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Electrical stimulation of the human claustrum

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ABSTRACT

To probe the causal importance of the claustrum in human subjective experience, we delivered electrical pulses either unilaterally or bilaterally within the core of this structure in five neurosurgical patients implanted with intracranial electrodes. Patients reported subjective experiences in various sensory domains and exhibited reflexive movements after real but not sham stimulations. However, none of the stimulations evoked loss of consciousness or lack of subjective awareness even with strong bilateral stimulations. Our study is the first to probe the effects of electrical perturbation of human claustrum through electrodes implanted within the claustrum itself and provide novel causal information about the human claustrum.

1. Introduction

The claustrum is a thin, elongated band of gray matter surrounded by thick bundles of white matter in the proximity of basal ganglia and insula (Fig. 1). Anatomical studies have shown that the claustrum has a remarkably widespread bihemispheric, reciprocal connectivity to cortical and subcortical structures [1,2]. While much is known about the claustrum's anatomy, its function remains mysterious and is subject to hotly debated discussions and even dedicated societies (e.g., Society for Claustrum Research). Our elusive understanding of the claustrum is mainly because of its position and small size, which renders it extremely difficult to study. As a result, selective claustral lesions, for example, due to stroke, cannot occur without also involving its nearby surroundings [3]. Animal studies have shown that the claustrum may be involved in multisensory integration [4,5], although this is a matter of debate [6,7]. However, animal studies are limited for testing hypotheses about the role of claustrum in subjective experience or the generation of a unified conscious percept as it has been debated extensively in the extant literature [8]. A useful approach to test the causal importance of the claustrum in human subjective experience is with direct electrical brain stimulation (EBS) in neurosurgical patients implanted with intracranial electrodes. This method is routinely used in the clinical realm to delineate functional brain areas and to study research questions [9,10]. Indeed, a recent study from one patient with epilepsy demonstrated impaired awareness when an area close to (but not within) the claustrum was stimulated unilaterally with strong and prolonged stimulations [11]. In the present study, we tested if awareness could be impaired with unilateral and bilateral stimulations directly applied within the claustrum in 5 subjects.

2. Material and methods

2.1. Participants

Data reported here were obtained from 5 patients (3 females) with pharmacologically resistant epilepsy undergoing invasive electrophysiological monitoring at the Stanford Medical Center, California (see Table 1 for basic demographics). All patients provided verbal and written consent prior to participation in this study, which was approved by the Stanford Institutional Review Board for human research. Only participants that could reliably give report about their internal sensations were included. None of the subjects had seizure foci in the vicinity of the stimulated areas (Table 1).

2.2. Electrode localization

Subjects in this study had electrodes implanted in multiple regions of their brain including targets within the insula. These insular electrodes were implanted with oblique (dorsal-posterior to ventral-anterior) trajectory traversing through the claustrum. The depth electrodes used in this study were 0.86–1.1 mm in diameter, 2.29–2.41 mm in length, and 3–6 mm in intercontact spacing (AdTech, Racine, WI). For the electrode localization and visualization process, we used the freely available iElvis toolbox (https://github.com/iELVis/iELVis [12]). Briefly, the electrodes were manually localized using the software BioImage Suite (http://www.bioimagesuite.org) on a

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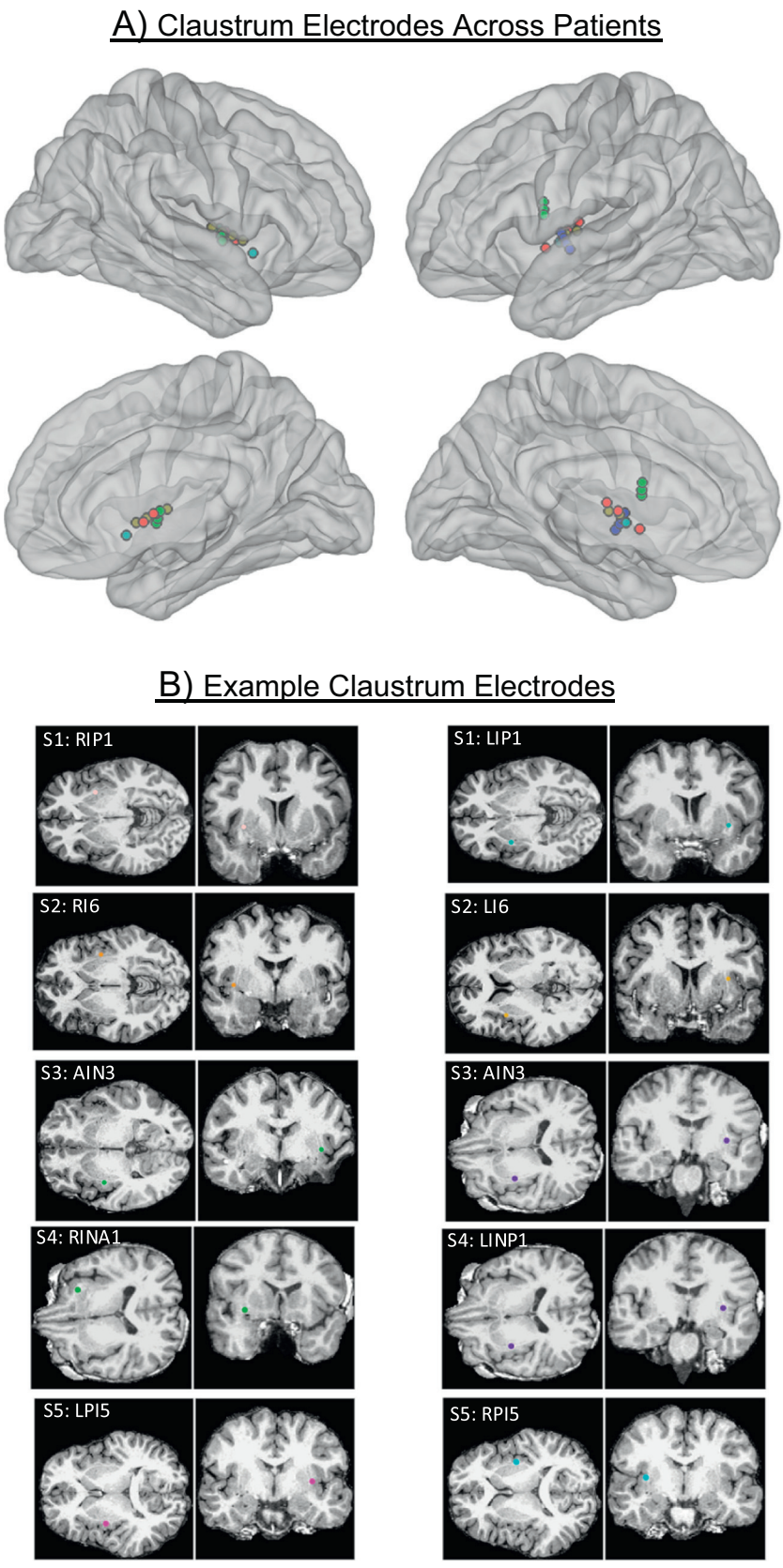

Fig. 1. Locations of the stimulated claustrum electrodes. A) All stimulated electrodes across the five patients plotted on the standard MRI. Electrodes in different patients are plotted with different colors. B) Example electrodes for each patient on the subject's anatomical MRI.

Table 1
Demographics and clinical information.

Subject ID	Gender	Age	Handedness	IQ	Seizure onset	Prior surgery
S1	F	21	R	105	Right insula	No
S2	F	31	R	72	Left temporal	No
S3	F	28	R	Low average	Left frontotemporal	Yes
S4	M	50	R	High functioning[a]	Bitemporal	No
S5	F	19	R	91	Left posterior temporal	No

[a] No neuropsychological testing available. Patient is working in an intellectually demanding job.

postimplant computed tomography scan (CT) that was coregistered using an affine transformation (FLIRT, www.fmrib.ox.ac.uk/fsl) to a preimplant high-resolution T1 magnetic resonance imaging scan (MRI). The electrodes were then visualized on 2D MRI slices and visually inspected by the authors. Subjects were included in this study if any of the implanted electrodes overlapped with the claustrum, if these electrodes were not within the seizure onset zone and did not show epileptiform discharges. For visualization of all included electrodes across subjects, the electrode coordinates were mapped to the Freesurfer average brain (http://surfer.nmr.mgh.harvard.edu) (Fig. 1, Supp. Table 2).

2.3. Electrical brain stimulation (EBS)

Electrical brain stimulation is routinely applied clinically to identify eloquent cortex to be spared from surgical resection. For this study, we used EBS parameters that are routinely used in such clinical mapping procedures with a S12X Grass cortical stimulator (Grass Technologies, Pleasanton, CA). We delivered current-regulated, bipolar electrical pulses between two adjacent electrodes as well as simultaneously within the claustrum in each hemisphere. Unilateral stimulations targeted a confined area between the two adjacent electrodes within the same hemisphere. By contrast, bilateral stimulations were performed in 4 out of 5 patients with anode and cathode electrodes in separate hemispheres. Given that the electrical charge was delivered as alternating current, the anode and cathode locations interchanged with the chosen frequency of the pulses (i.e., 50 times a second).

We used charge-balanced alternating square-wave pulses with 0.2 to 0.3 ms width per phase, at 50 Hz, with stimulation amplitudes between 2 and 10 mA, 3 and 41 μC/cm^2, and for 0.5 to 2 s while the patient was either quietly resting or performing simple tasks such as reading, talking, recalling past events, or moving limbs. Without informing the patient, sham trials (0 mA stimulation) were intermixed with real stimulation trials. Intracranial electroencephalogram (EEG) was monitored across all implanted electrodes for the presence of after-discharges and seizures (see Table 2 for the exact number of electrodes in each subject). Stimulation-induced behavior and subjective experiences reported by each patient were recorded during the procedure and later used to transpose the conversation between experimenters and the patient.

3. Results

3.1. Electrical brain stimulation

For details of each subject's electrode coverage, please see Supplementary Figs. 1-1 to 1-5. For a detailed listing of the patient reports, observed effects, and sequence of applied stimulation parameters, see Table 3 (and in more detail in Suppl. Tables 2-1 to 2-5). For the total of electrodes implanted in each patient and their hemispheric and lobar locations, please refer to Table 2.

3.2. Size of electrical field generated by the stimulations

Using our recent computations from the human occipital cortex, we calculated the size of electrical field generated by the stimulations (Fig. 2). Increasing stimulation current leads to larger electrical fields affecting a relatively large area of the brain (up until ~36.5 mm^2) and increases the probability of nonspecific perturbation of adjacent areas of the brain beyond the targeted pool of neurons [13].

3.3. Subjective reports

Subject 1: 2 of the total of 140 implanted electrode contacts traversed through the claustrum (1 in each hemisphere). Initial monopolar stimulation of the left claustral target up to 6 mA during wakefulness did not cause any changes in patient's experience, besides a tingling sensation over her right temple. Bipolar stimulation of the claustral contact referenced to 1 cm dorsal contact (in the vicinity of the white matter) caused a loss of muscle tone in the right arm and leg and an arrest of tongue movements at 6 mA stimulation most likely due to involvement of the adjacent corticospinal tracts. The same effect was observed for the left arm and leg during bipolar stimulation at 6 mA of the right claustrum contact. The patient was fully aware of these effects and denied changes in her thoughts or emotions. Simultaneous stimulation of bilateral claustra up to 8 mA caused no major changes. The patient was able to continue reading an article from her phone during stimulation. She was asked to recall and tell a memory from the past and was able to continue during the stimulation itself and later reported that her ability to remember the event was not disrupted, but maybe, she felt her muscles were more relaxed.

Subject 2: 6 of the total of 120 implanted electrode contacts traversed the claustrum (3 in each hemisphere). Electrical brain stimulation of these claustral contacts at 4 mA and 6 mA caused a "strange" skin sensation from the face running down the leg. During this induced sensation, the patient denied having loss of awareness and stated that she was aware of objects and people around the bed. Stimulations at 8 mA at the same site induced a sense of pressure in the mouth and a squeeze of his left hand but denied change in

Table 2
Electrical stimulation parameters and locations.

Subject ID	Electrodes/contacts per hemisphere	Contacts/lobe	Bilateral stimulation	# of electrodes in claustrum	# of electrode pairs stimulated	Stimulation amplitudes (mA)	PD (ms/phase)/SD (s)/SF (Hz)
S1	6R 8L/60R 80L	44T, 6I, 80F	Yes	2	4	2–8	0.2/1–2/50
S2	6R 6R/60R 60L	59T, 17I, 41F	Yes	6	6	4–10	0.3/0.5–1/50
S3	6L/56L	31T, 9I, 15F	No	4	3	2–6	0.2–0.5/1–2/50
S4	8L 8R/80L 80R	33T, 5I, 20P, 88F	Yes	6	1	4–9	0.2/1/50
S5	9L 3R/86L 30R	41T, 25I, 33P, 2O, 11F	Yes	6	5	1–4	0.2/1/50

PD = pulse duration; SD = stimulus duration; SF = stimulation frequency.
T = temporal, I = insular, P = parietal, O = occipital, F = frontal.

Table 3
Abbreviated table of patient responses and stimulation parameters (for the complete table please see Supplementary Tables 1–1 to 1–5).

Subject	Electrode	Current (mA)/SD (s)	Time elapsed	Prompt	Examiner observation	Poststimulation response
Subject 1 stimulation parameters: 50 Hz, 200 μs/phase	LPI1-RAH10	4/1	0:00:22	How about now? One, two, three.	Patient had eyes closed, sitting up in bed, hands folded in front of her. No effect of stimulation noted.	Any pain? *No.* Any change in your thought, emotion? (Patient shakes head).
	LPI1-RAH10	6/1	0:00:46	If you can hold your eyes open and try read this stuff over here (examiner giving instruction "6 mA")? *Ok. Read it out low?* Sure. What about now? Did anything change?	Eyes open during stimulation. No effect noted.	*I felt tingling over here.* (pointing to the right temple). Ok, you felt tingling right there. But, no other change, right? *I'm sorry I was supposed to be reading.* No, it's ok. Is your vision changing? *No.*
	LPI1-LIP3	6/1	0:03:25	[Inaudible]…in your leg area as well. Bring your legs up. *Both of them?* Both of them. Hands up and do like this (open and close both hands). Go.	Right arm immediately drops and she stops open and closing her hand with stimulation.	*This is weird*. This is weird.
	LPI1-LIP3	6/1	0:03:42	Smile, stick out your tongue, do like this (move tongue from side to side).	Tongue stopped moving with stimulation. Patient was looking straight ahead and eyes did not move (observed by one dedicated examiner at the foot of the bed).	You stopped. *Aha (acknowledges).* That's good. What type of feeling did you get? *My whole right side was relaxed and [inaudible: uncontrolled?] until you stopped.*
	LPI3-LPI4	4/1	0:05:30	Up, exercise (the patient has arms and legs elevated and opens–closes the hands repeatedly).	The right arm and leg drop to the bed immediately with stimulation, hand movements also arrest.	But nothing in your thoughts of emotions?
	Sham	Sham	0:08:15	What about your legs? Bring them up. *With my arms too?* Yes. I am going to repeat it. This is 6. One, two, three.	Arms and legs were raised, the patient continued open/closing the hands.	Anything changed? *No.* I was trying to trick you. Sham. We have to do it. *Ok.* Pretend to do it to see if the patient is actually making it up. *Ok, nothing happened.*
	LPI1-RPI1	6/1	0:08:33	Bring it up (legs and arms lifted off the bed, open/closing hands). One, two, three.	Slight slowing of the hand movements and looked like the legs slightly wavered.	What happened? *Ahm mostly my legs, both dropped a little bit. Both hands a little as well, but not as much as before with the right side.* In your mental state did anything change? You were yourself? *I felt like myself.*
	LPI1-RPI1	8/1	0:11:59	(The patient is instructed to read an article off her phone). *Ok,* Ready, whenever you are ready read out loud. (starts reading) *[inaudible]… politics news,* (reads current date, while getting stimulated).	No interruption in speech or dysarthria.	The patient continues reading a politics article.
	LPI1-RPI1	8/2	0:13:04	Recall a memory of your past. Something that has happened to you and your mom knows what happened. Just talk to your mom, in a natural way, recall that memory. *Ahm, do you remember, when you and me* (starts being inaudible on video, stimulation started).	Continued reading, although inaudible in parts on video. Stops reading then says "Ok, I relaxed there".	(In the last 500 ms of stimulation she starts saying) *Ok, I relaxed there, my whole* (points to the right side of the body) Did you notice anything. *Just relaxed.* Relaxed, but your mental thinking, was it disturbed or no? (Shakes head). You were able to talk with your mom. (Patient nods)... *I felt like my head was relaxed but my memory was still there. I was still talking but my head, shoulders, and arms, anything but just relaxed, ahm, muscley. I don't know another word. But, muscle wise it relaxed. But everything was still intact.* Got it, thank you.
	RPI1-RPI2	6/1.5	0:15:11	Let's go to 6 mA and then we're done. Hands up, legs up, and you do this function (open close hands), can you tap with your legs as well? I know it is very hard. (Patient does it while having both arms and legs elevated). One, two, three.	Left arm and leg drop to the bed.	Ok, ok, that's it.
	LPI1-RPI1	8/2	0:16:03	Go to LPI1 and RPI1 again. So we are at 8 mA. Legs up, exercise again, (to 2nd observer: can you look at the eyes as well?), arms as well, and do like this as you were doing (tapping the feet and open/closing hands). Ready? One, two, three. (starts stimulating 3.5 s later).	Legs start loosing tone at stimulation onset, legs stop tapping within first second. Arms start dropping and hands stop open/closing after 1 s of stimulation. No eye movements.	What happened now? *It took a little time but they both dropped.*
Subject 2 stimulation parameters: 50 Hz, 300 μs/phase	RI6–RI7	6/1	0:00:54	Ok so now you notice that your friend is here. I can *I can see all the computers.* You are aware that you're here? *Yes* You didn't lose yourself [during prior stimulation]? *Shakes head no* How about now?	Patient had eyes open and hands to the side. No visible effect of stimulation.	*…I still see the computers and the the colors and water..like my mind isn't going… It was what just happened. The first one was lighter and the second one was stronger but I still notice that you guys were here…*So it was almost like a seizure-like feeling? *Yes. That would be coming from both sides definitely that would be a seizure.*

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Table 3 (*continued*)

Subject	Electrode	Current (mA)/SD (s)	Time elapsed	Prompt	Examiner observation	Poststimulation response
	RI6–RI7	8/1	0:01:58	How about if you just relax and just pay attention to where you are, what's happening to you. And I will not tell you when I…but I want you to tell me when you feel like something happened ok?	During stimulation, patient curls left hand around an object and squeezes it.	*[mumbles]* What happened? *[more mumbling]…pressure on my mouth, pressure on my teeth.* Pressure on your teeth? *Yea.* Got it. Ok. So you were fully aware?… *I was paying attention to the board and I felt that pressure [holds mouth] right here.*
	RI 7–8	10/1	0:03:01	Ok. Let's go on to the next one. …Ok, I won't tell you when I do things, you'll tell me when I did I it. Ok.	Patient had eyes open and hands to the side. No visible effect of stimulation.	Ok what happened? *My knee went that way [points to the left].*
	RI 7–8	10/1	0:04:20	Ok. How about now? I'm going to 10 (mA). *You want my hands up?* Yes hands up, all the way up. And with your left hand, can you do like this [starts curling hands into fist and out] Yea keep doing it.	Patient is sitting up and curling her left hand into a fist and out of a fist. No visible effect of stimulation.	Ok. *I felt something in my hand (left hand) and here [holds left ear]* I see ok. Did you feel like you are yourself? *Yes.* Did anything weird like you being someone else? *No.* Did you lose your awareness or consciousness? *No.* Did you feel like your friend is sitting here with you? *Yea I can see the computers here and I notice the…(something inconspicuous) over there.* Alright.
	L1 9–8	4/0.5	0:06:56	Keep your hands up. Good, let's see what happens ok?	Patient is sitting up with eyes open and both arms held above her body. No visible effect of stimulation.	Did anything change? *Yea I felt some numbness in my arm.* Which arm? *This one (Moves right arm)* Right side? *Yea I felt some…*Numbness? *I felt some warm…* Warm feeling? *Yea.* Ok.
	L1 8–7	4/0.5	0:07:31	Can you do like this? [motions testee to curl fingers in and out of fists] Ready?	Patient curls both of her hands in and out of a fist. The curling motion becomes slower during and after the stimulation.	Anything change? *Yea now I felt it in this one also* In both sides? What happened? *I felt like when I squished my hands felt it block* Say it again? *When I squished hand I felt it blocked. I will not squish like all the way in.*
	L1 6–7	6/1	0:07:56	Let's pay attention. One, two, three.	Patient is sitting up with eyes open and legs out. No visible effect of stimulation.	What happened? *Inaudible talking and pointing to back of neck.* Ok.
	R17-L17	10/1	0:12:45	Let's take that one more time. *Should I start it again, or* Uh yea. Start again and without stimulation go from 20 down. *20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10,*	No change in speech observed.	*(After stimulation), 9, 8, 7, 6, 7* Ok. *That's where I went up there again.* What happened? *I felt like my. I felt like I was getting out of my voice and then couldn't talk. Like when they say I'm having a seizure.* Ok alright.
Subject 3 stimulation parameters: 50 Hz, 200 μs/phase	AIN3–AIN4	2/1	0:23:30	[Look out for] if your conscious has changed. Ok. Like if your friend is still here or if you're aware you're yourself. Are you aware you're in bed…who we are, like your surroundings. You have a picture in your mind right? If any of that changes, then [let me know] ok? *Patient nods* (examiner gives instructions for parameters) one, two, three	Patient has eyes open and is sitting up with hands around her knees and legs close to her. No visible effect of stimulation.	Anything change? *I had my aura.* You had your aura? … *I feel like I knew what was going on.* Ok. *Like a claustrophobic…worries me.* Still right now? *Yea.…* Put your head back and your legs out. You're not seeing any seizure activity right now. Are you still feeling it? *No I'm fine now.* Can you tell me what exactly happened? *So, when you guys were talking the stuff I heard… my aura was slowly progressing. I started feeling claustrophobic. I'm looking at my focal point … and it almost looked like it was [starts waving her hand left to right].* Ok can you open your eyes and see if that moving is still there? *It's still there.*
	Sham	Sham	0:25:59	Ok well I'm going to stimulate one more time. You tell me if you're noticing any change in your awareness, in your consciousness. Your friend is still there, right? *Yes.* How about now. One, two, three. (didn't stimulate).	Patient is sitting up with hands together and legs out with feet crossed.	Any change? *Patient looks around. No* Not at all? You are yourself? *Everything is a little more blurry but [inaudible murmurs]*
	AIN4–AIN5	2/2	0:26:29	Ok. How about now? One, two, three (starts stimulation on three)	Patient is sitting up with hands together and legs out with feet crossed. During stimulation, the head sighs lower.	*I think I feel worse.* What happened? *Well, I think it took the breath out of me. I'm getting these hot flashes… [incomprehensible statements]*Did your consciousness change? *What do you mean by that?* Like who you are and where you are change. *It just felt [incomprehensible statement]…wanted to get out.* Alright.

Table 3 (*continued*)

Subject	Electrode	Current (mA)/SD (s)	Time elapsed	Prompt	Examiner observation	Poststimulation response
	AIN5–AIN6	4/2	0:29:03	(Examiner gives instructions to look for an article) Tell us about that [referring to a politician] being content with him? Yea. *Well I think…*	Slight pause in speech but speech overall was not affected by stimulation.	*(Continuing after stimulation) …already screwed our country over but now it's time for someone to clean it up.* Did you notice any change? *I did. I was somewhat annoyed at what I wanted to say.* Really? We didn't notice it. That's interesting.
	AIN5–AIN6	4/2	0:29:39	Can you remember what she's reading and try to repeat what she's reading? (examiner starts reading) "We will add fact check in search engine results much like Facebook".	Patient was sitting up with right hand clenching in and out of a fist. Knees were pushed up and legs were close to the body. No visible effect of stimulation.	*Can you repeat it?* Is it hard? *I remember the part where like "We will add" and something like a Facebook account somehow.*
	AIN5–AIN6	4/2	0:31:09	So are you relaxed completely? *Yea.* Why don't you bring arm and just do like this (starts clenching fingers in and out of a fist). Keep doing it fast and count from ten backwards. *10, 9, 8*	Patient was sitting up with right hand clenching in and out of a fist. Knees were pushed up and legs were close to the body. The clenching motion was not affected by the stimulation and no other effect of stimulation was visible.	(After stimulation) *7, 6, 5, 4.* Ok anything change? *Yea.* What happened? *It was hard for me to remember which way was up or down.* Hard for you to say the numbers? *Yea (incomprehensible statement)*
Subject 4 stimulation parameters: 50 Hz, 200 μs/phase	RINA2-LINP1	4/1	0:00:08	Ok.	Patient was sitting upright with eyes open and arms out. There was no visible effect of the stimulation.	Any change? *No.*
	*Sham	Sham	0:00:16	I'm most interested to know if I'm changing anything in your mental state. I know it is very vague but I want to be as vague as possible so you can report to us any change whatsoever in your level of awareness especially. *Right.* Do you think we are altering your awareness? Are you aware that we are standing by your bedside? We are who we are and you who you are, etc. *I am aware of that.* Ok. One, two, three	Patient sitting up, with eyes open and hands slightly curled on the table in front of him. No visible effect of stimulation.	Any change? *No.*
	RINA2-LINP1	8/1	0:01:27	(Starts stimulation again)	Patient sitting up, with eyes open and hands slightly curled on the table in front of him. No visible effect of stimulation.	How about now? *Uh, I'm going to say no.* What's the hesitation about? *It felt like for a minute like I had an urge to lift my right leg.* Urge to lift your right leg. Ok.
	RINA2-LINP1	9/1	0:03:03	Can you lift your right leg? (gives instruction to another person to lift and hold the right leg) I'm going to do it one more time. Now lift your leg up. Ok ready, one, two, three	Patient is sitting up with eyes closed and hands rested together. Right leg is held up in the air and shakes slightly in place during stimulation.	What happened? *Um. It felt like a jerk a little*
Subject 5 stimulation parameters: 50 Hz, 200 μs/phase	LPI5-RPI4	2.25/1	0:00:00	Ok, you tell me what you notice.	Any abnormal sensation? *I felt like a… when I take like a deep breath*	Patient sitting upright, arms crossed in front, and eyes open.
		Sham	Sham	Sham	Any change? Patient shakes head no	Patient sitting upright, arms crossed in front, and eyes open.
	LPI5-RPI4	2.25/1	0:00:45	How about now?	*Yea.* What happened? *Feels like I've had something acidy in here [points at throat]* Was it unpleasant or pleasant? *Unpleasant.* Were you aware of yourself, us, the room, your mom or did you lose awareness and consciousness? *I was aware.*	Patient sitting upright, arms crossed in front, and eyes open.
	LPI5-RPI4	1/1	0:01:30	Ready?	How about now? Did you notice it? *[Patient shakes head no]* Not at all?	Patient sitting upright, arms crossed in front, and eyes open.
	LPI5-RPI4	1.5/1	0:01:44	Ok.	How about now? Did you notice it? *I feel a little bit in here [pointing at throat]* Was it less intense or more intense? *Way less.*	Patient sitting upright, arms crossed in front, and eyes open.
		Sham	Sham	Sham	Anything? *[Patient shakes head no]*	Patient sitting upright, arms crossed in front, and eyes open.
	LPI6-RPI5	2/1	0:03:42		How about now? … *Yea like a knife jabbing pain but it felt like through my mouth… I feel like it was a bit of both. At first it was jabbing and then it was burning.* In your mouth, throat, then stomach? *Yea.* Did you notice any anxiety or mood change? U*m, I'm a little anxious overall.*	Patient sitting upright, arms crossed in front, and eyes open.
	RPI 1–2	2/1	0:05:44	Ready? So tell me how you feel?	Anything change? *Um, felt like a small dizziness.*	Patient sitting upright, arms crossed in front, and eyes open.
		Sham	Sham	Sham	Anything? [Patient shakes head no].	Patient sitting upright, arms crossed in front, and eyes open.

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Table 3 (*continued*)

Subject	Electrode	Current (mA)/SD (s)	Time elapsed	Prompt	Examiner observation	Poststimulation response
	RPI 2–3	2/1	0:07:17	We are going to RPI 2 and 3. 1, 2, 3.	Did you notice anything? *[Shakes head no]* No? Great.	Patient sitting upright, arms crossed in front, and eyes open.
	RPI 2–3	4/1	0:07:27	How about now?	*Yea inside my body felt weird my left side.* Your left side of body felt weird in what sense? *Felt like a sudden rush and then I had that burning feeling.* Still? You're still nauseous?	Patient sitting upright, arms crossed in front, and eyes open.
	Sham	Sham	0:11:59	Ok tell me how you feel ok?	Any change? *No.*	

awareness. During stimulation at the neighboring contacts at 10 mA, he only felt "something strange" in the left hand and ear. Electrical brain stimulation to the left claustrum at 4 mA caused a numbness and warmth sensation in the right arm, but the stimulation of adjacent electrode with 4 mA stimulation elicited an unclear sensation on the right arm above the wrist. Stimulation of more dorsal segments of claustrum on the left with amplitude up until 6 mA had no subjective or objective effects. Bilateral stimulation at amplitudes up until 10 mA caused a sensory sensation around the left knee but no other changes. For instance, the subjects could perfectly continue counting backwards from 20 during stimulation.

Subject 3: In total, 4 of the 56 implanted electrode contacts were located in the left claustrum. When stimulating the most ventral parts of the claustrum (up to 6 mA), the patient experienced an aura of anxiety without EEG correlate of epileptiform activity, which outlasted the time of stimulation. The adjacent dorsal stimulations up to 2 mA caused hot flashes, and stronger stimulations could not be applied. Stimulation of the most dorsal contacts, at 4 mA, did not cause any symptoms, and her behavior of clenching her fist and counting back continued uninterrupted but she felt it was hard to say the numbers.

Subject 4: 6 of the total of 160 implanted electrode contacts were located in the claustrum (3 on each side). Only bilateral stimulation was applied in this patient. Bilateral stimulation up to 6 mA did not have any subjective or objective effects. At 8 mA, the patient felt the urge to lift the right leg, and at 9 mA, the leg jerked during stimulation, and the patient stopped toe-tapping movements. The patient did not like the experience, and we did not continue the stimulation. Again, the subject was probed directly, and she denied any change in her awareness of the situation and of her surroundings.

Subject 5: 6 of the total of 116 implanted electrode contacts were located in the claustrum. Upon bilateral stimulations, the patient experienced an unpleasant acid-like sensation in the throat with currents at 1–2 mA, but did not experience a change in awareness. When stimulating more dorsal electrodes, he experienced a jabbing/burning pain in the mouth at 2 mA. Stronger stimulations could not be applied.

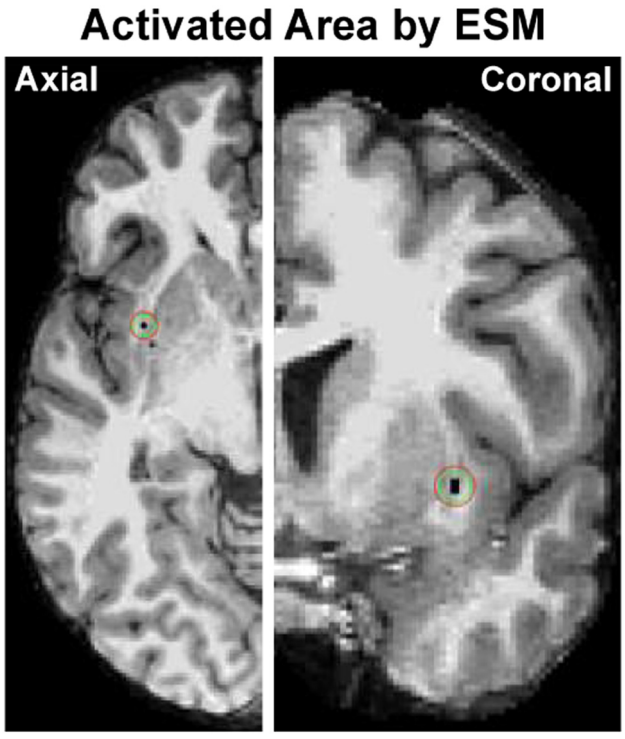

Fig. 2. Approximate brain area activated per amount of charge deposited, calculated based on data from [13]. The yellow outline corresponds to the location of claustrum seen in the coronal plane. The pink ellipse shows the size of the depth electrode (LPI1 in subject 1, overlaid on the subject's own MRI). The red circle shows the estimated activated area with the highest used stimulation parameters (50 Hz, biphasic, 0.2 ms/phase, 8 mA, for 2 s). The green circle shows the activated area with the most commonly used parameters (50 Hz, biphasic, 0.2 ms/phase, 4 mA, for 1 s). Note the close proximity of the claustrum, the insula (I) and the putamen (P).

4. Discussion

Direct electrical stimulation of the claustrum was delivered in a total of 19 contact pairs across five subjects. Simultaneous bilateral stimulation was applied in 4 patients at a total of 5 contact pairs. Stimulations mainly caused sensory-motor effects ranging from acute loss of tone in the contralateral limbs; sensation of numbness and warmth on the contralateral arm; urge to lift the right leg with bilateral stimulation; or arrest of tapping movement. Given the location of descending corticospinal tracts and ascending thalamocortical pathways and the vicinity of basal ganglia structures, it is most likely that these effects were merely due to the perturbation of the neighboring white matter structures rather than the claustral neurons per se (Fig. 2).

Our findings are in contrast with a previous case report in which a "transient loss of consciousness" was elicited in a patient by electrical stimulation of an electrode that was located between the left anterior-dorsal insular cortex and the claustrum [11]. In that study, the effect was described as arrest of reading, blank staring, unresponsiveness to auditory or visual commands, and slowing of spontaneous respiratory movements with the patient returning to baseline as soon as the stimulation stopped. Occasionally, the induced impairment of consciousness was associated with "scanty, perseverative, and incomprehensible verbal output consisting of one or two syllables, with a confused look on

the face." The authors hypothesized that while the stimulation likely caused a functional interruption of the anterior insula, claustrum, or both, the effect on awareness could possibly be attributed to claustral stimulation since prior reports of electrical stimulation in the insula did not cause such impairments [14]. The discrepancy between our findings and the report by Koubeissi and colleagues could be explained by several methodological differences: 1) We stimulated the claustrum directly while their observations were attributed to the stimulation of the claustrum even though the actual electrical charge was delivered in the extreme capsule, between the anterior insula and the claustrum. Additionally, it is possible that stimulation between the electrode in the extreme capsule and the frontal lobe as in their study may have caused a field crucially different to fields elicited in our study. 2) Most stimulated locations in our report were more posterior and inferior compared with their stimulated location, although some electrodes (i.e., see Supplementary Fig. 1, Subject 2, LI6 to 8) were also located dorsal and anterior relative to the insula. 3) Most of the maximal currents used in our study were lower compared with their study, but in one patient, we used a comparable peak current and current density (Subject 2, 0.3 ms/phase, 10 mA), however, only for 1 s, while Koubeissi et al. stimulated for 3 to 5 s [11]. However, as we noted in our subjects, direct claustral stimulations with relatively lesser currents were perceived as unpleasant because of severe motor jerking, loss of tone, or strong somatosensory sensations that were induced most likely because of the spread of current to neighboring ascending and descending pathways. Our patients would not have been able to tolerate currents as high as 14 mA for several seconds if the stimulation targets the claustral area directly. Additionally, applying higher currents increases the probability to obtain effects due to stimulation of surrounding tissue of the claustrum. 4) The patient in the study by Koubeissi et al. had a prior resection of the temporal lobe in the same hemisphere, and the patient's epileptic tissue was near the stimulation site, while none of our subjects had been through lobectomies. However, it is unlikely that their observed effects were due to stimulation-induced seizures since effects did not outlast stimulation, and the effect of prior lobectomy remains unclear.

In closing, we are mindful that our bedside clinical electrical stimulation procedures only provide a short glimpse to the immediate effects of electrical charge delivery to the claustrum. As such, our experiment was not designed to decipher the importance of the claustrum for human consciousness per se but merely attempted to follow up on a previous recent EBS study that generated a significant interest within the scientific community. We are fully aware that our failure of inducing loss of awareness with electrical stimulation of the claustrum does not necessarily mean that the claustrum is not important for human consciousness or subjective awareness. This issue needs to be determined with well-designed controlled experiments outside the limited settings in which we obtained our observations.

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Conflict of interest

None.

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