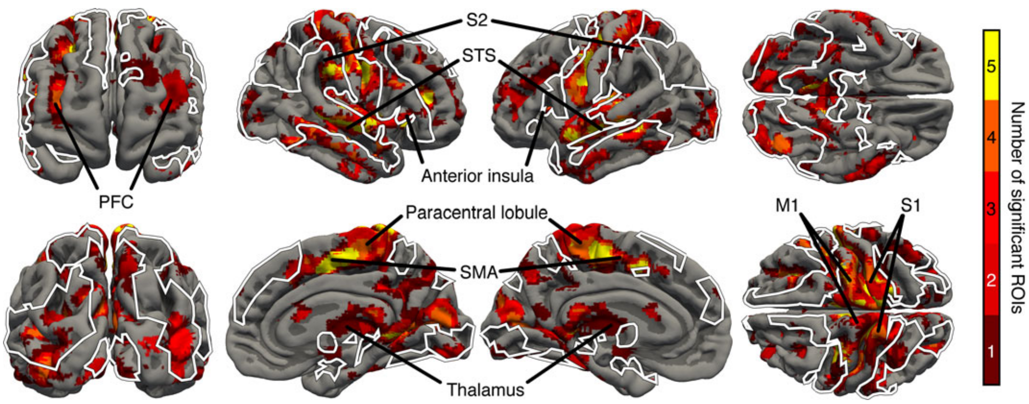


Figure 4. Cumulative maps showing the number of ROIs (out of 13) whose [^{11}C]carfentanil BP_{ND} was correlated ($P < 0.05$, FWE-corrected at cluster level) with BOLD responses to seeing others in pain in each brain area. White outline shows regions where BOLD signal correlated with the intensity of vicarious pain (Fig. 3). PFC, prefrontal cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; M1, primary motor cortex; SMA, supplementary motor area; STS, superior temporal sulcus. The results are shown on fsaverage pial surface from FreeSurfer.

Fusion Analysis of PET and fMRI Data

We next tested how regional MOR and D_2R availabilities influence BOLD responses to seeing others in pain. In the full-brain GLM analyses, regional MOR availabilities in caudate, OFC, posterior insula, postcentral gyrus, STS, putamen, and rostral ACC were negatively correlated with the BOLD responses in thalamus, sensorimotor regions (S1, S2, M1, paracentral lobule, SMA), anterior insulae, lateral PFC, and STS (Figs 4 and 5*a*; see http://neurovault.org/collections/BHAGGQLK/ for the unthresholded *t*-maps). Results using the global MOR availability closely mirror these findings (Supplementary Fig. 1). These effects overlapped most clearly with the main effect of vicarious pain in anterior insula, somatosensory cortex, thalamus, STS, and striatum (total overlap 28%; see Supplementary Fig. 2). Positive correlations did not exceed the a priori statistical threshold in any region. However, at more lenient thresholding ($P < 0.05$ uncorrected, cluster size >1000) MOR availability in thalamus was correlated with the BOLD responses in OFC (Fig. 5*b*). While not found using the global MOR availability, this effect was also detected in the a priori ROI analysis (see below). Finally, D_2R availability did not predict BOLD responses to seeing others in pain in any brain region, even when more lenient statistical threshold (uncorrected $P < 0.05$, cluster size >3000) was used.

These results were corroborated by the ROI-wise correlation analyses (Fig. 6), which revealed that cerebral MOR availability (particularly in caudate, putamen, and rostral ACC) correlates negatively with BOLD responses (e.g., in postcentral gyrus, posterior insula, and precentral gyrus) to seeing others in pain. In addition, cerebral MOR availability correlated positively with the BOLD responses in OFC. This association pattern was remarkably consistent across the ROIs in which [^{11}C]carfentanil BP_{ND} was estimated in (Fig. 6). Again, D_2R availability was not correlated with BOLD responses.

Discussion

Our results show that haemodynamic responses during vicarious pain depended on cerebral MOR but not D_2R availability in regionally selective manner: MOR availability was negatively correlated with BOLD responses in sensorimotor (S1, S2, M1, paracentral lobule, SMA) regions as well as parts of the emotion circuit (insula, thalamus), whereas positive correlation was found in OFC involved in multiple socioemotional functions, such as mentalizing and social bonding (Powell et al. 2012; Schurz et al. 2014; Nummenmaa et al. 2015). These data provide the first in vivo evidence about the neuromolecular pathways involved in processing of vicarious pain, suggesting that MORs

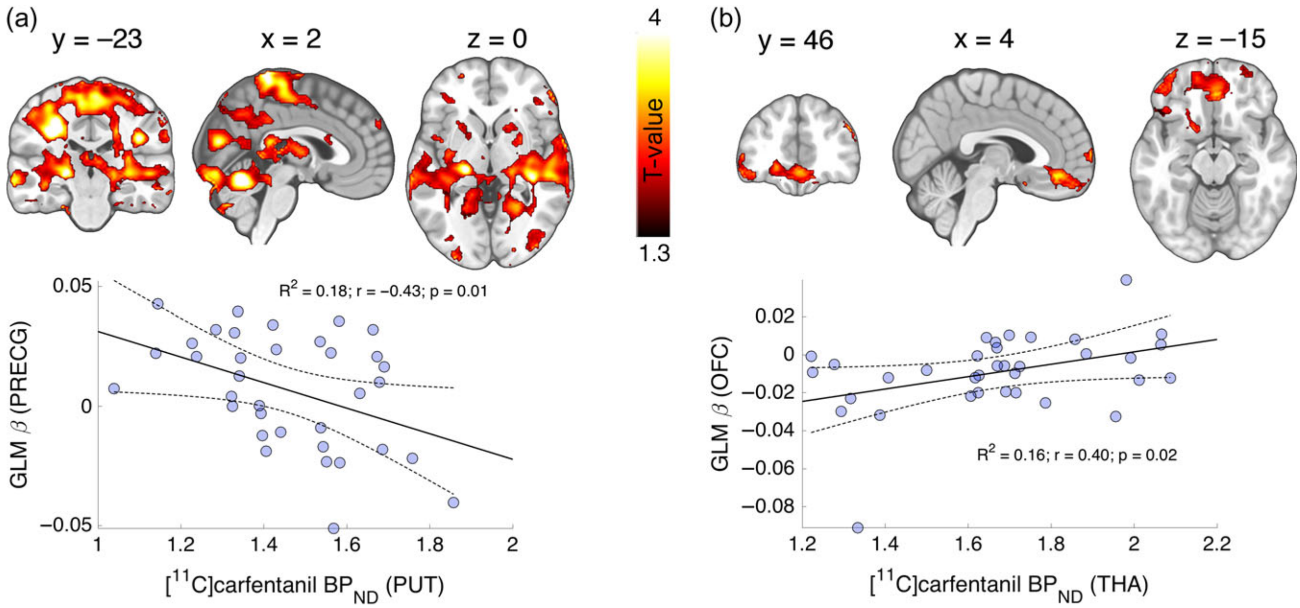
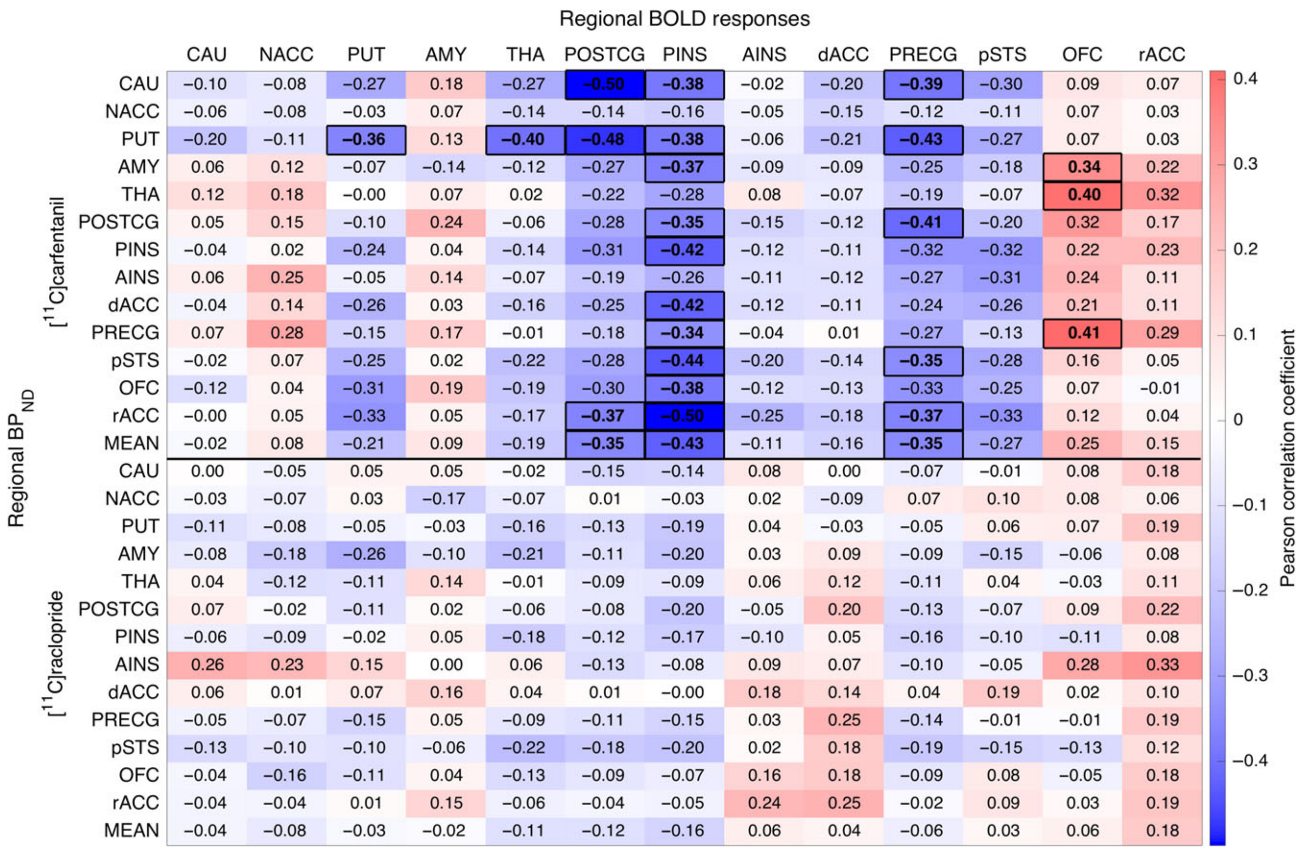


Figure 5. (*a*) Brain regions showing negative correlation between MOR availability in putamen and BOLD responses during vicarious pain ($P < 0.05$, FWE-corrected at cluster level). PCG, precentral gyrus. (*b*) Brain regions showing positive correlation between thalamic MOR availability and BOLD responses in orbitofrontal cortex during vicarious pain ($P < 0.05$, uncorrected, cluster size >1000). The scatterplots show least-square regression lines with 95% confidence intervals. Data are shown for thalamus and putamen because MORs are abundantly expressed in these regions and because there BP_{ND} had consistent associations with the BOLD responses. The results are shown on MNI-152 template mni152_2009bet.nii.



Tracer	Regional BPND	CAU	NACC	PUT	AMY	THA	POSTCG	PINS	AINS	dACC	PRECG	pSTS	OFC	rACC
[11C]carfentanil	CAU	−0.10	−0.08	−0.27	0.18	−0.27	**−0.50**	**−0.38**	−0.02	−0.20	**−0.39**	−0.30	0.09	0.07
	NACC	−0.06	−0.08	−0.03	0.07	−0.14	−0.14	−0.16	−0.05	−0.15	−0.12	−0.11	0.07	0.03
	PUT	−0.20	−0.11	**−0.36**	0.13	**−0.40**	**−0.48**	**−0.38**	−0.06	−0.21	**−0.43**	−0.27	0.07	0.03
	AMY	0.06	0.12	−0.07	−0.14	−0.12	−0.27	**−0.37**	−0.09	−0.09	−0.25	−0.18	**0.34**	0.22
	THA	0.12	0.18	−0.00	0.07	0.02	−0.22	−0.28	0.08	−0.07	−0.19	−0.07	**0.40**	0.32
	POSTCG	0.05	0.15	−0.10	0.24	−0.06	−0.28	**−0.35**	−0.15	−0.12	**−0.41**	−0.20	0.32	0.17
	PINS	−0.04	0.02	−0.24	0.04	−0.14	−0.31	**−0.42**	−0.12	−0.11	−0.32	−0.32	0.22	0.23
	AINS	0.06	0.25	−0.05	0.14	−0.07	−0.19	−0.26	−0.11	−0.12	−0.27	−0.31	0.24	0.11
	dACC	−0.04	0.14	−0.26	0.03	−0.16	−0.25	**−0.42**	−0.12	−0.11	−0.24	−0.26	0.21	0.11
	PRECG	0.07	0.28	−0.15	0.17	−0.01	−0.18	**−0.34**	−0.04	0.01	−0.27	−0.13	**0.41**	0.29
	pSTS	−0.02	0.07	−0.25	0.02	−0.22	−0.28	**−0.44**	−0.20	−0.14	**−0.35**	−0.28	0.16	0.05
	OFC	−0.12	0.04	−0.31	0.19	−0.19	−0.30	**−0.38**	−0.12	−0.13	−0.33	−0.25	0.07	-0.01
	rACC	−0.00	0.05	−0.33	0.05	−0.17	**−0.37**	**−0.50**	−0.25	−0.18	**−0.37**	−0.33	0.12	0.04
	MEAN	−0.02	0.08	−0.21	0.09	−0.19	**−0.35**	**−0.43**	−0.11	−0.16	**−0.35**	−0.27	0.25	0.15
[11C]raclopride	CAU	0.00	−0.05	0.05	0.05	−0.02	−0.15	−0.14	0.08	0.00	−0.07	−0.01	0.08	0.18
	NACC	−0.03	−0.07	0.03	−0.17	−0.07	0.01	−0.03	0.02	−0.09	0.07	0.10	0.08	0.06
	PUT	−0.11	−0.08	−0.05	−0.03	−0.16	−0.13	−0.19	0.04	−0.03	−0.05	0.06	0.07	0.19
	AMY	−0.08	−0.18	−0.26	−0.10	−0.21	−0.11	−0.20	0.03	0.09	−0.09	−0.15	−0.06	0.08
	THA	0.04	−0.12	−0.11	0.14	−0.01	−0.09	−0.09	0.06	0.12	−0.11	0.04	−0.03	0.11
	POSTCG	0.07	−0.02	−0.11	0.02	−0.06	−0.08	−0.20	−0.05	0.20	−0.13	−0.07	0.09	0.22
	PINS	−0.06	−0.09	−0.02	0.05	−0.18	−0.12	−0.17	−0.10	0.05	−0.16	−0.10	−0.11	0.08
	AINS	0.26	0.23	0.15	0.00	0.06	−0.13	−0.08	0.09	0.07	−0.10	−0.05	0.28	0.33
	dACC	0.06	0.01	0.07	0.16	0.04	0.01	−0.00	0.18	0.14	0.04	0.19	0.02	0.10
	PRECG	−0.05	−0.07	−0.15	0.05	−0.09	−0.11	−0.15	0.03	0.25	−0.14	−0.01	−0.01	0.19
	pSTS	−0.13	−0.10	−0.10	−0.06	−0.22	−0.18	−0.20	0.02	0.18	−0.19	−0.15	−0.13	0.12
	OFC	−0.04	−0.16	−0.11	0.04	−0.13	−0.09	−0.07	0.16	0.18	−0.09	0.08	−0.05	0.18
	rACC	−0.04	−0.04	0.01	0.15	−0.06	−0.04	−0.05	0.24	0.25	−0.02	0.09	0.03	0.19
	MEAN	−0.04	−0.08	−0.03	−0.02	−0.11	−0.12	−0.16	0.06	0.04	−0.06	0.03	0.06	0.18

Figure 6. Results of the ROI analysis. Rows show ROIs for PET data, columns for fMRI data. Colourbar indicates the correlation between the regional BP_{ND} and BOLD-fMRI-betas for each region. Statistically significant associations are shown in boldface and black outline. AMY, amygdala; CAU, caudate; dACC, dorsal anterior cingulate cortex; NACC, nucleus accumbens; OFC, orbitofrontal cortex; POSTCG, postcentral gyrus; PRECG, precentral gyrus; PUT, putamen; rACC, rostral anterior cingulate cortex; THA, thalamus; AINS, anterior insula; PINS, posterior insula; STS, superior temporal sulcus.

but not D_2Rs contribute to the experience. Even though recent studies suggest that functional (as measured with fMRI) neural basis of first-hand and vicarious pain experience differ (Krishnan et al. 2016), our study highlights that they however rely on the same neurotransmitter mechanism.

Opioidergic Basis of Vicarious Pain Experience

Intensity of seen pain in the videos correlated positively with BOLD signals in thalamus, anterior insulae, S2, superior PFC, as well as precuneus, occipital cortex and pSTS, thereby replicating our previous findings using the same experimental setup (Lahnakoski et al. 2012). The results also accord with prior work showing that these regions are consistently engaged during vicarious pain (Singer et al. 2004; Jackson et al. 2005; Saarela et al. 2007). Thalamus, insula, S2 and PFC are also activated by noxious stimuli (Tracey and Mantyh 2007) and likely underlie the affective mirroring of pain. On the contrary, PFC and STS are important regions linked to empathy and representing others' internal states in general (Morishima et al. 2012; Rameson et al. 2012).

Our main new finding is the negative correlation between cerebral MOR availability and BOLD responses to seeing others in pain, observed in sensorimotor regions (S1, S2, M1, SMA, paracentral lobule), anterior insula, posterior insula, PFC, and STS. This pattern was consistent across the ROIs where MOR availabilities were estimated, likely reflecting the widespread spatial autocorrelation of MOR availability across the brain (Tuominen et al. 2014). These results extend the similarities between first-hand and vicarious pain to neuromolecular level by showing that the endogenous opioid system—a key modulator of nociceptive processing (Heinricher and Fields 2013)—also affects vicarious pain.

Prior PET studies have linked lowered MOR availability, specifically in striatum and frontal cortex (subgenual ACC, ventromedial PFC), to heightened pain sensitivity (Hagelberg et al. 2012; Pecina et al. 2015). Our data show that low MOR availability in these same regions (caudate, putamen, rostral ACC) is also associated with heightened BOLD responses to seeing others in pain. Importantly, prior studies have established that an individuals sensitivity to first-hand pain predicts their sensitivity to vicarious pain (Danziger et al. 2006; Derbyshire et al. 2013). Similarly, pharmacological work has confirmed that pain suppression decreases and pain facilitation increases the negative emotional experiences associated with seeing others in pain (Bos et al. 2015; Rutgen, Seidel, Riecansky, et al. 2015; Rutgen, Seidel, Silani, et al. 2015; Mischkowski et al. 2016). Altogether these observations suggest that individuals who have low threshold to noxious stimuli tend to react strongly also to others' pain, and that endogenous opioid system provides a neuromolecular link between these 2 phenomena.

In contrast to the sensorimotor responses, the BOLD responses in OFC were positively correlated with cerebral MOR availability. OFC has an important role in mentalizing (Schurz et al. 2014), and orbitofrontal cortical volume correlates with an individual's social network size (Powell et al. 2012). While not directly linked to mentalizing, MORs have been linked to various forms of prosocial behavior, including pair bonding and sociability (Panksepp et al. 1980; Moles et al. 2004; Nummenmaa et al. 2015; Karjalainen et al. 2016; Manninen et al. 2017). Thus, future studies could dissociate whether orbitofrontal brain activity during pain observation reflects mentalizing, and whether MORs regulate this function.

Haemodynamic responses during vicarious pain in the regions processing the affective dimension of pain reflect the empathic concerns of the observer (Singer et al. 2004; Saarela et al. 2007), while the somatosensory regions represent the intensity of observed pain (Bufalari et al. 2007). The presently observed negative correlation between cerebral MOR availability and BOLD responses in both systems thus suggests that individuals with high baseline MOR availability may have attenuated emotional responses to others' pain, and in general they may be less likely to catch others' negative emotions. Indeed, individuals with high cerebral MOR availability have reduced regional cerebral blood flow during negative emotions (Liberzon et al. 2002). Furthermore, opioidergic neurotransmission in dACC, thalamus, and basal ganglia reduces the negative emotional experience associated with first-hand pain (Zubieta et al. 2001). Together with the present data, these observations indicate that high cerebral MOR availability may constitute a resiliency factor that protects individuals from excessive personal distress triggered by negative social signals, such as witnessing others in pain. Future studies could test whether individuals with high cerebral MOR availability are less concerned about others' distress, and whether they would be less willing to engage in helping others—a property shown to correlate with activation of the anterior insula (Hein et al. 2010).

No Evidence for D_2R Involvement in Vicarious Pain

In contrast to the MOR, we found no correlation between D_2R availability and BOLD responses to seeing others in pain. Abundant evidence shows that the D_2Rs process nociceptive signals (Scott et al. 2006, 2007, 2008) and striatal D_2R availability correlates positively with an individual's sensitivity to sensory pain (Hagelberg et al. 2002; Pertovaara et al. 2004; Martikainen et al. 2005; Scott et al. 2006). However, our data suggest that dopaminergic processing of first-hand pain may be decoupled from vicarious pain. It is possible that the D_2R activation does not relieve subjective discomfort as effectively as MOR does activation (Taylor et al. 2016) and therefore has weaker effects on how individuals perceive others' pain.

Limitations

The main limitation of our study is that we did not measure BOLD responses to first-hand pain and could thus not directly compare the contribution of MOR and D_2R systems on first-hand versus vicarious pain. However, prior studies have consistently shown that MOR availability reliably predicts pain sensitivity (Hagelberg et al. 2012; Pecina et al. 2015). Another limitation is that we only scanned females and our results thus may not generalize to males. Our subject selection was, however, designed to maximize statistical power of the study. First, because the spatial distribution of MOR availability is different in in females and males (Gabilondo et al. 1995; Zubieta et al. 1999, 2002), it was better to include subjects of one sex only. Second, females report higher pain ratings to others' pain (Robinson and Wise 2003) and experience and portray stronger emotions and emotional mimicry than males (Grossman and Wood 1993), predicting stronger brain responses, in general, during painful situations. We also note that the fMRI and PET data were acquired on average 3 weeks apart. However, the short-term test–retest reliability is known to be excellent for $[^{11}C]$carfentanil scans (Hirvonen et al. 2009), and $[^{11}C]$raclopride estimates are consistent even with multiple-month-intervals (Nordström et al. 1992; Hietala et al.

1999). Thus, the temporal gap between PET and fMRI scans is unlikely a significant confound in the present study.

Conclusions

Our data provide the first in vivo evidence for opioidergic contribution to vicarious pain. Baseline MOR availability correlated negatively with haemodynamic responses to seeing others in pain in regions supporting negative affect of pain and sensorimotor mirroring of others' pain. On the contrary, MOR availability was positively correlated with orbitofrontal haemodynamic activity, possibly reflecting the region's role in mentalizing and socioemotional functions. Despite its well-established role in nociceptive processing, D_2R system was not significantly associated with vicarious pain. We propose that high MOR availability may protect against excessive distress resulting from negative social signals, and that variation in endogenous opioid system may thus explain why some individuals react more strongly to seeing pain than others.

Supplementary Material

Supplementary data is available at *Cerebral Cortex* online.

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