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# Intrainsular Functional Connectivity in Human

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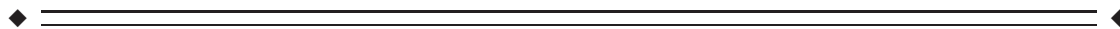
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**Abstract:** *Objectives:* The anatomical organization of the insular cortex is characterized by its rich and heterogeneous cytoarchitecture and its wide network of connections. However, only limited knowledge is available regarding the intrainsular connections subserving the complex integrative role of the insular cortex. The aim of this study was to analyze the functional connectivity within- and across-insular subregions, at both gyral and functional levels. *Experimental design:* We performed intracerebral electrical stimulation in 10 patients with refractory epilepsy investigated with depth electrodes, 38 of which were inserted in the insula. Bipolar electrical stimulation, consisting of two series of 20 pulses of 1-ms duration, 0.2-Hz frequency, and 1-mA intensity, was delivered at each insular contact. For each stimulated insular anatomical region, we calculated a rate of connectivity, reflecting the proportion of other insular contacts, showing significant evoked potentials. *Results:* Statistically significant evoked potentials were recorded in 74% of tested connections, with an average latency of  $26 \pm 3$  ms. All insular gyri were interconnected, except the anterior and posterior short gyri. Most connections were reciprocal, showing no clear anterior to posterior directionality. No connection was observed between the right and the left insula. *Conclusions:* These findings point to specific features of human insula connectivity as compared to non-Human primates, and remain consistent with the complex integration role devoted to the human insula in many cognitive domains. *Hum Brain Mapp* 35:2779–2788, 2014. © 2013 Wiley Periodicals, Inc.

**Key words:** insular; functional connectivity; intra-cranial electrical stimulation; evoked potential; human



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## INTRODUCTION

The insula is a functionally complex region providing multimodal integration and involved in various verbal and nonverbal activities, including speech motor control [Bohland and Guenther, 2006], emotional processing [Adolphs et al., 2003; Calder et al., 2000; Wicker et al., 2003], pain perception [Alkire et al., 2004; Mazzola et al., 2006; Ostrowsky et al., 2002], somatosensory integration [Burton et al., 1993], auditory processing [Bieser, 1998], as well as taste and olfactory perceptions [Kurth et al., 2010; Small, 2010; Stephani et al., 2011; Yaxley et al., 1990].

The anatomical organization of the insular cortex is characterized by its rich and heterogeneous cytoarchitecture [Mesulam and Mufson, 1985] and its wide network of connections [Augustine, 1996]. However, only limited knowledge is available regarding the intransular connections subserving the complex integrative role of the insular cortex. The nonhuman primate insula demonstrates abundant bidirectional intransular connections, with stronger connectivity from anterior to posterior regions [Mesulam and Mufson, 1982b; Seltzer and Pandya, 1991], providing a potential pathway for conveying olfactory and gustatory information into the posterior insula [Mesulam and Mufson, 1982b]. In human, a single *in vivo* probabilistic tractography has suggested strong within-subregion structural connectivity in both anterior and posterior insular regions [Cloutman et al., 2011].

Human cerebral functional connectivity can be studied in patients with refractory epilepsy undergoing intracerebral EEG (icEEG) investigation, by applying electrical stimulation to cortical areas and recording evoked potentials (EPs) in distant-connected structures [Brazier, 1964; Buser and Bancaud, 1983; Buser et al., 1992; Catenoix et al., 2005, 2011; Lacruz et al., 2007; Matsumoto et al., 2004, 2007; Rosenberg et al., 2009; Rutecki et al., 1989; Wilson et al., 1990]. This approach offers a high level of temporal and spatial resolution for mapping brain connectivity, providing complementary findings to those depicted by currently available neuroimaging methods. Taking advantage of clinical icEEG investigations of the insula, we applied this method to analyze the functional connectivity within- and across-insular subregions, at both gyral and functional levels.

## MATERIALS AND METHODS

### Patients

Ten patients with drug-resistant partial epilepsy contemplating epilepsy surgery and undergoing icEEG were included in this study. Inclusion criteria included the presence of at least two different electrodes inserted within the same insula to look at EP in at least one insular electrode while stimulating another. Exclusion criteria included the presence of any morphological abnormality of the insula.

All patients gave their informed consent to participate in this study.

### Stereotaxic Implantation of Depth Electrodes

icEEG was performed according to the technique described by Talairach and Bancaud [1973], a procedure used routinely in our department [Guenot et al., 2001]. The brain regions to be investigated were determined for each patient, based on individual presurgical data, and most likely origin of seizure onset. In general, about one-third of the electrodes target the most likely zone of seizure onset, whereas the remaining electrodes probe alternative but less likely hypothesis, or help to define the borders of the epileptogenic zone. As a result, a significant number of recording contacts are not affected by the epileptic process. This is particularly true for the insula, which is often investigated owing to its potential to mimic temporal or frontal lobe seizures [Isnard et al., 2001, 2004, Ryvlin et al., 2006], but eventually found not to be affected in the majority of cases.

Electrodes were implanted perpendicular to the midsagittal plane with the patient's head fixed in the Talairach's stereotaxic frame, providing Talairach's coordinates for each electrode in relation to the anterior commissure/posterior commissure plane. The exact location of each electrode and recording lead was further verified with a postimplantation MRI. The two deepest contacts of the electrodes targeting the insula were located within that structure, whereas the next more lateral contact was located either in the sylvian fissure or within the deepest portion of the adjacent operculum (temporal, frontal, or parietal). For the purpose of illustrating our findings on a single template, we used the above information to position the insular leads inserted in our 10 patients onto a T1-MRI sagittal slice of the insula derived from a normal subject.

In total, 11–16 semi-rigid intracerebral electrodes were implanted per patient, either unilaterally ( $n = 8$ ) or bilaterally ( $n = 2$ ). Each electrode was 0.8 mm in diameter and included 5, 12, or 15 contacts 2 mm in length, 1.5 mm apart (Dixi, Besançon, France), depending on the target region. No morbidity was related to cerebral electrode implantation.

### Brain Stimulation and EP Recordings

Intracerebral EP recordings were performed using a video-EEG monitoring system (Micromed, Treviso, Italy) that allowed to simultaneously record up to 128 contacts at a sampling rate of 1,024 Hz. As part of the clinical icEEG investigation, electrical brain stimulation is systematically performed to assess the epileptogenicity and functionality of the implanted brain regions. This procedure includes 50, 1, and 0.2 Hz stimulations, with 50 and 1 Hz, aiming at triggering ictal signs, epileptic discharge, or full-blown seizure [Kahane et al., 1993, 2004; Munari et al.,

1993], whereas 0.2 Hz are used to trigger abnormal cortical-evoked responses (i.e., delayed to more than 100 ms or repetitive) suggestive of an underlying epileptogenic cortex [Valentin et al., 2002; 2005a,2005b; van't Klooster et al., 2011]. In addition to their clinical utility, 0.2-Hz stimulations also allow to measure physiological early responses, referred to as EPs in this study, and reflecting brain connectivity [Brazier, 1964; Buser and Bancaud, 1983; Buser et al., 1992; Catenoux et al., 2005, 2011; Lacruz et al., 2007; Matsumoto et al., 2004, 2007; Rosenberg et al., 2009; Rutecki et al., 1989; Wilson et al., 1990].

We performed 0.2-Hz stimulations at least 4 days after electrodes implantation, once the patient has fully recovered from the surgical procedure. We used bipolar stimulation of adjacent contacts from the same electrode, known to deliver current within 5 mm around the stimulated bipole [Nathan et al., 1993]. Electrical stimulation was delivered using a current-regulated neurostimulator (Micromed, Treviso, Italy), with parameters ensuring patient's safety and effective generation of EPs [Catenoux et al., 2005, 2011; Gordon et al., 1990]. We used monophasic pulse of 1 ms width and 1 mA intensity, resulting in an electrical charge of 1  $\mu\text{C}$ . The latter was delivered over a contact surface of 0.05  $\text{cm}^2$  (0.8 mm diameter  $\times$  2 mm length  $\times$   $\pi$ ), resulting in a total charge density of 20  $\mu\text{C}/\text{cm}^2/\text{phase}$ , thus significantly lower than the maximum safe value of 60  $\mu\text{C}/\text{cm}^2/\text{phase}$  [Gordon et al., 1990]. Two consecutive series of 20 pulses were delivered at each pair of contacts.

### Data Analysis

EEG data were analyzed with the software package for electrophysiological analysis (ELAN-pack) developed at the DYCOG laboratory of Lyon Neuroscience Research Centre (CRNL, Lyon, France) [Aguera et al., 2011]. We first performed an automatic detection of the pulse artifact generated on the stimulated contacts, and systematically verified the accuracy of the generated marker. Thanks to the very reproducible shape and amplitude of artifacts, as well as the associated high signal-to-noise ratio, this procedure did not suffer from false-positive or -negative detection. We used the stimulation marker for averaging each block of 20 consecutive pulse stimulations, and then calculated grand averages from the two blocks. Both visual and statistical analyses were used to conclude on the presence of significant EPs over each recording contact. EPs were first selected on the basis of visual analysis if detected and found comparable in each of the two consecutive series. Statistical analysis of the selected EPs was then performed using the nonparametric statistical function of ELAN-pack for single trails (Wilcoxon test), with significance set at  $P < 0.001$ . In brief, this statistical analysis compared each time point of the poststimulation period to the 1,000-ms prestimulation baseline, providing a curve, superimposed on the EP, illustrating the  $P$ -value associated with each

component of the EP. The first 10-ms poststimulations were not evaluated owing to the presence of residual stimulation-induced artifact. EPs were considered significant when they reached the statistical threshold of  $P \leq 0.001$  during at least five consecutive milliseconds. The latency of the first peak of each significant potential was measured on the grand average of the two series.

Insular regions targeted by intracerebral electrodes were divided into those involved at seizure onset, those invaded during seizure propagation, and those not affected by the epileptic discharge.

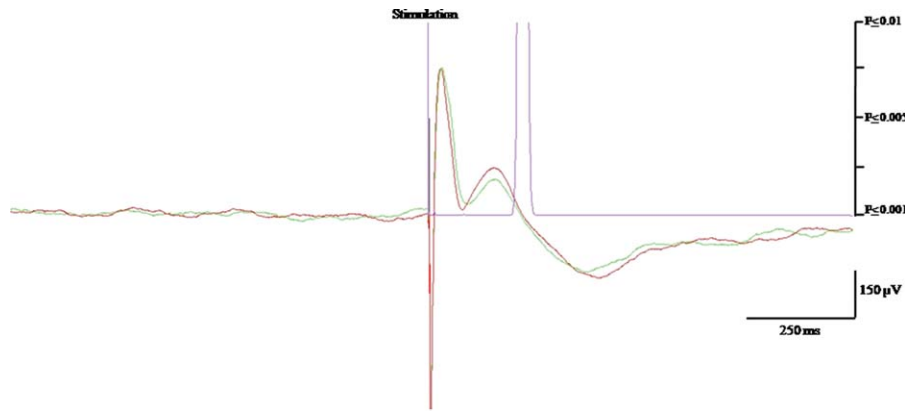
For each stimulated insular anatomical region, we calculated a rate of connectivity, reflecting the proportion of other insular contacts showing significant EPs. This analysis was performed at the gyral level, considering the three short and two long gyri as five distinct anatomical regions, and at a functional level, distinguishing the social-emotional, cognitive, chemical sensory, and sensorimotor insular subregions as identified in a recent functional neuroimaging meta-analysis of the human insula [Kurth et al., 2010].

## RESULTS

A total number of 38 electrodes were implanted into 12 insulae (two patients had bilateral implantation), with 29 electrodes placed in the left insula (76%) and 9 in the right (24%). The gyral location of these electrodes was as follows: three electrodes in the anterior short gyrus (ASG), four in the middle short gyrus (MSG), seven in the posterior short gyrus (PSG), 10 in the anterior long gyrus (ALG), and 14 in the posterior long gyrus (PLG). In terms of functional areas, three electrodes sampled the cognitive, four the chemical sensory, and 26 the sensorimotor subregions of the insula, whereas no electrode was placed in its social emotional portion (five other electrodes fell out of the functional areas determined by Kurth et al., 2010).

Stimulations were performed at all 38 insular electrodes, giving rise to significant EPs on other insular contacts in 74% of the 110 tested connections. Recorded EPs typically corresponded to biphasic early responses, with an average  $\pm$  SD latency of the first peak of  $26 \pm 3$  ms (range, 20–31 ms) (Fig. 1).

The epileptogenic zone was located in the temporal lobe in five patients, frontal lobe in three, parietal lobe in one, and in the insula in one. In this patient, four out of the six insular electrodes were involved at seizure onset, whereas the other two were affected only during seizure propagation. Another two patients with a temporal or a frontal epileptogenic zone had one of their insular electrodes affected by seizure propagation. The proportion, morphology, and latency of EPs triggered or recorded at each insular contact were comparable for those involved at seizure onset, those affected during seizure propagation, and those not affected by the epileptic discharge. Thus, further results pooled data triggered and recorded at all insular contacts.



**Figure 1.**

Illustration of EP recorded after insular stimulation. Negative polarity is upward. The green and red color superimposed curves are the average of two 20 trials, showing similar N1 and N2 peaks. The purple curve represents the *P*-statistic whose value is 0.001 threshold (i.e., significant response) when reaching the abscissa.

**Gyral Connectivity**

Connectivity between the different insular gyri is shown in Table I and Figures 2 and 3. The ASG demonstrated limited connectivity (30%, *N* = 20), with most of its connections targeting the adjacent MSG and being unidirectional from anterior to posterior. The MSG showed a 67% rate of connectivity (*N* = 24), distributed over all other gyri, with most connections being reciprocal. The PSG showed the highest rate of connectivity (88%, *N* = 40), primarily targeting the MSG and the two long gyri with

reciprocal connections. ALG showed a 71% rate of connectivity (*N* = 52), mostly with the adjacent PSG and PLG. All connections with PSG were reciprocal, whereas nearly half of those with PLG were unidirectional. Among the latter, half were anterior to posterior, whereas the other half were posterior to anterior. PLG showed a 75% rate of connectivity (*N* = 60), which qualitative pattern was similar to that described for ALG.

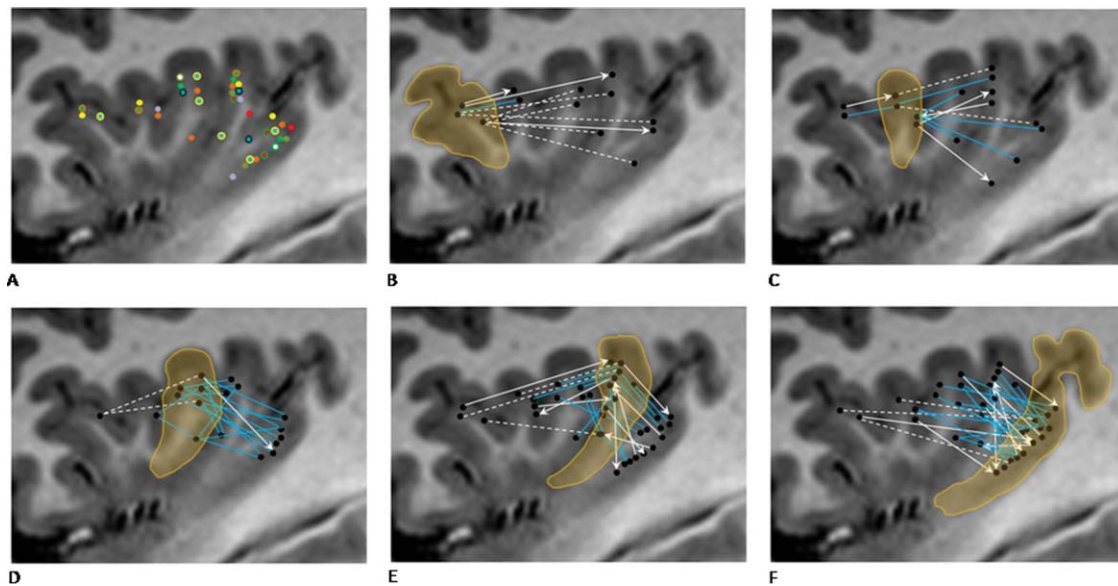
Latencies of the earliest EP varied from  $21 \pm 1$  ms for connections between the anterior short and long gyri, to  $34 \pm 1$  ms for connections between the MSG and the PLG (Table I).

**TABLE I. Connectivity across and within the five insular gyri**

		Recorded leads				
		Anterior short gyrus	Middle short gyrus	Posterior short gyrus	Anterior long gyrus	Posterior long gyrus
Stimulated leads	Anterior short gyrus	–	100% ( <i>N</i> = 2) $24 \pm 7$	0 ( <i>N</i> = 2)	33% ( <i>N</i> = 3) $21 \pm 1$	33% ( <i>N</i> = 3) $28 \pm 1$
	Middle short gyrus	50% ( <i>N</i> = 2) $24 \pm 5$	–	100% ( <i>N</i> = 2) $30 \pm 1$	50% ( <i>N</i> = 4) $26 \pm 5$	75% ( <i>N</i> = 4) $34 \pm 1$
	Posterior short gyrus	0 ( <i>N</i> = 2)	100% ( <i>N</i> = 2) $29 \pm 4$	100% ( <i>N</i> = 4) $30 \pm 4$	50% ( <i>N</i> = 6) $25 \pm 3$	75% ( <i>N</i> = 10) $28 \pm 4$
	Anterior long gyrus	0 ( <i>N</i> = 3)	50% ( <i>N</i> = 4) $26 \pm 1$	100% ( <i>N</i> = 6) $27 \pm 5$	–	77% ( <i>N</i> = 13) $27 \pm 2$
	Posterior long gyrus	0 ( <i>N</i> = 3)	50% ( <i>N</i> = 4) $27 \pm 5$	90% ( <i>N</i> = 10) $28 \pm 3$	77% ( <i>N</i> = 13) $26 \pm 5$	100% ( <i>N</i> = 8) $28 \pm 2$

For each tested connection: 1) top number represents connectivity rate (responding connections/total tested connections); 2) middle number in brackets (*N*) is the number of connections tested; 3) bottom number is the mean latency in ms ± SD. Empty cells: no tested connection.





**Figure 2.**

Electrode location and gyral connectivity pattern. **A:** Electrode location in all 10 patients; different color is used for each patient. **B:** ASG connectivity. **C:** MSG connectivity. **D:** PSG connectivity. **E:** ALG connectivity. **F:** PLG connectivity. For graphs B–F, gyrus of interest is encircled and highlighted, blue solid lines indicate bidirectional connectivity, white solid arrows indicate unidirectional connectivity, and dotted white lines indicate lack of detectable connection.

Intragyrus connectivity could be tested only across a limited number of connections within the PSG ( $N = 4$ ) and the PLG ( $N = 8$ ), showing 100% connectivity rate in these two gyri.

### Functional Connectivity

Connectivity between the different functional areas of the insula is shown in Table II and Figure 4. The cognitive subregion had a low rate of unidirectional anterior to posterior connectivity with sensorimotor areas (29%) ( $N = 7$ ). In contrast, the chemical sensory and sensorimotor areas demonstrated a 63% rate of reciprocal connectivity ( $N = 8$ ). Only two connections could be tested between the cognitive and the chemical sensory areas, showing bidirectional connectivity. Connectivity within the same functional subregion could be assessed only within the sensorimotor area where 93% of connections proved functional ( $N = 44$ ).

Latencies of the earliest EP were  $25 \pm 3$  ms for connections between the cognitive and the chemical sensory regions,  $28 \pm 5$  ms for connections between the chemical sensory and the sensorimotor regions, and  $27 \pm 3$  ms for connections within the sensorimotor area (Table II).

### Contralateral Connectivity

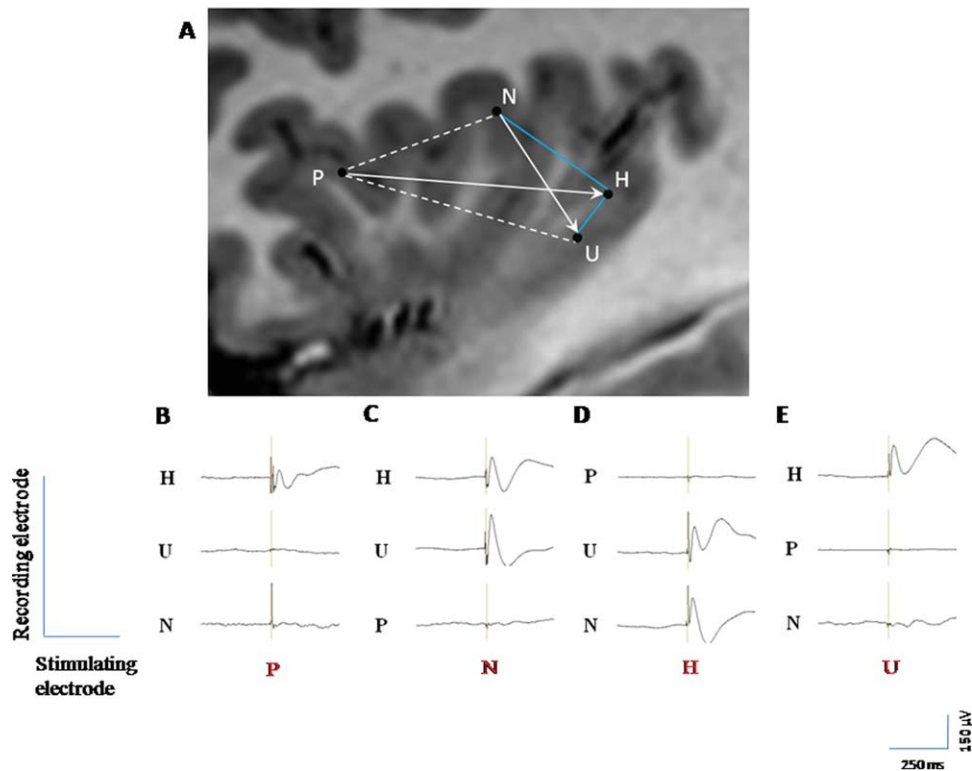
Two patients had bilateral insular implantation, both of whom had three electrodes on one side and a single

electrode on the opposite side. In one patient, contralateral electrode had an exact counterpart in the opposite insula, located in the same portion of the PLG. However, no EP could be recorded over the 12 potential interhemispheric connections tested in these patients.

## DISCUSSION

This study provides the first human electrophysiological data exploring intra-insular functional connectivity, offering clues to the complex integrative role of the insular cortex.

Our knowledge of intra-insular connectivity primarily relies on data from nonhuman primates [Mesulam and Mufson, 1982b; Seltzer and Pandya, 1991]. The relevance of these to human insular connectivity is suggested by the similarities observed in the gyration, cytoarchitecture, and functional anatomy of the insula between the two species, leading some authors to suggest that the human insula has a plan of organization virtually identical to that of the rhesus monkey [Mesulam and Mufson, 1982a]. Both display an anterior–posterior pattern evolving from agranular to granular cortices through a transitional dysgranular area and a similar pattern of connectivity with extrainsular regions, primarily governed by the cytoarchitectonic features of the connected brain regions [Cerliani et al., 2011]. In line with the above observations, a recent *in vivo* tractography study reported an anterior–posterior connectivity predominating over the transitional area of the human



**Figure 3.**

Illustration of the various patterns of connectivity in one patient. **A:** Connectivity between four insular leads located within the ASG (P), PSG (N), and PLG (H, U). Blue solid lines indicate bidirectional connectivity, white solid arrows indicate unidirectional connectivity, and dotted white lines indicate lack of detectable connection. **(B–E)** EPs evoked by stimulating P, N, H, and U leads, respectively.

**B:** the stimulation of P (ASG) generate EPs only over H (PLG), which are not reciprocal; **(C)** the stimulation of N (PSG) generate EPs over H and U (PLG), only one of which is reciprocal (H); **(D)** the stimulation of U (PLG) generate EPs only over H, which are reciprocal; and **(E)** the stimulation of H (PLG) generate EPs over N (PSG), and U (PLG), both of which are reciprocal.

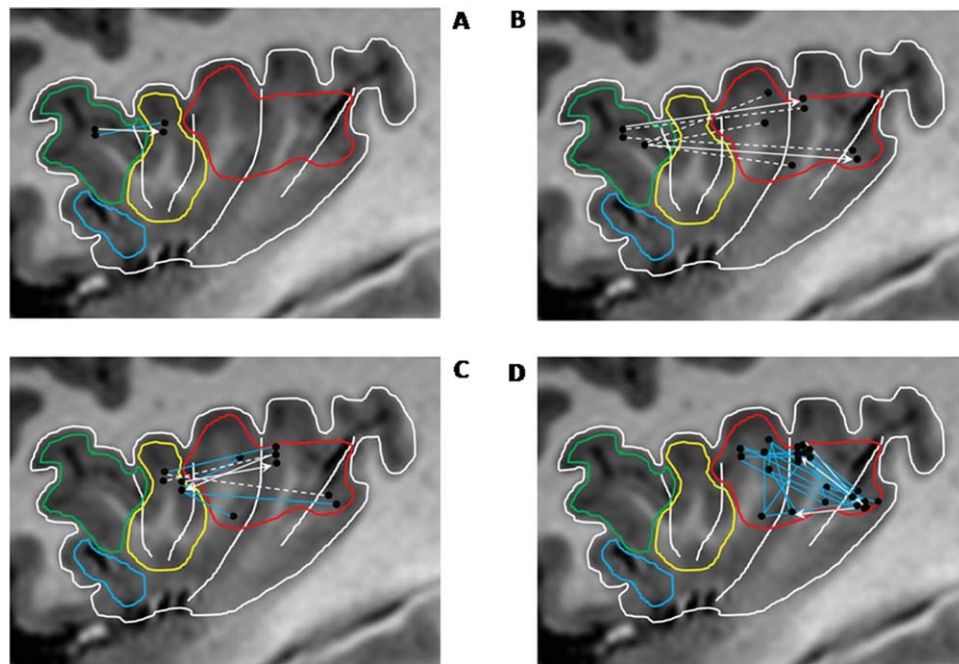
insula, particularly between the PSG and the ALG [Cloutman et al., 2011]. However, whether intrainular functional connectivity overlaps with these structural data remained to be determined. So far, only indirect evidence of intrainular functional connectivity in human is available, based on the pattern of insular activation observed in a meta-analysis of 1,768 functional neuroimaging experiments [Kurth et al., 2010].

Our study used direct electrical stimulation of the human insula and associated EPs to assess intrainular functional connectivity. This method has been used to study the connectivity of various brain regions such as the mesial temporal structures [Brazier, 1964; Buser and Bancaud, 1983; Catenoix et al., 2005, 2011; Lacruz et al., 2007; Rutecki et al., 1989; Wilson et al., 1990], temporal neocortex including language areas [Matsumoto et al., 2004], frontal cortex [Buser et al., 1992; Lacruz et al., 2007], motor system [Matsumoto et al., 2007], and thalamic medial pulvinar nucleus [Rosenberg et al., 2009]. Based on this method, we showed that the human insula was characterized by rich connections between various insular gyri

**TABLE II. Connectivity across and within main insular functional subregions**

		Recorded leads		
		Cognitive	Chemical sensory	Sensory-motor
Stimulated leads	Cognitive	–	100% (N = 2) 25 ± 3	29% (N = 7) 27 ± 4
	Chemical sensory	50% (N = 2) 26 ± 2	–	63% (N = 8) 28 ± 5
	Sensory-motor	0 (N = 7)	63% (N = 8) 28 ± 2	93% (N = 44) 27 ± 3

For each tested connection: 1) top number represents connectivity rate (responding connections/total tested connections); 2) middle number in brackets (N) is the number of connections tested; 3) bottom number is the mean latency in ms ± SD. Empty cells: no tested connection.



**Figure 4.**

Connectivity across- and within-main insular functional subregions. **A:** Connectivity between cognitive and chemical sensory regions. **B:** Connectivity between cognitive and sensorimotor regions. **C:** Connectivity between chemical sensory and sensorimotor regions. **D:** Connectivity within sensorimotor region. Functional regions as

delineated by Kurth et al. [2010]: social-emotional (blue), cognitive (green), chemical sensory (yellow), and sensorimotor (red). For graphs (A–D), blue lines indicate bidirectional connectivity, white arrows indicate unidirectional connectivity, and dotted white lines indicate lack of detectable connection.

which can be summarized as follows: (1) all gyri were interconnected, except the ASG and PSG for which limited sample size hampers any firm conclusion; (2) most intra-insular connections proved reciprocal, in contrast with the anterior-posterior preferential direction described in the macaque monkey [Mesulam et al., 1982b; Seltzer and Pandya, 1991].

This latter finding raises the possibility that the human insula might have specific functional characteristics, but could also reflect differences between structural and functional methods on the one hand, and nonhuman and human studies on the other hand. Indeed, divergences between human neuroimaging functional and structural connectivity data have been reported in other brain regions [Damoiseaux and Greicius, 2009; Zhang et al., 2010]. The anterograde transport methods used in monkeys have offered the possibility to clearly determine the directionality of connections within the insular cortex of nonhuman primates [Mesulam et al., 1982b], an information not assessable with in vivo tractography in human. Whether connection directionality can be reliably tested by intracerebral electrical stimulation studies remains disputed. Indeed, such stimulation is thought to primarily trigger action potentials within bypassing axons with both orthodromic and antidromic propagation [Matsumoto

et al., 2004; Wilson et al., 1990; Zhang and Oppenheimer, 2000]. Although this mechanism might well occur, it remains that 14% of the connections observed in our study were unidirectional, demonstrating that bidirectional stimulation of the same axons is not a generalized feature of corticocortical EPs. Furthermore, some insular contacts showed bidirectional connectivity with one insular region and unidirectional connectivity with another (Fig. 3). Finally, latencies of reciprocal EPs usually differ between the two directions, supporting the view that distinct neurons are stimulated to produce EPs of varying latency.

Although our data suggest a more reciprocal intra-insular connectivity pattern than that described in monkeys, some anterior-to-posterior preferential direction was observed for connections in the ASG and cognitive subregion. However, only a few electrodes were available in those regions, with very low connectivity rates, hampering any robust conclusion regarding this finding.

The morphology of EPs recorded in our series was consistent with those observed in the above studies. Latencies of the first detectable peak were consistent with the average values found in the previous studies, typically ranging between 20 and 30 ms [Matsumoto et al., 2004, 2007]. However, 10–20 ms latencies were reported for short-distance EPs recorded within the motor and language



brain regions [Matsumoto et al., 2004, 2007], in line with the observation that EP latency increases with the distance between the stimulating and the recording electrodes [Matsumoto et al., 2012]. Thus, latencies of intrasular EPs could be considered excessive according to the relatively short distance of the tested connections. One possibility could be that we failed at detecting earlier EPs occurring during the first 10-ms poststimulation owing to stimulus-induced artifact. Another hypothesis would be that different from the motor and language networks that require fast processing monosynaptic pathways, the more integrative role of the insula is subtended by slower polysynaptic connections.

This hypothesis is consistent with the greater connectivity rate observed within the insula as compared to other brain regions assessed using the same methodology [Lacruz et al., 2007; Wilson et al., 1990], as well as with the dense connections observed between the insular cortex and many cortical and subcortical regions [Brooks et al., 2005; Büttner-Ennever and Gerrits, 2004; Mesulam and Mufson, 1985; Pritchard and Norgren, 2004]. The human insula is thought to ensure a complex integration role in many cognitive domains such as emotional processing [Adolphs et al., 2003; Calder et al., 2000; Wicker et al., 2003], somatosensory and pain integration [Alkire et al., 2004; Burton et al., 1993; Ostrowsky et al., 2002; Mazzola et al., 2006], taste and olfaction [Kurth et al., 2010; Small, 2010; Stephani et al., 2011; Yaxley et al., 1990], and auditory and speech processing [Bieser, 1998; Bohland and Guenther, 2006]. Specifically, we observed dense functional connectivity within the sensorimotor portion of the insula, a region thought to provide a primary interoceptive representation of the physiological condition of the body which is then associated with input from multiple other sources in the mid-insula [Craig, 2010]. Rich bidirectional connectivity between cortical areas that receive sensory afferents from different sources might underlie the build-up of complex representation of sensory environment.

On the other hand, the low connectivity pattern observed between the ASG or cognitive subregion of the insula and the other insular regions is surprising. Indeed, it has been proposed that multimodal sensory information converge to the most anterior portion of the insula in a way that would contribute to self-recognition and emotional awareness [Craig, 2009]. Conversely, other authors view that the anterior insula as an anatomically and functionally distinct structure from the remainder of the insula [Nelson et al., 2010]. In any event, our study suffers from clear undersampling of the most anterior portion of the insula, and further data will be needed to conclude on this issue.

Some other limitations need to be acknowledged. First, icEEG-based studies of functional connectivity are necessarily performed in patients with epilepsy whose brain connections might be altered, especially within the epileptogenic zone. In fact, no difference between the EPs recorded from contacts included in the epileptic network

and those recorded from nonepileptic tissue was observed as described previously by others [Lacruz et al., 2007; Wilson et al., 1990]. Furthermore, the insula was not part of the epileptogenic zone in nine out of our 10 patients, with the majority of tested contacts not even affected by the epileptic discharge during its propagation. Thus, we believe that our main findings are likely to apply to healthy individuals. A second issue relates to the sampling limitations of electrodes implanted orthogonally in the insula, with vascular constraints hampering access to the ventral portion of the anterior insula, leaving this region unexplored by our study. In addition, the clinical profiles of our patients resulted in greater need for exploring the PSG and PLG rather than the anterior and MSG, leading to less robust findings for these latter structures.

We failed to detect contralateral EPs to insular stimulation although we could test 12 potential connections in two patients, including one pair of electrodes placed in a very homologous position within both insulae. This negative finding is in contrast with the evidence of structural and functional connections between the two insulae in human [Anderson et al., 2011; Ebisch et al., 2010]. Although most likely reflecting insufficient insular sampling, our findings also suggest that the density of connections between the two insulae is much lower than that observed within each insula.

Overall, electrically induced corticocortical EPs demonstrate that the human insula is characterized by rich reciprocal connections within and between its mid and posterior aspects, in particular throughout the regions underlying sensorimotor integration. Further studies should confirm these findings in larger population and look at their relationship to the insular afferents and efferents as delineated by the same electrophysiological method.

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