Spatial segregation of somato-sensory and pain activations in the human operculo-insular cortex.

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Abstract :

The role of operculo-insular region in the processing of somato-sensory inputs, painful or not, is now well established. However, available maps from previous literature show a substantial overlap of cortical areas activated by these stimuli, and the region referred to as the "secondary somatosensory area (SII)" is widely distributed in the parietal operculum. Differentiating SII from posterior insula cortex, which is anatomically contiguous, is not easy, explaining why the "operculo-insular" label has been introduced to describe activations by somatosensory stimuli in this cortical region. Based on the recent cyto-architectural parcellation of the human insular/SII cortices (Eickhoff et al., 2006, Kurth et al., 2010), the present study investigates with functional MRI (fMRI), whether these structural subdivisions could subserve distinct aspects of discriminative somato-sensory functions, including pain. Responses to five types of stimuli applied on the left hand of 25 healthy volunteers were considered: *i)* tactile stimuli; *ii)* passive movements; *iii)* innocuous cold stimuli; *iv)* non-noxious warm and *v)* heat pain.

Our results show different patterns of activation depending on the type of somato-sensory stimulation. The posterior part of SII (OP1 area), contralateral to stimuli, was the only sub-region activated by all type of stimuli and might therefore be considered as a common cortical target for different types of somato-sensory inputs. Proprioceptive stimulation by passive finger movements activated the posterior part of SII (OP1 sub-region) bilaterally and the contralateral median part of insula (PreCG and MSG). Innocuous cooling activated the contralateral posterior part of SII (OP1) and the dorsal posterior and median part of insula (OP2, PostCG). Pain stimuli induced the most widespread and intense activation that was bilateral in SII (OP1, OP4) and distributed to all sub-regions of contralateral insula (except OP2) and to the anterior part of the ipsilateral insula (PreCG, MSG, ASG). However, the posterior granular part of insula contralateral to stimulus (Ig area) and the anterior part of SII

bilaterally (OP4) were specifically activated during pain stimulation. This raises the question whether these latter areas could be the anatomical substrate of the sensory-discriminative processing of thermal pain.

Highlights :

- Five different somatosensory stimuli were applied on the left hand of 25 volunteers.
- fMRI activations were described in the operculo-insular (OI) cortex.
- Somato-sensory functions were consistent with structural subdivisions.
- Each type of stimuli evoked a specific co-activation of several sub-regions.
- Spatial segregation of somatosensory and pain activations were found in the OI cortex

Abreviations : (see figure 1)

- OP1, OP2, OP3, OP4 : the four cyto-architectonic sub-divisions of parietal operculum
- Ig : granular sub-divisions of the posterior insula
- Id1 : dysgranular sub-division of the posterior insula
- PostCG : post-central insular gyrus
- PreCG : pre-central insular gyrus
- MSG : middle short insular gyrus
- ASG : anterior short insular gyrus

INTRODUCTION

The role of operculo-insular (OI) cortex in the processing of painful sensations is now well established on the basis of various experimental approaches, such as single unit recordings (Robinson and Burton 1980a, 1980b), anatomical studies in monkeys (Burton and Jones 1976; Mesulam and Mufson, 1982; Mufson and Mesulam, 1984; Craig, 1995), laser evoked potentials in humans, as recorded on the scalp (Valeriani et al., 2000; Vogel et al., 2003) or with intra-cerebral electrodes (Frot and Mauguiere, 2003), and direct electrical stimulations of the human cortex with depth-intracerebral electrodes (Ostrowsky et al., 2002; Mazzola et al., 2006; Afif et al., 2008; Nguyen et al., 2009, Stephani et al., 2010).

Functional imaging studies carried out in humans in the recent years, consistently reported that the cortical regions located in the upper bank of the lateral sulcus, including the second somatosensory area (SII), and the insular cortex are activated by nociceptive stimuli (reviews in Peyron et al., 2000; Treede et al., 2000; Garcia-Larrea et al., 2002; Vogel et al., 2003; Apkarian et al., 2005). These two supra-sylvian pain areas are also involved in the processing of innocuous somato-sensory inputs such as cold (Craig and al., 2000; Hua et al., 2005), warm (Moulton et al., 2005; Tseng et al., 2010), tickling (Carlsson et al., 2000), non-painful cutaneous stimuli (Ferretti et al., 2004) or passive movements (Mima et al., 1999; Alary et al., 2002; Druschky et al., 2003).

However, functional imaging studies showed a substantial overlap in all these activations, noxious or not, within SII or insula (Coghill et al., 1994). In addition, differentiation between the functional properties of the contiguous SII and posterior insular cortices proved very difficult in both functional imaging and electrophysiological studies. Since the exact anatomical border between the granular cortex of the inner part of the operculum and the posterior granular insular cortex cannot be delineated on individual MRI,

the SII/insular region (especially in its posterior extent), has been considered as a single functional entity in most pain imaging studies (for reviews, see Peyron et al., 2000; Apkarian et al., 2005).

Using an observer-independent approach, Eickhoff et al. (2006a, b) and Kurth et al. (2010) solved this anatomical limitation by identifying distinct cytoarchitectonic areas in the human parietal operculum and posterior insula. Although this new scheme does not contradict previous classification of SII or insular cortex based on its granularity, it further develops these maps by demonstrating the existence of several individual areas within these major zones. Given the variety of functions ascribed to the SII-insula cortices based on functional imaging studies and connectivity, it seems reasonable to hypothesize that these subdivisions based on anatomical substrates might reflect a diversified mosaic of structurally and functionally distinct areas in the human operculo-insular cortex. Since the cyto-architectonic probabilistic maps of the OI cortex have been computed by Eickhoff et al. (2006a, b) and Kurth et al. (2010) in the standard MNI space, the present study was carried out to investigate with functional MRI (fMRI), whether these structural subdivisions could subserve distinct aspects of discriminative somato-sensory functions, including pain. We therefore investigated responses to i/ heat pain in comparison to ii/ non-noxious warm and iii/ innocuous cold stimuli, iv/ tactile sensations and v/ passive movements, taking into account the recent advances in insular and SII structural anatomy. Since recent studies showed some degree of somatotopic organization both in SII (Krubitzer et al., 1995) and in insula (Brooks et al., 2005; Mazzola et al., 2009), all types of stimuli were strictly applied on the same body area (the left hand) to avoid topographic changes of activation location due to this somatotopic organization.

SUBJECTS AND METHODS

1. Subjects

Twenty-five healthy right handed volunteers (12 male, 13 female, mean age 30.5 years, range 19-39 years) participated in this study, which was approved by the local ethics committee. Written informed consent was obtained from each subject prior to investigations.

2. Stimulation procedures

Volunteers laid supine inside the MRI scanner. Five runs were performed, with a paradigm alternating rest and one of the five somato-sensory stimuli (tactile; passive finger movement; cold; warm; heat pain). Each session was made of 24 blocks of 7 seconds during which one of the five somato-sensory stimulations was delivered. Rest blocks had a randomized duration from 5s to 25s (mean= 15s; SD= 6.7s). The order of the sessions (i.e. of the stimulations) was arranged in a randomized order. The experimenter wore a headphone. A high-pitched sound was delivered to announce that stimulation was imminent. Then two low-pitched sounds were produced to indicate respectively the beginning and the end of the stimulation time. Stimulation onsets were randomized.

All stimuli were applied to the left hand, on its dorsum for cutaneous stimuli. Passive movements were applied to the 2nd finger, so that they concerned the same somatotopic territory for all modalities. Tactile stimulation was obtained by rubbing a soft brush back and forth from proximal to distal (0.5 Hz). Cold stimulation consisted in the application a plastic pack filled with a frozen fluid. The surface temperature checked at the beginning and the end of the sessions, showed a good stability (mean temperature ± SD = 1.8°C ± 2.4). For warm and heat pain stimulations, we used hand heaters for outdoor use, consisting in a plastic pack filled with a fluid having good thermic inertia, that could be heated at 37°C and 47°C

respectively (mean temperature ± SD = 36.8°C ± 0.3 and 47.9° ± 0.2). Passive flexor and extensor movements of the metacarpo-phalangeal joint were applied mechanically by the experimenter and were paced at a frequency of 0.5 Hz. The intensity of pain in all conditions was assessed individually before and after the experiment by a visual analogic scale (VAS).

Even though an experimental paradigm alternating the five different stimuli in a same run would have been ideal, we prefered to perform five sessions with one single stimulus alternating with rest. Reasons for this choice were that it was impossible, in a same run, to manage five different and intermingled stimuli with only 5-25 seconds intervals. In addition, such intermingled stimuli might have interfered together as they were applied at only 5-25 seconds intervals whereas the aim of this study required to avoid any interference between stimuli (for example cold inhibiting warm and heat pain sensations (Yarnitsky and Ochoa, 1990; Craig et al., 2000)). For similar reasons, we deliberately selected an experimental paradigm without psychophysical assessement to minimize cognitive activities that may be associated with sensory or pain intensity scoring (Kong et al., 2006; Lötsch et al., 2011).

3. Acquisitions

MRI images were acquired in a 1.5 tesla scanner (Symphony Maestro Class, Siemens, Erlangen, Germany). For functional images, an EPI (Echo Planar) sequence was used with following parameters: 28 slices, 4 mm thick without gap, with a 64x64 matrix and a 256x256 mm^2 field of view (FOV); TR/TE = 2700/55 ms. Each subject had 5 EPI runs of 200 images each (9 min 5s) corresponding to the five types of stimuli. Anatomic scans were acquired using a 3D T1 sequence (MPRAGE) including 160 sagittal slices and the following sequence parameters: TI/TR/TE; 920/1700/4.35 ms, matrix = 256x256 voxels, FOV= 256x256 mm^2.

4. Image analysis

fMRI data were analyzed using Statistical Parametric Mapping (SPM8 - Wellcome Department of Cognitive Neurology, UK ; http://www.fil.ion.ucl.ac.uk/spm/) and some SPM compatible toolboxes : MarsBar (Brett et al., 2002), Anatomy (Eickhoff et al., 2005) and WFU_PickAtlas (Maldjian et al., 2003). Moreover, in order to build anatomical Regions of Interest (ROI), MRIcroN (http://www.cabiatl.com/mricro/) and AAL regions (Automated Anatomical Labels; Tzourio et al., 2002) were used.

4.1 Preprocessing

The two first volumes of each run were discarded. To correct for subject's movements, all scans of each individual were realigned to each other in a two pass procedure: they were first aligned to the first image and then, to the mean image. Data from all subjects showed motion corrections during each run less than 3 mm and 2°. A mean functional volume was constructed for each subject from the realigned images. This image was coregistred on the anatomical image and then transformation parameters applied to all EPI images. The anatomical image was normalized to the MNI template brain image using the segmentation step. The parameters estimated from this normalization process were then applied to all functional images. Since the interindividual variability was about 5 mm after normalization (Crinion et al., 2007), we considered that a smoothing of 6 mm FWHM was appropriate for the voxel wise analysis (SPM 8). For the ROIs study that focused on small ROIs, a 6 mm smoothing would have blurred the resolution necessary for segregating distinct insular sub-regions, and therefore a 3 mm kernel was used for that analysis. The resulting voxel size in standard stereotaxic coordinates was 2x2x2 mm^3. For localization purpose, the anatomical images of all subjects were averaged.

4.2 ROI definition

Since the aim of the present study was to investigate specifically the operculo-insular responses to different noxious and non-noxious stimuli, we did not consider the results of SPM analysis outside the a priori defined ROIs. SII and insula were investigated with a template of ROIs that were defined within the MNI space and their localization was checked on the normalized anatomical image of each subject (Figure 1).

4.2.1 Insula

The insula region was defined as the union of the AAL's (Automated Anatomical Labels; Tzourio et al., 2002) insula and the post-insula of the Anatomy toolbox (Id1, Ig1 and Ig2). When superimposed on the mean image of the group, the right insula provided in AAL needed to be 2 mm-shifted towards the left and 2D-dilated by 2 mm in both directions. After unification of ROIs, 7 sub-regions were defined inside each insular cortex. The insular central sulcus was drawn on the mean T1 image, separating the anterior from the posterior insula. Inside the posterior insula four sub-regions were delimited: Id1, Ig1, Ig2 (as defined by Kurth et al., 2010 and Eickhoff et al., 2005) and the Post Central Gyrus (PostCG) caudal to the central sulcus. Within the anterior insula, the anterior insular sulcus and the precentral insular sulcus were drawn defining three gyri: anterior short gyrus (ASG), middle short gyrus (MSG) and precentral insular gyrus (PreCG) (see figure 1). As the actual resolution for our data was of approximately 7 mm (size of the resolution element estimated by SPM between 23 and 26 voxels), we measured the size of each ROI and concluded that three of the cytoarchitectonic subregions delineated by Kurth et al. (2010) and Eickhoff et al. (2006a,b) were definitely too small to be anatomically segregated and studied. We therefore dropped Id1 from the list of the studied ROIs and pooled together Ig1 and Ig2 (becoming the ROI labeled Ig) (see figure 1C). Hence, whatever the considered plane (sagittal, coronal or axial), all ROIs had an over-all size

≥1 cm and could be separated from each other with a spatial resolution of 7mm.

4.2.2 Parietal opercular cortex

The opercular cortex was defined as the combination of the four sub-regions described by Eickhoff et al., (2006a, b): OP1, OP2, OP3 and OP4. Actually, the OP2 and OP3 subdivisions of the opercular cortex include part of the postero-superior portion of the insular cortex, covering at maximum its upper third. Therefore we considered OP2 and OP3 as parts of posterior insula and assimilated the OP1 and OP4 cytoarchitectonic regions as anatomical homologue of the functionally defined human SII area (Eickhoff et al., 2006b) (figure 1).

A global opercular-insular ROI was built including all of the pre-cited insular and opercular ROIs, i.e. 9 ROIs in the right hemisphere and 9 in the left (figure 1C(d)).

5. Statistics

First level analysis (individual level) used a general linear model (GLM, Friston et al., 1995). Then, three random-effects analyses were performed at the group level. Studies were limited to SII and insula areas on both sides, after having checked that the global activation profile in response to heat pain stimuli met the standard of what has been described as the "pain matrix" in previous pain fMRI studies, including anterior cingulate cortex and primary somato-sensitive area (Peyron et al., 2000).

5.1 Individual analysis

The functional MRI signal in response to stimulation was modeled using a general linear model approach. The regressors of interest were constructed by convolving the stimulus input function with a canonical hemodynamic response function (HRF) and its time derivative. The

estimated time courses of the head motion parameters were included as covariates of no interest to further control for the artefacts linked to subject movements. Data were high-pass filtered (cut-off period was set to 128 s). Serial correlations were corrected using an autoregressive model. Contrast images were obtained for each subject and each run for the canonical HRF regressor, reflecting the contrasts of interest (beta-weight for stimulation vs rest). These individual contrast images were entered into second level (random-effects) analyses to study group activations.

5.2 Voxel wise group analysis

SPM8 was used to test group activations inside the global operculo-insular ROI. In order to describe activations by each stimulation compared to rest, five one-sample t-tests were performed on the contrast images of the individual analysis. In order to describe common activation for all conditions, a one-way ANOVA was designed with 5 dependant groups with unequal variance, each group was consisted in individual contrast images of each stimulation.

Statistical results were reported with an uncorrected threshold ($p < 0.001$) at the voxel level, and a FWE corrected threshold ($p < 0.05$) at the cluster level.

5.3 ROI group analysis

For each condition, the value of the voxels (beta weight) of each ROI was extracted on the contrast individual images and the median was calculated.

In order to describe the within group activation in each of the 18 regions of interest, a one-sample t-test was performed using MarsBar for the 5 conditions. ROIs were considered as significantly activated if their associated p-value was < 0.05 after Bonferroni correction for the 18 comparisons.

In order to assess if some ROIs responded differently to the different types of stimuli, a two-ways ANOVA was performed with stimulus (5 levels) and ROI (9 levels) as factors. The dependant variable was the individual median value extracted for each sub-region. Interactions between factors and post-hoc Scheffe tests were done using SPSS. Significant threshold was set to p=0.05.

In order to look for correlations between activations and pain intensity, the mean VAS score of each volunteer was correlated to the median value of each ROI after noxious stimulation. Significant correlations were reported if the Spearman (non-parametric) test p value was < 0.05.

RESULTS

Because of storage failure and corrupted data, data were missing for passive movement, pain or warm in three, four and five subjects respectively, and for the two conditions brush and cold in two subjects.

1. Psychophysics

Not surprisingly, brush, warm, cold and passive movements stimuli were scored nil on pain VAS scale (VAS = 0), while noxious heat was scored 6.7 ± 1.1 and 6.9 ± 1.3, respectively before and after experiment, showing both a rather intense pain sensation and a stability of pain perception during the whole session.

2. Cortical activations

All types of stimuli applied on the left hand led to statistically significant increases in fMRI signal intensity in several cortical areas.

2.1 Voxel wise group analysis (see figure 2; Table 1s)

For non-painful stimulations, skin brushing was associated with the largest activation in the contra- and ipsilateral SII and in the posterior part of the contralateral insula. Passive movement of fingers activated the contra- and ipsilateral SII and the middle-anterior part of contralateral insula. After cold stimulations, only the contralateral SII and the extreme posterior part of contralateral insula (at the boundary with SII cortex) were activated. Warm activated the contralateral SII and bilateral insula predominantly in the anterior part. Heat pain resulted in a broad activation of bilateral SII and insular cortex.

A conjunction analysis was performed, as an indicator of which areas were activated in all stimulation modalities. Shared activation consisted in a single significant cluster of 163 voxels ($p<0.001$ FWE corrected, maximum peak coordinates: x = 54, y = -26, y = 22), of which 89% were located in OP1 according to Anatomy toolbox (see figure 3).

2.2 ROI analyses (see Figure 4)

Statistics for significantly activated ROI are given in Table 1.

Brush stimulation activated predominantly SII (bilateral OP1), contralateral OP2-OP3 and PreCG insular region. Passive movements of fingers activated only the OP1 part of SII bilaterally, and the PreCG and MSG of contralateral insula. Cold stimulations activated OP1-OP2 and PostCG contralateral sub-regions. Warm activated contralateral OP1 SII sub-region,

the anterior half of the contralateral insula (PostCG, PreCG, MSG, ASG) and the medium-anterior part of the ipsilateral insula (PreCG, MSG). Heat pain induced an activation of bilateral OP1/OP4, all sub-regions of contralateral insula except OP2 (OP3, Ig, PostCG, PreCG, MSG, ASG) and the anterior part of the ipsilateral insula (PreCG, MSG, ASG).

3. Comparisons between different stimulation types and correlations with pain scores for each ROI (see figure 5; Table 2s)

The two-ways ANOVA indicated that there is a strong interaction between stimuli and ROIs (right: $F(32) = 4.2$, $p < 10^{-3}$; left: $F(32) = 2.6$, $p < 10^{-3}$). As illustrated in figure 5, activation of all SII and insular regions except OP2 and OP3 contralateral to stimulus, was more intense after pain stimulation than after any other stimulation types, but these differences did not reach significance levels for all stimulus types in all regions (see table 2s for details). Only contralateral OP4 in SII and PreCG, MSG, ASG in the anterior part of insula bilaterally were significantly more activated by pain stimulation than by any other modalities ($p<0.05$). Activation in OP1 was more intense after passive movement stimulation than after warm and cold stimulation bilaterally, and than after brush stimulation in the right OP1 ($p<0.05$).

The mean pain VAS score of each volunteer correlated to the intensity of activation of the contralateral OP4 only ($p = 0.035$; $r = 0.45$).

DISCUSSION

All of the five types of somato-sensory stimulations that we tested activated at least one region in SII and insular cortex. Using an approach based on cytoarchitectonic subdivisions of these areas, our results show that it does exist different patterns of activation. These findings suggest that several different networks of sub-regions in the SII-insula cortex are activated depending upon the nature of somato-sensory stimulation. Contralateral OP1, corresponding to the posterior part of SII, was the only sub-region to be activated by all type of stimuli and might therefore be a common unspecific cortical target for different types of somato-sensory inputs.

Proprioceptive stimulation by passive finger movements also activated the contralateral OP1 sub-region in addition to ipsilateral OP1 and the contralateral median part of insula (PreCG and MSG). Previous functional imaging studies have shown that passive movement activates an extensive cortical somatosensory network, including SI and SII regions (Mima et al., 1999; Alary et al., 2002; Druschky et al., 2003). The involvement of insula is less described although some studies mentioned the role of perisylvian regions (Druschky et al., 2003; Kavounoudias et al., 2008). This implication of the median part of insula is consistent with anatomical connections of this region with both sensory (especially SII) and motor (BA6, SMA) areas (Mesulam and Mufson, 1982). Interestingly, both brush and passive movements activated OP1 bilateraly, and PreCG contralateral to stimulus. This reminds of similarities between the two stimuli in terms of stimulated peripheral fibers (large myelinated fibers), but also suggests that discrimination between the two stimuli may be integrated in contralateral MSG and in the medial subdivision of SII (i.e. OP2 and OP3) that are activated by only one of these two types of stimuli.

The activation induced by innocuous cooling was restricted to the contralateral posterior part of SII (OP1), the dorsal posterior part of insula (OP2) and the median posterior part of insula (PostCG). This finding fits well functional anatomy in monkeys indicating that dedicated thalamic nucleus (VMpo) relays topographic, discriminative thermoreceptive-specific spino-thalamic projections to the dorsal margin of middle/posterior insular cortex (Craig et al., 1995). Likewise, cold allodynia can occur in subjects with isolated ischemic lesions of the posterior insular/retroinsular cortex (Veldhuijzen et al., 2010). This region has already been reported to be activated using Positron Emission Tomography (PET) after graded innocuous cooling stimuli in humans (Craig et al., 2000) and a rostro-caudallly organized somatotopic map of innocuous cooling activation was also described using fMRI in the dorsal posterior insular cortex (Hua et al., 2005). So, this region corresponding to the cytoarchitectonic OP2 operculo-insular sub-region seems to be reliably and consistently activated by cold stimuli suggesting a possible primary cortex for cold sensation.

Consistent with our results, non-noxious thermal and noxious heat stimuli have been shown to activate SII and insula cortices (Casey et al., 1996; Craig et al., 2000; Peyron et al., 2000; Brooks et al., 2002; Hua et al., 2005). Warm and heat pain conditions had bilateral representations, in the contralateral but also the ipsilateral insula. Pain representation largely goes beyond that of warm both in the posterior contralateral insula (OP3, Ig) and in the ipsilateral anterior insula (ASG). These findings are in agreement with several functional imaging studies comparing activations in processing innocuous versus noxious contact heat showing responses to noxious but not to innocuous stimuli in the contralateral posterior insula (Moulton et al., 2005; Tseng et al., 2010) or increased activation during noxious compared to innocuous thermal stimuli in bilateral anterior insula and contralateral posterior insula (Peltz et al., 2011).

Pain stimuli induced the most widespread and intense activation bilaterally in SII (OP1, OP4),

contralaterally to stimulus in all sub-regions of insula (except OP2) and ipsilateral to stimulus in the anterior part of insula (PreCG, MSG, ASG). To avoid the risk of interactions between stimuli processing at peripheral or central levels, for example cold inhibiting warm and heat pain sensations (Yarnitsky and Ochoa, 1990; Craig et al., 2000), we did not intermingle stimuli of different types in the same run of acquisition. As a consequence we cannot not rule out the possibility that a thresholding effect could participate to the different spatial activation patterns that we observed. However, to strengthen the statistical power of the study we increased both the number of subjects and of repetitions for each stimulus to a level compatible with description of differences between statistical maps of activation.

Our paradigm did not include on-line scoring of intensity, salience or emotional valence of somatosensory stimuli. This choice was made to avoid the risk of collecting cognitive activations (of no-interest in our study) that are known to occur for instance during somatosensory or pain scoring (Kong et al., 2006; Lötsch et al., 2011). Therefore we cannot exclude that part of differences in activation maps between the different stimulation conditions could reflect differences in additional factors differentiating the painful vs. non-painful quality of the stimuli, such as differences in the intensity of the eliciting somatosensory input, as well as in their salience.

Contralateral posterior insula was activated only after pain stimulation and especially Ig sub-region that seems to be specific of the pain condition as it was not activated by any of the other stimuli. Interestingly, this finding fits well functional anatomy in monkeys indicating that dedicated thalamic nucleus (posterior part of the ventral medial nucleus VMpo) relays topographic, discriminative nociceptive-specific lamina I spino and trigemino-thalamic projections to the dorsal margin of middle/posterior insular cortex (Craig et al., 1995). Likewise, direct electrical stimulation of posterior insula can elicit pain in restricted parts of the body (Ostrowsky et al., 2002; Mazzola et al., 2006) with a certain degree of

somatotopic organization (Mazzola et al., 2009; Brooks et al., 2005). A recent study found that cortical stimulation sites where electrical stimulation elicited pain sensation were located in Ig1-Ig2 insula sub-regions (corresponding to our Ig sub-region), fitting with our results (Stephani et al., 2010). Considering human models of lesions, it has been recently shown that a selective lesion in the posterior insula and the inner parietal operculum could be associated with an increase of cold and warm detection thresholds but also with spontaneous pain and allodynia (Garcia-Larrea et al., 2010).

In agreement with intracerebral LEPs recordings (Frot et al., 2007), recent functional imaging studies suggested that posterior insula is involved in the encoding of pain intensity (Bornhövd et al., 2002), and/or stimulus intensity (Craig et al., 2000; Moayedi et al., 2009; Owen et al., 2010). Even though the experimental paradigm was not designed to address this question (only one intensity of pain stimulus was delivered for each patient), the finding that inter-individual VAS scores correlated with activity in OP4 sub-region (ie the anterior part of SII) suggests that this subdivision plays an important role in pain intensity processing. Very recently, functional connectivity studies have shown that during noxious thermal stimulation, posterior insula is strongly connected to areas known as being involved in sensory-discriminative processing such as S1 (Petzl et al., 2010). All these data converge on a sensory discriminative role of the posterior insular cortex in pain processing, mainly in Ig cytoarchitectonic sub-region, and of the anterior part of SII (OP4 sub-region).

Cutaneous warmth and heat pain were the only stimuli associated with the activation of the very anterior part of insula (ASG). Interestingly, warm and pain are known to be associated with emotional and affective components often related to the anterior insula whereas activations of posterior insula were shown to correlate with the intensity, but not with the pleasantness/unpleasantness, of the thermal stimuli (Schreckenberger et al., 2005; Carlsson et al., 2006; Rolls et al., 2008; Owen et al., 2010). Brooks et al. (2002), reported a clear

lateralized response after pain stimulation in a small region of the posterior insula, which was contralateral to the hand stimulation and did not depend on the attentional context of the experiment, contrary to more anterior insular activations. Most of the recent imaging studies report a simultaneous co-activation of anterior insula and anterior cingulate cortex or/and prefrontal cortex, postulating complementary limbic sensory and motor processing functions (Craig et al., 2000; 2009; Oshiro et al., 2009; Petzl et al., 2010). This functional segregation between a posterior insula supporting sensory-discriminative functions and a rather emotional and cognitive anterior insula is also supported by studies assessing the empathy for pain. Using fMRI, Singer et al. (2004) investigated pain without self-stimulation (ie: pain inflicted to a beloved person) compared to self-pain. They found that bilateral anterior insula was activated both when subjects received pain and when viewing a loved one experiencing pain. In contrast, activity in the posterior insula (and SII) was specific to painful self-sensation. Thus, activation of the anterior insula occurs in a pain context but does not need a self-experience of pain while the posterior insula and SII are involved in self-pain intensity coding (Singer et al., 2004; Apkarian et al., 2005; Botvinick et al., 2005; Jackson et al., 2006; Kong et al., 2006).

In a fMRI study Ferretti et al. (2004) described a functional topography of SII cortex for non-painful and painful stimulation of median and tibial nerves. Contrary to our results, they suggested that the posterior region within SII could be more specifically involved in the processing of noxious stimuli. In their recent meta-analysis, Eickhoff et al. (2006b) compared the functionally defined SII region (in fMRI and PET studies) to their cytoarchitectonic map of the parietal operculum, showing a good fit with OP1 and OP4 sub-regions. They suggested that pain-related activations might be more caudal than non-pain related activations. However, they also emphasized the wide distribution the region referred as the "SII region" in the parietal operculum, and the difficulty in differentiating contiguous SII and posterior insula

cortices in functional imaging studies. Spatial discrepancies between the different studies raise doubt about whether they indeed reflect activations of the same area, so that no definite conclusion can be drawn regarding functional segregation (Eickhoff et al., 2006b). The present study, designed to answer specifically this question argues against such a posterior localization of nociceptive relative to other activities in SII. Whereas the posterior part of SII (OP1) is activated by all type of somato-sensory inputs, the activation of its anterior part (OP4 sub-region) seems to be specific to pain stimuli.

Therefore, our results argue in favor of a modality dependant activation by somatosensory and pain stimuli within the SII-insula cortices. By studying for the first time five distinct modalities of somatosensory stimulations in the same group of subjects we identified different patterns of activation, as the co-activation of several sub-regions in the SII-insula cortex, depending on the type of somato-sensory stimulation. Pain-related activations consisted of a wide SII and insula activation but OP4 and Ig areas seem to be specifically activated during pain stimulation. This may raise the question whether these areas could be the anatomical substrate of thermal pain sensory-discrimination.

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