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HUMAN SII AND POSTERIOR INSULA DIFFERENTLY ENCODE LASER STIMULI

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Running title :

Somatosensory processing in operculoinsular cortex.

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Abstract

The SII area and the posterior insular region are activated jointly by thermal stimuli in functional imaging studies. However, controversy remains as to a possible differential encoding of intensity between these two contiguous areas. Using CO₂ laser stimulations, we analysed the modifications induced by increasing thermal energy on evoked potentials recorded in epileptic patients, with electrodes implanted within SII and posterior insular cortices. Although increasing stimulus intensities enhanced both SII and insular responses, the *dynamics* of their respective amplitude changes were different. SII responses were able to encode gradually the intensity of stimuli from sensory threshold to barely painful levels, but tended to show a ceiling effect for increasing pain intensities. In contrast, the posterior insular cortex failed to detect non-noxious laser pulses but encoded faithfully stimulus intensity variations at painful levels, without showing saturation effects for intensities above painful threshold. According to these results, one can assume that insular cortex could be more involved in the triggering of affective recognition of, and motor reaction to noxious stimuli, whereas SII would have a more dedicated role in finer-grain discrimination of stimulus intensity, from non-painful to painful levels.

Keywords : CO₂ laser, Evoked potentials, Posterior Insular cortex, Intra-cerebral recordings, SII area, Thermal and painful stimuli

1. Introduction

According to numerous electrophysiological and functional imaging studies conducted in humans in the recent years, pain perception is subserved by the coordinated activity of multiple cortical regions. Although the activated areas are not strictly the same throughout studies, and their relative importance may be dependent upon the specific stimulus parameters and the experimental conditions used, it appears clearly that the cortical regions located in the upper bank of the lateral sulcus, including the second somatosensory area (SII), and the insular cortex, are the sites most consistently activated by all kind of nociceptive stimuli (reviews in Peyron et al 2000, Schnitzler and Ploner 2000, Treede et al 2000, Garcia-Larrea et al 2003, Vogel et al. 2003). Neurophysiological studies in monkeys have demonstrated the existence of nociceptive neurons in both SII and the insula (Robinson and Burton 1980a,b, Dong et al. 1989, 1994, Dostrovsky and Craig 1996, Zhang et al. 1999), and nociceptive regions of thalamus in non-human primates send axons to the parietal operculum, the mid- and posterior insular cortex as well as the retroinsula (Burton and Jones 1976, Jones and Burton 1976, Mufson and Mesulam 1984, Mesulam and Mufson 1985, Burton and Carlson 1986, Friedman and Murray 1986, Stevens et al. 1993, Craig 1995). In humans, regions containing nociceptive neurons in and around the thalamic ventral caudal nucleus (Lenz et al. 1993, 1994) have been shown to project to the insular cortex and the parietal operculum (Van Buren and Borke 1972). These supra-sylvian nociceptive-related areas appear also implicated in the processing

of innocuous somatosensory information. In functional imaging studies, there is a substantial overlap in activity evoked by noxious and innocuous stimuli within SII (Coghill et al. 1994, Chen et al. 2002), and our previous data recorded with intra-cerebral electrodes in humans showed that both noxious CO₂ laser and innocuous electrical stimulations evoke responses within the same sub-regions of SII (Frot et al. 2001). One recent study has provided fMRI evidence suggesting that a posterior region within SII could be specifically involved in the processing of noxious stimuli (Ferretti et al., 2004). However, these authors used electrical stimuli that activated simultaneously noxious (A-delta) and non-noxious (A-Beta) afferents (Gracely 1994); therefore, no definite conclusion could be drawn as to whether these separate areas also participate to the encoding of inputs coming from specific activation of nociceptors.

A major obstacle for the understanding of this region's role in pain perception is our limited knowledge on response properties of perisylvian nociceptive neurons (Treede et al. 2000). Previous studies, including ours, usually compared stimulations of different modalities (mostly electrical or tactile vs noxious heat), and therefore could not assess specifically whether the SII-insular cortex is able to encode intensity *within the thermo-algesic modality*. Moreover, differentiating between the functional properties of the contiguous SII and posterior insular cortices has proven very difficult in both functional imaging and electrophysiological studies. Most human imaging pain studies have considered the SII-insular region, especially in its posterior extent,

as a functional entity with common response characteristics (see Peyron et al 2000, Garcia-Larrea et al 2003 for reviews). However, Ferretti et al. (2004) suggested a functional dissociation within opercular sub-areas, and Frot and Mauguière (2003) demonstrated by intracortical recordings that the responses of the opercular and the contiguous insular cortices could be distinguished on the basis of their different latency and morphology. Therefore, their functional capacities to encode stimulus intensity may also be different.

Studies of the response properties of SII-insular activities have been notoriously inconsistent. Timmerman et al. (2001) and Bornhövd et al. (2002) studied the response modes of SII and insula to progressively increasing stimulus intensities, and did not disclose any difference in their respective modes of response; they considered therefore that both areas responded exclusively to painful stimuli. On the contrary, Davis et al (1998) found that innocuous thermal stimuli often activated the posterior insula, but never SII. In contrast to all of them, Coghill et al (1999) found consistent SII activation at innocuous intensities (35 to 46 °C), and described a significant correlation between thermal intensity and contralateral SII activity (while the anterior insula tended to be activated only at painful levels). Very recently, Iannetti and coworkers (2005) described a positive relationship between the amplitude of SII-insular responses and the subjective pain magnitude to laser pulses. However, these authors used exclusively stimuli in the painful range, and therefore could not investigate whether pre-pain sensations were also coded by these early responses.

In this study we attempted to characterize response properties of the SII and posterior insular regions by studying local intracortical evoked potentials directly recorded within these two cortices, and using increasing levels of thermal energy, ranging from non-noxious to noxious levels. Stimuli were delivered by means of a laser beam, thus avoiding skin contact and co-activation of mechanoreceptors, and therefore the cortical activation could be safely ascribed to stimulation of epidermal thermo-nociceptors exclusively.

2. Materials and Methods

Patients

All of the ten patients included in this study had refractory temporal lobe epilepsy and were investigated using stereotactically implanted intracerebral electrodes before functional surgery. Among other sites, these patients had electrodes chronically implanted in the SII-insular cortex for the recording of their seizures. The decision to explore this area resulted from the observation during scalp video-EEG recordings of ictal manifestations suggesting the possibility of seizures originating in SII and/or insula (see Isnard et al. 2000, 2004 for a complete description of the rationale of electrode implantation). This procedure, performed routinely before epilepsy surgery in patients implanted with depth electrodes, is completed by the functional mapping of potentially eloquent cortical areas using evoked potentials recordings and cortical electrical stimulation (see Ostrowsky et al. 2002 and Mazzola et al. 2005 for a description of the stimulation procedure). According to French regulations concerning invasive

investigations with a direct individual benefit, patients were fully informed about electrode implantation, stereotactic EEG (SEEG), evoked potentials recordings, and cortical stimulation procedures used to localize the epileptogenic and eloquent brain areas and gave their consent. The CO₂ laser stimulation paradigm was submitted to, and approved by, the local Ethics Committee.

Two patients out of the 10 recorded were excluded from the study because paroxysmal epileptic discharges originated in the recorded SII and/or insula. For the other patients (8 cases) several spontaneous seizures could be recorded during the SEEG, all of which originated in the mesial structures of the temporal lobe. In these patients ictal discharges propagated outside the mesio-temporal cortex and involved most frequently the temporal pole, the temporal neo-cortex, the cingulate gyrus, and the orbito-frontal cortex. In three patients the supra-sylvian operculum showed a rhythmic spike-wave activity during the spread of the discharges and in two of these patients this type of activity was also observed in the insular cortex.

The possibility remains that, in these three patients, the supra-sylvian opercular and insular cortices could have shown some degree of interictal hyper-excitability modifying their responsiveness to somatosensory or pain inputs. However this possibility seems unlikely for the following reasons; i) none of the patients included in this study showed ictal discharges onset in the operculo-insular cortex and no low voltage fast activity was recorded in this cortex during spontaneous seizures; ii) focal bipolar electric stimulations delivered

through the contacts used for LEP recordings did not show any evidence of focal hyper-excitability manifesting by the occurrence of after-discharges at stimulus intensities of 1-3 mA currently used for functional mapping (reported in Mazzola et al. 2005); iii) latency and amplitude of somatosensory and pain EPs recorded in the operculo-insular cortex concerned by the spread of ictal mesial activities were not different from those recorded in patients whose seizures did not propagate to these cortical areas.

CO₂ laser evoked potentials (LEPs) were thus recorded from a total of 63 opercular and 30 posterior insular sites in 8 patients (22-59 years, mean age 33 years, 4 females, 4 males). LEPs were obtained at the end of the SEEG monitoring period of two weeks, once relevant seizures had been recorded. At that time, patients were under monotherapy with one of the major anti-epileptic drugs (carbamazepine, phenytoin, valproate, lamotrigine or topiramate) with daily dosages at, or slightly under the minimum of their therapeutic usual range.

Electrode implantation

Intracerebral electrodes were implanted using the Talairach's stereotactic frame. As a first step, a cerebral angiography was performed in stereotactic conditions using an X-ray source located 4.85 meters away from the patient's head, thus eliminating the linear enlargement due to X-ray divergence, so that the films could be used for measurements without any correction. In a second step, the relevant targets were identified on the patient's MRI, previously enlarged at scale one-to-one. As MR and angiographic images were at the

same scale, they could easily be superimposed, thus minimizing the risk of any damage to cerebral veins or arteries during implantation. The electrodes were orthogonally implanted using the Talairach's stereotactic grid; each electrode had 10 to 15 contacts, each of 2 mm length, separated by 1.5 mm, and could be left in place chronically up to 15 days. Because of the physical characteristics of the contacts (stainless steel), it was impossible to perform MRI with electrodes in place. Scale 1:1 skull radiographies superimposed to scale 1:1 angiographies were used to perform the implantation within the stereotactic frame of Talairach and Tournoux (1988). The electrode tracks and the contacts of each electrodes could be plotted onto the appropriate MRIs slices of each patient (MRIcro® software; Rorden and Brett 2000). Each of the contacts was then localized in the Talairach space using its stereotactic coordinates: x for the lateral medial axis, with x=0 being the coordinate of the sagittal inter-hemispheric plane; y for the rostro-caudal (anterior-posterior) axis, y=0 being the coordinate of the vertical anterior commissure (VAC) plane and z for the inferior-superior axis, z=0 being the coordinate of the horizontal anterior commissure-posterior commissure (AC-PC) plane (see also Frot and Mauguière 1999, 2003, Frot et al. 1999, 2001) .

In the SII region, electrodes were implanted caudal and rostral to the VAC plane (y=0). The deepest contacts of the electrodes implanted in SII or the first temporal gyrus explored the insula proper. Four patients were implanted by a single opercular electrode exploring either the pre- (2 cases) or the post-rolandic (2 cases) SII cortex. In the 4 other patients both

the frontal and the parietal SII were each implanted by one electrode. Five patients had one electrode implanted in the first temporal gyrus, the deepest contacts of which exploring the insula proper. Thus our data were collected using a total number of 17 electrodes, 12 of them having contacts in SII and 15 of them in the posterior insula (figure 2 and table 2). Thirty contacts explored the posterior insular cortex, distributed along the rostro-caudal axis, 14 mm rostral and 19 mm caudal to the VAC plane (y coordinates). Sixty-three contacts explored the SII area, distributed along the rostro-caudal axis, 14 mm rostral and 23 mm caudal to the VAC plane (y coordinates).

Stimulation procedure, recording and signal averaging

The LEP recordings were performed between 10 and 15 days after electrodes implantation. During the recordings, the patients laid relaxed on a bed in a quiet room. Cutaneous heat stimuli were delivered by a CO₂ laser (10.6 µm wavelength, beam diameter 3 mm, Optilas®, Evry, France) thus avoiding skin contact and co-activation of mechanoreceptors. Therefore the cortical activations linked to laser stimuli could be safely ascribed to the specific stimulation of epidermal thermo-nociceptors.

CO₂ laser pulses were applied at 4 different intensities in each subject. The power output being fixed, the amount of thermal energy delivered depended on the duration of the pulse. Pulse duration was set up according to subjects' subjective reports, rated on a visual analog scale (VAS) with an anchor point corresponding to pain threshold. The printed scales consisted of 10-cm horizontal lines where the left extreme was labelled "no

sensation" and the right extreme "maximal pain", and an anchored level 4 was at pain threshold (Lickert-type scale).

The different stimuli and related subjective sensation were as follows:

- (a) **I0 (Intensity 0)** : below sensory threshold (pulse duration: 5-15 ms, mean energy density: 7 mJ/mm², no sensation);
- (b) **I1 (Intensity 1)** : above sensory threshold (pulse duration: 15-45 ms, mean energy density: 19 mJ/mm², producing a detectable non painful sensation reported for more than 90% of stimulations; For 1/3 of patients this sensation was a warmth sensation and for the others 2/3 a slight non painful pinprick sensation; VAS 1.6 ± 1.09);
- (c) **I2 (Intensity 2)** : pain threshold (pulse duration: 25-80 ms, mean energy density: 33 mJ/mm², producing a pricking sensation, like a hair pulling or a drop of hot boiling water on the skin; VAS 3.9 ± 1.46);
- (d) **I3 (Intensity 3)** : 20% above pain threshold (pulse duration: 35-110 ms, mean energy density: 46 mJ/mm², producing a pricking sensation described as clearly painful; VAS 5.4 ± 1.6).

The subjects were instructed to draw a vertical mark at the appropriate position on the VAS to indicate the perceived pain intensity. This procedure mostly aimed at differentiating between pain threshold (I2) and clearly painful sensation (I3). It was checked that VAS rates were significantly higher for this latter intensity (Student's t test for paired data, $p < 0.05$).

Two separate runs of 12 to 16 stimulations applied to the superficial radial nerve territory on the dorsum of the hand were delivered at each intensity value, the order of intensities being randomized. The interstimulus interval varied randomly between 10 and 25s. The laser beam was slightly moved between two successive stimuli to avoid habituation and especially to avoid peripheral nociceptor fatigue (Schwarz et al. 2000).

On-line recordings were performed using a sample frequency of 256 Hz and a band pass filter of 0.03-400 Hz (Micromed®, St Etienne des Oullières, France) both in bipolar and reference modes. The reference electrode was chosen for each patient on an implanted contact located in the skull.

Epoching of the EEG, selective averaging and record analysis were performed offline using the Neuroscan® software. The continuous EEG was cut in epochs (each epoch of EEG began 100 ms before the stimulus and ended 900 ms after). A 100 ms prestimulus baseline correction was performed. Analysis was performed both on single epochs and on averages. Averaging was performed to reduce the background EEG noise so as to facilitate analysis of stimulus-locked activity (evoked potentials); epoch averaging was done after rejecting epochs with epileptic transient activities. Finally, the two runs for a given stimulation intensity were averaged after having checked that the averaged waveforms were reproducible.

Amplitude measurements

Given the high signal/noise ratio obtained in intracortical recordings, the LEP amplitudes at

insular and opercular sites could be measured on individual single sweeps, without the need of averaging. A total of 112 single responses were analysed for each intensity level.

Statistical analysis

Amplitudes and latencies of responses were submitted to repeated measures analysis of variance (ANOVA, Statistica 6®), with 3 within-subject factors : Intensity (I0, I1, I2 and I3), localization (Pre vs post-central operculum for SII, and pre vs post vs ventral insula for the insular cortex) and epoch order. The Geisser-Greenhouse (G-G) procedure was applied to correct degrees of freedom (Geisser and Greenhouse 1958). The G-G correction was used whenever a significant violation of the sphericity assumption was detected in repeated-measures ANOVA (with more than 2 *df*) (see Vasey and Thayer 1987). Significance was accepted at $p < 0.05$. Post hoc comparisons t tests were performed with a threshold significance at $p < 0.05$. Correlation between VAS and intensity was assessed using a linear regression model. To define the dynamics of the responses amplitudes as a function of stimulus intensity obtained in SII and insula, data were fitted with a polynomial function [$f(x) = b_0 + (b_1 * x) + (b_2 * x^2) + (b_3 * x^3)$]. Paired t tests were performed between the coefficients determining the increase (b_1) and shape (b_2 and b_3) of the fitted curves (see Timmermann et al. 2001).

3. Results

3.1 Psychophysical responses

By definition (see Experimental Procedures), all the subjects rated 0 (no sensation) on the visual analogue scale (VAS) when the intensity

was under the perception threshold (I0). Subjective intensity rates to I2 corresponded well to a barely painful sensation (boiling water drop on the skin), while I3 (maximal intensity) was unanimously considered as painful and quite unpleasant, albeit tolerable (mean 5.4 /10). This latter stimulus intensity being at the upper limit of tolerance, higher intensities were not used for evident ethical reasons. There was a positive significant linear correlation between the subjective VAS reports and the stimulus intensities ($r=0.87$, $p < 0.001$) (Fig 1).

3.2 Polarity, latency and voltage of SII-insular LEPs

Two distinct evoked potential components contralateral to the stimulation site were recorded along all the electrode tracks implanted in SII, anterior and posterior to the rolandic fissure. They consisted of a negative wave (Nop, for “negative-opercular”) followed by a positive one (Pop), the latencies of which are given in table 1. Similarly, a biphasic negative (Ni, for “negative-insular”) - positive (Pi) components were recorded on contacts located in the posterior insular cortex. Note that absolute latency values could not be used for comparison due to latency pre-normalisation across patients (see figures 3 and 5). However, latency differences among electrode plots within a single patient remained valuable despite normalisation. Calculation on relative latencies showed a significant delay of the insular response relative to the opercular one (t tests, $p < 0.05$, Table 1). No earlier response peaking before these Nop-Pop and Ni-Pi were observed along the electrode tracks implanted respectively in SII and in the insula (Figures 2 and 3).

3.3 Stereotactic localization of the SII-insular LEPs

The maximal amplitude of the N/P deflection was taken to determine the electrode contact likely to be the closest to the source.

The SII LEPs were recorded along the trajectory of all electrodes penetrating the SII cortex within a rectangle bounded by vertical planes 14 mm anterior and 23 mm posterior (y coordinates) to the vertical anterior commissure (VAC) plane, and between horizontal planes 2 mm below and 21 mm above (z coordinates) the horizontal anterior commissure-posterior commissure (AC-PC) plane. These responses were picked up with maximal amplitude on contacts located between 33.25 and 52.75 mm from the mid-sagittal vertical plane (x coordinates) (Table 2 and Figure 4).

The insular LEPs were recorded by the 2 or 3 deepest contacts of the electrodes penetrating the opercular and temporal cortex between vertical planes 14 mm rostral and 19 mm caudal (y coordinates) to the VAC plane, and between horizontal planes 4 mm below and 20 mm above (z coordinates) the AC-PC plane. The contacts recording these responses with maximal amplitude were distributed between 29 and 38.25 mm from the median line (x coordinates) (Table 2 and Figure 4).

3.4 Statistical analysis

Effect of electrode localization on SII-insular LEPs

In the patients whose SII or posterior insular cortices were explored by several electrodes (two or three, see Experimental Procedures) along the antero-posterior axis (y), repeated-

measures ANOVA showed no effect of electrode location on the latency or amplitude of LEP components (Table 3). This reflected a certain level of homogeneity of the responses recorded by the different electrode tracks, at least in the sub-regions of SII and insular cortices we explored. In support of this, (i) there was no waveform difference in SII or insular responses recorded along different electrode tracks; (ii) polarity reversals along the different electrode tracks in a given patient always occurred at the same depth, and (iii) the dynamics of SII and insular responses to variations of stimulus intensity (see below) were always similar along the different electrode tracks. Therefore, we considered that when several electrodes with different antero-posterior (y) coordinates were located in SII or in the insula, they all recorded responses originating from the same source.

Effect of stimulus intensity

- on source localisation

For each patient, the electrode contacts yielding maximal SII or insular responses were the same for all intensities. Therefore, the sources location of these responses did not appear to be modified by the intensity changes. However, due to our restricted spatial sampling, especially along the antero-posterior (y) and vertical (z) axes, we cannot draw any definitive conclusion on this point.

- on SII and insular LEPs latencies and amplitudes

Repeated-measures ANOVA showed no significant effect of intensity on the latencies of insular or SII LEPs (Table 3). There was no effect of epoch order on response latencies either, i.e., for all intensity conditions both

insular and SII response latencies remained stable between consecutive epochs (Table 3). Repeated-measures ANOVA showed a significant effect of stimulus intensity on SII and insular LEP amplitudes (Table 3). For a given stimulation intensity, there was no effect of the epoch order on response amplitudes, i.e., both insular and SII response amplitudes remained stable between consecutive epochs (Table 3). SII and insular responses showed a highly significant increase of their amplitude between the two extreme intensities (I0 and I3; t-tests, $p < 0.001$) (figure 5).

- on the dynamics of SII and insular LEP amplitudes change

Although increasing stimulus intensities enhanced both SII and insular responses, the *dynamics* of their respective amplitude changes were different. In SII, a significant increase of the LEP was observed as soon as the stimulus intensity reached the sensory threshold (between I0 and I1, $p < 0.001$), as well as between sensory and pain thresholds (I1 to I2, $p < 0.001$) while amplitudes rapidly reached a plateau for intensities above pain threshold (no significant amplitude difference between I2 and I3, $p = 0.1$). In the insula, no significant amplitude increase was observed for low stimulation intensities ($p = 0.1$ between I0 and I1), LEP amplitudes also increased between sensory and pain threshold intensities ($p < 0.05$ between I1 and I2) and, contrary to what was observed in SII, continued to increase significantly at higher intensities over pain threshold ($p < 0.001$ between I2 and I3). Figure 3 illustrate this point in the whole set of patient's responses, and notably the fact that increasing slightly the stimulus intensity above perception threshold produced clear potentials

within SII but no response above noise in the contiguous posterior insula. As a consequence, the stimulus-response function of posterior insula and SII appeared different, and were fitted with different polynomial functions [$f(x) = b_0 + (b_1 * x) + (b_2 * x^2) + (b_3 * x^3)$], which had an exponential profile in the insula and an S-shaped profile in SII (figure 6). The coefficients determining the increase (b_1) and shape (b_2 and b_3) of the fitted curves were significantly different between SII and insula (SII: $b_1, 56.4 \pm 28.7$; $b_2, -30.7 \pm 13.3$; $b_3, 5.09 \pm 1.4$; Insula: $b_1, -16.03 \pm 19.6$; $b_2, -0.06 \pm 8.3$; $b_3, 0.31 \pm 1.11$; mean \pm SE; paired t-tests for b_1 , b_2 and b_3 in SII and insula: $p < 0.05$, see Figure 5). Note that when SII and insular data were pooled together, the stimulus-response function of the coupled areas yielded a linear function similar to the VAS reports (right part of figure 6).

4. Discussion

Intracranial recordings provide a unique opportunity to explore, with good spatio-temporal resolution, the activity of most cortical structures, even those buried in the depth of sulci and hence of difficult access using scalp or epicortical recordings. Using this technique, we previously showed that the responses to painful stimuli in SII and insula could be distinguished on the basis of response latencies and of stereotactic source coordinates (Frot and Mauguière 2003). In the present work, we further specify that, although increasing stimulus intensities enhanced both SII and insular responses, the *dynamics* of their responses as a function of thermal stimulus intensity are significantly different. The SII responses were able to encode

gradually the intensity of laser thermal stimuli from sensory threshold to barely painful levels, but tended to show a ceiling effect for increasing pain intensities. In contrast, the posterior insular cortex failed to detect responses changes for very low levels of stimulus intensities (around sensory perception threshold), but encoded stimulus intensity variations in the painful range without showing saturation effects for the highest painful intensities used.

Very recently, Iannetti and coworkers (2005) described a positive relationship between the amplitude of SII-insular responses and the subjective pain magnitude to laser pulses. These authors founded their analysis on the behaviour of the scalp 'N1' response, which most probably reflects lumped opercular and insular sub-components (Valeriani et al. 2000, Garcia-Larrea et al. 2003); therefore, sustained increase at painful ranges may have reflected the insular, rather than the SII contribution to scalp N1. Indeed, as Iannetti et al. (2005) used exclusively stimuli in the painful range, they could not investigate whether pre-pain sensations were also coded by these early scalp responses.

No evoked responses were recorded in the insular cortex explored by our electrodes for stimulus intensities at, or just above, sensory threshold (I1), while clearly recordable insular potentials were recruited at pain threshold (I2). However, as we did not test intermediate intensities, firm conclusions cannot be drawn on the insular encoding properties for intensities between sensory and pain levels. It is however noteworthy that no significant LEPs could be recorded in posterior insular cortex for

the non-noxious levels for which clear evoked responses were recorded in the neighbouring opercular (SII) cortex. This is in agreement with the results of an elegant study of Bornhövd et al. (2002) on the dynamics of fMRI signals in response to progressively intense laser pulses. In accordance with our data, their results suggested that neither anterior nor posterior insular responses distinguished among non-painful intensities, but showed a positive relationship for painful trials such as we observed in our insular recordings. This point deserves some considerations since, contrary to our study and Bornhövd's data, several previous studies described activations within insular cortex for non noxious stimuli (Coghill et al. 1994, Craig et al. 1996, 2000; Davis et al. 1998, Becerra et al. 1999, Maihöfner et al. 2002). The stimulation technique used may be at the origin of this discrepancy since our study and the one of Bornhövd used laser stimulations (brief thermal pulses: 1 to 110 ms; small skin surface stimulated: about 30 mm²), while the others studies performed stimulations of much longer durations (several hundreds of ms up to minutes) involving larger skin areas (thermode: up to several cm²). These diverging results are by themselves very interesting. They suggest that insular activation by non noxious stimuli may be possible if thermal non noxious stimuli are applied long enough on sufficient extended skin areas. This could indicate the necessity of some time/surface dependent recruitment processes in order to obtain some responses within the posterior insular cortex. Since reports of posterior insular activation by non-noxious stimuli (innocuous warmth) most often used mixed mechanical and thermal stimuli, sometimes moving, it is difficult to ascertain

which of stimulus duration, stimulus surface or mechanical contact (or a combination of the three) was the most important contributor to eliciting the insular response. A good compromise to obtain good time/surface recruitment without losing thermal specificity could perhaps be achieved in a near future by specific stimulation of warmth (C-fiber) receptors without skin contact, using a recently described laser technique (Cruccu et al. 2003). However, there have not been to date any reports of intracranial responses obtained using such a technique.

Bornhövd et al. (2002) also described similar activation patterns in SII and posterior insula, while our intracranial data showed different dynamics of response between the insula *proper* and SII responses. In our study, SII responses were able to distinguish between low levels of innocuous stimulus intensities (see figures 3 and 5). Given the limited spatial resolution of fMRI relative to SEEG studies, it is possible that different signals from SII and posterior insular cortices could not be disentangled in Bornhövd et al.'s study (2002), and that their SII-insular fMRI signal reflected almost exclusively the insular response. A similar inability to detect SII response changes to small intensity levels was reported by Timmermann et al. (2001) using MEG. The stimulus-response function described by these authors in SII cortex showed no significant activation change at low stimulus intensities (subthreshold and perception threshold), while a sharp increase in source activation was observed for stimuli above pain threshold. As was the case with fMRI, surface MEG recordings are probably not precise enough to dissociate activation patterns emanating from

SII and posterior insula, even when using dipolar modelling. One could assume that Timmermann et al.'s results may have actually reflected a mixed signal largely dominated by the insular responses. In accordance with this view, a recent meta-analysis of dipole-modelling studies of cortical pain responses indicated that scalp-derived modelled dipoles reflect a 'lumped' activation of several sources in the suprasylvian region, including both SII and the insula (Garcia-Larrea et al. 2003, Apkarian et al. 2005). Survey of the literature therefore suggest that separation of SII and insular encoding properties may be beyond the reach of both haemodynamic functional imaging (PET / fMRI) and scalp electrocortical recordings (EEG / MEG), at least until the signals emanating from these two very closely located regions can be reliably segregated by these techniques (see reviews in Peyron et al. 2000, Derbyshire 2000, Jones et al. 2002, Garcia-Larrea et al. 2003, Apkarian et al. 2005).

The present work is, to our knowledge, the first demonstrating that the closely located SII and posterior insular cortices differently encode gradual thermal stimulus intensity changes, and thus points out different functional organizations in these two areas. The fact that insular responses continued to increase when SII potentials tapered makes it clear that posterior insula cannot receive thermal information *exclusively* from the SII regions explored in this study. This is consistent with anatomical and physiological data suggesting that these two cortices are part of partially different networks for somatosensory processing. In monkeys, SII cortex receives its major thalamic input from the ventroposterior

inferior thalamic nucleus (VPI) (Stevens et al. 1993, Friedman and Murray 1986) whereas the posterior insula (granular and dysgranular parts of insular cortex) is afferented by a variety of thalamic nuclei including, in addition to the VPI, the suprageniculato-limban complex, the basal ventromedial, the medial pulvinar and the posterior nuclei (Burton and Jones 1976, Mufson and Mesulam 1984, Friedman and Murray 1986). Neuronal units in monkey's VPI are known to be essentially somatic, and contain a majority of non-nociceptive and wide-dynamic-range (WDR) neurons that could encode gradually the stimulus intensity from non-painful to painful ranges (Apkarian et al. 1991, Apkarian and Shi 1994). Conversely, thalamic nuclei sending projections on insular cortex contain much more polymodal neuronal units that respond to a variety of stimuli including not only somatosensory, but also auditory and visual stimulations (Berkley 1973, Hicks et al. 1984, Benedek et al. 1997). Given the properties of its thalamic afferent neurons, the insular cortex appears to be clearly multimodal as compared to SII. This conclusion is reinforced by stimulation data showing that contrary to SII stimulation, which produces almost exclusively somatosensory responses, insular stimulations produces nearly 40% of non-somatosensory responses including viscerosensitive, auditory, speech, vestibular and olfacto-gustatory responses (Isnard et al. 2004 and Mazzola et al. 2005).

At a cortical level, the patterns of insular and SII cortical connections support this hypothesis. Insula receives afferents from a wide variety of cortices including somatosensory areas (SI and SII), auditory

cortex, orbitofrontal cortex, amygdala, cingulate gyrus and other limbic areas (Mesulam and Mufson 1982, Augustine 1985, Friedman et al. 1986, Augustine 1996). In contrast, projections to SII area arise exclusively from SI and 7b parietal areas, posterior insular and retroinsular cortices (Friedman et al. 1986). The massive amount of afferents from associative cortices to the insula implies that this region is involved in numerous types of information processing. Such continuous and multimodal input should represent a 'background activity' that could hamper the precise encoding of stimulus attributes unless they are sufficiently salient. This is in agreement with our intracortical recordings, as well as the results reported by Bornhövd et al. (2002) using fMRI, suggesting that insular activity is modulated mostly by brief thermal stimuli of high intensity, while it remains poorly modified by stimuli at or near sensory threshold. Efferent insular projections are massive to limbic and memory-related areas (amygdala, cingulate cortex) (Augustine 1985, 1996). This probably explains that insular responses do not show a saturation effect for intensities above painful threshold. It is not surprising that posterior insular cortex, which is directly connected to areas contributing to the emotional processing of painful events (Büchel et al. 1999, Bornhövd et al. 2002) and the orienting reactions towards the noxious stimulation (Büchel et al. 2002) encodes for stimuli well above painful threshold.

According to our results we can assume that the encoding of thermal stimuli above pain threshold includes a maximal response in SII and a pain level related response in the insula.

It can be hypothesized that pain sensation results in part from this coupled activation of both areas. This assumption is supported by the fact that when SII and insular data were pooled together, the stimulus-response function of the coupled areas yielded a linear function similar to that of the subjective intensity perception (compare figure 1 and the right part of figure 6).

For evident ethical reasons, we fixed the highest stimulus intensity (I3) at 20% over the pain threshold level and thus did not explore subjective pain levels higher than 6-7/10 on VAS ratings. Consequently we cannot ascertain whether the 'ceiling effect' that we observed for painful pulses in SII remained so for very highly painful intensities. A similar ceiling effect was however suggested by Ferretti et al. (2004) in a recent fMRI study. These authors showed that the activity of an anterior part of SII, largely overlapping the location of our SII electrodes, failed to increase between non-painful and painful stimulations. These authors also suggested that a more posterior part of SII could be more specifically involved in pain encoding, since the BOLD signal at this location kept increasing between pre-pain and pain stimuli. None of our opercular electrodes was implanted posterior enough to explore this most caudal region in SII, so our recordings may reflect only part of the opercular pain network. On the other hand, contrary to us, these authors did not explore the SII responses to gradually increasing stimuli. Thus, in the eventuality that a posterior SII sub-region would be able to code for stimulus intensities over pain threshold, it would remain to determine whether it is nociceptive-specific, or made of wide dynamic

range cells able to code for stimulus intensities both below and above pain threshold.

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Tables & Figures

Table 1

N_P PEAK AMPLITUDES							
	I0	I1-I0	I1	I2-I1	I2	I3-I2	I3
SII	27.6 ± 3.2	11.9 ± 4.4	39.5 ± 4	22.4 ± 4.3	61.9 ± 4.8	11.3 ± 4.5	73.2 ± 4.9
Insula	23 ± 1.7	4.9 ± 2.4	27.9 ± 2.4	9.5 ± 3	37.4 ± 2.5	16.5 ± 3.1	53.9 ± 2.9

LATENCIES								
	I0		I1		I2		I3	
	N	P	N	P	N	P	N	P
SII	150.9 ± 9.7	215.9 ± 20.5	150.6 ± 12.5	211.5 ± 20.1	154.3 ± 20	215.7 ± 22.5	149.4 ± 22.3	210.03 ± 25.1
Insula	270.6 ± 37.6	403.3 ± 44.6	273.9 ± 39.4	400.2 ± 43.3	275.7 ± 41	401.8 ± 48.8	268.7 ± 40.6	398.5 ± 53.6

Latencies (ms) and amplitudes (µV) of responses

All the means are given with the standard errors.

Table 2

Patients		SII Cortex			Insular Cortex		
		x	y	z	x	y	z
AL	PrC	33.25	14	7	29	14	7
LS	PrC	41.75	2	12	38.25	2	12
	PoC	37.25	-16	20	32.75	-16	20
	T1				38.25	-12	-1
FF	PrC	38.75	-2	12	31	-2	12
	PoC	45.75	-23	21			
	T1				33	-10	-2
CM	PrC	45.75	-1	17	31	-1	17
VE	PrC	33.25	4	7	29	4	7
	PoC	42.25	-12	8	31	-12	8
DB	PoC	35.75	-12	18	35	-12	18
	T1				34	-9	-4
LM	PrC	52.75	2	-2	35.25	2	-2
	PoC	36.75	-12	14			
	T1				32.75	-19	-4
KL	PoC	37.75	-13	14	30	-13	14
	T1				32.75	-10	-1
Mean		40.1	-5.8	12.3	32.9	-6.3	6.7
SD		5.8	10.5	6.6	2.9	9	8.5

Coordinates (Atlas of Talairach and Tournoux) of contacts (in mm) where the maximal amplitudes of the N/P deflection in bipolar mode were recorded.

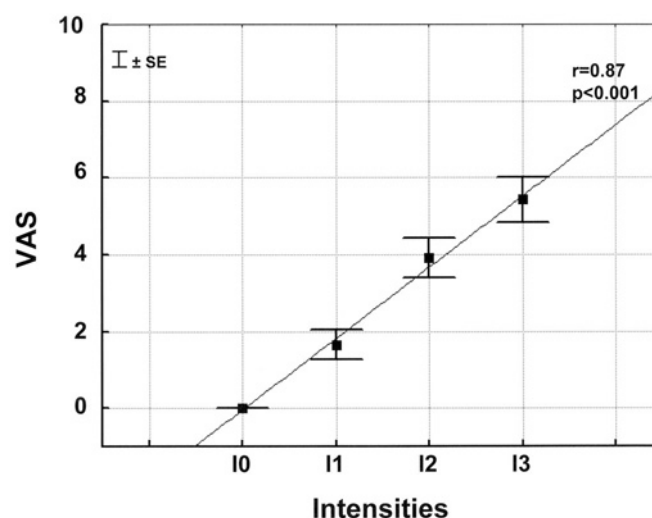
PrC: Pre-central SII cortex, PoC: Post-central SII cortex

Table 3

	SII		INSULA	
AMPLITUDE	Nop-Pop		Ni-Pi	
<i>Intensity</i>	F(3,40)= 2.9 <u>p< 0.05*</u>		F(3,48)= 4.8 <u>p< 0.01*</u>	
<i>Electrode Localisation</i>	F(1,40)=0.4 p= 0.5		F(2,48)= 0.4 p= 0.7	
<i>Epoch Order</i>	F(11,440)=0.7 p= 0.7		F(11,528)= 1.7 p= 0.07	
LATENCY	Nop	Pop	Ni	Pi
<i>Intensity</i>	F(3,40)= 0.3 p= 0.82	F(3,40)= 0.3 p= 0.85	F(3,48)= 0.06 p= 0.98	F(3,48)= 0.05 p= 0.98
<i>Electrode Localisation</i>	F(1,40)= 1.4 p= 0.2	F(1,40)= 1.9 p= 0.2	F(2,48)= 3.2 p= 0.06	F(2,48)= 0.2 p= 0.83
<i>Epoch Order</i>	F(11,440)= 1.6 p= 0.1	F(11,440)= 1.4 p= 0.2	F(11,528)= 1.8 p= 0.06	F(11,528)= 0.89 p= 0.54

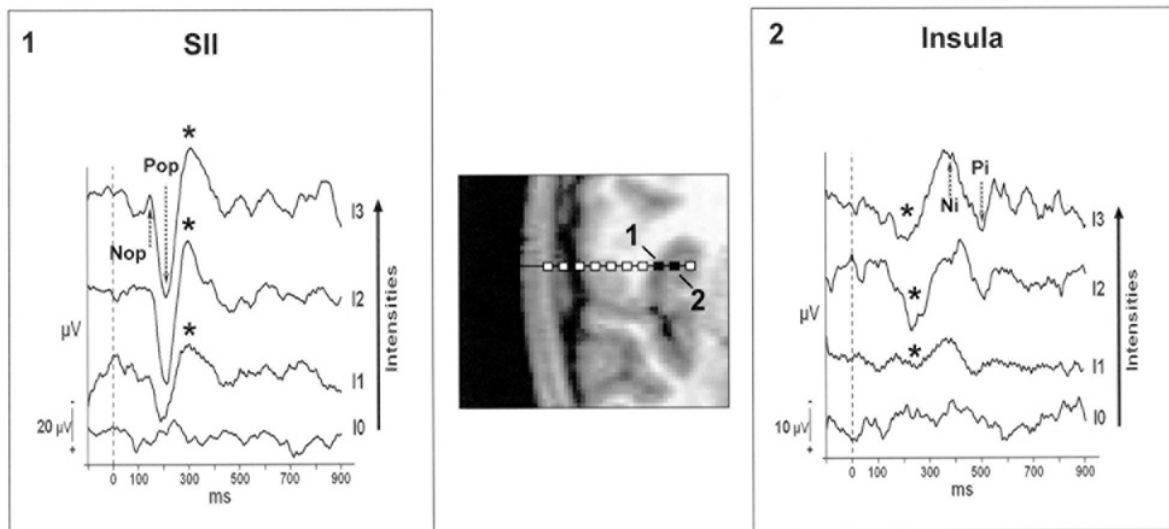
Statistical analysis (ANOVA) of the effects of intensity, electrode localization and epoch order on amplitude and latency of SII and insular LEPS.

Significant results were indicated by a *, were in bold and underlined. df: before the G-G correction.

Figure 1

Correlation between the subjective VAS reports and the stimulus intensities.

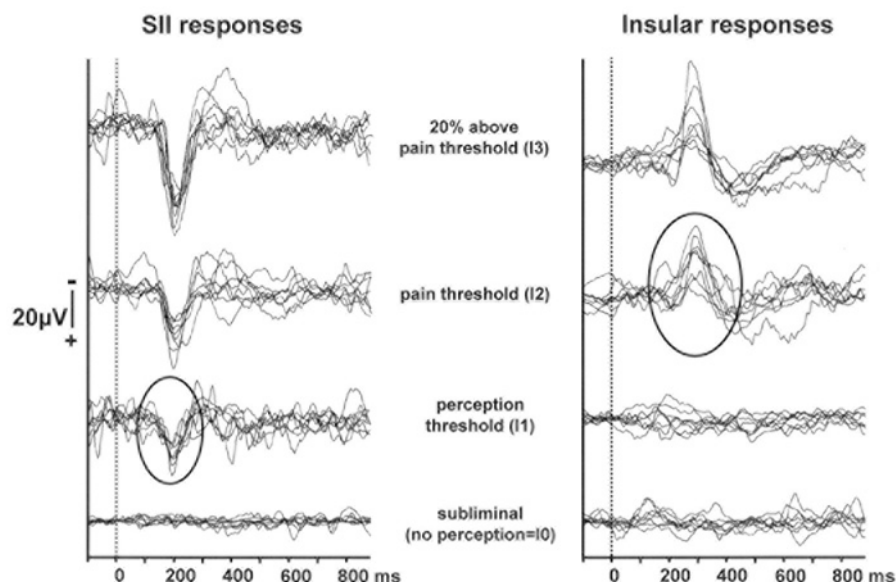
Figure 2



Effects of intensity on SII-insular responses – One patient.

LEPs recorded on two depth contacts located in SII (1) and insular (2) cortices of one patient, for each level of stimulus intensity (I0, I1, I2, I3). These LEPs were recorded in referential mode. Note that in SII (1) we recorded a late negative response indicated by a black star. This component did not appear to be generated in SII but rather corresponded to the diffusion of the Ni component of the insular LEPs, due to the proximity of the contacts 1 and 2. This was confirmed by recordings in bipolar mode where this late SII negative component disappeared (see figure 5). A similar phenomenon is present on insular recordings where a positive peak occurs at about 180ms (black star on 2).

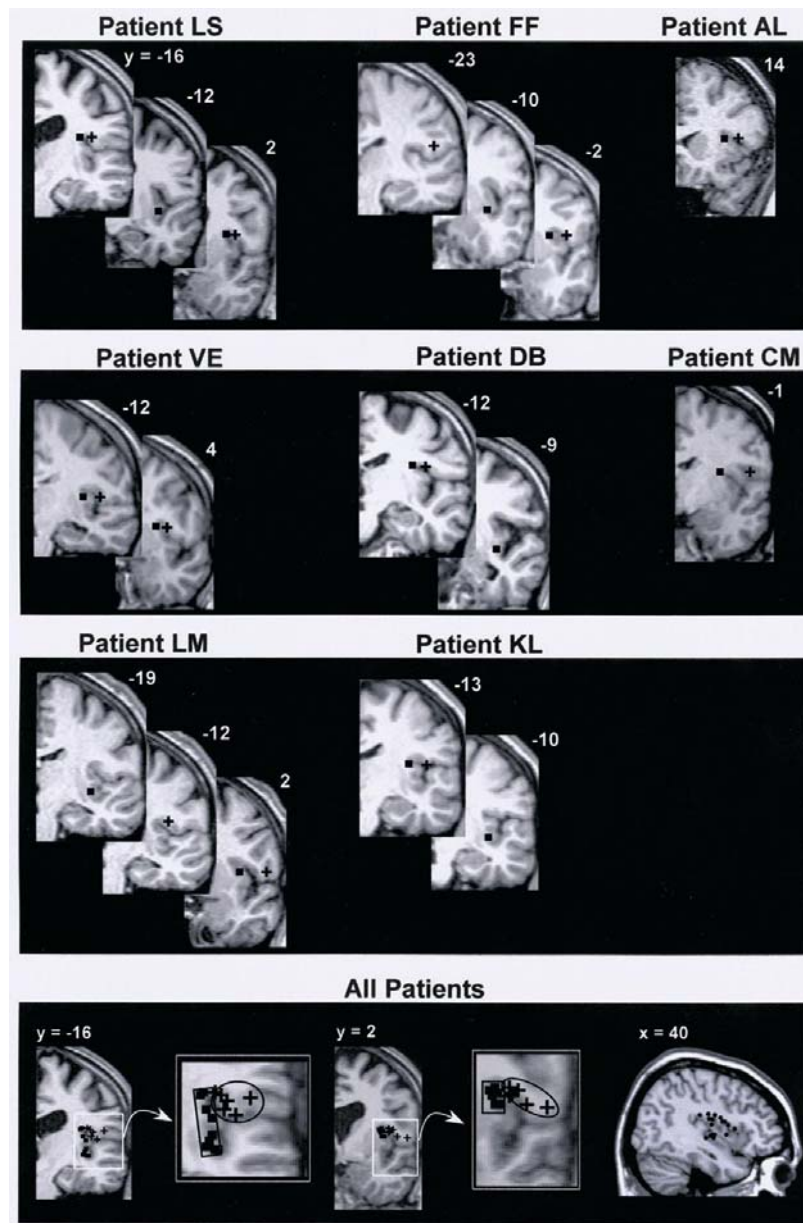
Figure 3



LEPs recorded on depth contacts located in SII and insular cortices of all patients (8 subjects) for each level of stimulus intensity.

All these responses have been latency-normalized according to the maximal LEP peaks (Pop for SII and Ni for insular responses). A response has been recorded for all patients in SII at I1 whereas the first evoked response in the insular cortex was recorded at I2 in the majority of cases.

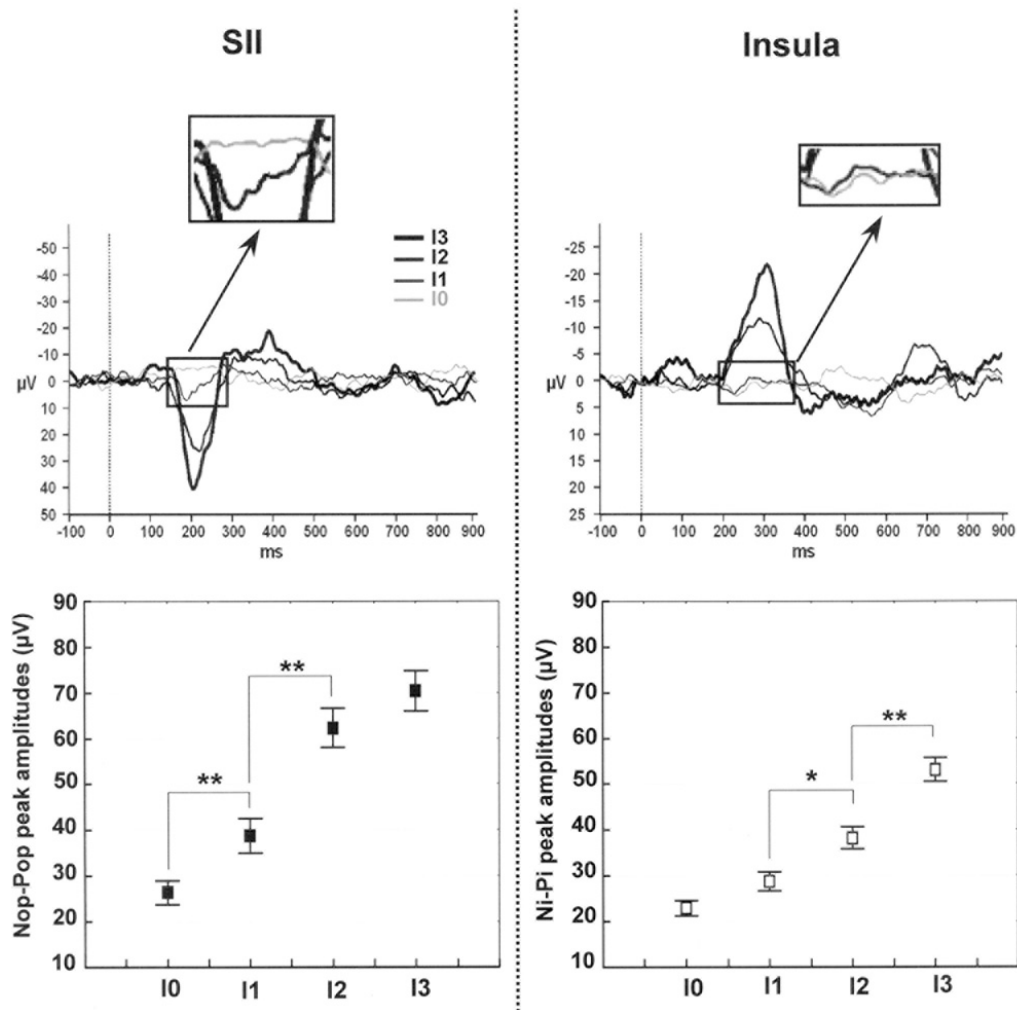
Figure 4



Location of the contacts where the maximal amplitudes of the N/P deflection in bipolar mode were recorded.

Black crosses: contacts located in SII; Black squares: contacts located in the insula. Contacts have been located on the 3D-MRI of each patient. y : antero-posterior coordinate (in mm) of the coronal plane according to the Talairach and Tournoux atlas.

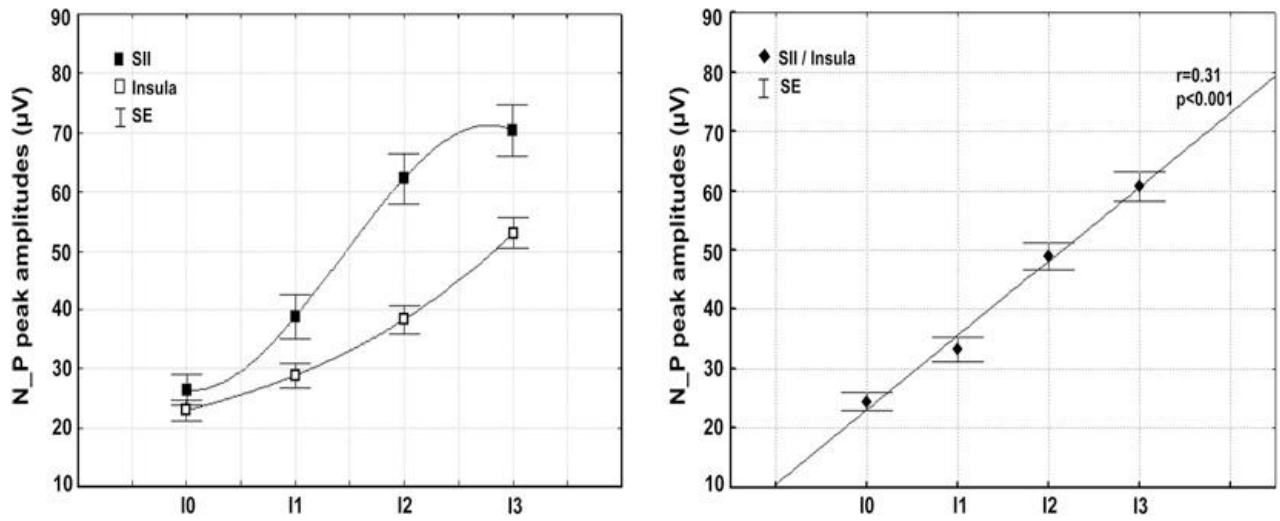
Figure 5



Effects of intensity on SII-insular responses

On the top of the columns are represented the latency-normalized grand average LEPs in bipolar mode from all the patients in SII (on the left) and insula (on the right) for each level of stimulus intensity. Below are represented the amplitude of SII and insular LEPs as a function of intensities (I0, I1, I2, I3). Error bars indicate standard error (SE). * p<0.05; ** p<0.001

Figure 6



Insula and SII peak amplitudes as a function of stimulus intensity

Using the polynomial function $f(x) = b_0 + (b_1 * x) + (b_2 * x^2) + (b_3 * x^3)$ curves were fitted on the stimulus response amplitude functions. The dynamics of response were significantly different in SII and in the insula: the fitting curve had an exponential profile for the insula ($r=0.98$, $p=0.02$) and a S-shaped profile in SII ($r=0.98$, $p=0.016$). Amplitudes were those measured between N and P peaks for both SII and insula. The right part of the figure shows the correlation between the amplitudes when SII and Insula data were pooled together.