Brain Stimulation 16 (2023) 1653–1665

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.journals.elsevier.com/brain-stimulation

Subjective states induced by intracranial electrical stimulation matches the cytoarchitectonic organization of the human insula

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ARTICLE INFO

Keywords:
Insula
Microstructure
Direct cortical stimulation
Intracranial recordings
Functional mapping

ABSTRACT

Functions of the human insula have been explored extensively with neuroimaging methods and intracranial electrical stimulation studies that have highlighted a functional segregation across its subregions. A recently developed cytoarchitectonic map of the human insula has also segregated this brain region into various areas. Our knowledge of the functional organization of this brain region at the level of these fine-parceled microstructural areas remains only partially understood. We address this gap of knowledge by applying a multimodal approach linking direct electrical stimulation and task-evoked intracranial EEG recordings with microstructural subdivisions of the human insular cortex. In 17 neurosurgical patients with 142 implanted electrodes, stimulation of 40 % of the sites induced a reportable change in the conscious experience of the subjects in visceral/autonomic, anxiety, taste/olfactory, pain/temperature as well as somatosensory domains. These subjective responses showed a topographical allocation to microstructural areas defined by probabilistic cytoarchitectonic parcellation maps of the human insula. We found the pain and thermal responses to be located in areas lg2/ld2, while non-painful/non-thermal somatosensory responses corresponded to area ld3 and visceroceptive responses to area Id6. Lastly, the stimulation of area Id7 in the dorsal anterior insula, failed to induce reportable changes to subjective experience even though intracranial EEG recordings from this region captured significant time-locked high-frequency activity (HFA). Our results provide a multimodal map of functional subdivisions within the human insular cortex at the individual brain basis and characterize their anatomical association with fine-grained cytoarchitectonic parcellations of this brain structure.

1. Introduction

The human insular cortex is a complex brain region characterized by its involvement in a multitude of different functions including interoception [1], pain [2], thermoception [3], chemical perception [4,5], social-emotional processing [6], salience detection [7,8], decision making [9], and outcome prediction [10,11] with a multifunctional interface across different functional delineations [12,13].

The central sulcus of the insula has been traditionally used as a landmark to divide this brain structure into the anterior and posterior subdivisions (Fig. 1). Neuroimaging studies have reported higher responses in the anterior insula during, for instance, conditions of interoceptive awareness and anxiety [14], salience detection [8], social-emotional processing [6,15], and outcome prediction [10,11] and the posterior insular activity has been linked to, for instance, thermal sensation [3] and speech [16], while pain and interoceptive cardiac attention have been linked to subregions across the entire insular cortex [12,17].

Intracranial direct cortical stimulation studies have provided another means for exploring the anatomical-functional relationship in the insula

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https://doi.org/10.1016/j.brs.2023.11.001
Received 13 September 2023; Received in revised form 23 October 2023; Accepted 3 November 2023
Available online 8 November 2023

(see Supplementary Table 1 for a summary of the findings from these studies). For instance, Penfield and Faulk (1955) [18] were the first to report subjective changes in somatosensory and visceral sensation from direct stimulation of the insular cortex. Since then, visceral [18–26], somatosensation [18,20–24,27,28], vestibular [20,21,28,29], pain [20,23,27,28,30,31], warmth [20–23,28,30,31], taste/olfaction [20,21,23,32,33], and auditory [20–22,24,28] sensations along with motor [21,22,28] and emotional responses [33,34] have been reported in the extant literature further proving the insular cortex to be a functionally heterogenous region [35]. In general, visceral sensations have been attributed to more anterior parts of the insula while somatosensation has been attributed to more posterior parts [35]. Vestibular responses were also reported to be localized in the dorsal posterior tip of the insula [29]. Pain was associated with the posterior and superior region of the insula [30] where thermosensations were also observed [30] while auditory responses were reported from the most posterior and inferior insular region near the Heschl's gyrus [20–22,24,28]. Gustatory and olfactory responses have been reported to be localized to the inferior middle short gyrus [20–22,24,28]. Lastly, emotional responses have been reported to be concentrated in the far anterior portion of the insular cortex [33].

In parallel to the imaging and stimulation studies of the insula, recent scientific developments have yielded novel insight in parcellating the brain according to its cytoarchitectonic organization [36]. This microstructural map of the brain has served as a new anatomical framework for delineation of functional architectural subdivisions within a given brain region [36,37]. For instance, based on cytoarchitectonic mapping [38], the human insula has been parcellated into 16 distinct areas, covering a wide range of different microstructural features [39–41]. The topography and borders of such areas hardly correspond to macroscopic landmarks [36,41] (Fig. 1).

A recent study by Mazzola and colleagues [29] reported a link between a specific cytoarchitectonic area in the posterior insula (Ig2) and a specific response to electrical stimulation (i.e., vestibular sensations). However, it remains to be determined whether various other responses to electrical stimulation and task performance can also be topographically delineated by the microstructural diversity across the entire mantle of the insular cortex. The present study was designed to address this important unknown using intracranial electrical stimulation, task-evoked iEEG responses, and an updated cytoarchitectonic map of the insula [39–41] within each patient's native anatomical brain space.

2. Material and methods

2.1. Informed consent

Patients signed informed consent for participation in our study, which is already approved by the Stanford University IRB. None of the procedures proposed in this study will introduce any additional risks to the patients.

2.2. Protections against risk

Implantation of intracranial electrodes followed the standard of clinical care practiced at our institution and was motivated solely by clinical needs. The decision to move forward with invasive monitoring and about the approximate location of electrodes is made in a consensus meeting held each week with neurosurgeons, epileptologists, neuropsychologists, psychiatrists, and radiologists gathering together to decide the course of action and the approximate sites of implantations. This is to ensure that the implantation of electrodes and their approximate locations are solely influenced by clinical needs and consensus.

Electrical brain stimulation is used routinely in our clinical practice with satisfactory safety profile and does not vary from the ones reported in the literature. The amount of electrical charge delivered per pulse (voltage and pulse width) is always kept within the safe limits. Each implanted electrode comes with one-page specs that provides information about charge density limits. We follow these limits carefully to stay below the safe limit (30 μC/cm 2/pulse as confirmed by earlier studies

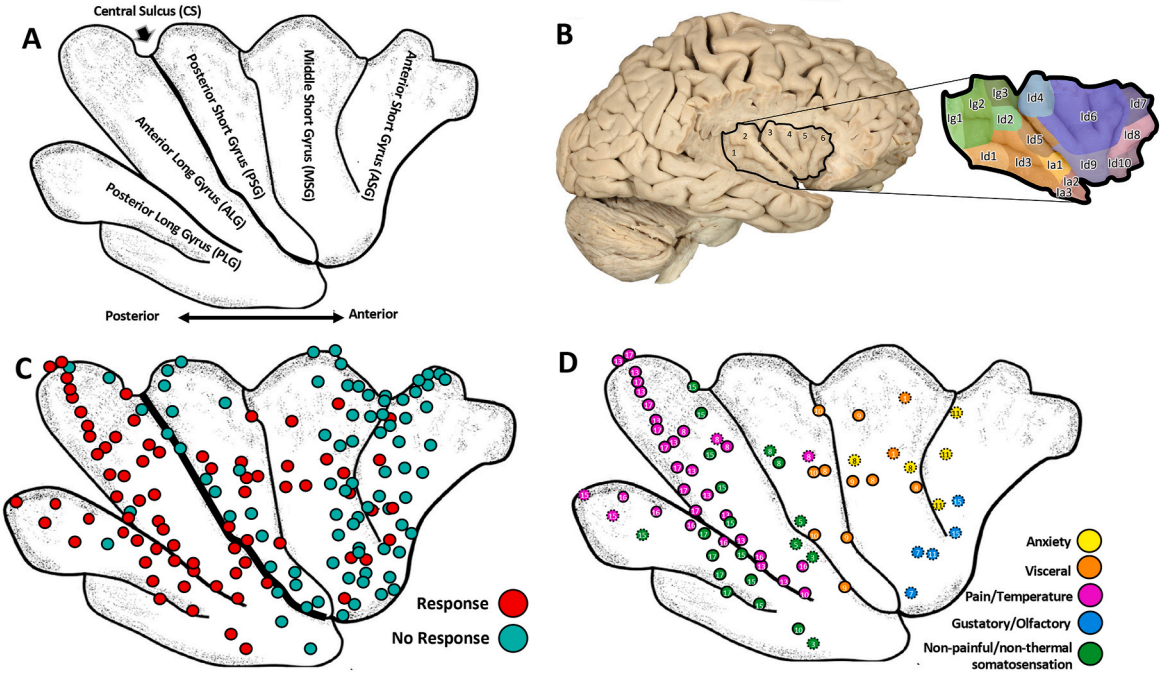


Fig. 1. Structure and Subjective Responses in the Human Insula A) Macrostructural organization of insula based on sulci and gyrus [41]. **B)** Depiction of the microstructural parcellation of the insula projected onto a brain's surface. Brain only used for visualization purposes derived from the body donor program of the Institute for Anatomy I, University of Düsseldorf, Germany. **C)** Locations of all single electrodes stimulated as pairs of bipolar electrodes in the insula. Red and teal colors represent electrodes whose stimulation did or did not cause any change in the reportable subjective change, respectively. **D)** Response type of each electrode and the subject numbers (corresponding to Table 1) are presented. All electrodes are shown in the right insula. Those from the left hemisphere are indicated by dotted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

[42,43]). To mitigate any unwanted risk, two physicians always are at the bedside during the stimulation experiments. The doctors have access to IV benzodiazepines (lorazepam, 2 mg) and would be able to administer it intravenously if the procedure of electrical stimulation induced a seizure.

2.3. Electrodes localization

Electrode contacts (0.86 mm diameter; 2.29 mm height; 3-5 mm center-to-center inter-electrode distance, AdTech Inc) were localized on the patient's structural MRI scan (T1-weighted) after fusion and co-registration with their post-implantation CT image. As part of the structural MRI image processing, cortical surfaces were reconstructed via FreeSurfer [44]. The co-registration of the MRI and CT images was performed with FreeSurfer and manually checked for accuracy. On the fused image, we used BioImage Suite[47] to pick up the electrode contacts which appear as the local brightest points on the CT scan. The coordinates of the electrode contacts (in FreeSurfer surface space, voxel space, and MNI space) were automatically extracted by iElVis toolbox [45]. The electrodes' coordinates in the native scanner space were linearly transformed from the voxel space. The anatomy of every contact's localization was carefully inspected and manually labeled by neurologist J.P., based on the individual brain's morphology.

Surgical Approach: Electrodes with placement and quantity in the insula were chosen based on the patients' theorized epileptic zone. This surgical approach using oblique insular electrodes has been previously reported elsewhere [46] with success in safety and sampling considering the unique location of the insula. Electrodes implanted followed a transfrontal and/or transparietal trajectory. This approach allowed for better coverage in the insula compared to the original lateral transopercula approach by having one electrode strip pass through multiple gyrus and/or the anterior-posterior separation of the insula while also limiting electrical interference from nearby areas [46]. This method has been reported safely across literature and resulted in no morbidities across our subjects.

2.4. Intracranial electrical stimulation (iES)

iES is routinely performed as a clinical procedure in preparation for surgical resection. Data reported here come from the clinical procedure performed on each participant typically in one session of iES. During this procedure, pairs of neighboring electrodes are selected for bipolar stimulation, in which an alternating square wave current is delivered between the 2 contacts. We used Nihon Kohden video EEG monitoring equipment (version WEE-1200 with 1000-Hz sampling rate), Nihon Kohden Cortical Stimulator (MS-120BK-EEG), and PE-210AK Stimulator Switchbox to deliver electrical pulses at various frequencies, amplitudes, and pulse widths. The clinician performing the procedure decides stimulation parameters, which typically have a duration of 1–3 s and a frequency of 50 Hz with current levels below the threshold for producing after discharges (i.e., 2–10 mA).

To mitigate the risk of bias, we have established a rigorous protocol in our institution for performing electrical stimulation procedures which is summarized as follows: 1) The patient, while seated in bed, faces the room ahead, and no investigator stands or sits in front of the patient. This ensures that the patient does not see the investigators' faces and, as a result, is not influenced by their facial expressions or cues. 2) The stimulator includes a checkbox for sham stimulation. When selected, the stimulation is delivered at 0 mA, and the patient is kept unaware of whether the stimulation is sham. The same level of secrecy applies to the current, frequency, and location of the electrodes, all of which are silently configured on the computer by JP. The patient neither hears nor sees the specific parameters chosen. All parameters, including their timing, are stored in the computer. After the procedure is completed, the exact order and parameters of stimulations are exported as a text file. 3) During the procedure, the clinician (here, JP) faces the computer and changes the location and parameters of the stimulation while another person behind him takes notes on the order of stimulations, their parameters, and a few keywords related to the patient's responses. These notes serve only as a preliminary report for quality assurance. 4) The patient, facing the room forward and the camera, reports everything happening in his/her subjective domain, and audio-video recordings capture the details of interactions, including investigator questions and the patient's responses. These reports are then transcribed verbatim by a high school student who is unaware of the procedure's purpose. If the patient exhibits a behavior (or a change thereof) the report writer describes it as seen on the video. 5) We compare the final report with the one prepared as the skeleton report (at the bedside). Any disparities are meticulously reviewed by watching the stored bedside videos. The report includes the precise probing questions and raw subjective statements offered by the patient.

For a given stimulation, any observed behavior (e.g., hand movement) was noted in addition to any subjective report (e.g., "I saw a bright flash of light") provided by the participant. The transcription procedures are taken by external raters who are blinded to the aims of the project. We examined the stimulated pairs depending on the profile of pairing and responses elicited: For instance, if stimulation of the A-B pair elicited Response 1 and Response 2 combined, but the stimulation of B–C pair caused only Response 2, then we assigned electrode A to Response 1 and electrode B to response 2.

2.5. Linking electrode localization and microstructural parcellation of the insula

To determine the exact location of electrodes on the microstructural map of the human insula [39–41], the cytoarchitectonic maximum probability maps of the insula as available in the fsaverage template of the Julich-Brain Atlas [38] were registered to the T1-weighted MRI scans of each subject using FreeSurfer. The previously extracted coordinates in FreeSurfer space allowed a precise allocation between electrode localization and microstructural areas. The topographical anatomy of microstructural areas after registration was carefully inspected by J.Q. The electrodes localizations were then transferred to a cytoarchitectonic insula template for visualization purposes. It was therefore possible to quantify and compare electrically induced functional sensations and intracranial recordings during behavioral tasks of the stimulated/tested areas. Areas were included in the analysis if i) they were stimulated across at least four different subjects ii) more than nine electrodes were assigned to them. Based on these criteria a total of 8 insular areas were selected: Ig2, Id2, Id3, Id5, Id6, Id7, Id8, Id9. After descriptive quantification, the results were tested against the null hypothesis that the number of responding electrodes for a given function per area would not differ from that of randomly drawn electrodes across the whole insular cortex. Therefore, we tested whether the electrically induced functional sensations were topographically independent of the microstructural parcellation. Given the nominal quality of our stimulation data, we used a binomial test to compare the true number of electrodes for a specific function per area against the expected number of electrodes ($EV = \sum P(Xi) \times X$) from sampling by chance out of our total electrode response pool independent of localization. A test for significance was only conducted if the true value exceeded the expected value from the random draw in order to reduce the number of multiple comparisons. The probability of drawing the true significant number of electrodes for a specific function per area by chance was then visually compared to a null distribution derived from random sampling from our entire electrode response pool with 10,000 iterations to estimate the effect size. The frequency of responding electrodes for a certain functional sensation within a significant microstructural area was also directly compared to the response frequency in all other areas using a binomial test (Supplementary Table 5).

For the Gradual-Onset Continuous Performance Task (GradCPT) data (see below), we directly compared the mean of high frequency

broadband activity for each area against a null distribution of means generated by random sampling from all values for the power of high frequency activity (HFA) with 10,000 iterations. We accepted the result as significant if the true mean exceeded 95 % of the random distribution ($p = 0.05$). HFA values of significant areas were additionally compared to all other areas applying a Mann–Whitney *U* test (Supplementary Table 5). We used the Benjamini-Hochberg false discovery rate (FDR) procedure to correct for the number of conducted tests. All statistics were carried out in R (version 4.2.1).

2.6. Gradual-onset continuous performance task (GradCPT)

Among patients in the electrical stimulation cohort, 9 performed at least 2 runs of the gradual-onset continuous performance task (gradCPT) [47] administered in 2–8 runs, each lasting 6 or 8 min, as described previously [48]. The task was administered at bedside via a laptop running Windows. Stimuli were presented using Psychophysics Toolbox in Matlab R2016b (MathWorks, Natick MA, USA). Transistor-transistor logic pulses were sent to an empty channel on the EEG montage to mark the onset times of each stimulus. In the gradCPT, grayscale visual images of either city or mountain scenes gradually transitioned from one to another for the duration of the task. Each transition lasted 800 ms, with image coherence gradually increasing at the trial onset and then decreasing toward the trial end. Scene categories were presented randomly with at a rate of either 10 % mountain and 90 % city or 25 % mountain and 75 % city (10 unique images, respectively, for each category). The order of these images was randomized during each run. Participants were instructed to press the space bar on the laptop when they noticed a city appearing but to withhold response when noticed a mountain appearing. Participants performed with their dominant hand, except in situations where there was discomfort of the dominant hand.

2.7. Intracranial EEG data acquisition and preprocessing

Data were collected using the Nihon Kohden recording system with a sampling rate of 1000 Hz. We used the in-house programmed preprocessing pipeline to denoise the intracranial EEG data before any statistical testing. The pipeline included notch filtering at 60, 120 and 180 Hz, data exclusion, and re-referencing. Excluded data included noisy and pathological channels, and bad trials. Pathological channels were either identified by clinicians or by the presence of pathological high-frequency oscillatory activity – as detected by our automatized algorithm [49]. Noisy channels were identified by the presence of extremely high raw amplitude (>5 standard deviation [SD] across all channels) and/or the prevalence of (>3 median of the distribution across all channels) spikes, i.e. jumps between consecutive data points larger than 80 μV. Trials with an extreme value (>3.5 SD across all trials) of the mean, absolute raw voltage (averaged across the sample timepoints in a trial), and an extreme value (>3.5 SD) of the standard deviation of the raw voltage (across the sample timepoints in a trial) were discarded. Following data screening, we re-referenced the data to the common average of all the non-noisy channels for the intracranial EEG recording analysis for a cognitive task. In addition, we applied bi-polar re-referencing to the intracranial recording for the brain stimulation experiment, given the bi-polar nature of the electrical stimulation.

The gradCPT iEEG data were preprocessed with a previously established pipeline [50]. Notch filtering was performed to attenuate power-line noise at 50 Hz and its harmonics (zero-phase, third order, butterworth filter with band-stop between 47 and 53, 97–103, and 147–153 Hz). Signals were then re-referenced from each channel to the common average signal across all channels, with the channels excluded from the common average if they met one of the following criteria: a) showed pathological activity during clinical monitoring (as noted by a neurologist); b) were manually labeled as outliers based on visual inspection of power spectra; c) had a variance greater or lesser than five times the median variance across all channels; or d) had greater than three times the median number of spikes across all channels, with spikes defined as 100 μV changes between successive samples. We performed time-frequency decomposition using a Morlet wavelet transform with frequencies bands log-spaced between 1 and 170 Hz (38 total values). For each frequency band, we rescaled each time sample by the log ratio of the whole run's power amplitude time series. (i.e., to account for band-specific 1/f decline of the power spectrum). Subsequently, power amplitude was averaged within the HFA (70–170 Hz) range, and minimal temporal smoothing was applied with a 50-ms Gaussian kernel.

We defined target-responsive electrodes among all implanted insular cortex sites within each patient, based on mountain- (i.e., target)evoked HFA responses during correct omission trials (withholding a button press when a target stimulus appears) relative to correct commission trials (pressing the button when a non-target appears), as done previously [48,50]. To define target-responsive electrodes, we used a nonparametric cluster-based permutation test implemented in Fieldtrip[54] to compare HFA power amplitude during target (mountains) relative to nontarget (city) trials, after combining trials across all task runs within each participant. The cluster-based permutation test was based on the time window of zero to +1500 ms relative to trial onset (i.e., beginning of stimulus fading in) [48,50]. An electrode was considered target-responsive if it showed a significant temporal cluster of increased HFA power amplitude for correct omission (mountain) relative to baseline (city) trials at the level of one-tailed Monte Carlo significance $p < 0.05$, corrected for multiple comparisons (across all insular cortex electrodes) within each subject.

3. Results

3.1. Subjects and electrodes

Data were collected from a pool of medically refractory epilepsy patients over the past 11 years that were implanted with stereoelectroencephalography (sEEG) for clinical evaluation. Patients were included if they underwent intracranial electrical stimulation of sEEG electrodes in the insula, and if post-operative computerized tomography (CT) and pre-operative magnetic resonance imaging (MRI) scans were available to examine the precise anatomical location of the insular electrodes. Our final cohort consisted of 17 patients (10 male and 7 female) with 142 bipolar pairs of electrodes stimulated within the insula. We only included bipolar pairs of stimulated electrodes if *both* contacts were located within the boundaries of insular cortex in the *native anatomical space,* as stimulation of a bipolar electrode pair induces electrical current between the two contacts. Table 1 provides more detailed subject demographics, electrode locations, and seizure onset zones (see Table 2).

3.2. Electrical stimulation of the insula

142 sites were stimulated in bipolar manner with one electrode as cathode and the adjacent electrode as anode to create an electrical field between and underneath the 2 electrodes. This was done to constrain the spread of electrical field outside the intended volume compared to unipolar stimulation where current can cause more spread [51]. Stimulations involved 60 (42 %) left hemisphere electrode pairs, 80 (56 %) right hemisphere electrode pairs and 2 (1 %) bilateral insular electrode pairs. Of these, stimulation of 57 pairs (40 %, 12 patients, 13 left, 42 right) produced a change in the subject's conscious experience (Supplementary Table 2 and Fig. 1). The subjective reports fell within five different non-overlapping categories of visceral sensation, pain/temperature sensation, non-painful/non-thermal somatosensation, gustatory/olfactory sensation, and "emotion" in the form of foreboding and anxiety/hyper-vigilance (Fig. 1, Supplementary Table 3).

Overall, electrodes located in posterior insula were more responsive (74 %) compared to those in the anterior insula (21 %). Pain/

Table 1
Cohort demographics.

Subject #	Age	Sex	Handedness	Electrode pairs stimulated	SOZ
1	50	M	L	2	R Hippocampus, L amygdala
2	26	M	R	2	R Hippocampus, R insula
3	30	M	R	2	R Hippocampus
4	31	F	R	2	L amygdala, L hippocampus, L superior temporal gyrus
5	28	F		1	amygdala, hippocampus, anterior temporal cortex
6	50	M	R	4	R hippocampus, L hippocampus
7	31	M	R	3	L hippocampus, L amygdala
8	19	F	R	8	L lateral temporal lobe
9	59	M	R	3	R mesial temporal lobe
10	23	M	R	7	R amygdala, R hippocampus, R insula
11	51	M	R	4	L and R posterior mesial temporal lobe
12	62	F	R	27	R mesial temporal lobe, R insula
13	33	M	R	17	R anterior mesial temporal lobe
14	36	F	R	16	L mesial temporal lobe
15	27	M	R	16	L Posterior Insula and L Hippocampus
16	25	F	R	17	R anterior hippocampus
17	47	F	R	11	bilateral temporal-insular

*Electrode pairs were counted as pairs of bipolar electrodes stimulated, SOZ = seizure onset zone.

temperature responses were elicited from stimulation of 30 pairs of electrodes (53 %) in 6 patients. Warmth and pain sensations were grouped together because subjects reported the two sensations together, consistent with prior findings [31]. Subjective reports describing "pain" explicitly such as "stabbing pain", "throat pain" and "painful squeezing" accounted for 8 of the 30 electrode pairs (4 patients). Temperature sensations such as "warm bath" or "hot boiling sensation" accounted for 22 of the 30 sites in this category. Of these, 28 sites (93 %) were in the right hemisphere. Non painful/non thermal somatosensory reports were seen in 14 pairs of electrodes (25 %) across 6 subjects from stimulation of both left and right insular sites. Visceral and autonomic sensations were reported from the stimulation of 8 pairs of electrodes (14 %) across 4 patients, and largely from sites in the middle short and posterior short gyri in the anterior insula. Most electrodes, except 2 (Subject 1), causing visceral sensations were from the right hemisphere insula. Foreboding and anxiety/hypervigilance states were reported from the stimulation of 4 pairs of electrodes (7 %) in 2 patients. All sites in both of the patients were within the left hemisphere. Subjective reports were described broadly as feeling "anxious" or "nervous", and hence grouped as emotion of anxiety. No other stimulations of the insular sites triggered any other emotional responses. Of note, electrode sites, whose stimulation caused emotional changes, were located solely in the anterior and middle short gyri of the anterior insula. Lastly, stimulation of 3 pairs of electrodes (5 %) in 3 subjects in sites located in the inferior middle short gyrus caused gustatory or olfactory sensations. All sites were within the left hemisphere. Auditory responses were seen with the stimulation of electrodes abutting the superior temporal gyrus outside the boundaries of the insula – of note, these electrode locations could fall within the insular boundaries if we had localized our sites in a standard – rather than native anatomical space.

Table 2
Summary of subjective reports.

Category	Left Hemisphere	Right Hemisphere	Other Notes
Painful/thermal sensation			
Subject #8	" throat pain, jabbing pain through my mouth, then burn like acid stomach"	" throat pain, jabbing pain through my mouth, then burn like acid stomach"	bilateral stimulation
Subject #8	"Burning throat"	"Stabbing pain on chest"	
Subject #10		"Kind of like a heat feeling. It flows through you"	
Subject #13		"warm feeling" "little bit of warmth" "getting unpleasantly hot" "unpleasant warm feeling"	Subject reported increased unpleasant feeling as electrode stimulation moved superior in the insula. Somatotopic organization in the inferior-superior direction started with warm in left arm and leg that moved to ribcage to shoulders and neck
Subject #15	"painful feeling of being squeezed on right arm"		
Subject #16		"unpleasant cramping and heat in left leg" "hot boiling sensation from left shoulder to left hand" "whole left side of the body getting warm from leg to arm" "painful squeezing on left side of body"	Subject reported painful/thermal sensation that moved from left arm and leg to entire side and body as electrodes moved higher in the insula
Subject #17		"hot temperature in my left arm and leg " "much stronger and hotter in temperature on my left side only" "felt temperature, couldn't describe sensation because heat was too strong- like a "hot iron" "too painful on the left side, hand in particular. Felt like touching a burning plate" "jolt of temperature"	
Non-painful/non-thermal sensation			
Subject #3	"head sensation", "kicked back. Like I woke up in the morning"		
Subject #5	"Pressure on the side",		
Subject #8	"feeling [like acid] in throat"	"feeling [like acid] in throat"	bilateral stimulation
Subjetc #10		"tingling in my forehead"	
Subject #15	"felt internal movement from shoulder and right arm"	"Little bit of tension in left arm, left leg" "felt like I was moving [my hand] but I wasn't in reality", stomach hurt, "feeling of dryness in the mouth" "feeling on left side of face, feeling taught/ contracted"tingling in my forehead"	

(*continued on next page*)

Table 2 (*continued*)

Category	Left Hemisphere	Right Hemisphere	Other Notes
Subject #17		"sensing as if I'm about to go in water" "a wave is coming up and touching- almost a wave type of pressure- on my left side, only from my leg up"	
Visceral Sensation			
Subject #1	"squirling, almost like a dry heave ", "nausea" feeling through chest and stomach		
Subject #8		"dizziness", "wave like a boat. Travelling from top of my body to the bottom"	
Subject #9		"felt a little dizzy, headrush through the center of my body" "A feeling of energy going up, feel something in the center (energy) change"	
Subject #10		"like I'm falling backwards" "tiny wave across my head"	
Anxiety			
Subject #8	"anxiety and nervousness"		
Subject #11	"beginning of anxiety", "anxious"		
Olfactory/Gustatory			
Subject #7	"weird smell" "taste through throat and nose"		
Subject #11	"taste change" "metallic, unpleasant (taste)" "orange taste"		
Subject #15	"taste of milk in mouth"		

In total, stimulation of 85 of the 142 (60 %) insular electrode pairs did *not* cause any reportable change. These "silent" electrodes were largely concentrated in the anterior insula, specifically in the anterior short gyrus.

3.3. Colocalizing the stimulated insular sites with microstructural cytoarchitectonic maps

Next, we examined the location of the stimulated sites according to new cytoarchitectonic maps of the human insula [39–41], combining localization of intracranial electrodes with microanatomical structural atlases [38]. For this, we *colocalized the stimulated insular sites with microstructural* cytoarchitectonic maps using standard registration procedures (see *Methods* for details). Pain/thermal responses were found to be primarily induced by the stimulation of posterior insular areas Ig2 (87 % of stimulated electrodes) and Id2 (50 % of stimulated electrodes) - consistent across 4 subjects. Although 37 % of pain/thermal sites (3 subjects) were localized to area Id3, some of these stimulated sites were located in close proximity to neighboring area Id2. Most of the non-painful/non-thermal responses, on the other hand, were observed especially in and around area Id3 (50 % of all stimulated electrodes in 3 subjects).

For areas of the anterior insula, area Id6 in particular could be linked to visceral sensation (18 % of all stimulated electrodes in 4 subjects). Gustatory and anxiety responses were rarely induced in general. The few anxiety-provoking stimulations showed a close topographical relationship to area Id6 (7 % of all stimulated electrodes in 2 subjects) (Figs. 2 and 3).

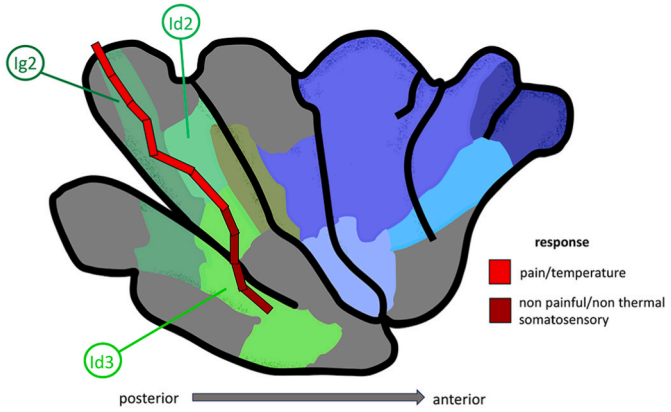
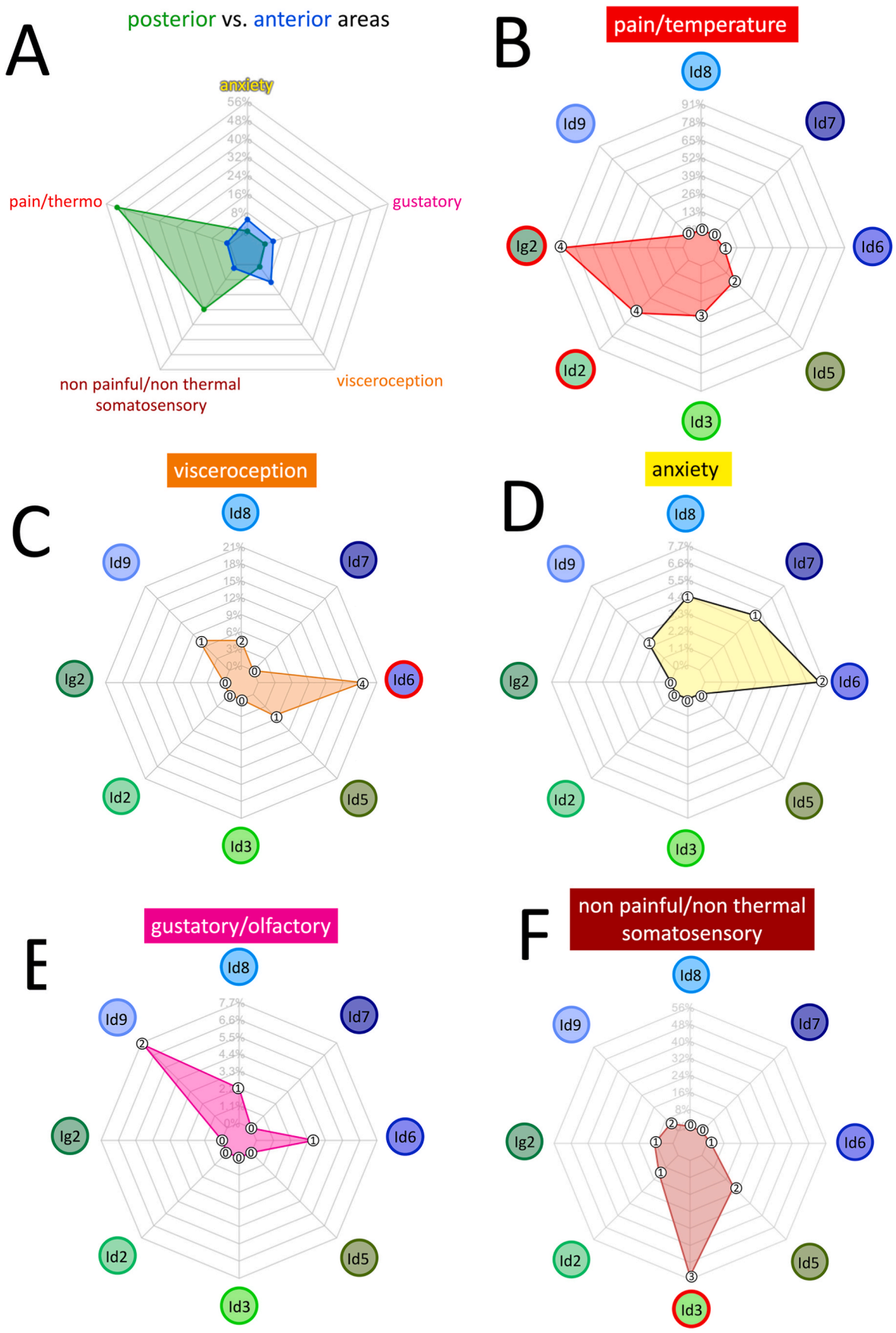


Fig. 2. Microstructural border between areas Id2 and Id3 reflected by stimulated responses in subject 16, the distinction between pain/thermal (light red) and non-painful/non thermal somatosensory sensations (dark red) was at the cytoarchitectonic border of area Id3. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Next, we tested the statistical validity of the findings described above by exploring whether the association between the microstructural identity of an area and the subjective responses elicited by the stimulation of electrodes in that area was merely by chance. Our null hypothesis was that the true number of category-specific responsive electrodes per area does not differ from the number of electrodes selected randomly by chance and therefore electrically induced functional sensations were independent of localization (see *Methods* for detail). As shown in Figs. 3 and 4, statistically significant findings were seen as a red circle surrounding each significant cytoarchitectonic area by response type. A statistically significant finding was observed in the anterior insular area Id6 for viscerceptive sensations (relying on 4 subjects) and in the posterior insular cortex areas Id2 and Ig2 for pain/thermal sensation (with the pain/thermal responses in area Ig2 being the most reproducible ones across all microstructural areas) (both findings relying on 4 subjects). Somatosensory responses were significant for area Id3 (relying on 3 subjects). A notable result was reported for Subject #16, demonstrating how the shift between pain/thermal and non-painful/non-thermal somatosensory response spectrum is exactly reflected by the microstructural boundary between areas Ig2/Id2 and Id3 (Fig. 2). Remaining posterior area Id5 did not show any significant responses. Supplementary Table 5 shows the results for directly comparing response frequencies for a certain functional sensation between significant and non-significant areas. The response frequency for previously reported significant areas Ig2 (pain/thermal sensations), Id6 (viscerception), and Id3 (feeling of touch) all significantly exceeded the response frequency for the respective functional sensation in all other areas. Only the pain/thermal response frequency in significant area Id2 was not exceeding the frequency of areas Ig2 and Id3 in the direct comparison. Altogether, these results demonstrate that electrically induced subjective sensations may be assigned to distinct microstructural areas.

3.4. Cognitive task responses in the anterior dorsal insula

We noted across subjects that the stimulation of area Id7 in the anterior dorsal part of the insula elicited no reportable subjective changes. We therefore wanted to explore whether this region invoked any cognitive task-related changes. Since this part of the insula is often highlighted in functional neuroimaging studies as a node of the cingulo-opercular network involved in cognitive control, we administered an experimental task known to evoke responses in this part of the insula [47,48,50] in the same human participants while recording intracranial



(caption on next page)

Fig. 3. Quantification of specific responses to electrical stimulation in relation to microstructural organization of the human insula A) Separation of response types in anterior insula (blue) and posterior insula (green). B) Pain/temperature responses were found primarily in areas Ig2 and Id2 as well as the superior part of area Id3, with area Ig2 having the highest number of pain/temperature responsive electrodes. C) Visceroceptive sensations were mainly located in area Id6. (D) The overall sparse number of responsive electrodes for anxiety (E) and chemical perception showed an approximate allocation to area Id6 (anxiety) and Id9 (gustatory). F) Non painful/non thermal somatosensory responses mostly appeared in and around area Id3. After statistical testing and correcting for multiple comparisons four areas (Ig2, Id2, Id3, Id6) showed significant results for a specific response (see red circles). The number of subjects on which the results are based is indicated by the encircled number in the polar plot. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

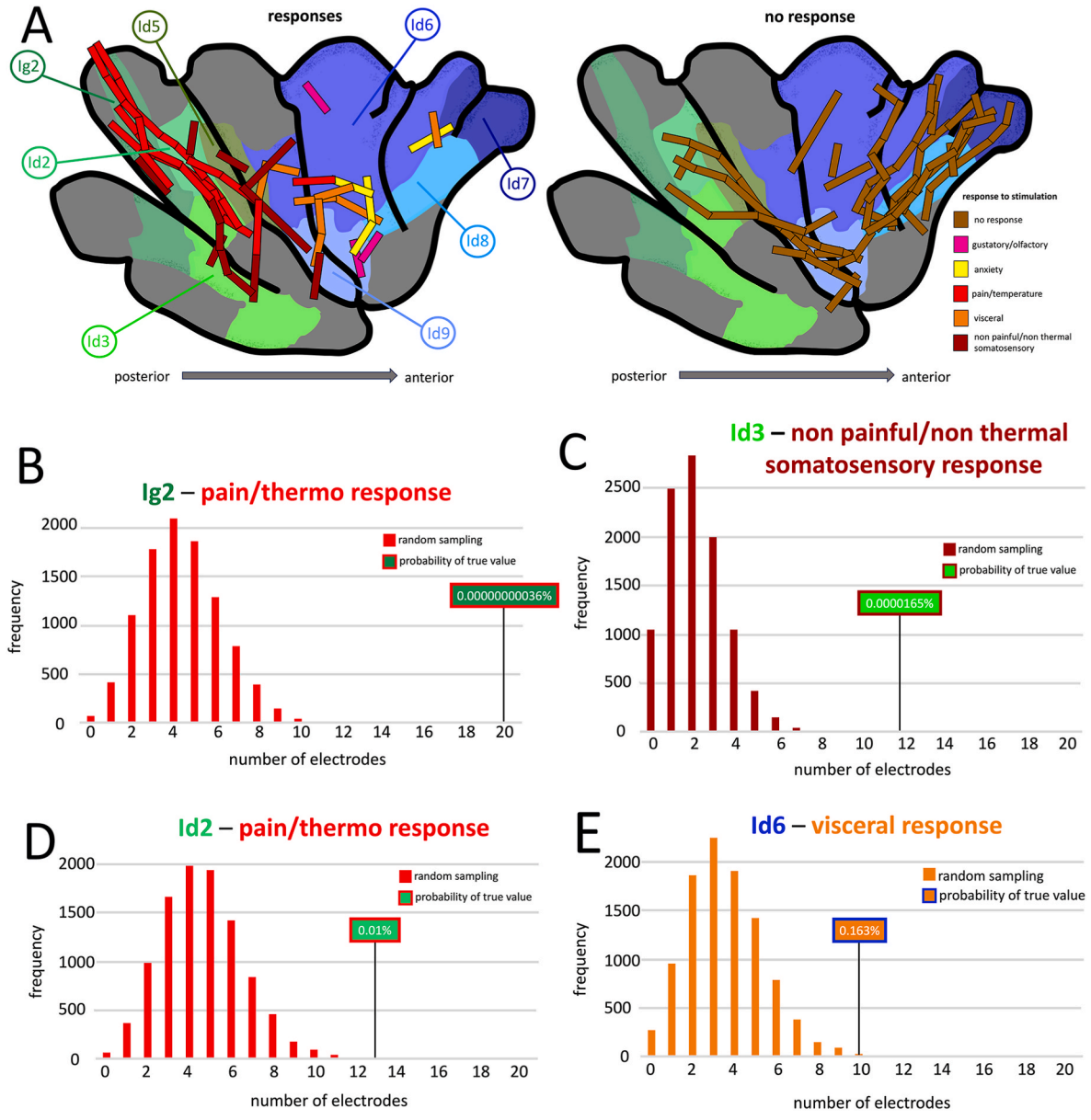


Fig. 4. Statistically significant associations between response profile and cytoarchitectonic identity A) All responses mapped onto cytoarchitectonic maps. The probability that the true number of responsive electrodes for significant areas was drawn by chance is $4{:}10^{12}$ for pain/thermal responses in area Ig2 B); $2{:}10^{7}$ for non painful/non thermal somatosensory responses in area Id3 (C); $1{:}10^{4}$ for pain/thermal responses in area Id2 (D) and $2{:}10^{3}$ for visceroceptive responses in area Id6 (E).

electrophysiological signals. We found a significantly higher mean and median values of high frequency activity (HFA, also known as high gamma activity) in area Id7 compared to all other areas of the insula (Fig. 5). In particular, sites within areas Id6, Id8 and Id7 showed significantly increased HFA power in response to salient (infrequent) stimuli relative to less salient (frequent) stimuli. Most electrodes with the highest HFA values were topographically located in or around Id7. In fact, the electrodes with increased HFA activity in area Id6 were all directly adjacent to area Id7 (Fig. 5). The results were tested for statical significance by comparing the true mean values for each area with means from a randomly drawn null distribution. Only the mean HFA value for area Id7 exceeded the 95 % threshold and was therefore considered significant. The direct comparison of HFA values between area Id7 and all other areas using a Mann-Whitney *U* test further

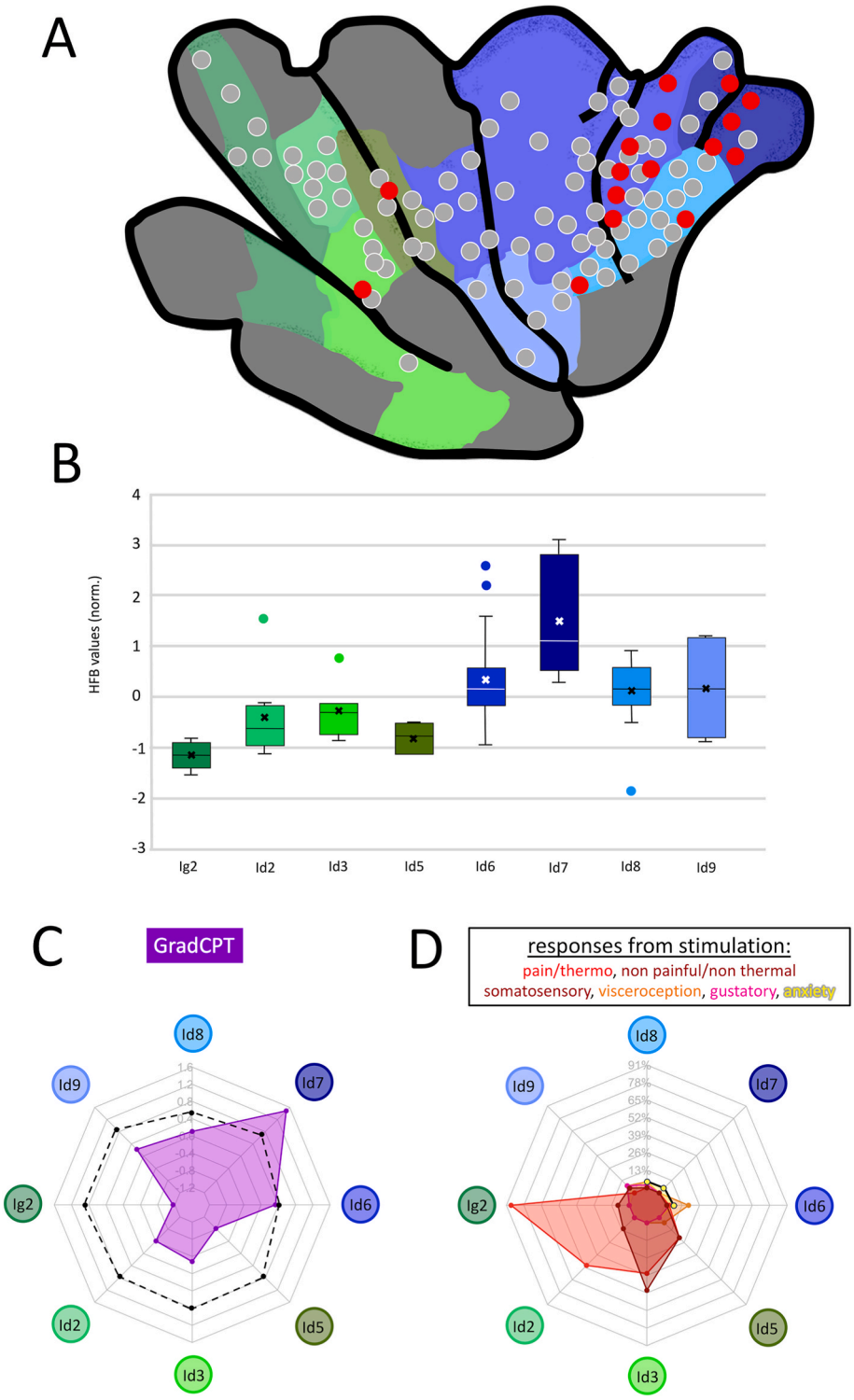


Fig. 5. Comparing the profile of neuronal population responses during a salience task with the map of microstructural areas A) Mapping of all electrodes recorded during gradCPT task (red electrodes are significant after statistical testing and grey electrodes are electrodes without significant change of HFA power during task condition compared to task baseline activity). Most electrodes were centered in and directly around area Id7 in the dorsal anterior insula. B) Boxplot, showing the mean (line) and median (x) for HFA power for each microstructural area. Especially, area Id7 showed increased parameters compared to the other areas. C) Comparing the true mean values for each area with means from a randomly drawn null distribution (dotted line is 95 % threshold). Only the HFA mean for area Id7 was considered significant. D) All responses per area from electrical stimulation. Area Id7 did not show any significant responses. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

reinforces the designated role of area Id7, as it demonstrates a significant elevation of HFA values in this area when compared to all other areas (Supplementary Table 5). Remarkably, area Id7 showed the highest HFA power of all measured electrodes across all subjects in which it was tested. This was true for subjects with both poor and good behavioral performance during the task (Supplementary Fig. 1). Altogether, these data suggest that area Id7 in the dorsal anterior insula is likely to represent a microstructural correlate for salience detection in the human insula.

4. Discussion

Over the past decades, considerable progress has been made in uncovering various functions of the human insula. Nevertheless, our comprehensive understanding of these functions' topography has been limited, predominantly relying on rough macroscopic reference points instead of intricate microstructural subdivisions. In our study, we offer evidence establishing connections between five cytoarchitectonic areas within the human insula and particular functional processes examined through direct intracranial electrical stimulation. Additionally, we furnish a map detailing insular subregions where the delivery of electrical pulses induced distinct subjective alterations in domains such as pain/temperature perception, visceral sensations, somatosensory experiences, gustatory/olfactory perceptions, and feelings of foreboding, anxiety, and hypervigilance. Moreover, the recordings of neural activity during an experimental task condition provided additional support for the connection between condition-specific neural responses and the cytoarchitectonic profile of the dorsal anterior insula. This observation also documented that a so-called cold region of the brain (traditionally known as a silent area [52]) whose stimulation does not induce any changes in the conscious state of the individual does indeed have specific functions that need to be probed with means other than electrical stimulation (e.g., recording neural activity during a specific cognitive condition).

We have recently confirmed that the subjective reports given by patients are highly reliable as the sham stimulations of the brain elicits very negligible effects [53]. Moreover, we have shown that lack of stimulation effects largely depend on the system-level profile of connectivity of the stimulated areas. Pulses of electrical stimulation in unimodal brain networks (e.g., sensory motor structures with relatively lower hierarchies in terms of their connectivity profiles) reliably elicit changes in the conscious state of the individual that are reported by the individual whose brain is stimulated. By contrast these effects become increasingly rare, heterogeneous, and complex in heteromodal and transmodal networks with more diffuse and higher hierarchical connectivity patterns [54].

Based on this observation, one can deduce that the insular regions in the responsive areas must have direct connections to unimodal sensory regions (including somatosensory and visceral sensations) while the non-responsive sites belong to a more heteromodal network with connections to higher association areas. This interpretation is compatible with several models of insular functional architecture proposed in the extant literature (for a review see Ref. [55]).

While our findings are generally consistent with previous research on the insula, they expand upon and enhance prior studies in several crucial aspects. Notably, our results offer a finer anatomical resolution, which is more pertinent to individual native anatomical coordinates rather than relying on group-based averaging of effects in the standard anatomical space.

Over the past decades, different fine-grained microstructural concepts of the human insula have been proposed [41]. However, the Julich-Brain parcellation used in this study provides two crucial advantages over the other maps: i) it is available in a standard reference space, allowing it to be used in multimodal topographical studies. ii) It is based on statistical, quantitative criteria. (for detailed overview see Ref. [41]). Our results align with several key characteristics of the Julich-Brain atlas. Specifically, our findings reveal a distinct division between different electrically induced functional sensations in anterior and posterior areas. In contrast, other microstructural maps lack this distinction, describing the areas as extending uniformly across the entire insular cortex without distinguishing between anterior and posterior areas [56,57]. Furthermore, our results provide evidence for an association between electrically induced functional sensations and specific areas of the Julich-Brain Atlas (see Supplementary Table 5), such as area Id6 and viscerceptive sensations, which are not described in other parcellations (for review see Refs. [41,58]).

Neuroimaging studies of the insula, as summarized in a recent large meta-analysis [12], have suggested that the entire dorsal anterior insula may be involved in detecting salience-relevant stimuli (Fig. 6A) [7,12,59]. In our study, however, we found only area Id7 to be significantly engaged during accurate detection of infrequent target (likely salient) visual stimuli (Fig. 5A). This region has been associated with the cingulo-opercular network implicated in task control [60,61] and is reliably activated during similar conditions in fMRI studies [47,62]. As such, even though Id7 was activated during processing of salient stimuli, it is possible that this region is not directly involved in visceral sensations during salience detection (a role supported by the salience network involving more ventral insular cortex regions [63,64]). Additionally, our finding of a link between pain and thermal perception and two distinct microstructural areas in the posterior insula differs from fMRI findings which suggest the entire dorsal insular cortex to be engaged [12,65] (Fig. 6B).

The stimulation of anterior areas - which have been shown to exhibit fMRI activity for pain processing – did not elicit any pain or thermal sensations. This discrepancy may be related to i) differences in the analysis of electrical stimulations data in the individual patients compared to group-based averaging of fMRI data, or ii) differences between correlative versus causal evidence in that the anterior insular areas may not be causally involved in pain processing but possibly operate as an integrative interface, incorporating, for instance, pain stimuli with higher cognitive functions [65]. For visceroception, the functional meta-analysis showed a partial overlap with microstructural area Id6, which was also significant for viscerceptive sensations in our analysis (Fig. 6D). The meta-analysis also revealed a multi-integrational hotspot in a comparable localization to area Id6, involving emotions, interoception, pain, awareness, and memory resources [12]. Our data support this multifunctionality, as stimulation of Id6 elicited foreboding and anxiety/hypervigilance responses as well as increased activity during the experimental condition of salience. In conclusion, our findings indicate that while some results align with outcomes from fMRI studies, there are also discernible differences in others, potentially offering anatomically more intricate and causal understanding of functional processing within the insula.

The majority of our stimulation findings are in line with previous literature: Pain sensations have been reported in the posterior-superior region of the insula [31] whereas another study [22] reported pain sensations from stimulation of the middle short gyrus (see later for our interpretation of such discrepancies). Past stimulation studies have also reported a visceral sensation [18–26] as a result of middle insular stimulations similar to what we are reporting here. Somatosensory responses [18,20–24,27,28] have been associated with the stimulation of the posterior insula. Olfaction and gustatory responses have also been reported in the inferior subregions of the anterior short gyrus [20,21,23,32,33].

Similar to prior observations, emotional responses were scarce. In a single center meta-analysis of retrospective iES data from 329 patients (3451 sites across the brain), a pleasant sensation was only reported with the stimulation of 0.6 % of sites in 2.7 % of the subjects [66]. Five of 329 patients who experienced positive sensations during stimulation of the anterior insula (precise anatomical location was not provided), felt positive about sensations such as warmth, floating, and shivering akin to the subjective symptoms of their usual seizures and the stimulated sites

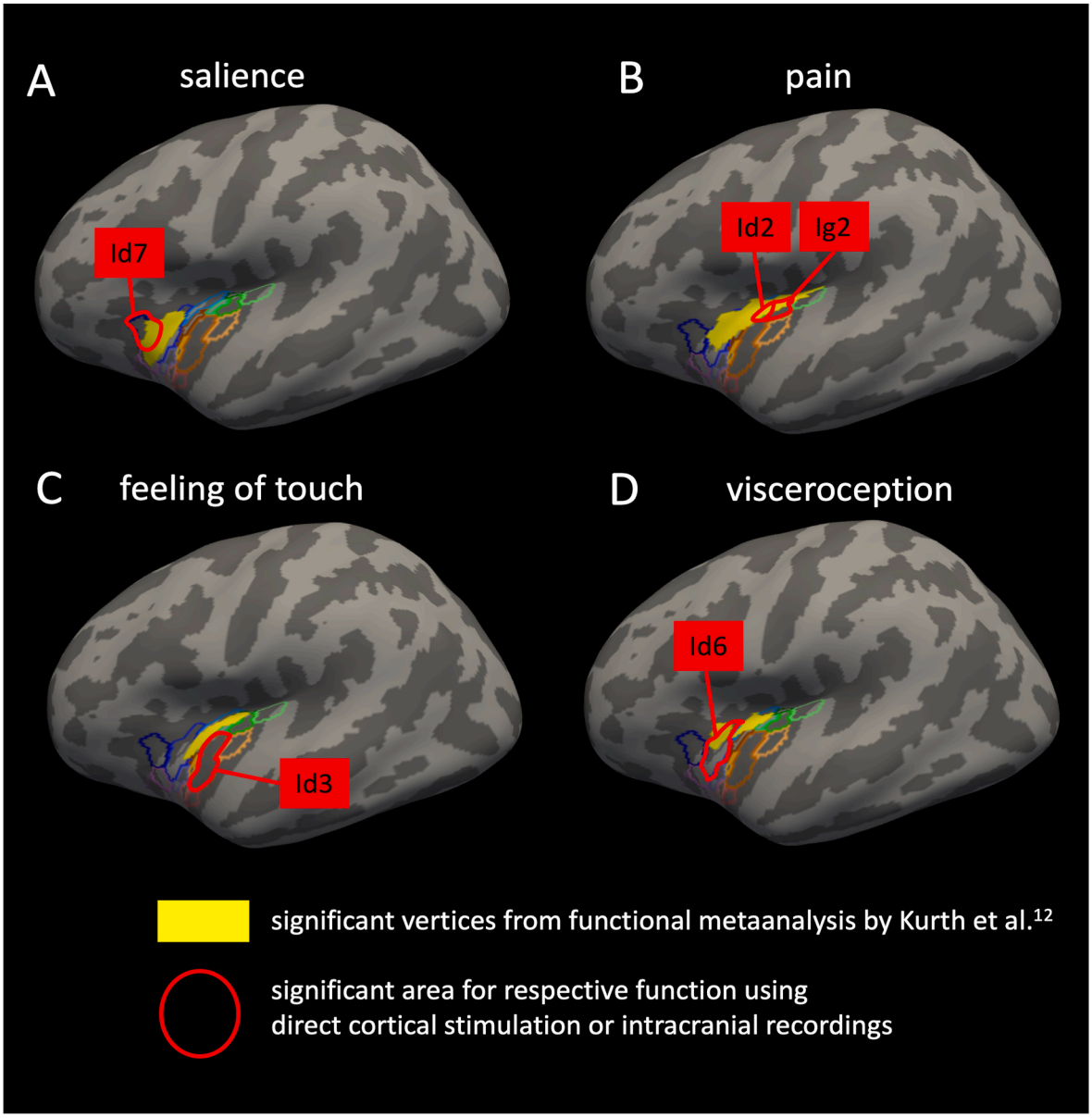


Fig. 6. Significant areas resulting from cortical stimulation/intracranial recordings for salience, pain/temperature, feeling of touch, and visceroception compared with findings from a functional meta-analysis [12]. **A)** Functional meta-analysis shows that all dorsal anterior insula areas (Id6, Id7, Id8) are part of the salience network. However, only area Id7 exhibited significantly enlarged HFB values during intracranial recordings of a visual attentional negation task. **B)** The functional meta-analysis revealed activation of the entire dorsal insula during pain processing. However, during cortical stimulation, only two areas in the dorsal posterior insula responded significantly with painful experiences. This suggests that primary pain and temperature processing might be localized in areas Ig2/Id2, while the anterior insula could be more involved in further cognitive processing of pain stimuli. **C)** Area Id3 exhibited a significant response to direct stimulation, eliciting a feeling of touch. However, in the functional meta-analysis, this area was not part of the observable activation pattern regarding tactile processing in the insula. **D**) The functional meta-analysis shows an activation of a large part of the anterior dorsal insula during interoceptive tasks, whereas in this study we were able to associate area Id6 in particular with visceroceptive experiences.

were also part of the epileptogenic zone in majority of these cases. The reproducibility of the responses was not systematically assessed in this study.

Some of the prior studies have reported vestibular [20,21,28,29] changes in the form of "dizziness" or "sensation of rotation" from the stimulation of the middle of the insula. These reports are not in line with the non-human monkey literature that places a vestibular subregion in the transitional area between the far dorsal posterior insula and the parietal operculum. Within our cohort (subjects 8, 9, and 10), we noted "dizziness" after stimulation of 3 electrode sites (6 %) in the middle insular subregions but we grouped these under the category of visceral sensations rather than vestibular. We did not note clear vestibular responses with stimulation of electrode pairs if both of the sites were located within the anatomical boundaries of the insula. It is possible that the insular vestibular area may indeed be a parietal opercular, rather than purely insular [46] subregion.

Some studies in the past have reported auditory responses [20–22, 24,28] arising from the stimulation of the ventral subregion of the posterior long gyrus of the insula. This region of the insula is located at the edge of the ventral posterior insula and posterior superior temporal gyrus (STG) that is known for their crucial roles in hearing. Thus, it is likely that electrodes whose stimulation caused auditory responses, may have been partially located in the posterior STG region. Through careful analysis of the anatomical data, we verified that auditory responses,

which were previously associated with the stimulation of inferior insular subregions, indeed only occurred when the adjacent cortices of the superior temporal gyrus were stimulated.

We believe the discrepancies between our findings and some of the other reports can be explained by the following methodological factors. First, some of the past studies used limited methods to localize the anatomical coordinates of the stimulated sites or relied purely on the location of the electrode sites on anatomical atlases rather than relying on the native anatomical space information – as we did here. We emphasize again that one should be cognizant of the shift in anatomical coordinates of electrodes when transferring from native to standard space. Second, all studies including ours relied purely on subjective reports of the patients and labeled the category of these reports to the best of their understanding. Language and subjective variabilities and the complex nature of some of the electrically-induced subjective states could be another source of variance across studies and populations of patients, Third, we excluded from our study data pertaining to sites that were partially outside the insula. For instance, in a couple of patients, we noted auditory responses elicited by stimulation of the most ventral part of the insula but in these cases, one or both of the electrodes in the pair of stimulated electrodes were located immediately outside the insula and within the boundaries of STG. Perhaps some of the past reports of auditory responses could be attributed to the stimulation of the structures adjacent to rather than within the insular boundaries.

5. Limitations

It is important to highlight that our study had its own set of limitations. We acknowledge that the electrical stimulation data obtained in our cohort were based solely on patient reports rather than a systematic exploration of sensory phenomena using standardized questionnaires. While our approach is considered the gold standard in Neurology, it does have inherent imprecision due to its sole reliance on subjects' level of participation in the procedure and their motivation, ability to articulate their subjective changes, and their level of self-awareness. Future electrical stimulation studies of the insula may optimize their approach by incorporating more optimized ways of probing the subjective effects of stimulation.

6. Conclusions

Apart from its diverse functions, the insula is linked to numerous disorders, including autism, depression, anxiety disorders, addiction [13], and chronic pain [65]. For some of those diseases, the insula has been considered a viable target for therapies like deep brain stimulation or transcranial magnetic stimulation [67–71]. For successful implementation of these therapies, precise knowledge about the localization of specific functions is crucial and a microstructural/functional model, as partly presented in this study, could serve as a reliable framework to guide such applications in the future. With this integrated model, functions can be precisely located within cytoarchitectonic insular areas [41], surpassing the approximate topography of macroscopic landmarks. Accessible as probability maps in a standard reference space [36], each microstructural area could then potentially be registered onto the patient's brain and used as a precise target point for interventions.

CRediT authorship contribution statement

Anna Duong: Writing – Original Draft, Visualization, Formal analysis, Investigation. **Julian Quabs:** Writing – Original Draft, Visualization, Formal analysis, Investigation, Methodology. **Aaron Kucyi:** Review & Editing, Formal Analysis, Methodology. **Zoe Lusk:** Data collection, Review & Editing, Visualization. **Vivek Buch:** Review & Editing, Methodology. **Svenja Caspers:** Writing – Review & Editing, Supervision, Methodology. **Josef Parvizi:** Writing – Review & Editing, Supervision, Project administration, Data Curation, Conceptualization, Methodology, Investigation, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2023.11.001.

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