Exp Brain Res (2015) 233:2517–2526
DOI 10.1007/s00221-015-4322-9

RESEARCH ARTICLE

Polymorphism in the μ-opioid receptor gene (*OPRM1*) modulates neural processing of physical pain, social rejection and error processing

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Received: 12 January 2015 / Accepted: 11 May 2015 / Published online: 28 May 2015

Abstract Variations of the μ-opioid receptor gene *OPRM1* have been shown to modulate pain perception with some evidence pointing towards a modulation of not only physical but also "psychological pain". In line with suggestions of a common neural network involved in the processing of physical pain and negative and distressing stimuli, like social rejection as a psychologically harmful event, we examined the influence of the A118G polymorphism on the neural processing of physical and non-physical pain. Using fMRI, we investigated a sample of 23 females with the more frequent AA genotype, and eight females with the relatively rare but more pain-sensitive AG genotype during electrical stimulation to the dorsum of the non-dominant hand. Non-physical pain was investigated using Cyberball, a virtual ball-tossing game, to induce experiences of non-self-dependent social rejection. A Go/NoGo task with an increased risk of self-dependent erroneous performance was used as a control task to investigate the effects of negative feedback as a more cognitive form of distress. Relative to A118G homozygous A-allele carriers, G-allele carriers showed significantly increased activation of the supplementary motor area/superior frontal gyrus and the precentral gyrus during electrical stimulation. Increased activation of the secondary sensorimotor cortex (SII) was found during social exclusion and during negative feedback. We demonstrate that brain regions particularly related to the somatosensory component of pain processing are modulated by variations in *OPRM1*. Influences were evident for both physical and psychological pain processing supporting the assumption of shared neural pathways.

Keywords Pain · fMRI · Neuroimaging · *OPRM1* · A118G · Social pain

Introduction

Pain can be interpreted as a basic alarm system of negative, threatening circumstances, which potentially compromise survival. Animal research and lesion studies in humans have suggested a neuroanatomy of pain processing that has meanwhile been well confirmed by different neuroimaging methods (e.g. Adolph et al. 2010; Hahn et al. 2013; Zhao et al. 2012; Peyron et al. 2000; Veldhuijzen et al. 2010; Wager et al. 2013). Besides somatosensory cortices and the thalamus, the anterior cingulate cortex (ACC) and the insula have been described as integral parts of the neural pain matrix involved in the processing of physical pain (Fomberstein et al. 2013). Activation in this network has also been identified in relation to the processing of stimuli that are painful in a more metaphorical sense, like social rejection, which may lead to social separation and may even threaten survival or gene transmission. Previous studies and two recent meta-analyses particularly identified the anterior insula, dorsal ACC and the ventrolateral prefrontal cortex but also supplementary motor and somatosensory cortices as a common neural basis for the processing of social pain (e.g. Eisenberger et al. 2003; DeWall and Baumeister 2006; Dewall et al. 2010; Kross et al. 2011;

Electronic supplementary material The online version of this article (doi:10.1007/s00221-015-4322-9) contains supplementary material, which is available to authorized users.

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Singer et al. 2004; Kawamoto et al. 2015; Cacioppo et al. 2013; Rotge et al. 2015). In this context, the dorsal ACC has been related to the affectively distressing perception of both physical and social pain, whereas the sensory component of pain seems to be represented in the posterior insula and the sensorimotor cortices (Eisenberger and Lieberman 2004). Directly contrasting physical and social pain, Wager et al. (2013) identified the ventrolateral thalamus, the secondary somatosensory cortex (SII) and the dorsal posterior insula as rather specific for physical pain processing. In addition to the sensory-discriminative and the affective-motivational components of pain processing, a more cognitive dimension of pain was described to be associated with the cognitive evaluation of pain including expectation and attentional processes (Price 2002). Particularly, parts of the dorsolateral prefrontal cortex have been reported to be associated with the processing of the cognitive aspects of pain (e.g. Atlas et al. 2014).

Accumulating evidence suggests that these different components of pain may equally be modulated by endogenous opioids or by exogenous treatments with opioids that alleviate physical pain effectively and also may influence reactions to socially threatening situations like social rejection or separation (Nelson and Panksepp 1998; Panksepp 2003). The primary mechanism for physical pain relief of the most commonly used exogenous opioids like morphine or fentanyl is their interaction with the μ-opioid receptor (*OPRM1*). The *OPRM1* encoding gene is located on chromosome 6q25.2. This receptor is also the primary receptor for endogenous opioid peptides (such as β-endorphin, enkephalin and endomorphin) (Zadina et al. 1997) with its activation being related to the reduction in pain experience (Bresin and Gordon 2013). The A118G single-nucleotide polymorphism (SNP) leads to an asn40-to-asp (N40D) substitution in the N-terminal region of the protein and distinctively effects the *OPRM1* expression. In previous studies, the infrequent G-allele was associated with reduced levels of *OPRM1* mRNA and protein relative to the A-allele (Zhang et al. 2005). Subjects, homo- or heterozygous for the G-allele, have shown enhanced sensitivity for physical pain perception (Sia et al. 2008, Fillingim et al. 2005). By contrast, homozygous A-allele carrier was reliably shown to require less analgesic medication after surgical interventions (e.g. Coulbault et al. 2006; Chou et al. 2006a, b). Some first evidence for the modulation of social threatening situations or social pain comes from a recent neuroimaging study. In this study, the G-allele of the A118G polymorphism was associated with enhanced dispositional sensitivity to social rejection and G-allele carriers displayed greater activity of anterior insula and ACC (Way et al. 2009). However, whether G-allele carrier status indeed modulated the neural processing of physical pain has not yet been investigated sufficiently.

Based on the previous evidence that the neural system processing physical pain is also involved in the processing of non-physical pain, we hypothesised that individual opioid receptor genetics would modulate the processing of physically as well as psychologically painful experiences. In addition to electrically induced physical pain and social rejection, we also investigated error processing with negative feedback in a cognitive task to cover a wider range of potentially distressing events. Previous studies have identified a network overlapping with the physical pain matrix as core regions involved in error processing, including the dorsal ACC, pre-supplementary motor area (pre-SMA) (Sosic-Vasic et al. 2012) and lateral inferior prefrontal cortex (Menon et al. 2001; Mathalon et al. 2003).

Materials and methods

Subjects

Thirty-one female subjects (mean age 22.2 years, SD 3.38), 28 of them right-handed, were recruited for this study. The project was approved by the Institutional Review Board of Ulm University, Ulm, Germany. Written informed consent was obtained. All procedures were performed according to the 1964 Declaration of Helsinki.

Recruitment of subjects took place within the framework of a larger investigation on non-suicidal self-injury (NSSI) which occurs in about 13.4 % of young adult people in non-clinical samples (Swannell et al. 2014). To account for gender differences and to reduce sample heterogeneity, only female subjects were included in our sample. Eight participants were genotyped as heterozygous G-allele carriers (AG), and 23 were homozygous for the A-allele (AA) of the A118G-SNP. All comparisons in this paper are reported with respect to these two genotype groups (AA vs AG). The rare G-allele was not found as a homozygous genotype, which is in line with allele frequencies reported in databases (www.hapmap.org/) with regard to the studied sample size. Thorough psychiatric examination revealed that 11 subjects of the AA genotype had a history of self-injury without any current psychiatric disease. Five of eight subjects in the G-allele group also had a history of self-injury. Three subjects in the AA group reported a lifetime history of borderline personality disorder (BPD), one subject had a history of depression (AG subgroup), and one subject reported mild symptoms of agoraphobia and specific phobia (AA subgroup). Other psychiatric diagnoses, current psychiatric symptoms or neurological illness were not present and would have been excluded. None of the participants displayed significant functional impairment in daily life due to current psychiatric symptoms. One of the participants of the G-allele carrier group was included

in the study despite regular methadone treatment which became evident only after closing the study. To control for hormonal influences on neural processing, data were acquired within 10 days after menstruation onset or after at least 14 days of continuous intake of oral contraception.

Genotyping

To analyse the single-nucleotide polymorphism (rs1799971, *ORPM1*-Gen c.118A > G), buccal cell samples were taken from each subject with commercial buccal cell collection brushes (Qiagen). DNA was extracted using a commercial isolation kit (Qiagen) according to the manufacturer's instructions. After PCR-amplification, the SNP analysis was performed in a laboratory for molecular genetic services (BioGlobe, Hamburg, Germany) on the MassARRAY® system (Sequenom) applying the iPLEX® method and MALDI-TOF mass spectrometry for analyte detection.

Psychometric measurements

Past and current psychiatric diagnoses were assessed using the Structured Clinical Interview for DSM-IV (SKID; Wittchen et al. 1999) and the Short Diagnostic Interview for Mental Disorders (Mini-DIPS; Margraf 1994). Additionally, participants completed the Becks Depression Inventory (BDI-II; German version: Hautzinger et al. 2006) before scanning and a German version of the Need-Threat Scale (Williams et al. 2000) after the last Cyberball game to assess feelings and emotional consequences of social rejection.

Experimental tasks

All subjects took part in three different tasks during fMRI scanning.

Electrical stimulation

Physical pain was induced via electric stimulation over the dorsum of the non-dominant hand as described in Adolph et al. (2010). Individual upper and lower boundaries of stimulus intensities were assessed prior to the functional scan. In a first step, the minimum stimulus intensity was assessed as the lowest level that the subject could reliably perceive the stimulus. Each stimulus consisted of a train of four electrical square pulses with 1 ms duration each (100 Hz). Subjects gave direct feedback and permission to increase stimulation intensity after each single step. Based on the minimum level (defined as level 1), the stimulation was increased stepwise to the individual maximum intensity that was already painful, but considered tolerable by the subject (defined as level 4). Intensity levels 2 and 3 were spaced equidistantly in-between levels 1 and 4. After the individual assessment, subjects were trained to correctly rate the stimulus levels 1–4 with stimuli provided at random and were asked to rate stimulus intensity by pressing buttons on a four button box during scanning. A total of 24 electrical stimuli (six per level) were administered during the scan, resulting in duration of about 10 min for the pain task. To control for effects of expectation and attention, a short signal tone was delivered 1.5 s before electrical stimulation.

Cyberball

To implement social rejection, the virtual ball-tossing game Cyberball was used in the same way as in other recent studies (Eisenberger et al. 2003; Williams et al. 2000). Participants took part in three separate rounds of Cyberball during fMRI scanning. The game was trained before the scanning session, and subjects were instructed to visualise all three game situations as real as possible. In order to assess a baseline of neural activity, in the first round, subjects were instructed to watch three playing characters (including the participant). As described previously (Eisenberger et al. 2009), in the next round, the participant was allowed to overtake one character which was included in the game. Without the subject's knowledge, the third round was pre-designed to model a situation of social rejection where the subject's character was consistently excluded from the game after some initial rallies with inclusion of the participant. Each condition (passive watching, inclusion, exclusion) was scanned for about 60 s with the exclusion condition being preceded by a short inclusion period of 16 s that was discarded afterwards. During inclusion and passive watching, the sequence of players was chosen at random. At the end of the total procedure, each participant was debriefed and informed that exclusion from the game had been planned and been performed by a computer and not by real participants. To control for sequence effects and interaction, electrical pain and the social exclusion task were assigned to subjects in alternating order.

Error processing task

After a short break outside of the scanner, subjects were trained with a combined Go/NoGo-Eriksen-Flanker-paradigm (Eriksen and Eriksen 1974) as described in previous fMRI studies on error processing (e.g. Sosic-Vasic et al. 2012). Five-letter strings composed of the letters R, U, P and V were presented for 200 ms after a fixation period of 1000 ms. Subjects were instructed to pay attention to the mid-standing letter (target) of the string. In Go trials, subjects had to respond to the target letter R with their index finger on a two button box and with their middle finger to

the target letter U. In NoGo trials, subjects were instructed to withhold a button press response upon appearance of the letters P and V. Target and flanker letters were combined either congruent (all five letters were the same) or incongruent. Incongruent Go trial targets were flanked by visually similar NoGo target letters (e.g. PPRPP), whereas incongruent NoGo trials consisted of a NoGo target flanked by visually similar Go letters (e.g. RRPRR). After stimulus presentation, a responding time window depending on individual reaction times was established for the response to the stimulus. After each trial, feedback (correct, wrong) followed the response. Delayed responses resulted in the feedback "faster" on the display. Each single trial lasted about 1.9 s with a total of 264 trials (66 trials per combination). Previous to scanning, the paradigm was trained with an exercise version in which individual reaction times were used to adjust maximum response times. From an average of reaction times on correct congruent and incongruent Go trials, a further 15 % were subtracted and this result was used as maximum responding time during the fMRI task.

Functional data acquisition

Image data were acquired using a 3.0 Tesla Magnetom ALLEGRA Scanner (Siemens, Erlangen, Germany). High-resolution anatomical T1-weighted images (1 × 1 × 1 mm voxels) were obtained (BW = 130 Hz/Pixel, TR = 2500 ms, TI = 1.1 s, TE = 4.57 ms, flip angle = 12°). For functional imaging, a T2*-sensitive gradient echo sequence was applied. During the physical pain and Cyberball paradigm, 35 transversal slices were recorded at a repetition time (TR) of 2000 ms with an image size of 64 × 64 pixels. The field of view (FOV) was 230 mm, and slice thickness was 2.5 mm with an interslice gap of 0.5 mm. Echo time (TE) was 33 ms with a flip angle of 90°. During the Go/NoGo task, functional image volumes comprised of 33 slices (TR = 2200 ms, TE = 39 ms). A total of 608 volumes were acquired. Number of volumes during the physical pain task was 305, 35 volumes each during Cyberball passive watching and inclusion and 43 volumes during Cyberball exclusion condition. Before each scanning session, a number of images were acquired to allow for T1 saturation effects and discarded from further analysis (electrical pain: six volumes, error processing: 10 volumes, Cyberball: passive watching and inclusion: five volumes, exclusion: 13 volumes to discard also the 16-s inclusion period).

Data analysis

Behavioural data

Inference of statistical significance of differences between genotype groups was computed in SPSS (IBM SPSS Statistics, version 21) using the "Wilcoxon–Mann–Whitney *U* test".

For the Go/NoGo task, numbers of correct and incorrect responses were assessed as well as number of Go trial responses within or beyond the individually predefined time window. We concentrated on the NoGo condition as a representative of negative feedback as a potentially psychologically painful situation.

fMRI Data

For image data preprocessing and statistical analyses, we used the Statistical Parametric Mapping Package (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK). Preprocessing included realignment and slice timing to correct for head movement artefacts and slice acquisition delay. All images were normalised to the standard MNI-template (Montreal Neurological Institute) and spatially smoothed using an 8 mm width at half maximum (FWHM) Gaussian kernel. To account for intrinsic autocorrelations, an AR(1) model was used and low frequency drifts were removed via high-pass filtering.

After preprocessing, session-separated, first-level analyses were performed for each subject estimating the variance of voxels for each trial according to a general linear model for each of the three different tasks. Regressors representing the six motion parameters were additionally added to the individual design matrix of each task and were integrated in the statistical analysis. Second-level group analyses concentrated on the interaction of genotype (two levels of genotype: AA and AG) and task conditions with focus on increased activity in brain regions within the AG group.

As in previous studies using the electrical pain task (see Adolph et al. 2010), we defined regressors for each of the four intensity levels. Onsets of electrical stimulation were each modelled as a stick function and convolved with the hemodynamic response function. Whole-brain analyses were calculated to test on pain-related activation patterns using differential contrast images for ascending pain intensity which were propagated to random-effects group analyses.

Functional analyses of the Cyberball data were conducted in the same way as in previous fMRI studies (Eisenberger et al. 2007b, 2009). Each round of the Cyberball game was modelled as one block of passive watching, inclusion or exclusion condition. Afterwards, linear contrasts for each condition were modelled and integrated in a between-subject analysis of variance (ANOVA). The contrast of interest was exclusion minus inclusion.

For the Go/NoGo task, regressors for the different combinations of the four factors (condition (Go/NoGo), type (congruent/incongruent), response accuracy (correct/incorrect) and deadline (within/beyond))

were defined and integrated in the general linear model. Missed Go events (errors of omission) were also modelled, resulting in 14 regressors for the first-level model. With regard to our hypothesis concerning error processing and as described previously (see Sosic-Vasic et al. 2012), we focused our analysis on the NoGo condition. A corresponding contrast of incorrect NoGo minus correct NoGo trials was defined and integrated in a second-level ANOVA. Data of two subjects of the AA group were not included in the analysis. One of these subjects did not complete the whole task, and the other had no erroneous responses in NoGo trials.

For the analysis of between-group differences, second-level whole-brain analyses for each of the three tasks were inclusively masked with the result of the contrast modelling increasing physical pain in the whole group of 31 subjects ($p < 001$; cluster extent: 18128 voxels) to ensure that clusters with an effect of genotype were indeed part of the neural pain matrix.

Initial voxel-level threshold was $p < 0.005$, uncorrected, together with an extent threshold of 10 contiguously significant voxels (see also Table 2). Results were considered significant at $p < 0.05$ FWE-corrected. FWE corrections were either implemented at the voxel level, or using small volume corrections, where the literature permitted to expect specific brain structures involved (Cyberball, Go/NoGo). Small volume corrections (9-mm spheres) on results from whole-brain analyses were applied using contralateral peak coordinates from an independent previous imaging study on brain regions activated by social rejection that overlaps with the physical pain matrix (MNI-coordinates SMA/superior frontal cortex: $x/y/z = -9/-1/62$; SII/inferior parietal lobule: $x/y/z = -62/-28/36$; Kross et al. 2011) for results of the Cyberball task. As the overlap of error processing and physical pain has not been investigated before, for analysis of the Go/NoGo data, we used peak coordinates within the SII/posterior insula identified to highly reliably relate to the processing of physical pain before (MNI-coordinates $x/y/z = -40/-20/13$; Wager et al. 2013) for small volume corrections (9-mm spheres) on results from whole-brain analyses.

Results

Demographical and behavioural data

Demographical and behavioural data together with associated statistics are summarised in Table 1. Genotype groups were comparable regarding age and the presence of mild depressive symptoms. Both groups evaluated electric stimulus intensities equally correct. Mean pain ratings did not differ significantly, but subjects with the G-allele rated marginally higher subjective physical pain intensities along with slightly higher ratings of rejection intensity. Mean minimum as well as maximum stimulus intensities did not differ significantly between the genotype groups. For the Go/NoGo task, we concentrated on the NoGo condition as a representative for negative feedback. As expected, during the Go/NoGo task, significantly more errors appeared upon incongruent than upon congruent NoGo trials ($p < 0.001$). Analyses of reaction times showed no significant differences between the genotype groups (see Table 1).

Table 1 Summary statistics of demographical and behavioural data

	AA	AG	Statistic
Age (years)	22 (3.43)	22.9 (3.58)	$p = 0.411$
BDI-II total score	8.14 (8.49)	13.5 (12.8)	$p = 0.320$
Physical pain			
Correct responses	16.36 (2.19)	15.00 (3.02)	$p = 0.298$
Mean pain rating	2.51 (0.22)	2.59 (0.33)	$p = 0.629$
Mean minimum stimulus intensity (mA)	4.76 (1.70)	4.93 (1.45)	$p = 0.809$
Mean maximum stimulus intensity (mA)	16.71 (7.44)	18.8 (6.54)	$p = 0.488$
Cyberball			
Need-Threat-Scale			
Rating of rejection intensity	7.52 (2.19)	8.88 (3.18)	$p = 0.275$
Rating of belongingness	13.30 (4.27)	14.0 (6.41)	$p = 0.982$
Go/NoGo			
Frequency of correct NoGo trials			
Incongruent	54.33 (9.3)	53.38 (11.27)	$p = 0.981$
Congruent	62.29 (5.25)	61.13 (6.36)	$p = 1.000$
Frequency of incorrect NoGo trials			
Incongruent	11.67 (9.3)	12.63 (11.27)	$p = 0.905$
Congruent	3.71 (5.16)	4.88 (6.36)	$p = 0.905$
Reaction time of incorrect NoGo trials (ms)			
Incongruent	441.47 (59.65)	405.89 (61.43)	$p = 0.179$
Congruent	418.25 (57.74)	443.37 (126.92)	$p = 0.630$
Reaction time of correct Go trials (ms)			
Incongruent	407.87 (47.31)	399.32 (72.45)	$p = 0.763$
Congruent	403.94 (39.67)	397.77 (69.69)	$p = 0.819$
Reaction time of incorrect Go trials (ms)			
Incongruent	388.83 (37.96)	392.46 (72.16)	$p = 0.903$
Congruent	391.17 (30.13)	403.79 (75.64)	$p = 0.689$

Values are mean ± SD (standard deviation) in rounded brackets; p values stem from Mann–Whitney U tests (or unpaired t tests for the Go/NoGo task) comparing AA and AG group

Table 2 Whole-brain genotype-dependent neural activation differences (AA < AG) during the fMRI tasks

Contrast AA < AG		BA	*x/y/z*	Cluster size (voxel)	*Z*	$p_{FWE\text{-}corr}$
Physical pain: increasing intensities						
SMA/Superior Frontal Gyrus	R	6	10/0/56	112	4.33	0.015
Precentral Gyrus	R	4	46/−12/48	274	4.13	0.033
Posterior Insula	R	41	36/−22/22	62	3.40	–[+]
Postcentral Gyrus	R	4	−22/−26/60	26	3.13	–[+]
	L	4	24/−22/58	17	2.85	–[+]
Lingual Gyrus	R	18	18/−86/−10	58	3.56	–[+]
Social pain: Cyberball (excl > incl)						
SMA/Superior Frontal Gyrus	L	6	−4/2/56	11	3.18	0.032[#]
SII/Inferior Parietal Lobule	L	2/40	28/34	19	3.35	0.024[#]
Precuneus	R		6/−44/56	38	3.01	–[+]
Negative feedback: NoGo (incor > cor)						
SII/Posterior Insula	L	2/41	−42/−18/16	28	3.10	0.044[#]

For each task, results are presented at the uncorrected voxel level of $p < 0.005$ with minimum cluster size of 10 contiguously significant voxels. To exert control on multiple comparisons, either whole-brain FWE corrections were used, or small volume corrections

[#] were applied in cases where the literature permitted the formulation of to be expected specific brain regions (see also "Materials and methods" section

[+] Significant only at uncorrected level

fMRI Data

Main effects of physical pain processing over all subjects of both groups together using the contrast modelling parametrically ascending stimulation intensities revealed reliable activation of brain regions associated with the neural processing of pain in former studies (Wager et al. 2013), including somatosensory cortices and the insula.

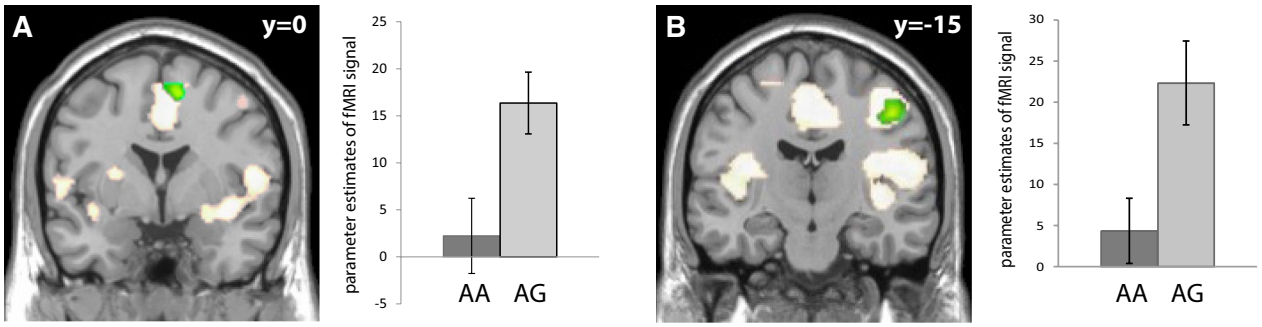
Whole-brain results for the different tasks irrespective of genotype group are shown in the supplementary material. During social exclusion compared to social inclusion in the Cyberball task, the whole-brain analysis showed significantly different activation of the ACC, insula, supplementary motor area (SMA)/superior frontal gyrus and cuneus as expected from previous investigations with the same task (e.g. Eisenberger et al. 2003; Way et al. 2009). Replicating results with the Go/NoGo task in a previous independent sample (Sosic-Vasic et al. 2012), we found enhanced activation upon processing of negative feedback (contrast: incorrect NoGo > correct NoGo trials) in the bilateral inferior frontal gyrus/anterior insula and the dorsal ACC (further results of whole-brain analyses are shown in the supplementary materials).

Results of analyses regarding increased task-dependent brain activation in the presumably more pain-sensitive carriers of the G-allele as compared to homozygous A-allele carriers are presented in Table 2 and Fig. 1. Increased brain activation within areas of the neural pain matrix was found in G-allele carriers for each of the three tasks. Physical pain processing was associated with increased activity in the right precentral gyrus and SMA/superior frontal gyrus for the AG genotype relative to the AA genotype.

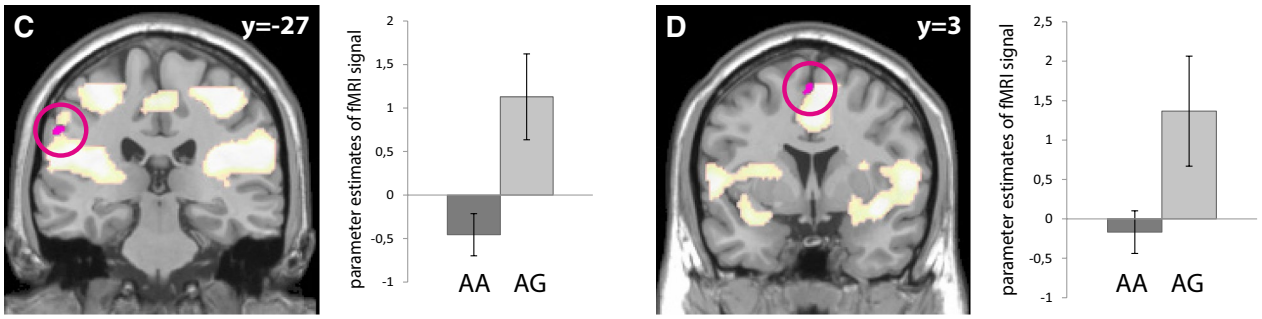
During social rejection, the AG genotype group exhibited relatively higher activation of the SMA/superior frontal cortex, SII/inferior parietal lobule, posterior insula, postcentral gyrus and the lingual gyrus. Significantly enhanced activity of the precuneus, the posterior insula, the postcentral and the lingual gyrus was found only at the uncorrected level, so it is not integrated into Fig. 1. During negative feedback processing upon commission of an erroneous NoGo response, the G-allele group demonstrated increased activation of the left SII/posterior insula as compared to the AA genotype.

Discussion

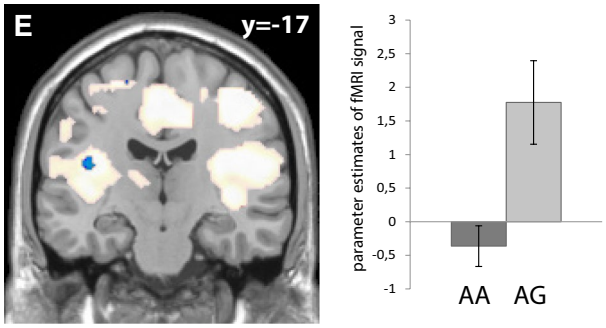
We investigated the influence of the A118G polymorphism of the μ-opioid receptor gene on the neural processing of different dimensions of distressing or painful experiences. Our data demonstrate increased brain activation within the pain matrix in carriers of the G-allele of the A118G polymorphism related to physical pain induced by electrical stimulation. Distressing events implemented by social exclusion and negative feedback upon erroneous responses were associated with increased neural activation of the AG group relative to the AA group, too. In particular, SMA and secondary somatosensory cortices (SII) showed higher neural activation, suggesting that within a network of brain regions associated with the processing of physical pain



Green: differential activation in the (A) SMA/superior frontal cortex (peak voxel 10 0 56) and the (B) precentral gyrus (peak voxel 46 -12 48) during ascending physical pain stimulation



Red: differential activation in the (C) SII/inferior parietal lobule (peak voxel -60 -28 34) and the (D) SMA/superior frontal cortex (peak voxel -4 2 56) during Cyberball social exclusion compared to inclusion condition



Blue: differential activation in the (E) SII/posterior insula (peak voxel -42 -18 16) during negative feedback (correct – incorrect nogo condition)

Fig. 1 Differential whole brain activation (AG-allele carriers > AA-allele carriers) during processing of physical pain stimulation (**a**, **b**), social rejection (Cyberball) (**c**, **d**) and negative feedback (Go/NoGo) (**e**); white to yellow coloured clusters depict brain regions that are part of the so called neural pain matrix (whole brain activation in all study participants upon increasing physical pain intensity)

(Wager et al. 2013), the μ-opioid receptor polymorphism may modulate neural representations of pain and other negative and potentially distressing circumstances like social rejection or negative feedback.

Pain is described as a multidimensional experience encompassing somatosensory-discriminative, affective-motivational components and cognitive processes (Price 2002; Wager and Atlas 2013). The relationship between physical and social pain has been discussed controversially in the recent literature (e.g. Iannetti and Mouraux 2011; Mouraux et al. 2011). Some evidence supports the assumption that the neural network commonly described as the pain matrix is rather ubiquitous (e.g. Iannetti and Mouraux 2010; Legrain et al. 2011) and that commensurabilities between physical and social pain would relate to a lack of specificity in the neural representation of pain. Directly comparing neural processing of physical and social pain, particularly the somatosensory cortex and posterior insula together with the ventrolateral thalamus were identified as a neural signature with high specificity for physical pain

as compared to networks generally coding salience (Wager et al. 2013). The same set of regions has been described to be also activated by social pain (Wager et al. 2013), although in some studies somatosensory cortex activation during social exclusion could not consistently be observed (Eisenberger 2008). One possible explanation relates to the assumption that socially harmful experiences more likely involve the affective but not the sensory components associated with physical pain perception. Alternatively, Kross et al. (2011) suggested that only rather powerful experiences of social rejection would recruit brain regions involved in both the affective and also the sensory components of physical pain. Previous findings have already indicated a dose-dependent relationship with involvement of somatosensory cortices that became activated only during rather intensive painful stimulation (Price 2000; Coghill et al. 1999; Peyron et al. 2000). In line with this, Kross et al. (2011) demonstrated reliable activation of the secondary somatosensory cortex and the posterior insula during a highly painful social rejection task in which participants viewed photographs of their ex-partners lost recently due to an unwanted break-up of the former friendship.

Increased activity of the SII and posterior insula in G-allele carriers also during social pain and negative feedback in the present study aligns with this dose-dependent relationship since the G-allele is thought to increase pain sensitivity so that the same dose of intensity leads to different heights of neural activations. It is, however, also of note in this context that a recent meta-analysis (Kross et al. 2011) of multiple studies contrasting high versus low intensity emotional stimuli demonstrated that SII/dorsal posterior insula activation was found only in relation to pain but not upon stimuli of merely intense emotion. (see also Phan et al. 2004; Waugh et al. 2010).

Activation of the supplementary motor cortex and adjacent medial cingulate cortex is highly consistent with previous reports of neural correlates of physical pain (e.g. Kwan et al. 2000; Misra and Coombes 2014; Wager et al. 2013). A very recent study (Misra and Coombes 2014) reported that brain regions associated with motor control and pain processing do overlap and activity in the SMA was increased when motor control and pain processing occurred simultaneously. The involvement of SMA proper and pre-SMA during pain processing has been related to a preparatory engagement of the motor system in planning or preparing a behavioural escape response to avoid more imminent pain (Misra and Coombes 2014; Wager et al. 2013). Enhanced activation of the SMA in the more pain-sensitive G-allele carriers might therefore reflect some greater anticipatory involvement of the motor system. It remains unclear, though, whether this would represent an increased effort to prepare escape, whether it represents some counteracting effort helping to resist impulses to move away from the painful stimulation (Gandolla et al. 2014), or—in the context of qualitatively different non-physical pain—whether it might represent prediction processes of the consequences of social exclusion or negative feedback (Atlas et al. 2014).

While the exact functional roles of increased activity upon physical and non-physical pain in carriers of the G-allele remain open, our data suggest that brain regions which are involved in the somatosensory processing of pain are modulated by the A118G polymorphism of the μ-opioid receptor gene. Regarding social exclusion, present findings could replicate the finding of one previous study on the same topic also demonstrating an association between G-allele carriers of the A118G polymorphism and social pain processing in the Cyberball task (Way et al. 2009) with relatively increased SMA activation in the more pain-sensitive G-allele carriers. Present findings are also consistent with another study demonstrating effects of this polymorphism on neural processing of pain under different doses of alfentanil, a commonly used opioid analgesic (Oertel et al. 2008). Increasing concentrations of opioids in this study had differential effects of the processing of pain depending on the different genotypes. In carriers of the G-allele of A118G, primary and secondary somatosensory cortices as well as posterior insular cortex showed significantly less pronounced decrease in activation in relation to alfentanil concentrations.

Despite the correspondence of present results with previous studies, one major limitation of our study is sample size, particularly of the AG group. This might account for the observation that behavioural data did not show significant group differences and for the absence of between-group differences in some specific regions of the pain matrix, e.g. dorsal anterior cingulate cortex or anterior insula (see Wager et al. 2013, Rotge et al. 2015, Cacioppo et al. 2013). This absence, however, must be treated with great caution before any functional interpretation in terms of differences in connectivity or signal propagation can be derived from the data. Especially, with respect to the risk of type-II errors, the study is clearly underpowered. Unfortunately, the base rate of carriers of the G-allele is relatively low which would afford to screen a multitude of subjects to obtain a reasonable sample size. Furthermore, methadone treatment of one of the participants of the G-allele group may have influenced the findings. However, the use of a synthetic opioid should bias results to reduced neural activity during pain stimulation due to its analgetic effects (Wise et al. 2002, 2004) but not to the opposite, that is enhanced pain-related activity, which might have had only a decreasing influence on average neural activity in the G-allele group.

Another limitation is the inclusion of left-handed subjects in the AA group. Although handedness has not been reported to affect pain processing in a similar extent as it has been reported for language or motor paradigms, we controlled

for this unfortunate effect by testing on between-group differences without those three left-handed subjects. The pattern of results remained stable as compared to the entire AA group, but would not have survived corrections for multiple comparisons at every instance. Therefore, present results have to be treated with caution and certainly await empirical replication in much larger and more homogenous samples.

Finally, as in previous studies with the Cyberball game (Eisenberger et al. 2003, 2006, 2007a), we used only one block for each condition without repetition. The use of several conditions, however, may increase reliability and statistical power and should therefore be considered in future studies together with further directions to improve the understanding of social rejection in fMRI research as summarised in a recent review (Kawamoto et al. 2015).

In conclusion, our results support the assumption of shared neural mechanisms regarding physical pain, socially distressing psychological events like social rejection and self-dependent intrinsic negative feedback (Bresin and Gordon 2013) that are modulated by *OPRM1*-receptor variations. Modulated activation appears to be particularly related to the processing of the somatosensory component of pain but awaits replication in larger, homogenous samples permitting greater specificity in associating the different aspects of pain processing with imaging results.

Acknowledgments Special thanks to Ursula Botzenhardt and Sabrina Skambraks for excellent technical assistance and to Prof. Dr. L. Bullinger for his laboratory support. In addition, many thanks to the team of BioGlobe Molecular Genetic Laboratory, particularly Dr. N. Storm, for excellent consulting and advanced service in genotyping. The study was conducted within the framework of a fellowship for individual doctoral training funded through the *Landesgraduierten-förderung* (LGFG).

Conflict of interest The authors MB, RCG, GG and BA declare that they have no conflicts of interest. PLP declares no competing interests. He is PI in a study for Lundbeck. He got research grants from the BMBF (German Ministries for Research and Education) and the BfArM (German Federal Institute for Drugs and Medical devices). He received travel grants from the DFG, DAAD and IACAPAP. He is not a stockholder or share-holder in the pharmaceutical industry.

Ethical standard All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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