

# Separating Brain Processing of Pain from that of Stimulus Intensity

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**Abstract:** Regions of the brain network activated by painful stimuli are also activated by nonpainful and even nonsomatosensory stimuli. We therefore analyzed where the qualitative change from nonpainful to painful perception at the pain thresholds is coded. Noxious stimuli of gaseous carbon dioxide ( $n = 50$ ) were applied to the nasal mucosa of 24 healthy volunteers at various concentrations from 10% below to 10% above the individual pain threshold. Functional magnetic resonance images showed that these trigeminal stimuli activated brain regions regarded as the “pain matrix.” However, most of these activations, including the posterior insula, the primary and secondary somatosensory cortex, the amygdala, and the middle cingulate cortex, were associated with quantitative changes in stimulus intensity and did not exclusively reflect the qualitative change from nonpainful to pain. After subtracting brain activations associated with quantitative changes in the stimuli, the qualitative change, reflecting pain-exclusive activations, could be localized mainly in the posterior insular cortex. This shows that cerebral processing of noxious stimuli focuses predominately on the quantitative properties of stimulus intensity in both their sensory and affective dimensions, whereas the integration of this information into the perception of pain is restricted to a small part of the pain matrix. *Hum Brain Mapp* 33:883–894, 2012. © 2011 Wiley Periodicals, Inc.

**Key words:** pain matrix; nociception; fMRI

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## INTRODUCTION

The perception of pain has been associated with the activation of a network of brain structures referred to as the “pain matrix” [Apkarian et al., 2005; Peyron et al., 2000]. However, the extent to which components of this network are required for the conscious experience of pain remains under discussion [Kupers and Kehlet, 2006]. Moreover, components of this pain matrix were also activated by non-nociceptive somatosensory, auditory, or visual stimuli [Baliki et al., 2009; Downar et al., 2000; Mouraux and Iannetti, 2009] and the amount of activation correlated with the saliency of the activating stimulus, that is, its ability to capture attention [Iannetti et al., 2008; Mouraux and

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Additional Supporting Information may be found in the online version of this article.

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Iannetti, 2009]. This led to the hypothesis that the pain matrix represents a sensory matrix of which pain is only a part [Mouraux and Iannetti, 2009]. Therefore, brain activations reflecting this qualitative change should be identifiable when taking out brain activations due to raising intensities of potentially noxious stimuli. This requires information about brain activations evoked by stimuli below pain threshold and above pain threshold, respectively, and about the activation reflecting stimulus intensity regardless of stimulus perception. Previous attempts to investigate pain analyzed brain activation in response to either painful and nonpainful stimuli without considering stimulus intensity related effects [Casey et al., 1994; Coghill et al., 1994; Talbot et al., 1991] or stimuli of graded intensity defined implicitly as either nonpainful or painful [Bornhövd et al., 2002; Coghill et al., 1999; Derbyshire et al., 1997; Tölle et al., 1999], or stimuli of a single intensity at the pain threshold while explicitly modeling the stimulus perception [Boly et al., 2007]. Neither design clearly avoids confusing pain perception with stimulus intensity processing. To obtain either information enabling this separation, in the present experiment, potentially nociceptive stimuli of different intensities around the individual pain threshold were applied to healthy volunteers, their painfulness was queried, and the associated brain activations were recorded using functional magnetic resonance imaging (fMRI).

## MATERIALS AND METHODS

### Subjects

Healthy, right-handed subjects (12 men, 12 women), aged 23.2–36.9 years (mean  $\pm$  standard deviation:  $29.0 \pm 0.7$  years) with normal body mass index ( $19.7\text{--}27.2$  kg/m<sup>2</sup>;  $23.3 \pm 2.4$  kg/m<sup>2</sup>) were enrolled after having given informed written consent. The study was conducted according to the Declaration of Helsinki on Biomedical Research Involving Human Subjects and had been approved by the Ethics Committee of the Medical Faculty of the Goethe-University, Frankfurt am Main, Germany. The subjects' health was ascertained by medical interview and a short examination. All medications, except oral contraceptives, were prohibited for 1 week, and alcohol for 24 h before the experiments taking place, which were performed between 9 a.m. and 2 p.m.

### Pain Stimulation and Experimental Design

Short pulses of gaseous CO<sub>2</sub> were administered to the mucosa of the subjects' right nostril by means of an "olfactometer" (OM/2, Burghart Instruments, Wedel, Germany), which allowed for the precise control of stimulus concentration, duration, and steepness of onset (stimulus duration 300 ms, stimulus rise time <50 ms; [Kobal, 1981,

1985]). To avoid stimulus habituation and adaptation [Hummel et al., 1994], a paradigm with long and regularly spaced intervals (interstimulus interval of 20 s) was preferred to a paradigm with short and randomly spaced intervals [Dale, 1999]. The CO<sub>2</sub>-stimuli triggered predominantly trigeminal A<sub>δ</sub>-fibers and, to a lesser degree, C-fibers [Steen et al., 1992] projecting into the nasal mucosa. They do this by activating transient receptor potential cation channels, subfamily V, member 1 (TRPV1), acid sensing ion channels [Ugawa et al., 2005], and proton sensing G-protein coupled receptors [Huang et al., 2007] expressed at the nociceptive nerve endings.

Subjects received 50 CO<sub>2</sub>-stimuli ranging from  $-10\%$  to  $+10\%$  v/v CO<sub>2</sub> (steps of  $0.5\%$  v/v CO<sub>2</sub>) of the individual pain threshold. Each stimulus was rated 4 s after its administration as either "unnoticed," "nonpainful," or "painful" (Presentation software, Neurobehavioral Systems, Albany, CA) [Bornhövd et al., 2002; Büchel et al., 2002]. The pain threshold was determined in a separate training session on the day before the actual measurements by applying 30 CO<sub>2</sub>-stimuli in the range from 20 to 50% v/v CO<sub>2</sub> under otherwise identical conditions as in the main experiment, that is, the subjects were placed in the MRI-scanner, and the blood oxygenation level dependent (BOLD) response to the CO<sub>2</sub>-stimuli was recorded. This temporally dissociated threshold determination was considered to provide sufficient guidance in the selection of the individual stimuli and allowed to keep the actual experiment short avoiding that subjects suffered fatigue that might have confounded the results. The pain threshold was determined by means of logistic regression of the responses unnoticed and nonpainful, coded as "0," or painful coded as "1." The CO<sub>2</sub> concentration at the pain threshold was  $44.4 \pm 3.0\%$  v/v CO<sub>2</sub> (mean  $\pm$  standard deviation). Thus, subjects received CO<sub>2</sub>-stimuli in a concentration range of 34–54 % v/v CO<sub>2</sub> (median range; minimum range: 29–49%, maximum range: 40–60 %) at the main study occasion. The main experiments started with administration of three CO<sub>2</sub> stimuli at individual pain threshold concentration to familiarize the subjects with the sensation. With each increase of the CO<sub>2</sub>-concentration of  $0.5\%$  v/v, the odds to rate a stimulus as painful increased by 1.42 times (Odds ratio; 95% CI = 1.36–1.48;  $\beta_{(\text{Concentration})}$ :  $0.35 \pm 0.02$ ,  $P < 0.01$ ; Model  $\chi^2(1) = 440.4$ ;  $R^2_{(\text{Nagelkerkes})} = 0.46$ ). The average number of administered unnoticed, nonpainful, and painful stimuli was  $12 \pm 8$ ,  $26 \pm 7$ , and  $12 \pm 5$ , respectively.

### Functional Imaging

The BOLD response to the CO<sub>2</sub> stimuli was recorded on a 3-T magnetic resonance head scanner (Siemens Magnetom Allegra, Siemens Medical Solutions, Erlangen, Germany) equipped with a 4-channel transmit-receive head coil. The subjects' head was immobilized using foam pads. Using a T<sub>2</sub>\*-weighted echo planar imaging (EPI)

sequence [parallel imaging method: GRAPPA (reduction factor  $R = 2$ ), repetition time 1530 ms, echo time 30 ms, flip angle  $90^\circ$ , matrix size  $64 \times 64$ , voxel size  $3 \times 3 \times 3 \text{ mm}^3$ ], 760 imaging volumes [29 axial slices (distance factor 40%) covering the whole brain] were acquired. Additionally, high resolution (voxel size  $1 \times 1 \times 1 \text{ mm}^3$ )  $T_1$ -weighted anatomical images were acquired for each subject using a three-dimensional (3D) magnetization prepared rapid gradient echo sequence.

Functional magnetic resonance brain image processing and statistical analyses were done with the statistical parametric mapping software SPM8 (Wellcome Department of Imaging Neuroscience, London, UK; <http://www.fil.ion.ucl.ac.uk/spm/> [Friston, 1995; Worsley and Friston, 1995]). All volumes of the EPI sequence were corrected for slice timing and realigned to the first volume [Friston et al., 1995]. The high-resolution  $T_1$ -weighted anatomical image was coregistered to the mean-EPI, segmented, and normalized using forth-degree B-spline interpolation. The resulting spatial normalization parameters were applied to the volumes of the EPI-sequence, which were subsequently smoothed with an isotropic 8-mm full-width-half-maximum Gaussian kernel.

First-level analysis used a general linear model [Friston, 1995] to partition the observed neurophysiological responses into components of interest, confounds, and errors. An event-related analysis estimated the BOLD responses evoked by the  $\text{CO}_2$  stimuli by modeling them as delta functions convolved with the canonical hemodynamic response function as implemented in SPM8.

$\text{CO}_2$ -stimuli-associated activations and deactivations, the latter also having been shown during painful stimulation [Kong et al., 2010], were analyzed in three steps. The first analysis aimed to ascertain that the painful  $\text{CO}_2$  stimuli activated the pain matrix as expected or led to deactivations of other brain regions. All painfully perceived  $\text{CO}_2$  stimuli were therefore modeled as a single regressor ("painful stimuli" regressor) in a categorical first-level (single subject) design. The unnoticed and nonpainful stimuli were additionally modeled as separate regressors within the design matrix but omitted from the second level analysis. The second and main analysis aimed at separating effects exclusively related to pain perception from stimulus intensity related effects or other stimulus inherent effects. All  $\text{CO}_2$  stimuli were therefore modeled as a single regressor of interest (" $\text{CO}_2$ -stimulus" regressor) in a parametric first-level design. Three first-order parametric regressors modulating the stick function, each orthogonalized with respect to the prior regressor, were added to the model in the order as follows (for a more detailed explanation of the regressor order, see Supporting Information): "stimulus intensity" ( $\text{CO}_2$  concentrations), "nonpainful perception" (Yes = 1/No = 0), and "painful perception" (Yes = 1/No = 0). This implemented a step-down regression that allowed to categorize the pain-related effects in (i) activations and deactivations occurring with each stimulus irrespective of its intensity and perception (" $\text{CO}_2$

stimulus" regressor), (ii) activations or deactivations depending on the physical stimulus intensity irrespective of stimulus perception (stimulus intensity regressor), (iii) activations or deactivations occurring when the stimulus was perceived as nonpainful (nonpainful perception regressor), and (iv) activations or deactivations occurring exclusively when the stimulus was perceived as painful (painful perception regressor). The assumption that stimulus-related brain activation increases linearly with increasing stimulus intensity regardless of its perception is based on reports of a linear relationship between the subjective intensity ratings and  $\text{CO}_2$  stimulus concentration in the present concentration range [Cain and Murphy, 1980; Frasnelli et al., 2003; Lötsch et al., 1997b]. The unnoticed stimuli were not explicitly modeled and served as implicit baseline in the model. The third analysis was performed to illustrate the different relationships between the  $\text{CO}_2$  stimulus concentrations and the corresponding brain activations that led to the separation of the different regressors in the second analysis. Therefore, the 50  $\text{CO}_2$  stimuli were arranged in 10 concentration regressors with each regressor representing the pooled activation of five  $\text{CO}_2$  stimuli in the range of 2% v/v  $\text{CO}_2$ . Subsequently, the mean percent signal change associated with each concentration was calculated for a 5-mm spherical search volume around selected peak coordinates determined for each regressor in the second analysis. This was done by means of the `rfxplot-toolbox` [Gläscher, 2009].

Additional regressors of no interest were modeled in each analysis within the design matrix including the first three  $\text{CO}_2$  stimuli administered for reacquaintance, the stimulus rating event and the six rotational and translational parameters from the rigid body transformation, obtained during image realignment. Low frequency fluctuations of the MR signal were removed with a high-pass filter (1/128 s frequency cutoff). The serial autocorrelation of the BOLD time series was modeled using a first-order autoregressive model. Voxelwise regression coefficients for all regressors were estimated using least squares within SPM8. After model estimation, the effects of interest were tested by using linear contrasts, generating statistical parametric maps for each subject.

The computed simple contrast maps derived from each participant were included in a random effects (RFX) analysis. Activations and deactivations associated with each regressor of interest were tested by means of simple positive (contrast weight: 1) and negative (contrast weight: -1)  $t$ -contrasts, respectively. The resulting statistical parametric maps (SPM $t$ ) were interpreted with regard to the probabilistic behavior of Gaussian random fields [Worsley, 1994]. Only voxels that were significant at the familywise error [Loring et al., 2002] corrected  $\alpha$  level ( $P < 0.05$ ) are reported. The localization of brain activations was aided by the anatomy toolbox, version 1.7, [Eickhoff et al., 2006, 2007, 2005]. Significant peak activations are reported as Montreal Neurological Institute coordinates (mm).

## RESULTS

Activations associated with the painful stimuli at pooled suprathreshold intensities were found in brain regions repeatedly attributed to the pain matrix, mainly contralaterally to the stimulated nostril (first RFX-analysis, painful stimuli regressor, *t*-contrast weight: 1). Specifically, activation was observed in the secondary somatosensory cortex, the postcentral ( $S_i$ ) and precentral ( $M_i$ ) gyrus, the inferior frontal gyrus, and the supplementary motor area. In addition, the insula lobe and middle cingulate cortex were bilaterally activated (Fig. 1/Panel A, Table I).

The stimulus-related activations observed in the pain matrix could be categorized in three different activation patterns (second RFX-analysis): (i) activations occurring with each stimulus irrespective of its intensity and perception ( $CO_2$  stimulus regressor, *t*-contrast weight: 1). This was observed bilaterally in the anterior insula and the supplementary motor area (Fig. 1/Panel B, Table II). (ii) Activations depending on the physical stimulus intensity irrespective of its perception ("stimulus intensity" regressor, *t*-contrast weight: 1). This was observed in the majority of brain regions including the bilateral posterior insula and superior temporal gyri, the contralateral secondary somatosensory cortex, amygdala, parts of the postcentral gyrus, and the middle cingulate cortex. (iii) Activations occurring exclusively when the stimulus was perceived as painful ("painful perception" regressor, *t*-contrast weight: 1). This was observed in only a small fraction of the pain matrix, mainly in the contralateral postcentral gyrus, precentral gyrus and middle insula, and in the ipsilateral putamen (Fig. 2). Exclusively nonpainful perception-related activation ("nonpainful perception" regressor, *t*-contrast weight: 1) was not observed.

The painful stimuli were further associated with deactivations in large areas of the brain (first RFX analysis, painful stimuli regressor, *t*-contrast weight: -1). However, in contrast to the activations, all deactivations occurred with each stimulus irrespective of its intensity and perception (second RFX analysis,  $CO_2$  stimulus regressor, *t*-contrast weight: -1). This was observed bilaterally in the fusiform gyrus, inferior occipital lobe, precuneus and rectal gyrus, contralaterally in the hippocampus, calcarine gyrus, posterior cingulate cortex, mid orbital gyrus, angular gyrus, middle temporal gyrus and middle frontal gyrus, ipsilateral in the lingual gyrus, thalamus, and superior medial gyrus (Fig. 3 and Table III).

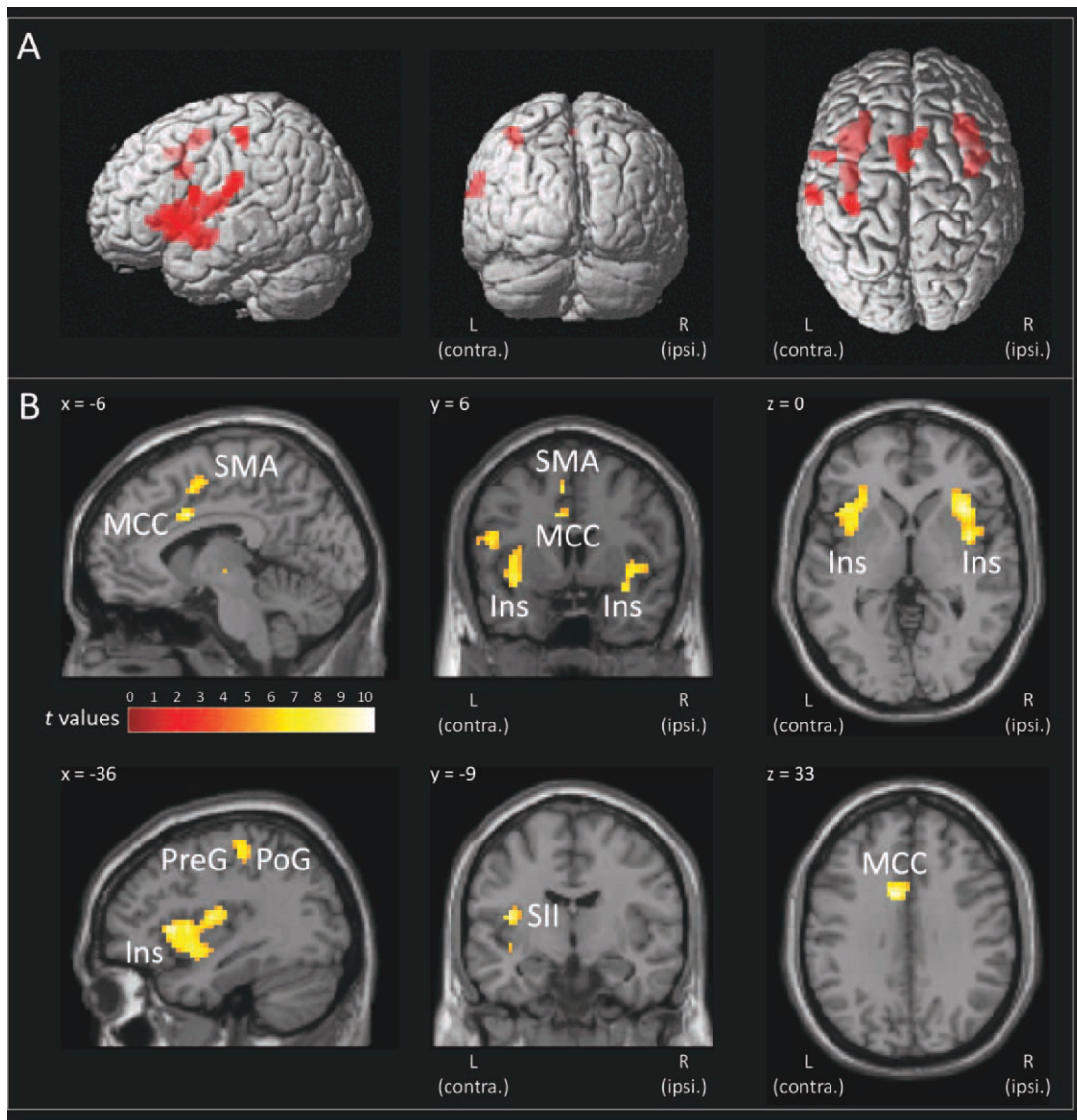
## DISCUSSION

The painful  $CO_2$  stimuli evoked activations in a network of brain regions frequently regarded as the pain matrix [Apkarian et al., 2005; Peyron et al., 2000]. However, when separating brain activations with respect to the underlying activation pattern, only a small part of the pain matrix remained specifically associated with the perception of

pain, whereas the major part reflected processing of stimulus intensity irrespective of stimulus perception and stimulus inherent processes that were neither related to stimulus intensity nor perception.

Only a small part of the pain matrix seems to be exclusively associated with the perception of pain. This included a small area in the contralateral part of the posterior insula, the postcentral gyrus, precentral gyrus, and ipsilateral putamen. Among these regions, an area in the posterior insula appears to provide sufficient neurophysiological properties for representing the qualitative change in stimulus evaluation occurring at the pain threshold. First, it is a major localization of pain-related brain activation and has been frequently reported in this context [Boly et al., 2007; Bornhövd et al., 2002; Casey et al., 1994; Coghill et al., 1999; Coghill et al., 1994; Derbyshire et al., 1997; Talbot et al., 1991; Tölle et al., 1999]. Second, the insula is extensively connected to other brain regions such as the prefrontal cortex, cingulate cortex, amygdala, parahippocampal gyrus, and secondary somatosensory cortex [Friedman and Murray, 1986; Friedman et al., 1986; Mesulam and Mufson, 1982; Mufson and Mesulam, 1982; Mufson et al., 1981]. It may therefore act as a relay integrating afferent nociceptive information with working memory, affect and attention, and may selectively gate nociceptive information at the cortical level to modulate varying levels of appreciation of the nociceptive stimulus [Starr et al., 2009]. Other reports substantiate this key role of the posterior insula for pain [Baliki et al., 2009; Frot et al., 2007], and it has been shown that pain can be induced by electrical stimulation of the insula [Mazzola et al., 2009].

The exclusively pain perception-related activation of the postcentral and precentral gyri, and the putamen appears to be less likely to represent the qualitative component of pain as these areas are typical motor areas. Nevertheless, pain-induced activation of motor areas has been repeatedly observed [Apkarian et al., 2000; Casey et al., 1996; Gelnar et al., 1999; Porro et al., 1998] and interpreted as the subjects attempt to terminate, reduce, or escape the presence of pain [Price, 2000]. In this respect it is further notable that the posterior insula has been suggested to be involved in directing pain-related motor responses [Henderson et al., 2007]. The putamen has been associated with immediate defense and withdrawal behavior from potential noxious external influence [Bingel et al., 2004, 2002]. Pain exclusive activation of the postcentral and precentral gyrus, however, was observed in the hand region of the body [Moore et al., 2000] hinting in the present context at a connection with the experimental rating task because it has not been observed when applying the  $CO_2$  stimuli without a subsequent rating task [Oertel et al., 2008]. It may reflect the subject's instantaneous certainty about the painful stimulus quality and the associated urge to press the painful button, which has been shown to activate the precentral gyrus even when movement was only imagined and not executed [Porro et al., 1996]. However, it should be noted that motor activity and pain may be closely



**Figure 1.**

Brain regions activated by painful CO<sub>2</sub> stimuli activated brain regions typically attributed to the pain matrix, including the insula lobe (Ins), secondary somatosensory cortex (SII), middle cingulate cortex (MCC), postcentral gyrus (PoG), precentral gyrus (PreG), inferior frontal gyrus, and the supplementary motor area (SMA). Statistically significantly activated voxels ( $P < 0.05$  familywise error corrected) of the painful stimuli  $t$ -contrast (contrast weight: 1) are presented overlaid

(red) on 3D surface renderings of a standard Montreal Neurological Institute brain (Panel A) and as colored overlay on the three orthogonal views of a structural standard T<sub>1</sub>-weighted MRI template (Panel B; L, Left hemisphere, contralateral to the presentation of the painful stimuli; R, Right hemisphere). The color depth of the displayed voxels reflects the respective  $t$  value of the voxel. Results reflect a 24-subject group analysis.

associated, especially when pain is produced by muscle contraction as in the human experimental model involving the RIII reflex [Willer, 1997], which has been shown by means of fMRI [Peyron et al., 2007]. In addition, activation

of brain motor areas is correlated with pain intensity [Bornhövd et al., 2002; Coghill et al., 1999], which further draws the attention to activation of motor areas as possible not pain specific confounders.

**TABLE I. Brain regions activated by painful CO<sub>2</sub> stimuli**

Effect	Left hemisphere <sup>a</sup>				Right hemisphere			
Region	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value
<b>Activations during painful CO<sub>2</sub> stimuli</b>								
Insula lobe	-33	18	6	10.36	39	15	-6	10.11
Insula lobe	-36	9	3	8.81	33	24	3	9.29
Insula lobe	-36	6	-6	8.41	39	-3	6	8.87
Insula lobe	-39	3	-9	8.37	—	—	—	—
MCC	-9	9	33	9.82	6	15	45	7.34
SII	-39	-9	12	9.55	—	—	—	—
Postcentral gyrus	-36	-27	51	7.95	—	—	—	—
Postcentral gyrus	-60	-21	27	7.77	—	—	—	—
Postcentral gyrus	-63	-18	21	7.43	—	—	—	—
Precentral gyrus	-51	3	18	8.35	—	—	—	—
IFG	-60	6	15	6.53	—	—	—	—
SMA	-6	6	51	7.46	—	—	—	—

<sup>a</sup>Contralateral to the stimulus application side. SII, secondary somatosensory cortex; MCC, Middle cingulate cortex; IFG, Inferior frontal gyrus; SMA, supplementary motor area.

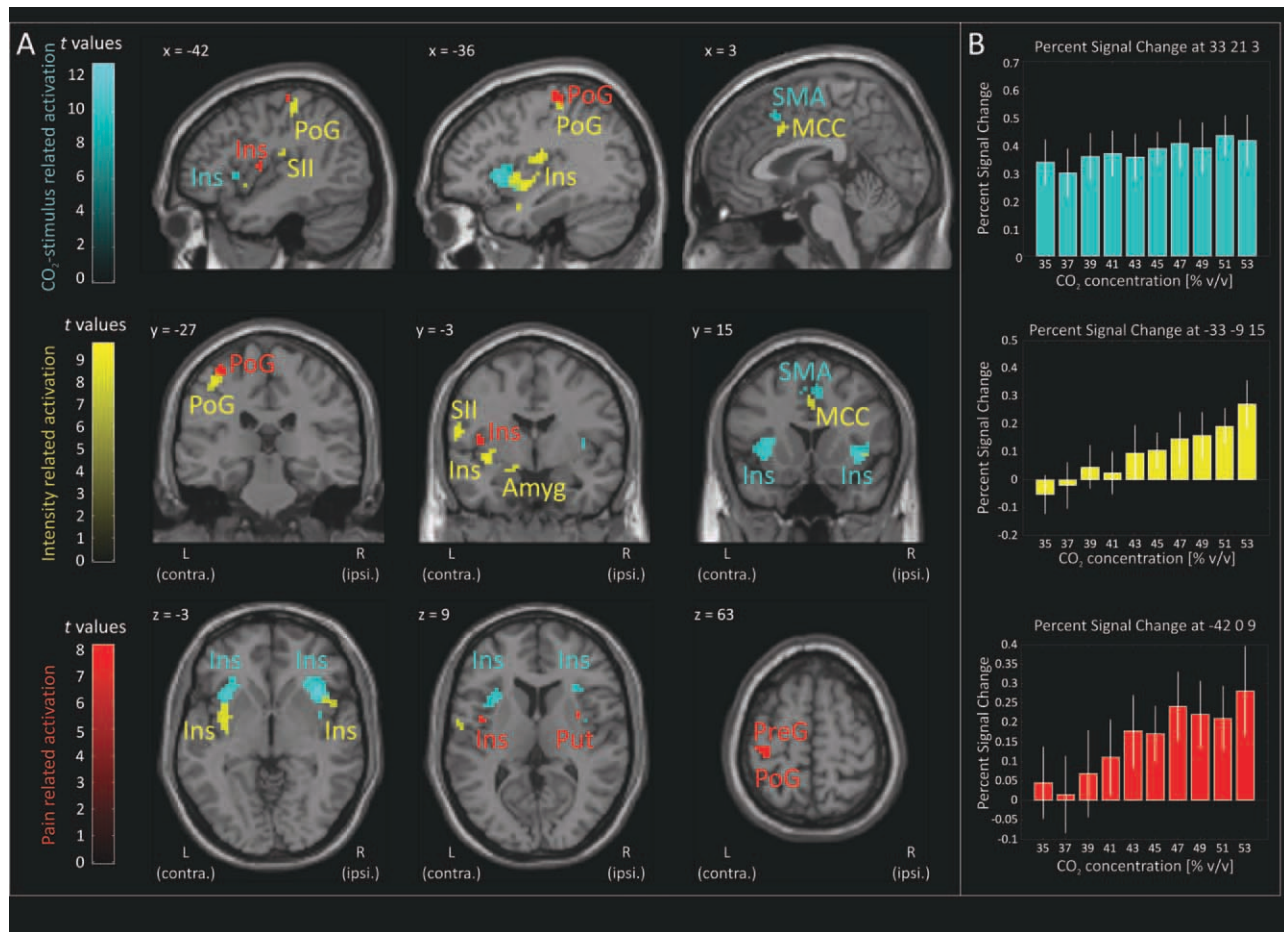
The table contains the anatomic location of the voxels with highest voxel level *t* in the respective region of a 24-subject group analysis. Reported voxels are thresholded at *P* < 0.05 familywise error (FWE) corrected. Coordinates are reported in MNI space.

**TABLE II. Brain regions activated by the CO<sub>2</sub> stimulus irrespective of its intensity and corresponding perception, brain regions coding for stimulus intensity irrespective of the corresponding perception, and brain regions coding exclusively for pain perception**

Effect	Left hemisphere <sup>a</sup>				Right hemisphere			
Region	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value
<b>CO<sub>2</sub>-stimulus-related activation</b>								
Insula lobe	-30	21	3	11.21	33	21	3	12.75
Insula lobe	-33	18	0	10.84	39	0	3	7.52
Insula lobe	-36	6	-9	6.54	—	—	—	—
SMA	-3	12	48	7.25	6	18	48	7.78
SMA	—	—	—	—	12	9	51	7.12
<b>Stimulus intensity-related activation</b>								
Insula lobe	-33	-9	15	9.70	45	12	-3	7.24
Insula lobe	-36	-3	-6	8.54	51	9	-3	6.94
Superior temporal gyrus	-57	-9	6	6.73	60	3	3	7.10
SII	-45	-18	21	7.65	—	—	—	—
SII	-48	3	12	6.71	—	—	—	—
Amygdala (LB)	-27	0	-21	8.05	—	—	—	—
Amygdala (SF)	-18	0	-15	7.15	—	—	—	—
Postcentral gyrus	-39	-27	54	7.67	—	—	—	—
Postcentral gyrus	-57	-3	15	7.60	—	—	—	—
Postcentral gyrus	-60	-15	27	7.07	—	—	—	—
Postcentral gyrus	-57	-12	30	7.02	—	—	—	—
Postcentral gyrus	-63	-21	15	7.50	—	—	—	—
MCC	0	12	39	7.37	—	—	—	—
<b>Pain perception-related activation</b>								
Precentral gyrus	-39	-24	60	8.14	—	—	—	—
Postcentral gyrus	-39	-33	57	7.12	—	—	—	—
Insula lobe	-42	0	9	7.83	—	—	—	—
Putamen	—	—	—	—	33	3	9	6.83

<sup>a</sup>Contralateral to the stimulus application side. SII, secondary somatosensory cortex; MCC, Middle cingulate cortex; SMA, supplementary motor area.

The table contains the anatomic location of the voxels with highest voxel level *t* in the respective region of a 24-subject group analysis. Reported voxels are thresholded at *P* < 0.05 family wise error (FWE) corrected. Coordinates are reported in MNI space.



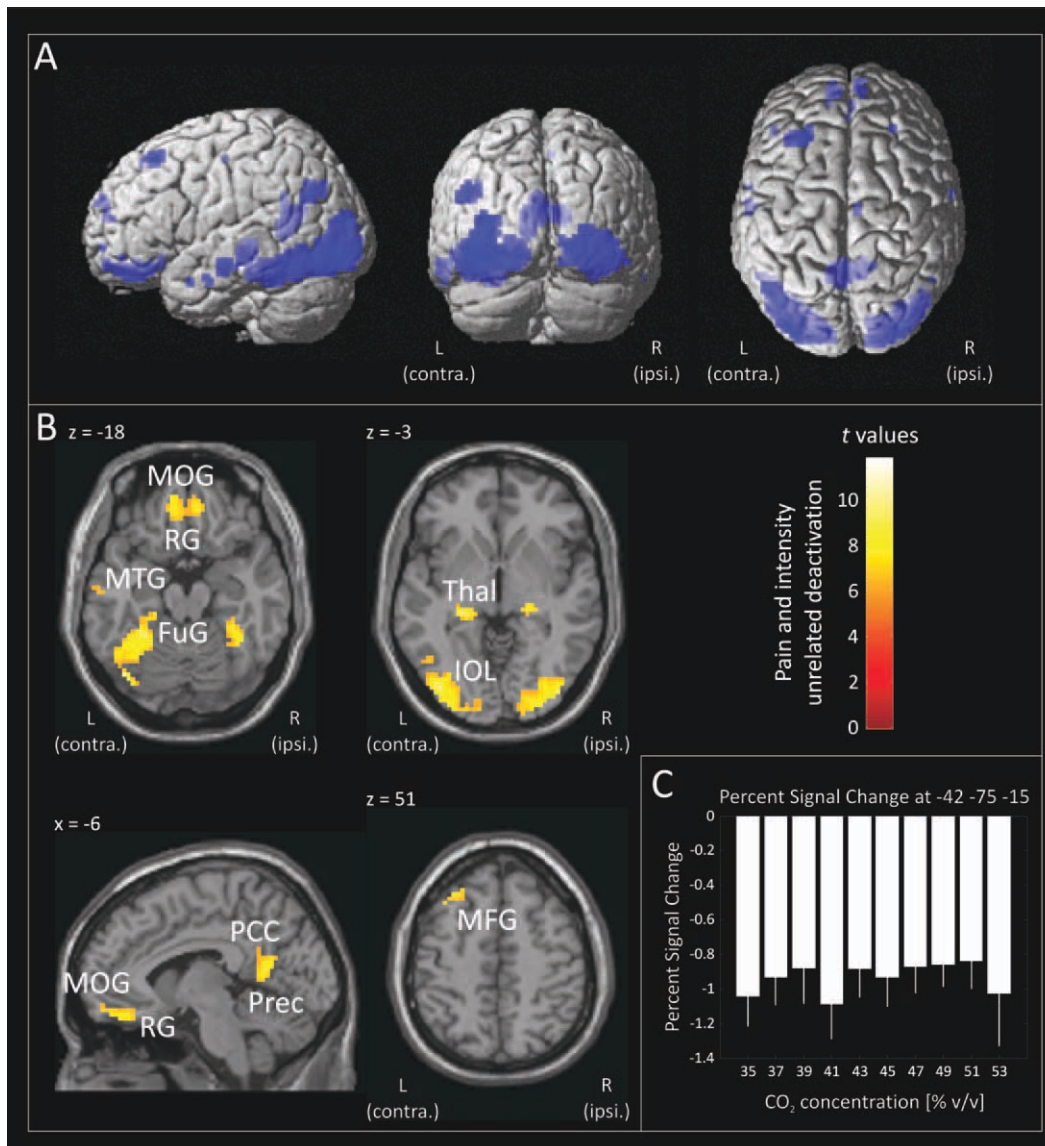
**Figure 2.**

CO<sub>2</sub>-stimulus related activation. Brain regions that were activated by the CO<sub>2</sub>-stimulus irrespective of its intensity and perception (cyan areas; “CO<sub>2</sub>-stimulus” regressor, *t*-contrast weight: 1) included the anterior insula (Ins) and the supplementary motor area (SMA). Regions coding for stimulus intensity irrespective of its perception (yellow areas; “stimulus intensity” regressor, *t*-contrast weight: 1) included the posterior insula, secondary somatosensory cortex (SII), amygdala (Amyg), post-central gyrus (PoG), superior temporal gyrus (STG), middle cingulate cortex (MCC). Regions displaying exclusively pain perception related activation (red areas; “painful perception” regressor, *t*-contrast weight: 1) included the postcentral gyrus, precentral gyrus (PreG), parts of the mid to posterior insula and the putamen (Put). In contrast, non-painful stimulus perception

was not associated with any specific brain activation (“non-painful perception” regressor, *t*-contrast weight: 1). Statistically significantly activated voxels ( $P < 0.05$  familywise error corrected) are displayed as colored overlay on a structural standard *T*<sub>1</sub>-weighted MRI template (Panel A; L, Left hemisphere, contralateral to the presentation of the painful stimuli; R, Right hemisphere). The color depth of the displayed voxels reflects the respective *t* value of the voxel. For each regressor, stimulus related brain activations corresponding to the different CO<sub>2</sub>-concentrations are reported as mean percent signal change in a 5-mm spherical search volume around selected familywise error-corrected peak coordinates (Panel B; PSC: Colored bars; 90% CI: white error bars). Results reflect a 24-subject group analysis.

In contrast to the small areas displaying exclusively pain perception-related activation, in the majority of brain regions regarded as the pain matrix activation correlated with the physical stimulus intensity irrespective of pain perception. This was observed in middle and posterior parts of the insula, SII, SI, the amygdala, and the midcin-

gulate cortex. These regions are associated with processing sensory (posterior insula, SII, SI) or affective (amygdala, midcingulate cortex) stimulus information [Coghill et al., 1999; Schneider et al., 2001; Vogt, 2005] suggesting that a nonpainful nociceptive stimulus is primarily assessed quantitatively with regard to its sensory intensity and



**Figure 3.**

CO<sub>2</sub>-stimulus-related brain deactivation. Brain regions were deactivated irrespective of the stimulus intensity and perception ("CO<sub>2</sub> stimulus" regressor, *t*-contrast weight:  $-1$ ). Deactivated regions included the fusiform gyrus (FuG), hippocampus (HipC), inferior occipital lobe (IOL), precuneus (Prec), posterior cingulate cortex (PCC), rectal gyrus (RG), mid orbital gyrus (MOG), thalamus (Thal), middle temporal gyrus (MTG), and middle frontal gyrus (MFG). Statistically significant deactivated voxels ( $P < 0.05$  familywise error corrected) are presented overlaid (blue) on 3D surface renderings of a standard Montreal Neurological Institute brain (Panel **A**) and as colored overlay on the horizon-

tal and sagittal plane of a structural standard *T*<sub>1</sub>-weighted MRI template (Panel **B**; L, Left hemisphere, contralateral to the presentation of the painful stimuli; R, Right hemisphere). The color depth of the displayed voxels reflects the respective *t* value of the voxel. Furthermore, stimulus related brain activations corresponding to the different CO<sub>2</sub> concentrations are reported as mean percent signal change in a 5-mm spherical search volume around a selected familywise error-corrected peak coordinate (Panel **C**; PSC: Colored bars; 90% CI: white error bars). Results reflect a 24-subject group analysis.



**TABLE III. Brain regions displaying a CO<sub>2</sub>-stimulus-associated deactivation that was not related to stimulus intensity or pain perception**

Effect	Left hemisphere <sup>a</sup>				Right hemisphere			
Region	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value
<b>Perception and intensity unrelated deactivation</b>								
Fusiform gyrus	-42	-75	-15	11.77	39	-51	-15	8.92
Fusiform gyrus	-27	-45	-18	9.49	—	—	—	—
Fusiform gyrus	-27	-51	-12	9.32	—	—	—	—
Fusiform gyrus	-36	-51	-12	9.09	—	—	—	—
Hippocampus	-24	-30	-6	10.42	—	—	—	—
Inferior occipital lobe	-33	-90	-6	9.62	36	-84	-12	10.27
Inferior occipital lobe	—	—	—	—	42	-84	-3	9.41
Inferior occipital lobe	—	—	—	—	27	-87	-12	9.14
Inferior occipital lobe	—	—	—	—	27	-93	-3	8.39
Lingual gyrus	—	—	—	—	24	-90	-9	8.61
Precuneus	-6	-57	15	8.63	9	-51	18	7.89
Calcarine gyrus	-9	-51	6	8.43	—	—	—	—
Posterior cingulate cortex	-3	-51	27	6.58	—	—	—	—
Rectal gyrus	-9	42	-21	8.61	9	45	-18	8.06
Rectal gyrus	-9	51	-18	7.74	—	—	—	—
Mid orbital gyrus	-9	57	-15	7.46	—	—	—	—
Angular gyrus	-48	-66	30	7.78	—	—	—	—
Thalamus	—	—	—	—	21	-27	0	9.08
Middle temporal gyrus	-60	-15	-12	7.73	—	—	—	—
Middle frontal gyrus	-30	30	51	8.33	—	—	—	—
Middle frontal gyrus	-27	27	54	8.17	—	—	—	—
Superior medial gyrus	—	—	—	—	9	57	18	6.8

<sup>a</sup>Contralateral to the stimulus application side. The table contains the anatomic location of the voxels with highest voxel level *t* in the respective region of a 24-subject group analysis. Reported voxels are thresholded at  $P < 0.05$  family wise error (FWE) corrected. Coordinates are reported in MNI space.

unpleasantness. It has recently been shown that these regions assess not only the magnitude of nociceptive stimuli but also of stimuli of various modalities, for example, auditory or visual stimuli [Baliki et al., 2009; Downar et al., 2000; Mouraux and Iannetti, 2009] and that activation in these regions correlated with the saliency of the activating stimulus, that is, its ability to capture attention [Iannetti et al., 2008; Mouraux and Iannetti, 2009]. These and our results suggest that major parts of the so called pain matrix represent primarily a sensory matrix that is not specifically coding for pain.

The remaining activations observed during the painful CO<sub>2</sub> stimuli were associated with the stimulus irrespective of its intensity and perception. This included the anterior insula and supplementary motor area. The anterior insula has been shown to be activated by a multitude of interoceptive stimuli including pain [Craig, 2009] and has been suggested to be the “neural correlate of consciousness” [Christensen et al., 2006] or the “neuronal correlate of awareness” [Craig, 2009]. Pain-related activation of the anterior insula was significantly attenuated when subjects were distracted from pain [Brooks et al., 2002; Craig et al., 2000] or did not have to rate the pain intensity [Schoedel et al., 2008]. The presently observed activation of the insula with each stimulus is therefore probably related to

the subjects’ attention required to fulfill the subsequent rating task. The anterior insula nevertheless is thought to be a key area of pain. Its pre-(pain)-stimulus functional connectivity with the brainstem periaqueductal gray recently has been shown to determine whether a noxious event is perceived as painful or not and to be modulated by pain-relevant personality traits [Ploner et al., 2010]. The supplementary motor area on the other hand plays an important role in the organization of movement [Cunnington et al., 2006] and therefore probably reflects the subjects preparation for the subsequent button press related to the rating task. Activations of both areas, however, are not pain exclusive although having been observed in many pain investigations [Apkarian et al., 2005; Peyron et al., 2000].

The painful CO<sub>2</sub> stimuli were also associated with brain deactivations. These were mainly observed in regions attributed to the core regions of the “default mode network” (DMN) [Buckner et al., 2008] including the posterior cingulate cortex, precuneus, hippocampus, angular gyrus, middle temporal gyrus, and mid orbital gyrus. However, deactivations also occurred in brain regions not primarily associated with the DMN including the inferior occipital lobe, calcarine gyrus, fusiform gyrus, lingual gyrus, rectal gyrus, middle frontal gyrus, and superior medial gyrus

and the thalamus. The DMN is suggested to be activated when subjects are left to think to themselves and deactivated during the execution of goal-oriented behaviors [Buckner et al., 2008]. The present deactivations occurred for all CO<sub>2</sub> stimuli and were not exclusively related to pain perception or stimulus intensity. They therefore probably reflect a sensory filter, which is switched off to allow for conscious stimulus perception. Recently, it has been shown that deactivations decreased with increasing pain intensity [Kong et al., 2010], which might indicate a protective function of this network that has presently not come into play because only slightly painful stimuli were applied. However, this is only a hypothesis and whether all deactivated regions act as sensory filter needs further clarification because other investigations have shown that pain stimulus-related deactivation depended on the stimulated tissue, that is, the signal intensity in the perigenual anterior cingulate cortex decreased during muscle but not cutaneous pain [Henderson et al., 2007; Svensson et al., 1997].

Looking through the published literature, reports of experiments can be found that use different approaches to investigate the different activation patterns underlying pain processing. For example, there are two fMRI studies that applied laser stimuli of graded intensity defined implicitly as either nonpainful or painful and subsequently identified brain regions coding for either stimulus perception, stimulus intensity, or pain intensity by fitting different stimulus response functions to the BOLD responses [Bornhövd et al., 2002; Büchel et al., 2002]. Thus, they found that activation of SII, the anterior insula, parts of the cingulate cortex, and the amygdala correlated with pain intensity instead of stimulus intensity as shown here. However, another study applying the same stimulus while taking intracranial recordings of evoked potentials showed concordantly that activation in the posterior insula coded for pain intensity but, in contrast, activation in SII correlated with stimulus intensity in the nonpainful and the painful range [Frot et al., 2007], which is similar to our results. In contrast to these reports, only binary pain ratings were acquired in the current study, which did thus not allow for separating between stimulus intensity- and pain intensity-related activation increases. However, such approach was adequate because we primarily aimed at identifying brain regions coding specifically for the change of the perception quality from nonpainful to painful. Activations in response to the stimulus intensity or other stimulus inherent effects were therefore statistically controlled for. Thus, the present experiment complements available fMRI data on pain by using a unique experimental design, which allowed for this separation. The only real discrepancy to previously published data is the presently observed stimulus inherent constant activation of the anterior insula, which has previously been found to correlate with pain intensity [Bornhövd et al., 2002]. A possible explanation for this discrepancy may be related to differences in the experimental design across studies, for exam-

ple, different pain stimuli displayed different activation and deactivation patterns that further depended on the stimulated tissue, that is, whether it originated from cutaneous or muscle tissue [Helmchen et al., 2008; Henderson et al., 2007; Svensson et al., 1997]. Most fMRI studies on pain used painful thermal or laser stimulation of cutaneous tissue [Apkarian et al., 2005]. The presently used pain model and application site provides, therefore, additional new information to the already available fMRI data on pain processing.

A potential problem of the current study was the comparably lower probability of “painful” stimuli (24% of the total number of applied stimuli). Infrequent innocuous and noxious sensory stimuli, in contrast to frequent stimuli, were shown to increase EEG-derived event-related brain potentials [Legrain et al., 2002] and also fMRI-derived BOLD responses [Menon et al., 1997]. This is usually observed when applying the stimuli according to an oddball paradigm. In contrast, in the present study, painful and nonpainful stimuli were mixed in a way that was similar to the succession of clearly distinguished suprathreshold CO<sub>2</sub> stimuli (55% v/v CO<sub>2</sub>, *n* = 8; 60%, *n* = 16; 65%, *n* = 16) applied in previous studies on effects of analgesics on event-related brain potentials [Hummel et al., 1995; Lötsch et al., 1997a]. In these studies, infrequent stimuli were not accompanied by increased P<sub>300</sub> amplitudes or other EEG signs of rareness processing.

The present results suggest that a potentially noxious stimulus is gated to the brain by deactivations in brain regions that had been labeled DMN. It reaches brain structures known as the pain matrix including areas processing qualitative information about sensory and affective dimensions of the stimulus. Although the most parts of the pain matrix represent quantitative processing of sensory input or stimulus inherent processes not specific for pain, the qualitative change in processing of potentially noxious stimuli at the pain threshold, where they become painful, is restricted to the posterior insular cortex. The results therefore suggest a focus on the posterior insular cortex to target pain.

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