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Opioidergic regulation of emotional arousal: A combined PET–fMRI study

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Abstract

Emotions can be characterized by dimensions of arousal and valence (pleasantness). While the functional brain bases of emotional arousal and valence have been actively investigated, the neuromolecular underpinnings remain poorly understood. We tested whether the opioid and dopamine systems involved in reward and motivational processes would be associated with emotional arousal and valence. We used *in vivo* positron emission tomography to quantify μ-opioid receptor and type 2 dopamine receptor (MOR and D_2R, respectively) availability in brains of 35 healthy adult females. During subsequent functional magnetic resonance imaging carried out to monitor haemodynamic activity, the subjects viewed movie scenes of varying emotional content. Arousal and valence were associated with haemodynamic activity in brain regions involved in emotional processing, including amygdala, thalamus, and superior temporal sulcus. Cerebral MOR availability correlated negatively with the haemodynamic responses to arousing scenes in amygdala, hippocampus, thalamus and hypothalamus, whereas no positive correlations were observed in any brain region. D_2R availability—here reliably quantified only in striatum—was not associated with either arousal or valence. These results suggest that the brain basis of emotional arousal is regulated by the MOR system, and that cerebral MOR availability influences brain activity elicited by arousing stimuli.

Introduction

Emotions are combinations of neural (Saarimäki et al. 2016) and bodily (Nummenmaa, Glerean, et al. 2014) states, arising in response to events that may influence well-being or survival. A bulk of psychophysiological and neurophysiological data support characterization of emotional responses in terms of two dimensions: one regulating the physiological and psychological *arousal,* and another associated with the appetitive–aversive dimension, often called *valence* (Lang 1995). High-arousal states are associated with increased activity of the sympathetic nervous system, indexed by increased skin conductance and enlarged pupil diameter (Lang 1995; Bradley et al. 2008), while high valence is associated with increased activity in zygomatic muscle (Lang 1995). On behavioural level, negatively valenced emotional states potentiate (Brown et al. 1951; Grillon et al. 1991) and positively valenced states attenuate (Vrana et al. 1988; Bradley et al. 1990) the startle response, an automatic defensive reaction to sudden and threatening stimuli. The magnitude of these effects also depends on the arousal state of the individual, with high arousal amplifying the effect (Bradley et al. 2001). Finally, the valence and arousal dimensions can dissociate a wide number of discrete emotional states (Schubert 1999).

According to functional neuroimaging studies, the brain regions supporting arousal and valence are mostly separate. Activity in the sensory cortices, amygdala, and thalamus typically increases as a function of arousal (Anderson et al. 2003; Small, Gregory, et al. 2003; Grabenhorst et al. 2007; Colibazzi et al. 2010; Costa et al. 2010). On the other hand, increasing valence is often associated with elevated haemodynamic activity in orbitofrontal cortex (Anderson *et al.* 2003; Small, Gregory*, et al.* 2003), likely reflecting this region's role in reward processing (Kringelbach 2005). Also other regions, including amygdala and thalamus, have been associated with valence (Lindquist et al. 2016). Strengthened functional connectivity between amygdala and thalamus during high arousal suggests that amygdala and thalamus may modulate emotional processing via their connections to sensory and higher-order association cortices (Nummenmaa, Saarimäki, et al. 2014).

While the psychological, physiological and functional neural correlates of emotional arousal and valence have been actively investigated, their neuromolecular bases remain elusive. Substantial evidence, however, associates the endogenous opioid and dopamine systems with reward and motivational processes both in animals and humans (Berridge and Kringelbach 2008; Nummenmaa and Tuominen 2017). Numerous drugs of abuse, including heroin, amphetamine, and cocaine, influence the mood via opioidergic or dopaminergic circuits (Koob 1992). Even if they both typically increase valence (Koob et al. 1989; Berridge 2003; Zacny and Gutierrez 2003; Wardle and de Wit 2012), opioidergic and dopaminergic drugs have opposite effects on arousal: Opioids increase parasympathetic activity, as is indicated by their pupil-constricting effects (Zacny and Gutierrez 2003; Zacny and Lichtor 2008; Zacny and Gutierrez 2011). Administration of cocaine, on the contrary, enhances sympathetic nervous system activity and is associated with dilated pupils and accelerated heart rate (Preston et al. 1992). Rat studies have revealed that opioid agonists (morphine) and partial agonists (buprenorphine) attenuate the fear-potentiated startle reflex (Glover and Davis 2008), whereas pharmacological facilitation of dopaminergic activity amplifies the acoustic startle response (Meloni and Davis 1999). In line with these physiological and behavioural observations, opioids are also typically perceived calming and relaxing (Zacny and Gutierrez 2003), while dopaminergic drugs are arousing (Kirkpatrick et al. 2013).

Human positron emission tomography (PET) studies have found opioidergic activation during positive emotions (Koepp et al. 2009), reward consumption (Peciña S. and Berridge 2000; Tuulari et al. 2017), and social interaction (Nummenmaa et al. 2016; Manninen et al. 2017). However, opioidergic activation has also been associated with negative emotions (Zubieta et al. 2003) and pain (Zubieta et al. 2001). Altogether these results suggest that opioidergic neurotransmission is not necessarily associated with only negative or positive valence, but instead with general changes in arousal. Finally, dopaminergic activity has been implicated in music-induced emotional states (Salimpoor et al. 2011) as well as in pleasure derived from eating (Small, Jones-Gotman, et al. 2003),

smoking tobacco (Barrett et al. 2004) and drinking alcohol (Boileau et al. 2003). While these experiments relate opioids and dopamine to affective processing, the contributions of these substances to the arousal and valence dimensions of human emotions remain unresolved.

Here we used fusion imaging with PET and functional magnetic resonance imaging (fMRI) to find out whether human opioidergic and dopaminergic circuits would be associated with arousal and valence dimensions of emotions. In PET, we used radioligands [^{11}C]carfentanil and [^{11}C]raclopride to estimate baseline availability (binding potential, BP_{ND}) of cerebral μ-opioid receptor (MOR) and type 2 dopamine receptor (D_2R), respectively. During the fMRI, the subjects viewed movie scenes with varying emotional content. Regional MOR and D_2R availabilities were correlated with the valence- and arousal-dependent haemodynamic responses to the movie scenes. Arousal was associated with haemodynamic activity in amygdala, while valence was associated with activity in posterior superior temporal sulcus and sensorimotor regions. MOR availability in the brain's affective circuits, most notably in insula and rostral anterior cingulate cortex, correlated negatively with arousal but not with valence-dependent haemodynamic responses. Neither arousal nor valence were associated with D_2R availability in striatum, the only brain region where receptor availability can be reliably estimated using [^{11}C]raclopride PET. These data suggest that cerebral MORs, but not striatal D_2Rs, are associated with emotional responses to naturalistic stimuli.

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 healthy females (mean ± SD age 44 ± 10 years, range 19–58 years). One subject was removed from the sample because her MRI revealed a previously undiagnosed 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 three imaging sessions. The PET scans were separated, on average, by four days and the PET and MRI scans on average by three weeks. All subjects signed ethics-committee-approved informed-consent forms, and were compensated for their time and travel costs. Portions of the PET–fMRI data unrelated to the current study have been published previously (Karjalainen et al. 2017).

PET imaging and analysis

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

Anatomical MR images (1 mm^3) were acquired with a Philips Gyroscan Intera 1.5 T scanner using a T1-weighted sequence. The PET images were motion-corrected and coregistered with the anatomical MR images. Subject-specific regional time-activity curves 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. Only voxels whose mean signal intensity exceeded the subject's mean reference tissue signal intensity were included in the ROIs to ensure that they would consist of only brain tissue.

Simplified reference tissue model (SRTM) was used to model the kinetics of both tracers (Lammertsma and Hume 1996). Tracer binding was quantified using nondisplaceable binding

potential BP_{ND} (Innis et al. 2007), which is the ratio of specifically bound radioligand to nondisplaceable radioligand in the tissue, thus reflecting receptor availability. ROI-level fitting was done using an in-house implementation of SRTM (Oikonen and Sederholm 2003), whereas voxel-level fitting was done using the basis-functions implementation of SRTM (Gunn et al. 1997) using the following parameter bounds: (θ_3^{min}(carfentanil) = 0.06/min, θ_3^{max}(carfentanil) = 0.6/min; θ_3^{min}(raclopride) = 0.082/min, θ_3^{max}(raclopride) = 0.6/min). These parameter values were defined so that averaging over voxel-level BP_{ND}-estimates within a ROI would produce the same result as first calculating a ROI-specific time-activity curve and then fitting the model to that.

ROI definition

Tracer binding was quantified in 9 ([^{11}C]carfentanil) and 3 ([^{11}C]raclopride) bilateral anatomical ROIs involved in emotional processing. The full-volume analysis did not influence the selection of the ROIs, as all of them were selected *a priori.* For both tracers, we defined striatal ROIs in caudate, nucleus accumbens and putamen. In addition, amygdala, dorsal anterior cingulate cortex, insula, orbitofrontal cortex, rostral anterior cingulate cortex and thalamus were used for [^{11}C]carfentanil. Because [^{11}C]raclopride has low specific binding outside striatum, extrastriatal [^{11}C]raclopride binding potential estimates are very noisy. Thus, extrastriatal regions were not analyzed for [^{11}C]raclopride. All ROIs were derived individually for each subject using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). Such ROI definition yields consistent BP_{ND} estimates with manually delineated ROIs in PET studies (Johansson et al. 2016).

fMRI data acquisition and analysis

Experimental design and stimuli

The experimental design has been previously described in detail (Lahnakoski et al. 2012; Karjalainen *et al.* 2017) and is summarized in Figure 1. Briefly, the stimuli consisted of 102 scenes (mean duration 12 s) extracted from mainstream Hollywood movies. They contained humans involved in various emotional and unemotional situations, as well as filler scenes without humans (e.g. scenery or

inanimate objects). The scenes were presented without breaks in a fixed order, and the total duration of the experiment was 21 min. During the fMRI, the participants were asked not to move and to watch the videos as attentively as they would be watching a movie or TV.

Dynamic emotional ratings for the movie scenes were collected in a separate experiment from 17 subjects (10 females) not participating in the neuroimaging study. While viewing each scene, the subjects moved a small cursor at the right side of the screen vertically to indicate their current level of arousal or valence. Ratings for valence and arousal were obtained on separate runs, and thus each participant viewed the clips twice. The ratings were sampled at 5 Hz. Pearson correlation coefficient between averaged arousal and valence ratings was –0.61. Because the ratings correlated strongly between males and females (Pearson correlation coefficients between average male and average female ratings were 0.67 for valence and 0.89 for arousal, respectively), we did not create gender-specific regressors to model the fMRI data. The online rating tool is available at https://version.aalto.fi/gitlab/eglerean/dynamicannotations.

MR image acquisition and preprocessing

Whole-brain functional data were acquired with the Philips Gyroscan Intera 1.5 T scanner using a $T2^*$-weighted echo-planar imaging sequence designed for measuring the blood-oxygen-level-dependent (BOLD) signal (TR = 3300 ms, TE = 50 ms, 90° flip angle, 192 mm FOV, 64 × 64 reconstruction matrix, 62.5 kHz bandwidth, 4 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 (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL) using the FEAT pipeline. The preprocessing consisted of slice-time correction, motion correction, two-step coregistration to a MNI152 2 mm template, and spatial smoothing using isotropic Gaussian kernel whose full width at half maximum (FWHM) was 8 mm. Low-frequency drifts in the BOLD

signals were estimated and removed using a 240-s Savitzky-Golay filter (Cukur et al. 2013). This procedure should not filter out meaningful signals that were considerably faster, as the stimulus movie clips lasted, on average, 12 s, and as the arousal and valence ratings often fluctuated significantly also within a clip. Finally, to control for head motion confounds, motion parameters were regressed out (Friston et al. 1996).

Statistical analysis

We modelled each individual's haemodynamic activity with the arousal and valence ratings of the movies using multiple regression, as implemented in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/). To produce the emotion regressors, the arousal and valence ratings were averaged across subjects participating in the behavioural study, downsampled to 1 TR, and convolved with the canonical haemodynamic response function (HRF). We controlled for low-level sensory confounds by using 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 sound intensity values for each time window. These time series were also convolved with the canonical HRF, downsampled to 1 TR, and finally included in the model as nuisance covariates. All the covariates were z-scored to make the beta coefficients comparable across predictors. Thus, the resulting regression coefficients for arousal and valence describe how, holding every other variable in the model constant, the BOLD signal would change if the corresponding ratings were increased by one standard deviation.

Because the methods relying on random field theory to correct for multiple comparisons have been questioned (Eklund et al. 2016), statistical significance of the estimates was assessed using nonparametric methods, as implemented in the SnPM toolbox (http://warwick.ac.uk/snpm) of SPM. 10 000 permutations were used to estimate the null distribution, the variance estimates were partially pooled using isotropic Gaussian kernel with FWHM of 8 mm, the cluster-defining threshold was set to $p = 0.05$, and only the clusters surviving family wise error (FWE) correction ($p < 0.05$) are reported.

PET–fMRI fusion analysis

To test if MOR and D_2R systems were associated with arousal or valence dimensions of emotions, the voxel-wise regression coefficients for arousal and valence were correlated with subjectwise [^{11}C]carfentanil and [^{11}C]raclopride binding potentials in each ROI (Figure 1). Separate models were run for each [^{11}C]carfentanil and [^{11}C]raclopride ROI, and models were also run separately for arousal and valence. We used the same nonparametric methods that were used to assess statistical significance of the main effects for arousal and valence. We also performed post-hoc analyses with the main results of the study. Only clusters surviving false discovery rate (FDR) correction ($p < 0.05$), as implemented in SPM12, are reported for these analyses.

Results

Main effects of arousal and valence

Arousal and valence were associated with robust haemodynamic responses in the brain's emotion, attention, and sensorimotor circuits (Figure 2). Arousal was positively correlated with haemodynamic activity in amygdala, thalamus, superior temporal sulci, anterior insula, inferior frontal gyri, and precuneus. The valence ratings were positively correlated with activity in posterior superior temporal sulci and sensorimotor regions. Haemodynamic activity correlated negatively with emotional arousal mainly in posterior cingulate cortex and in higher-order visual regions. We found no statistically significant clusters for negative valence; however, the cluster closest to statistical significance (p = 0.07) contained voxels in posterior cingulate cortex, thalamus, and associative visual regions.

The unthresholded *t*-maps can be found from https://neurovault.org/collections/GUBRIMXH/. The neurovault collection also contains unthresholded *t*-maps resulting from models including only arousal or valence. Because we wanted to dissociate the effects of arousal and valence from each other, all the conclusions of this study, however, rely on the full model that contains both emotion dimensions as well as brightness and

sound intensity as nuisance variables. Thus, the arousal-only and valence-only models are only shown for the sake of comprehensiveness.

Fusion analysis

We next tested whether regional MOR and D_2R availabilities would correlate with the arousal- and valence-dependent haemodynamic responses. Regional MOR availabilities in insula and rostral anterior cingulate cortex were negatively correlated with the haemodynamic responses in amygdala, hippocampus, thalamus and hypothalamus (see Figure 3). In other words, the more MORs subjects had in insula and rostral anterior cingulate cortex, the weaker were their haemodynamic responses in these regions to arousing movie scenes. This relationship is visualized at ROI level in Figure 4. For the purposes of creating the visualization, the arousal responses were quantified in voxels whose BOLD-fMRI signal correlated positively with arousal, i.e. the selection of voxels was independent of the subsequent fusion analyses, ensuring noncircular analysis (Kriegeskorte et al. 2009).

Due to strong spatial autocorrelation in cerebral MOR availability (Tuominen et al. 2014), we expected that all the regional MOR availabilities would correlate similarly with the haemodynamic responses. For this reason, we conducted a post-hoc analysis where we thresholded the statistical maps using cluster-level FDR-correction. These analyses supported our expectations, as five (caudate, insula, orbitofrontal cortex, putamen, and rostral anterior cingulate cortex) out of the nine regions produced statistically significant clusters in strongly overlapping regions, most notably in thalamus and hypothalamus but also in the hippocampus–amygdala complex. We found no statistically significant clusters for positive correlations between MOR availability and arousal. Similarly, MOR availability was not associated with valence, and D_2R availability in striatum was not associated with arousal or valence. All the unthresholded *t*-maps related to the fusion analyses can be found from https://neurovault.org/collections/GUBRIMXH/.

Discussion

Our main finding was that basal cerebral MOR availability is associated with haemodynamic brain activity during emotional arousal. More specifically, baseline MOR availability, particularly in insula and rostral anterior cingulate cortex, correlated negatively with haemodynamic responses to highly arousing scenes in amygdala, hippocampus, thalamus, and hypothalamus. Striatal D_2R availability, in turn, was not associated with either arousal or valence. The present data provide the first *in vivo* evidence about opioidergic regulation of emotional arousal in the human brain, agreeing with prior psychopharmacological work on the role of MOR system in alertness (Zacny and Gutierrez 2003; Zacny and Lichtor 2008; Zacny and Gutierrez 2011). Altogether these data suggest that the endogenous opioid system contributes to emotions by regulating the arousal dimension (Nummenmaa and Tuominen 2017).

Brain systems encoding arousal and valence

Arousing scenes increased haemodynamic activity in amygdala, thalamus, superior temporal sulcus, and inferior frontal cortex, according with prior fMRI experiments (Anderson *et al.* 2003; Small, Gregory*, et al.* 2003). The effects in auditory and visual cortices presumably reflect increased attention to the arousing scenes (Lang et al. 1998; Nummenmaa et al. 2012). Haemodynamic activity was decreased during arousing scenes in posterior cingulate cortex, suggesting that the activity of the default-mode network (Raichle 2015) is higher during low than high arousal. Positive valence was linked to activity of the posterior superior temporal sulcus, a region contributing to processing of facial expressions (Calder and Young 2005) and also involved more generally in social perception (Nummenmaa and Calder 2009; Lahnakoski *et al.* 2012; Isik et al. 2017). We also replicated a previous finding that had linked positive valence to increased activity of the somatosensory cortex (Viinikainen et al. 2012). Previous work has shown that haemodynamic activity in primary somatosensory cortex can discriminate between distinct emotion states (Saarimäki *et al.* 2016). Furthermore, damage of the primary somatosensory cortex or its inactivation by transcranial magnetic stimulation impairs the ability to recognize emotions (Adolphs et al. 2000; Pourtois et al. 2004), and

synchronous activity in the primary somatosensory cortex across individuals may underlie emotional contagion (Nummenmaa *et al.* 2012). We found no statistically significant clusters for negative valence; however the largest observed cluster (9991 voxels, 80 cm^3, $p < 0.08$) also contained voxels in posterior cingulate cortex, suggesting decreased activity in the default-mode network during pleasant scenes.

Opioidergic regulation of emotional arousal

High cerebral MOR availability was associated with weak haemodynamic responses to emotionally arousing events (Figures 3 and 4). The association was strongest for MOR availability in insula and in the rostral anterior cingulate cortex; however parametric analyses with more lenient (yet still multiple comparison corrected) statistical threshold revealed comparable negative associations in five out of nine ROIs. Overall, cerebral MOR availability was associated with haemodynamic responses to arousing events in amygdala, hippocampus, thalamus, and hypothalamus. These data suggest that individuals with high MOR availability have blunted responses to arousing movie scenes. The results are consistent with the sedative side effects of opioid drugs (Benyamin et al. 2008), the role of the opioidergic system in pain modulation (Heinricher and Fields 2013), as well as the calming and anxiolytic effects of MOR agonists (Colasanti et al. 2011). Thus, individual differences in the endogenous opioid system may explain inter-individual differences in how people react to both aversive and appetitive stimuli, both of which are known to activate the MOR system (Zubieta *et al.* 2003; Koepp *et al.* 2009).

We can only speculate about possible mechanisms underlying the association between baseline MOR availability and the arousal responses. If high receptor availability reflects high receptor density or affinity (as we believe it does for [^{11}C]carfentanil), then individuals with high MOR availability should have more responsive or higher-capacity MOR systems. The more postsynaptic receptors are available, the more efficiently should the opioid peptides released in response to arousing stimuli bind to the receptors, and the stronger should the effects of

neurotransmission be. It is well known that opioids have inhibitory effects at cellular level (Henderson 2015). Consequently, the higher the MOR availability, the stronger this inhibitory, calming effect could be. The psychological implication is that individuals with high MOR availability could be less disturbed by emotionally salient stimuli.

High cerebral MOR availability was associated with attenuated haemodynamic activity in amygdala and hippocampus during elevated emotional arousal. The hippocampus–amygdala complex is critically involved in emotional memory (Kensinger and Corkin 2004; McGaugh 2004). For example, activation of hippocampal neurons during fear conditioning is sufficient for creating false fear memories in mice (Ramirez et al. 2013). Our data thus suggest that low hippocampal–amygdala activity during emotionally arousing events in individuals with elevated MOR availability might counter against establishment of emotional memories. These findings dovetail nicely with clinical studies showing that acute opioid administration following a traumatic event decreases the likelihood of post-traumatic stress disorder (Saxe et al. 2001; Holbrook et al. 2010). The results are also in line with studies in healthy volunteers showing that high cerebral MOR availability is linked to impulsiveness (Love et al. 2009), approach behavior (Karjalainen et al. 2016) and secure attachment style (Nummenmaa et al. 2015).

High MOR availability was associated with weak arousal-related haemodynamic activity also in hypothalamus, a region with MOR-dense nuclei (Mansour et al. 1987) and the central component of the hypothalamic–pituitary–adrenal (HPA) axis driving the stress-response and regulating physiological arousal (Chapotot et al. 2001). β-endorphin, a potent endogenous MOR agonist, suppresses activity of the HPA axis by inhibiting neurons releasing corticotropin-releasing hormone in the paraventricular nucleus of the hypothalamus (Wand et al. 1998; Grisel et al. 2008). On the contrary, MOR antagonists disinhibit the HPA axis (Schluger et al. 1998; Wand *et al.* 1998; McCaul et al. 2000), triggering cortisol release whose magnitude depends on the genetic variant of the MOR gene (Wand et al. 2002) as well as baseline MOR availability in hypothalamus (Wand et

al. 2011). Our finding accords with the established opioidergic control of the HPA axis, suggesting that the MOR system regulates stress responses in emotionally arousing situations.

No evidence for opioidergic contribution to valence

While MOR availability was consistently associated with arousal-related BOLD responses, we found no dependence between valence-related haemodynamic activity and cerebral MOR availability. This result may seem at odds with pharmacological evidence regarding the effects of the opioid system on valence: Sufficiently high doses of the opioid antagonist naloxone often cause dysphoria (Pickar et al. 1982; Cohen et al. 1983), suggesting that tonic opioid-receptor occupation (Meucci et al. 1989; Spanagel et al. 1992; Skoubis et al. 2005) is required for maintaining basal positive mood (Narayanan et al. 2004). Furthermore, naloxone administration attenuates pleasure ratings related to winning and magnifies negative feelings associated with losing in gambling (Petrovic et al. 2008), suggesting that the opioid tone also influences phasic affective responses. These findings show that pharmacological manipulation of the opioid system influences the valence dimension of emotions. However, we focused solely on the MOR system, as [^{11}C]carfentanil binds very specifically to MOR compared with other opioid receptor subtypes (Frost et al. 1985). In contrast, naloxone also binds significantly to delta and kappa opioid receptors (Goldstein and Naidu 1989). Accordingly, future studies should resolve if delta or kappa opioid receptors, but not MORs, modulate the valence system (Lutz and Kieffer 2013).

No evidence for association between striatal D_2R availability and arousal or valence

In contrast to the MOR system, we found no association between striatal D_2R availability and arousal or valence. This finding was unexpected, given the mesolimbic dopamine system's major role in approach and avoidance motivation (Berridge 2007). Many dopaminergic drugs, such as amphetamine, are strongly arousing (Kirkpatrick *et al.* 2013), suggesting the neurotransmitter's involvement in physiological arousal. Previous human PET studies have shown that sleep-deprivation downregulates and caffeine consumption upregulates striatal D_2Rs (Volkow et al. 2012; Volkow et

al. 2015), showing that baseline D_2R availability in striatum is associated with human alertness. These data are not necessarily contradictory. It is possible that striatal D_2R availability influences basal alertness, but that dynamic arousal changes are caused by other mechanisms. For example, dopaminergic neurons in dorsal raphe nucleus fire in response to arousing stimuli regardless of their valence, and their activity mirrors wakefulness in mice (Cho et al. 2017), suggesting that extrastriatal dopaminergic circuits may influence emotional arousal also in humans. This discrepancy could be resolved in future with PET using e.g. [^{11}C]FLB 457 (Halldin et al. 1995), a tracer that is better suited for imaging extrastriatal dopamine receptors.

Previous human PET studies have also shown that striatal binding potential, as measured with [^{11}C]raclopride, changes in response to various affective stimuli, reflecting dopamine release (Drevets et al. 2001; Jonasson et al. 2014; Volkow et al. 2014). These experiments suggest that dopaminergic neurotransmission in striatum does contribute to emotions. However, we found no association between baseline availability of striatal D_2Rs and haemodynamic activity following emotional movie scenes. Thus, even if affective stimuli trigger dopamine release in striatum, our data suggest that individual differences in baseline D_2R availability in striatum do not explain inter-individual differences in brain activity elicited by emotional stimuli.

It is also possible that the D2R system was not associated with arousal or valence because the stimulus set shown during fMRI, despite containing a wide number of different naturalistic scenes, did not activate striatum strongly. This possibility is worth noting because striatum contains a dense population of dopaminergic neurons, and because, in contrast to our findings, striatal activity can, in some cases, correlate with arousal (Colibazzi T et al. 2010). With a different task for eliciting emotions, effects for arousal and valence might have occurred also in striatum. Subsequently, we could have seen effects for associations between D2R availability and arousal or valence in striatum. However, our current data provide no evidence for dopaminergic regulation of arousal or valence.

Limitations

We assumed that baseline receptor availability, as indexed by nondisplaceable binding potential, reflects neuroreceptor density. However, also concentration of endogenous ligands and the affinity of the radioligand to the receptor influence binding potential (Innis *et al.* 2007). No single-injection PET study can distinguish between these factors. However, genetically driven underexpression of MOR (Zhang et al. 2005) is associated with decreased [^{11}C]carfentanil BP_{ND} (Weerts et al. 2013; Peciña M. et al. 2015). Moreover, a multitude of data show that regions with high MOR availability, such as thalamus and striatum, also have high MOR density in both rats (Mansour *et al.* 1987) and humans (Bonnet et al. 1981; Pfeiffer et al. 1982). Both of these independent observations are in line with our assumption. Second, we did not measure directly the subject's physiological arousal. Consequently, we cannot assess if the haemodynamic responses to arousing scenes correlate with measures such as pupil size or skin conductance. Extensive literature, however, shows that subjective arousal ratings correlate with physiological indices of arousal (Lang 1995; Bradley *et al.* 2008). Arousal was also associated with brain activity similarly as in previous studies (Anderson *et al.* 2003; Small, Gregory*, et al.* 2003), indicating that our stimulation model likely elicited the intended arousal responses. Third, we scanned only females, and consequently the results may not generalize to males. We did this choice to maximize statistical power: First, because the spatial distribution of MORs is different in females and males (Zubieta et al. 1999; Weerts et al. 2011), single-sex design was optimal with respect to statistical power. Second, females typically experience and portray stronger emotions and emotional mimicry than males (Fischer and LaFrance 2014), making neuroimaging studies on emotions more sensitive in female populations. Fourth, we note that the fMRI and PET data were acquired, on average, three weeks apart. However, short-term test–retest reliability for [^{11}C]carfentanil scans is excellent (Hirvonen et al. 2009), and for [^{11}C]raclopride the estimates are consistent even at intervals of multiple months (Nordström et al. 1992; Hietala et al. 1999). Thus, the temporal gap between the scans is unlikely a significant confound. Finally, although large for a PET–

fMRI fusion study, the sample size of 35 is slightly lower than recent recommendations arising from worries about false positive findings in fMRI studies (Button et al. 2013; Poldrack et al. 2017). Even if the reliability of [^{11}C]carfentanil scans is known to be excellent (Hirvonen *et al.* 2009), the low number of subjects should be taken into account when assessing the relevance of the present results.

Conclusions

Our data provide the first *in vivo* evidence for opioidergic regulation of emotional arousal in highly natural conditions. Cerebral MOR availability correlated negatively with haemodynamic responses to arousing movie scenes in amygdala, hippocampus, thalamus, and hypothalamus, i.e. in brain regions that are involved in regulation of arousal. However, MOR availability was not correlated with valence. Similarly, D_2R availability—here reliably measured only in striatum—was not correlated with arousal or valence. We propose that cerebral MORs may buffer against high arousal, generalizing the MOR system's role beyond regulation of pleasure, pain, stress, and anxiety.

Conflict of interest

The authors declare no conflict of interest.

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Figures

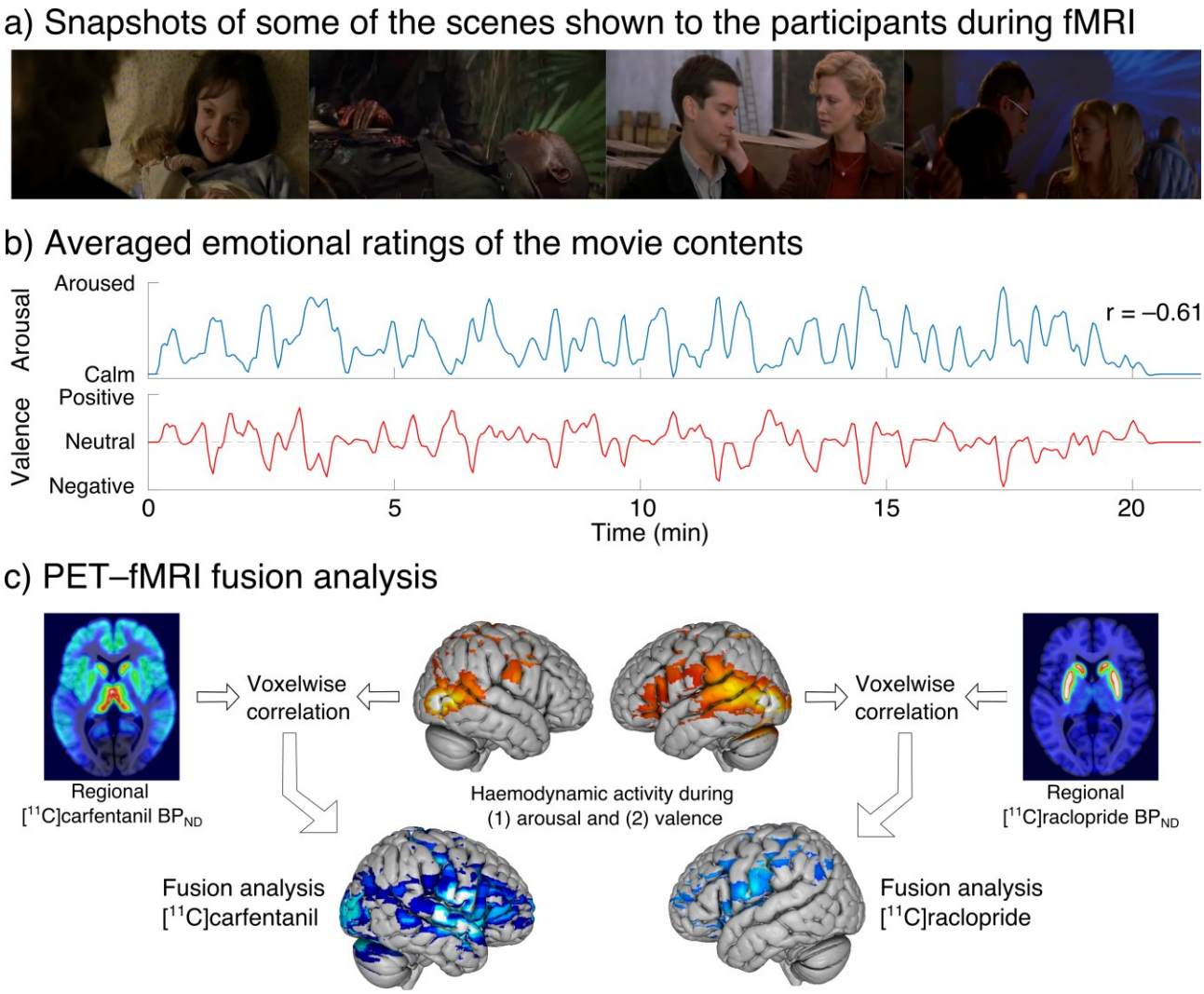

Figure 1. Experimental design and overview of the data analysis. (*a*) Snapshots of some of the movie scenes shown to the participants during fMRI. Subjects watched 102 short movie clips that varied in emotional content. (*b*) Averaged emotional ratings of the movie clips were obtained in a separate experiment. *(c)* PET–fMRI fusion analysis. The average arousal and valence ratings were first used to model subjectwise emotional haemodynamic activity. To evaluate the contributions of opioid and dopamine systems to arousal and valence, regional availabilities of μ-opioid receptor (MOR) and type 2 dopamine receptor (D_2R) were used to predict the individual regression coefficients corresponding to arousal and valence.

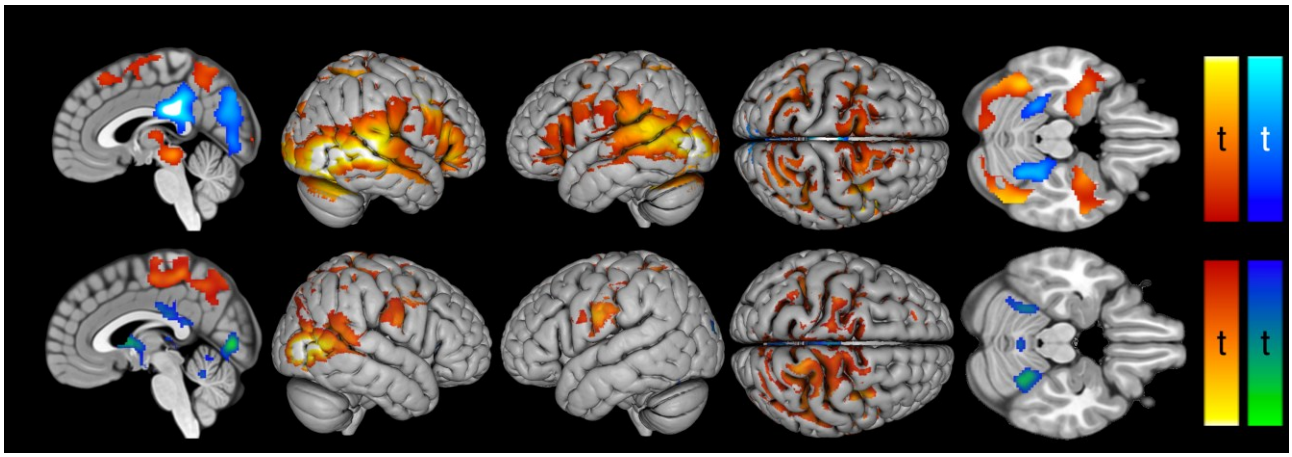

Figure 2. Brain regions where haemodynamic activity was positively (hot) and negatively (cold) correlated with emotional arousal (top row) and valence (bottom row). The data are thresholded at $p < 0.05$, FWE-corrected at cluster level, except for negative valence that is thresholded at $p < 0.08$ to visualize the strongest nonsignificant activation pattern.

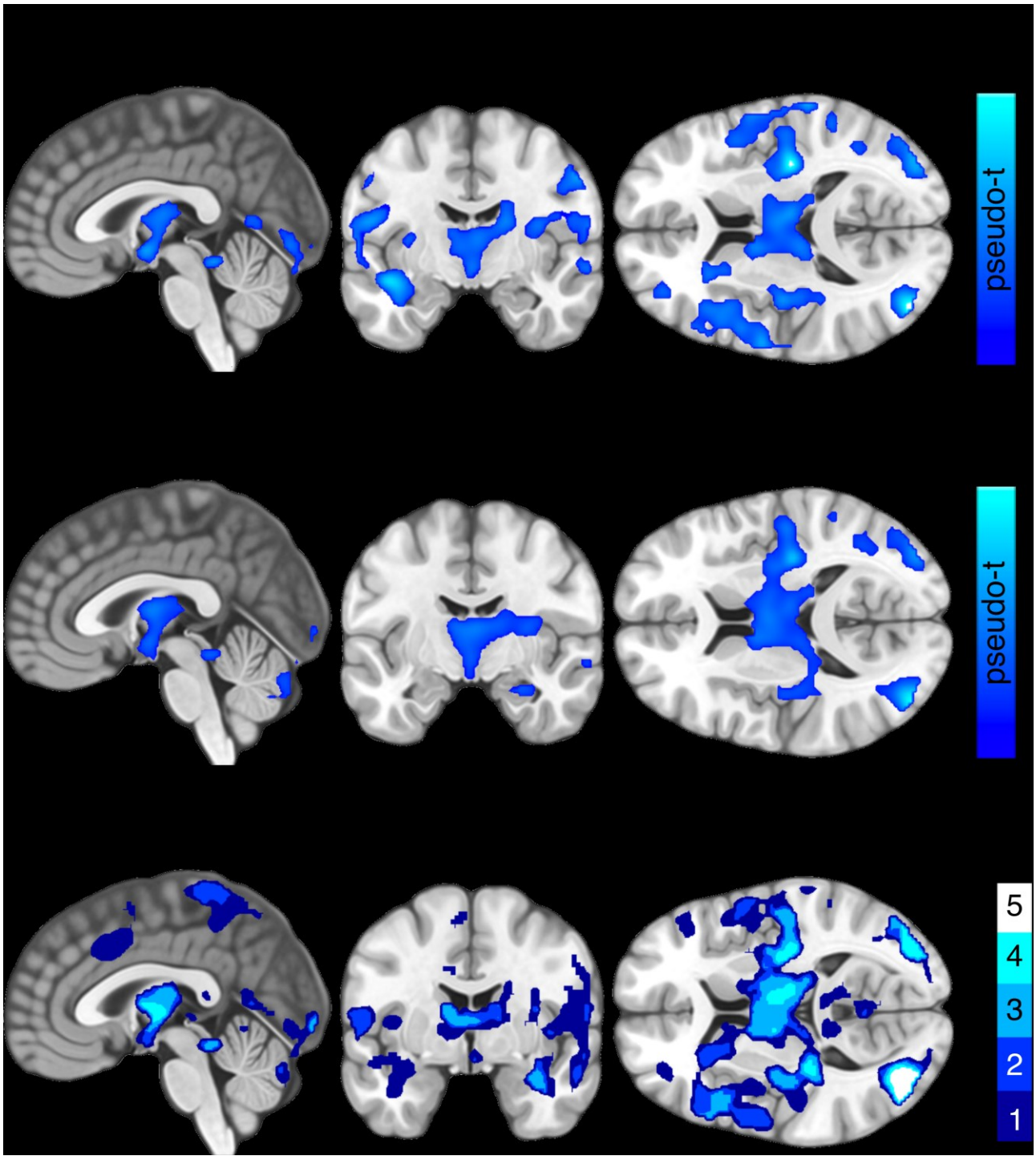

Figure 3. Negative correlation between cerebral MOR availability and haemodynamic responses to arousing scenes. a) Brain regions where the arousal-dependent haemodynamic responses correlated with MOR availability in insula (FWE-corrected at cluster level, $p < 0.05$). b) Brain regions where the arousal-dependent haemodynamic responses correlated with MOR availability in rostral anterior cingulate cortex (FWE-corrected at cluster level, $p < 0.05$). c) Brain regions where the arousal-dependent haemodynamic responses correlated with MOR availability in at least one of the ROIs. The cumulative maps show the number of ROIs (out of 9) whose [^{11}C]carfentanil BP_{ND} was negatively correlated ($p < 0.05$, FDR-corrected at cluster level) with haemodynamic responses to arousing scenes. The underlying statistical maps represent pseudo-t maps, i.e. variances of the regression coefficients in neighboring voxels influence each other.

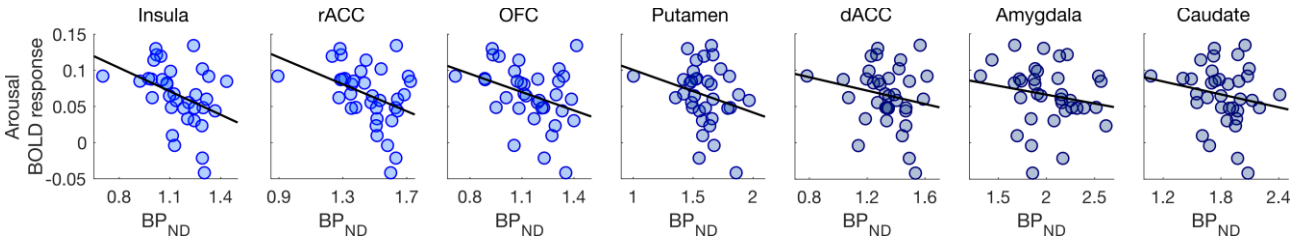

Figure 4. Relationships between individual arousal responses and regional [^{11}C]carfentanil binding potentials (BP_{ND}) in seven representative ROIs. The vertical axis represents individual arousal responses in the voxels whose BOLD-fMRI signal positively correlated with arousal (Figure 2). The horizontal axes represent [^{11}C]carfentanil binding potentials in the regions specified by the titles. Note that the scatter plots are shown for visualization purposes only, and the conclusions of the study rely on the full volume analyses.