Multiple somatotopic representations of heat and mechanical pain in the operculo-insular cortex: a high-resolution fMRI study

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Running title: Multiple pain representations in the operculoinsular cortex

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BOLD effect</td>
<td>blood oxygen level dependent (effect); correlate of cerebral activation</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<td>FSL</td>
<td>FMRIB’s Software Library</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<td>ROI</td>
<td>region of interest</td>
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<td>SI</td>
<td>primary somatosensory cortex</td>
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<td>SII / PV</td>
<td>secondary somatosensory cortex / parietal ventral area</td>
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Summary

Whereas studies of somatotopic representation of touch have been useful to distinguish multiple somatosensory areas within SI and SII regions, no such analysis exists for the representation of pain across nociceptive modalities. Here, we investigated somatotopy in the operculo-insular cortex with noxious heat and pin prick stimuli in eleven healthy subjects using high-resolution (2x2x4 mm) 3T fMRI. Heat stimuli (delivered using a laser) and pin prick stimuli (delivered using a punctate probe) were directed to the dorsum of the right hand and foot in a balanced design. Locations of the peak fMRI responses were compared between stimulation sites (hand vs foot) and modalities (heat vs pin prick) within four bilateral regions of interest: anterior and posterior insula, frontal and parietal operculum. Importantly, all analyses were performed on individual, non-normalised fMRI images. For heat stimuli, we found hand-foot somatotopy in the contralateral anterior and posterior insula (hand 9 mm ± 10 mm anterior to foot, mean ± SD, p<0.05) and in the contralateral parietal operculum (SII; hand 7 mm ± 10 mm lateral to foot, p<0.05). For pin prick stimuli we also found somatotopy in the contralateral posterior insula (hand 9 mm ± 10 mm anterior to foot, p<0.05).

Furthermore, the response to heat stimulation of the hand was 11 mm ± 12 mm anterior to the response to pin prick stimulation of the hand in the contralateral (left) anterior insula (p<0.05). These results indicate the existence of multiple somatotopic representations for pain within the operculo-insular region in humans, possibly reflecting its importance as a sensory-integration site that directs emotional responses and behaviour appropriately depending upon the body site being injured.
Introduction

The cortical representation of innocuous somatosensory stimuli has been the subject of investigations for many decades. Detailed electrophysiological studies of receptive field somatotopies revealed multiple representations of the body within the primary (SI) and secondary (SII) somatosensory cortex in monkeys (Kaas 1983, Krubitzer et al. 1995, Fitzgerald et al. 2004), and these somatosensory subdivisions exhibited different functional properties. Functional imaging studies with tactile stimuli in humans have supported these subdivisions within SI and SII (Gelnar et al. 1998, Disbrow et al. 2000, Eickhoff et al. 2006a&b, Young et al. 2004).

The cerebral representation and processing of nociceptive stimuli has been studied quite extensively with the evolution of neuroimaging techniques. An expansive set of regions, including S1, thalamus and distinct divisions of the insular, prefrontal and anterior cingulate cortices amongst other, has been described as relevant (for review see Apkarian et al. 2005, Tracey & Mantyh 2007). Surprisingly few studies have investigated the somatotopy for nociceptive stimuli, probably because it was anticipated to be identical to that for touch. The somatotopic maps for pain and touch are similar in the thalamus, where the face is represented medially and the foot laterally (Lenz et al. 1988, 1994, 1997), and in SI, where the face is represented laterally and the foot medially (Tarkka and Treede 1993, Andersson et al. 1997, DaSilva et al. 2002, Bingel et al. 2004).

For cortical processing of painful stimuli, the operculo-insular cortex plays an important role (Treede et al. 2000). Nociceptive areas within this region include several parts of the insula deep inside the lateral sulcus, and those parts of the frontal and parietal lobes that cover the insula (called the opercula). This region receives nociceptive input as early as or even earlier

For the somatotopy in the operculo-insular cortex there are two conflicting concepts: all tactile representations in the parietal operculum (including SII and parietal ventral area PV) are oriented similar to SI, i.e. the face laterally and the foot medially (Fitzgerald et al. 2004). In contrast, nociceptive input to the dorsal insula has been suggested to derive from the posterior part of the proposed ventral medial thalamic nucleus (VMpo; Craig et al 1994, Craig and Dostrovsky 1997) with a completely different somatotopy: face anterior and foot posterior (Craig 1995). Some studies with painful stimuli confirmed the anterior-posterior somatotopy (Vogel et al. 2003, Brooks et al. 2005, Baumgärtner et al. 2006b, Henderson 2007), whereas others showed a medio-lateral somatotopy (Bingel et al. 2004). As a combination of these two concepts, a parallel projection of spinal cord neurons to both the insula and the operculum (SII) has been demonstrated very recently by a viral tracing study in monkey (Dum et al. 2009).

A better understanding of the somatotopic representation of painful stimuli in the operculo-insular cortex may resolve conflicting concepts of its organization. Therefore we addressed these two questions: (i) whether multiple somatotopical maps exist in the operculo-insular area, and (ii) whether different types of cutaneous pain (heat and pin prick) share similar cortical representations.
Materials and Methods

Twelve subjects (8 males and 4 females; mean age 28 years, range 26-34 years) participated in the study after giving fully informed consent, which conformed to the guidelines of the Declaration of Helsinki (1996) and had been approved by the local ethics committee.

Laser stimulation

Infrared laser pulses selectively activate heat-sensitive Aδ- and C-nociceptors in the skin (Treede et al. 1995). They evoke a very brief pin prick-like and/or burning sensation and the input transmitted via type II A-fiber mechano-heat nociceptors (type II AMH) rapidly activates the operculo-insular cortex (Tarkka and Treede 1993, Xu et al. 1997, Iannetti et al. 2004). In the present study, nociceptive heat stimuli were generated by an infrared neodymium yttrium aluminium perovskite (Nd:YAP) laser (El.En., Florence, Italy, www.elengroup.com) with a wavelength of 1.34 µm. The laser beam was transmitted via optic fiber into the scanner room and directed to the skin area that was to be stimulated (hand or foot dorsum). The diameter of the laser beam was set at 6 mm (irradiated area ~ 28 mm²) by focusing lenses. Laser pulses produced by Nd:YAP stimulators do not induce damage to the irradiated skin that is sometimes produced by the widely-used, high-intensity CO2-laser pulses (Crucu et al., 2003; Iannetti et al., 2003).

Pin prick stimulation

Painful mechanical stimuli were applied using a hand-held 256 mN pin prick probe that has a flat cylindrical tip (diameter 250 µm), and evokes a pin-prick sensation primarily mediated by activation of a different type of Aδ-nociceptors (type I AMH, Slugg et al. 2000, Magerl et al. 2001). These mechanical stimulators have been proven to be an adequate tool to induce pin prick pain in psychophysical and clinical investigations (Greenspan and McGillis 1994,
Greenspan et al. 1997, Ziegler et al. 1999, Baumgärtner et al. 2002) and are commonly used as part of the protocol for quantitative sensory testing in the German Research Network on Neuropathic Pain (Rolke et al., 2006).

**Experimental paradigm**

Each experiment consisted of 4 different stimulation conditions: laser stimulation of the right hand, laser stimulation of the right foot, pin prick stimulation of the right hand, and pin prick stimulation of the right foot. In a psychophysical session prior to the fMRI experiment, the intensity of both laser and pin prick stimuli was adjusted in order to achieve a similarly perceived intensity of both heat and mechanically induced pain at both stimulated sites (hand, foot): For the hand, we used a 256-mN pin prick probe and 1.5-J laser pulses; for the foot, we used the same 256-mN pin prick probe and 2-J laser pulses. During this psychophysical session, subjects were asked to rate verbally the intensity of the perceived pricking pain on a numerical rating scale ranging from 0 to 10 (0= no pricking pain, 10 worst pricking pain imaginable).

During the fMRI recording, stimuli were delivered in blocks of 10 repeats. Within each block, stimuli of the same modality were delivered to the same body region every 11.5 s, with a break of 23 s separating the last stimulus of one block from the first stimulus of the next block. Each of the 4 stimulation conditions was repeated four times (16 blocks) resulting in 40 stimuli of the same modality (laser or pin prick) to the same body region (hand or foot); in total, 160 stimuli were delivered to each subject. The order of the blocks was balanced across subjects. As an example, a whole stimulation sequence for one subject is shown in Fig. 1. Subjects were instructed to focus their attention on the stimuli, without any specific discrimination task, and to give an average intensity rating of each block of stimuli using
their fingers 12.5 s after the last stimulus of that block, when the experimenter inside the scanner lightly touched their left ankle. To avoid nociceptor fatigue or sensitization, the laser beam or the pin prick stimulator was slightly moved after each stimulus.

**MR Image Acquisition**

Functional MRI scanning was performed continuously on a 3 Tesla Varian INOVA MRI system. A head-only gradient coil (Magnex SGRAD MKIII) was used with a birdcage radiofrequency head coil for pulse transmission and signal reception. A high resolution, gradient-echo, echo-planar imaging sequence was used for functional scans (TE =45 ms, 12 contiguous 4-mm axial slices, flip angle $87^\circ$, in-plane field of view 256 x 256 mm, image matrix 128 x 128) with a repetition time (TR) of 3 s over 680 volumes, corresponding to a total scan time of 34 min. By examining sagittal and coronal scout images, the 12 axial slices were adjusted to cover the maximum superior-inferior extent of the insula (Özcan et al. 2005). Furthermore, at the end of the functional scan, for each subject a T1-weighted, high-resolution structural image (70 contiguous 3-mm axial slices, in-plane field of view 256 x 192 mm, matrix 256 x 192) was collected for verification of anatomical structures.

**Region of interest (ROI) analysis**

As our intention was to analyse the somatotopy for the two types of nociceptive stimuli focussing on a confined area with high spatial resolution rather than the whole brain, we anatomically defined four ROIs within the operculo-insular cortex in each hemisphere (8 ROIs in total). Following the approach described by Bense et al (2001) and Afif et al. (2009), the insular cortex was divided into an anterior part, containing the 3 short (anterior) gyri, and a posterior part, containing the two long (posterior) gyri. Similarly, the opercular cortex was divided into a frontal and a parietal part, using the central sulcus as the separating structure.
As the quality of the EPI scans was high enough to recognize necessary anatomical
landmarks, such as the boundaries of the insular cortex and insular sulci, the sylvian fissure
and the central sulcus, this ROI definition was performed on the functional, BOLD-sensitive
images of each subject.

In order to obtain a measure of the structural brain variability of the subjects and to have
anatomical landmarks to relate the activations to, we measured five landmarks in each
hemisphere (Fig. 2) using the BOLD-sensitive images: the anterior and posterior poles of the
insula (on the transversal slice where the insula appeared longest), the center of the curvature
of the insula, the sulcus between the 3rd and fourth insular sulcus (anatomical separation of
anterior and posterior insula) and the location where the central sulcus ends (separation
frontal vs. parietal operculum). Locations of landmarks and of BOLD signal increases were
measured relative to the anterior commissure (AC). Thus, the origin of axes in our data is
identical to that in the brain atlases of the Montreal Neurological Institute and Talairach.

**Data Analysis**

Image analysis to reveal significant brain activity based on changes in BOLD signal was
performed using FEAT (part of FSL, www.fmrib.ox.ac.uk/fsl). Prior to statistical analysis,
the following pre-processing steps were applied to each subject's time series of fMRI
volumes: motion correction (FLIRT; Jenkinson et al., 2002), spatial smoothing using a
Gaussian kernel of full width at half maximum of 2 mm, subtraction of the mean of each
voxel time course from that time course, and nonlinear high-pass temporal filtering
(Gaussian-weighted least-squares straight line fitting, with a high-pass filter cut-off of 50 s).
The fMRI signal was then linearly modeled (Worsley and Friston, 1995) on a voxel-by- voxel
basis using a general linear model approach, with local autocorrelation correction (Woolrich et al., 2001).

**Group analysis**

Before the analysis of single subject data, a group analysis was carried out using a mixed-effect approach, thus generating group-representative statistical maps of brain responses to laser and pinprick stimulation. For the purpose of the group-level analysis, registration of low-resolution functional images to the corresponding high-resolution structural images was performed for each subject (Jenkinson and Smith, 2001), and followed by registration to a standard brain (MNI template, Collins et al., 1994). The raw Z statistic images from the group analysis were thresholded at Z scores >2.3. A cluster-based approach (threshold p<0.05) was used to correct for multiple comparisons (Worsley et al., 1992).

**Single-subject analysis**

As the quality of the EPI scans was high enough to recognize necessary anatomical landmarks, no image coregistration procedures were applied in this analysis, not even the alignment of functional to structural scans was necessary to assign activations to the ROIs chosen.

Single subject data were initially thresholded at Z = 2.3 and cluster corrected (minimum number of contiguous voxels constituting a cluster > 4). For each subject, the coordinates of the voxel with the highest Z score (i.e. most significantly activated) in each of the 8 ROIs (directly identified on the non-normalized functional images) were noted. This way, significant activations were found in 344/352 ROI analyses (11 subjects x 8 regions of interest x 4 conditions = 352 analyses). Lowering the statistical threshold to Z = 2.0 yielded
two additional activations. The whole procedure of identification of the peak voxel within each of the ROIs was done separately by two experimenters (UB and GDI) to double-check the results. The few cases (11 out of 346) where different locations had been selected by these experimenters were re-examined until agreement regarding the location of the peak voxel was reached.

To check for the presence of hand-foot somatotopy, differences in coordinates (x, y, z) between hand and foot peak Z-score activation were calculated for each subject. Significance was tested using paired Student’s t-tests separately for x, y and z coordinates (in mm) for all subjects; presence of somatotopy was assumed in cases where the difference between hand and foot peak activations within an ROI was significantly larger than 0 mm for at least one axis. Differences in location below 2 mm in the horizontal plane and 4 mm in vertical direction were not considered as valid even in case they were significant, because this was below the scanning resolution (2x2x4 mm). Differences in the location of activations induced by the two stimulation modalities (heat or pin prick) within the same stimulation area (hand or foot) were tested using the same approach.

Results

Pain ratings

During the fMRI experiment the average pricking pain intensity ratings (on 0 to 10 scale) were as follows: laser hand = 2.8 ±1.1; laser foot = 3.2 ±1.3; pin prick hand = 1.7 ±0.7; pin prick foot = 2.1 ±0.7. Two-way ANOVA revealed a significant main effect of the modality (higher pain ratings for laser stimulation compared to pin prick stimulation; p<0.01), no significant main effect for stimulus site, and no significant interaction. Average pain ratings remained stable throughout the experiment (Kruskall-Wallis test, p>0.3).
**fMRI activations**

**Group analysis**

The initial group analysis using a significance threshold of 2.3 (cluster threshold of 4 voxels) yielded significant activations in various brain areas. In Figure 3A, three transversal slices 4 mm apart from each other in superior-inferior (z-coordinate) direction show voxels with significant activations (z-score >2.3, see color bars) following stimulation of the hand or foot with laser (LH, laser hand; LF, laser foot) or pinprick stimuli (PH, pin prick hand; PF, pin prick foot). At a glance, there is a scattering of voxels visible without clear hand-foot somatotopy within the operculo-insular and frontal regions, with the widest distribution for laser hand stimulation (upper row) and the smallest number of significant voxels for pin prick foot stimulation (bottom row). The area which is active most reliably in all of the 4 modalities (laser hand, laser foot, pinprick hand, pinprick foot) is the posterior insula of the left (contralateral) hemisphere. On the right panel, Figure 3B, hand and foot stimulation is shown on the same brain slice for laser (top) and pin prick stimulation (bottom). The significance threshold was raised from 2.3 to 3.8, which unmask a different representation for hand and foot in the posterior insula. The hand representation (red voxels for laser, dark blue voxels for pinprick stimuli) is shown to be anterior to the foot representation (orange voxels for laser, light blue voxels for pinprick stimuli; white voxels: overlap).

**Single subject ROI analysis**

The quality of the functional EPI scans was high enough to localize peak activations within each of the 8 ROIs in almost every case (346 of 352 analyses). The peak Z-scores ranged from 2.2 to 8.5. Differences in location and Z scores of peak voxels for all ROIs between
locations of stimulation (hand and foot) in each of the two modalities are shown in Table I. Results for each for the single dimensions are shown separately (X: medial-lateral, Y: anterior-posterior, Z: superior-inferior) as well as in three-dimensional distance (3D). An asterisk marks significant differences.

As an example of the spatial scattering of individual cortical activations in three-dimensional space, Figure 4 illustrates the individual peak voxel activations of laser hand (red dots) and laser foot (yellow dots) stimulation within the ROI “left SII” projected into the brain of one of the participants of this study. The average localizations (large symbols) show a shift of hand and foot representation in medial-lateral direction, with the hand representation further lateral. The overlap of the distributions of red and yellow symbols demonstrates why the individual analysis with pairwise comparisons within subjects is likely to yield sharper separations with a better spatial resolution than the resolution that can be achieved by means of a group analysis.

For heat stimulation (Table Ia), we found a significant somatotopy in: (1) contralateral (left) SII/PV, with the hand 6.9 mm lateral to the foot, and a three-dimensional distance of the x, y and z coordinates (square root \(x^2 + y^2 + z^2\)) of 7.4 mm; (2) contralateral (left) posterior insula, with hand 8.5 mm anterior to the foot and a 3D distance of 9.1 mm; and (3) contralateral (left) anterior insula, with hand 9.3 mm anterior to the foot, and a 3D distance of 10.0 mm (Fig. 5, left panel).
For pin prick stimulation (Table Ib), we found a significant somatotopy only in the contralateral (left) posterior insula, with hand 4.4 mm anterior to the foot, and a 3D distance of 9.1 mm (Fig. 5, right panel).

For hand stimulation (Table IIa) we found a significant difference between the location of the responses to laser and pin prick stimulation in the contralateral (left) anterior insula, with the response to laser stimulation 11.0 mm anterior to the response to pin prick stimulation, and a 3D distance of 11.4 mm (Fig. 6, left panel). For foot stimulation (Table IIb) we could not identify significant differences between stimulation modalities. Differences in location (in mm) and Z scores of peak voxels for all ROIs between modalities of stimulation (heat and pin prick) in each ROI are shown in Table II.

In all comparisons (both between body sites and between modalities), the Z-scores were constant across subjects within the conditions tested, and the differences in Z-scores were not significantly different from zero (Tables I and II).
Discussion

Activation of the operculo-insular cortex has been demonstrated in the vast majority of studies investigating the brain responses to nociceptive stimuli in humans (Peyron et al 2000, Apkarian et al 2005). Using high-resolution fMRI based on individual, untransformed anatomical and functional data, we now demonstrate a somatotopic representation of nociceptive stimulation in three operculo-insular ROIs. There were spatially separate cortical responses to hand and foot stimulation in the contralateral posterior insula following both heat and pin prick stimulation, and in the contralateral anterior insula and parietal operculum following heat stimulation only. The mediolateral somatotopy in the parietal operculum was consistent with that of tactile representation in SII or PV (Disbrow et al. 2000). The posterior-anterior somatotopy in the insula was consistent with the predicted somatotopy for cortical projection areas of the proposed thalamic nucleus, VMpo (Craig 1995). Furthermore, we found differences in location of cortical responses to heat stimulation versus pin prick stimulation in contralateral anterior insula following hand stimulation, and contralateral parietal operculum following foot stimulation.

Specificity of nociceptive stimuli and specificity of the areas activated

We can assume with enough certainty, that the stimuli applied in this study were specific to activate nociceptive fibers. This is clearly the case for the laser stimulus, which has been shown to activate Aδ- and C-nociceptors, only a tiny fraction of C-warm-fibers (approx. 10%), and no Aβ-fibers as demonstrated in single fiber recordings (Bromm and Treede 1984, Bromm et al. 1984, Treede et al. 1995). The contribution of the C-warm fibers to the centrally conducted signal seems negligible, since 80% of this fraction stops firing at all at
temperatures in the higher noxious range (45-50°C; LaMotte and Campbell 1978, Darian-Smith et al. 1979).

The pin-prick stimulus, on the other hand, is less specific for nociception, since it co-activates Aβ-, Aδ- and C-fibers. Whereas the surface of the mechanical probe seems to be hardly relevant to single unit responses of low threshold mechanoreceptors (Aβ-fibers), the prickliness is differentially coded by a population of Aδ- and polymodal C-nociceptors (Garnsworthy et al. 1988). Among these, probe size (diameter) and force are better reflected in activity of Aδ- than in activity of C-nociceptors with one sub-population having high discharge rates at the threshold where mechanical stimuli are just recognized as sharp, and another sub-population with high discharge rates at higher intensities (Greenspan and McGillis 1994, Garell et al. 1996).

Taken together, the stimulation used in our study was nociceptive, and thus, the brain areas activated, like the frontal operculum, SII/PV, and anterior and posterior insula (and other regions outside our ROIs) were responsive to noxious stimuli. Since we did not compare brain responses following noxious versus non-noxious stimulation in this study, we cannot conclude directly that these brain areas are, in turn, specific for nociceptive processing. The fact that for tactile stimuli so far only a lateral-medial representation has been identified in the area of SII/PV suggests, that the double anterior-posterior somatotopy in the insula might be specific for nociception. On the other hand, there is evidence for polymodality of regions within the operculo-insular cortex coming from a number of studies (Davis et al. 1998, Downar et al. 2000, Matsuhashi et al. 2004).

Somatotopy in the opercular cortex
In functional imaging studies of the human brain, usually the entire parietal operculum and sometimes even parts of the frontal operculum are labelled SII. According to electrophysiological and imaging studies in monkeys and humans of the parietal operculum (Krubitzer et al. 1995, Disbrow et al. 2000, Fitzgerald et al. 2004), this large area can be separated into two or three functionally distinct regions with separate representations of the body: PV (anterior part, facing the posterior insula), SII proper (more posterior than PV), and possibly a third area further posterior. Cytoarchitectonically, the human parietal opercular region has recently been divided into four areas (OP1 – OP4, Eickhoff et al. 2006a), where OP1 corresponds to SII and OP4 to PV. A meta-analysis of imaging studies investigating the responses to nociceptive and non-nociceptive stimuli found the clusters responding to nociceptive stimuli within OP1, the posterior part of the parietal operculum (Eickhoff et al 2006b), and the clusters responding to non-nociceptive stimuli more anterior, at the border of OP1 and OP4. The fMRI peaks within the posterior operculum (SII/PV) as identified in the single subject analysis of the present study (Table 1, Fig. 5) were located mostly in OP1 or at the border of OP1 and OP4, and thus are consistent with being in SII proper.

The somatotopy that we found in SII is consistent with that known for tactile representation in SII (Disbrow et al. 2000). A similar somatotopy, with hand representation lateral to foot representation in SII, had previously been reported in a fMRI study using laser stimulation (Bingel et al 2004). However, possibly due to the limited spatial resolution, they were not able to ascertain whether the peak of activity following foot stimulation was located in SII or posterior insula. We found distinct locations for foot representation both in SII proper and posterior insula and hence demonstrated the presence of a nociceptive homunculus for thermal stimuli in SII. Ferretti et al (2004) found two activation areas for hand and foot in SII/PV; within each activation area, nociceptive stimuli were represented more posteriorly.
than non-nociceptive stimuli. These authors reported a significant somatotopy for the non-nociceptive stimuli only; however, they used two different intensities of electrical stimulation, and brain responses were detected using a 1.5 T scanner, thus raising the questions of specificity of the afferent neuronal populations activated and spatial resolution of the imaging technique. Our data indicate that apart from tactile information, as has been demonstrated by other investigators (Disbrow et al. 2000, Ferretti et al. 2004), also nociceptive information is projecting to SII as part of the “classical” lateral pain pathway and is somatotopically organized.

Only few nociceptive neurons have been identified in SII proper (Robinson and Burton 1980), and the largest published series of nociceptive neurons in that region is from a more posterior area (Dong et al. 1989). The scarcity of monkey electrophysiological data may be due to inadequate search stimuli: both type I and II AMH are not activated by gentle mechanical search stimuli, and electrical stimulation is necessary to study these primary afferents (Treede et al. 1998).

Although in the frontal operculum we observed significant responses to the nociceptive stimulation, we did not find significant hand-foot somatotopy in this area. We could thus not reproduce the posterior-anterior somatotopy in the frontal operculum observed by Vogel and coworkers (Vogel et al. 2003). One possible explanation could be that in that their source analysis study, the somatotopy of the dorsal insula was projected into the operculum. On the other hand, invasive depth recordings have confirmed a generator source within the frontal operculum, so this generator may be non-somatotopically organized.
Somatotopy in the insular cortex

As there is not yet an established standard on how to segregate the insula into functionally different ROIs, we chose to use gross anatomical features. The separation into an anterior part, containing the 3 short gyri, and a posterior part, containing the 2 long gyri, separated by the central insular sulcus, has been used previously by Ostrowsky et al. (2000) during invasive mapping and by Bense et al. (2001) in an fMRI study. Efforts to establish a stereotactic template of the insula have also been made (Afif et al. 2009). According to Brodmann (1909), the human insula contains a dorsogranular field and a ventrorostral agranular field, however, he did not assign any area numbers to it. Furthermore, monkey data show different cytoarchitecture between anterior and posterior insula, possibly with three distinct subregions (Augustine 1985). Very recently, first data on cytoarchitectonic mapping of the human posterior insula obtained from analysis of post mortem brains has become available (Kurth et al. 2009). These data suggest an even finer separation of the insula than previously supposed, but this work is currently still in progress.

We found two separate representations in the insula, both with the posterior-anterior somatotopy predicted for VMpo projection areas. A similar somatotopy with hand representation anterior to foot representation in dorsal posterior insula had previously been reported in a fMRI study using contact heat stimulation (Brooks et al 2005) and was very recently demonstrated by an intra-operative stimulation study using laser stimuli (Mazzola et al. 2009). Another study with painful stimulation of muscles also reported hand representation anterior and lateral of foot in dorsal posterior insula (Henderson et al. 2007).
These data underline the importance of the (posterior) insular cortex as a projection target for
the spinothalamic pathway through lamina 1 and the proposed VMpo thalamic nucleus (Craig
and Zhang 2006). In addition to the previous studies, we found the same anterior-posterior
gradient of hand and foot representation in both parts (anterior and posterior) of the
contralateral (left) insula. In response to pin prick stimuli a similar somatotopical
organization was found in the same direction within the posterior insula. If one follows the
suggestion by Craig (2009b), this somatotopy in the posterior insula could be a VMpo
projection with re-representation in the anterior part of the insula.

The findings of enhanced activity of the left frontal operculo-insular cortex during a
discrimination task using EEG source analysis (Schlereth et al. 2003) and increased activity
of the anterior insula (bilaterally) during a memory task of intensity discrimination using
fMRI (Albanese et al. 2007) following hand stimulation, supports the finding of hand-foot
somatotopy in this region since stimulus discrimination requires an anatomical map of the
body.

Separate representation of noxious heat and pin prick stimuli in the anterior insula?

An unexpected finding was the significantly different representation of noxious heat and pin
prick stimulation of the hand in the anterior insular cortex, with heat pain representation
located on average 11mm more anterior compared to the representation of mechanical pain.
We did not observe the same phenomenon for stimulation of the foot. One possible
explanation could be that a larger hand representation yields stronger fMRI activations,
making it easier to detect differences. However this seems implausible, since z-scores
between hand and foot activations differed less than 0.5 and were non-significant (Tables 1A&B), and pain ratings were even slightly lower following hand stimulation.

The anterior part of the insula has been assigned to the processing of emotion as well as pain anticipation and human awareness (Craig 2009a, Damasio et al. 2000, Ploghaus et al. 1999). It has been further characterized as center for interoception that integrates somato-visceral input from the body together with autonomic input and emotional awareness to drive adequate behavioural and motor responses (Craig 2009b, Critchley 2005). Different self-induced emotions, mostly negative, lead to different activations within the anterior insulas (left and right; Damasio et al. 2000). Although the subjects in our study had to rate the pricking aspect of the evoked pain sensation which better emphasizes the sensory than the affective dimension, since sensory and affective pain dimensions are closely interrelated, one may speculate that the interoceptive input is different with the result of different representations within the anterior insula for noxious heat and mechanical stimuli. The different representation of heat pain could be due to different levels of attention, emotion, or anticipation induced by a higher level of potential threat or negative affect associated with the laser stimulus. Since sensory inputs from the hands are more important than from the feet in everyday-life, and it appears reasonable why we found this segregation for the hands only.

**Conclusion**

By evaluating four subregions of the operculo-insular cortex separately we identified at least three representations of nociceptive inputs within this region. We have therefore contributed to a resolution of a controversy in the previously published literature that tried to establish the single representation of pain in that region. Our data indicate that both the classical lateral...
pain pathway from VPI to SII, as well as the pathway from VMpo to dorsal posterior insula have cortical projections that are somatotopically organized, but at 90° angle from each other. Due to the limited temporal resolution of fMRI it was not possible to ascertain whether the projection target in the anterior insula is processing information in parallel to the posterior insula or is activated following the posterior part in a sequential way.

**Acknowledgements**

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mechanically evoked cutaneous pain: Effects of laterality and repeated testing.


Table 1. Differences in location and Z-scores of fMRI responses to hand and foot stimulation.

A. Laser stimulation

<table>
<thead>
<tr>
<th></th>
<th>Left SII</th>
<th>Right SII</th>
<th>Left PI</th>
<th>Right PI</th>
<th>Left AI</th>
<th>Right AI</th>
<th>Left FOP</th>
<th>Right FOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>x (mm)</td>
<td>6.9*</td>
<td>-5.0</td>
<td>2.2*</td>
<td>-2.0</td>
<td>0.9</td>
<td>0.4</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>y (mm)</td>
<td>-2.4</td>
<td>-5.2</td>
<td>8.5*</td>
<td>4.6</td>
<td>9.3*</td>
<td>4.0</td>
<td>-8.2</td>
<td>2.9</td>
</tr>
<tr>
<td>z (mm)</td>
<td>1.1</td>
<td>0.0</td>
<td>-2.2</td>
<td>-2.4*</td>
<td>-3.6</td>
<td>-3.3</td>
<td>1.2</td>
<td>2.5</td>
</tr>
<tr>
<td>3D (mm)</td>
<td>7.4</td>
<td>7.2</td>
<td>9.1</td>
<td>5.6</td>
<td>10.0</td>
<td>5.2</td>
<td>8.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Z-score</td>
<td>0.2</td>
<td>-0.6</td>
<td>-1.2</td>
<td>-0.8</td>
<td>-0.4</td>
<td>-0.3</td>
<td>-0.5</td>
<td>-0.4</td>
</tr>
</tbody>
</table>

Note – all differences are obtained by subtracting the foot values from the hand values; stimuli were delivered to the right side of the body; * indicates p <0.05 (paired t test).

B. Pin prick stimulation

<table>
<thead>
<tr>
<th></th>
<th>Left SII</th>
<th>Right SII</th>
<th>Left PI</th>
<th>Right PI</th>
<th>Left AI</th>
<th>Right AI</th>
<th>Left FOP</th>
<th>Right FOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>x (mm)</td>
<td>1.3</td>
<td>-4.2</td>
<td>0.9</td>
<td>-0.2</td>
<td>0.4</td>
<td>1.1</td>
<td>-0.9</td>
<td>3.2</td>
</tr>
<tr>
<td>y (mm)</td>
<td>-0.5</td>
<td>-5.8</td>
<td>8.9*</td>
<td>5.6</td>
<td>-2.6</td>
<td>4.7</td>
<td>0.4</td>
<td>-2.2</td>
</tr>
<tr>
<td>z (mm)</td>
<td>-0.4</td>
<td>2.5</td>
<td>-1.5</td>
<td>-1.8</td>
<td>-1.6</td>
<td>-2.9</td>
<td>-4.0</td>
<td>-0.8</td>
</tr>
<tr>
<td>3D (mm)</td>
<td>1.4</td>
<td>7.6</td>
<td>9.1</td>
<td>5.9</td>
<td>3.1</td>
<td>5.7</td>
<td>4.1</td>
<td>4.0</td>
</tr>
<tr>
<td>Z-score</td>
<td>0.5</td>
<td>0.1</td>
<td>-0.4</td>
<td>-0.4</td>
<td>0.5</td>
<td>0.1</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Note – all differences are obtained by subtracting the foot values from the hand values; stimuli were delivered to the right side of the body; * indicates p <0.05 (paired t test).

Table 2. Differences in location and Z-scores of fMRI responses to laser and pin prick stimulation.

A. Hand stimulation

<table>
<thead>
<tr>
<th></th>
<th>Left SII</th>
<th>Right SII</th>
<th>Left PI</th>
<th>Right PI</th>
<th>Left AI</th>
<th>Right AI</th>
<th>Left FOP</th>
<th>Right FOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>x (mm)</td>
<td>0.2</td>
<td>-0.2</td>
<td>-0.9</td>
<td>1.3</td>
<td>-0.4</td>
<td>-1.5</td>
<td>1.4</td>
<td>-0.6</td>
</tr>
<tr>
<td>y (mm)</td>
<td>0.5</td>
<td>-1.5</td>
<td>-1.5</td>
<td>-0.5</td>
<td>-11.0*</td>
<td>0.7</td>
<td>5.2</td>
<td>-3.6</td>
</tr>
<tr>
<td>z (mm)</td>
<td>1.5</td>
<td>-1.8</td>
<td>0.4</td>
<td>1.1</td>
<td>-2.8</td>
<td>-0.7</td>
<td>0.4</td>
<td>-1.2</td>
</tr>
<tr>
<td>3D (mm)</td>
<td>1.6</td>
<td>2.3</td>
<td>1.8</td>
<td>1.8</td>
<td>11.4</td>
<td>1.8</td>
<td>5.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Z-score</td>
<td>-0.4</td>
<td>-0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>-0.3</td>
<td>-0.2</td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Note – all differences are obtained by subtracting the laser values from the pin prick values; stimuli were delivered to the right side of the body; * indicates p <0.05 (paired t test).

B. Foot stimulation

<table>
<thead>
<tr>
<th></th>
<th>Left SII</th>
<th>Right SII</th>
<th>Left PI</th>
<th>Right PI</th>
<th>Left AI</th>
<th>Right AI</th>
<th>Left FOP</th>
<th>Right FOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>x (mm)</td>
<td>-5.5§</td>
<td>-0.8</td>
<td>-2.2</td>
<td>2.2</td>
<td>1.3</td>
<td>0.7</td>
<td>-1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>y (mm)</td>
<td>1.3</td>
<td>-0.4</td>
<td>-1.8</td>
<td>-1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-2.9</td>
<td>-1.0</td>
</tr>
<tr>
<td>z (mm)</td>
<td>0.0</td>
<td>0.4</td>
<td>1.1</td>
<td>2.4</td>
<td>-1.8</td>
<td>0.4</td>
<td>1.8</td>
<td>-3.2</td>
</tr>
<tr>
<td>3D (mm)</td>
<td>5.6</td>
<td>1.0</td>
<td>3.0</td>
<td>3.4</td>
<td>2.2</td>
<td>0.8</td>
<td>3.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Z-score</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.5</td>
<td>0.5</td>
<td>0.7</td>
<td>0.9</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Note – all differences are obtained by subtracting the laser values from the pin prick values; stimuli were delivered to the right side of the body; * indicates p <0.05 (paired t test). § p=0.0508.
Captions to figures

Figure 1: Experimental design. The figure illustrates the timing of actions performed during the fMRI experiment. Laser heat and pin prick stimuli were delivered to the skin of the dorsum of the hand and of the foot by an experimenter inside the scanner room. Stimuli of the same modality and to the same body site were delivered in blocks of 10, with an inter-stimulus interval of 11.5 s. At the end of each block, the subject was asked to provide an average intensity rating for that block. The order of blocks was balanced across subjects.

Figure 2: Variability in structural anatomy and definition of regions of interest on EPI scans. The left panel shows the variability in structural anatomy of the population explored in this study. The individual brains were aligned to the anterior commissure (AC, origin of axes), in order to display variability of structural anatomy of the insular and opercular regions on a single brain. As AC is approximately located in the centre of the brain as well as of the ROIs, this Talairach-like alignment was chosen to minimize the influence of inter-individual anatomical differences. Each dot represents the average location of the landmarks used to define the 8 regions of interest (ROIs). On each hemisphere, the medial dots indicate the anterior pole of the insula, the middle of the curvature of the insula, the sulcus between the 3rd and 4th insular gyri (the anatomical separation of anterior and posterior insula, Bense et al 2001) and the posterior pole of the insula. The lateral dot indicates the location where the central sulcus ends (separation between the frontal and the parietal operculum). The single dot on the midline represents the posterior commissure (PC). Axis scaling is in mm, error bars represent the standard error of the mean. The right panel illustrates how the 8 ROIs (in yellow) were determined in a representative subject. FOP: frontal operculum; AIC: anterior insular cortex; PIC: posterior insular cortex; SII: secondary somatosensory cortex.
Figure 3: Group Analysis. Left (A): At the significance threshold of Z= 2.3, three transversal slices through the operculo-insular region are shown (left to right; 4 mm below AC level, 4 mm above AC level, 12 mm above AC level) in four lines for the four modalities (laser hand, laser foot, pinprick hand, pinprick foot). Although especially for the laser stimuli, the brain activations are scattered throughout the operculo-insular and frontal cortices, the brain area that is activated most consistently across the four different stimulation modalities is the left posterior insula. Right (B): Brain activation of both hand and foot stimulation is shown on the same slice (laser: top; pin prick: bottom). At an elevated threshold of Z=3.8, a differential somatotopic representation within the posterior insula in anterior-posterior direction can be seen: the hand representation is anterior to the foot representation. Top: Laser hand (red pixels) versus foot (orange pixels). Bottom: pinprick hand (dark blue) versus foot (light blue), overlapping pixels in white. Note that on group analysis level, the only somatotopic representation that can be identified is in the posterior insula. The left side of the brains is shown on the left.

Figure 4: Three-dimensional clustering of the individual cortical responses obtained within SII following laser hand (red) and foot (yellow) stimulation. To gain an impression of the variability of cortical representations within the secondary somatosensory cortex, the individual activations of all subjects (n=11) were projected onto one subject’s 3D reconstruction of the brain. The individual coordinate systems were aligned to the anterior commissure and the AC-PC plane. Despite the overlap between the scatterings of the red and yellow symbols, a systematic difference in medial-lateral direction is visible, and the average localizations for hand and foot representation are illustrated by the large symbols. Grid width: 1 cm.
Figure 5: Somatotopic representation of hand and foot following noxious stimulation

The left panel shows the average location of the activations in response to heat (laser) stimulation of the hand (red squares) and foot dorsum (orange diamonds), in each ROI. As indicated by red ellipsoids, the location of the activations was significantly different (circled in red) in the contralateral anterior insula, posterior insula, and parietal operculum (SII; for a quantitative description of location differences see Table 1A). The right panel shows the average location of the activations in response to pin prick (punctate probe) stimulation of the hand (dark blue squares) and foot dorsum (pale blue diamonds), in each ROI. The location of the activations was significantly different in the contralateral posterior insula (for a quantitative description of location differences see Table 1B).

In all panels, error bars represent the standard error of the mean. The left side of the brains is shown on the left. AIC: anterior insular cortex; PIC: posterior insular cortex; SII: secondary somatosensory cortex

Figure 6: Different location of activations in response to heat and pin prick stimulation

The left panel shows the average location of the activations in response to heat (laser) and pin prick (punctate probe) stimulation of the hand dorsum, in each ROI. Red squares represent heat stimulation, blue squares represent pin prick stimulation. The location of the activations was significantly different in the contralateral anterior insula (circled in red; AIC: anterior insular cortex; for a quantitative description of location differences see Table 2A). The right panel shows the average location of the activations in response to heat (laser) and pin prick (punctate probe) stimulation of the foot dorsum. Orange diamonds represent heat stimulation, pale blue diamonds represent pin prick stimulation. For a quantitative description of location differences see Table 2B.
In all panels error bars represent the standard error of the mean. The left side of the brains is shown on the left.