Deep continuous theta burst stimulation of the operculo-insular cortex selectively affects Aδ-fiber heat pain

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Key points

- Deep continuous theta burst stimulation (cTBS) of the right operculo-insular cortex delivered with a double cone coil selectively impairs the ability to perceive thermonociceptive input conveyed by Aδ-fiber thermonociceptors without concomitantly affecting the ability to perceive innocuous warm, cold or vibrotactile sensations.
- Unlike deep cTBS, superficial cTBS of the right operculum delivered with a figure-of-eight coil does not affect the ability to perceive thermonociceptive input conveyed by Aδ-fiber thermonociceptors.

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- The effect of deep operculo-insular cTBS on the perception of Aδ-fiber input was present at both the contralateral and the ipsilateral hand.
- The magnitude of the increase in Aδ-heat detection threshold induced by the deep cTBS was significantly correlated with the intensity of the cTBS pulses.
- Deep cTBS delivered over the operculo-insular cortex is associated with a risk of TMS-induced seizure.

Abstract

Previous studies have suggested a pivotal role of the insular cortex in nociception and pain perception. Using a double-cone coil designed for deep transcranial magnetic stimulation, our objective was to assess (1) whether continuous theta burst stimulation (cTBS) of the operculo-insular cortex affects differentially the perception of different types of thermal and mechanical somatosensory inputs, (2) whether the induced after-effects are lateralized relative to the stimulated hemisphere and (3) whether the after-effects are due to neuromodulation of the insula or neuromodulation of the more superficial opercular cortex.

Seventeen participants took part in two experiments. In experiment 1, thresholds and perceived intensity of Aδ- and C-fiber heat pain elicited by laser stimulation, non-painful cool sensations elicited by contact cold stimulation and mechanical vibrotactile sensations were assessed at the left hand before, immediately after and 20 minutes after deep cTBS delivered over the right operculo-insular cortex. In experiment 2, Aδ-fiber heat pain and vibrotactile sensations elicited by stimulating the contralateral and ipsilateral hands were evaluated before and after deep cTBS or superficial cTBS delivered using a flat figure-of-eight coil. Only the threshold to detect Aδ-fiber heat pain was significantly increased 20 minutes after deep cTBS. This effect was present at both hands. No effect was observed after superficial cTBS.

Neuromodulation of the operculo-insular cortex using deep cTBS induces a bilateral reduction of the ability to perceive Aδ-fiber heat pain, without concomitantly affecting the ability to perceive innocuous warm, cold or vibrotactile sensations.

Introduction

There is increasing – but somewhat conflicting – evidence that the operculo-insular cortex plays an important role in pain perception (Starr *et al.*, 2009; Davis *et al.*, 2015; Segerdahl *et al.*, 2015; Feinstein *et al.*, 2016; Liberati *et al.*, 2016). This has led some authors to propose that neuromodulation of the operculo-insular cortex could constitute a mean to alleviate chronic pain (Ciampi de Andrade *et al.*, 2012; Galhardoni *et al.*, 2015; Segerdahl *et al.*, 2015; Moisset *et al.*, 2016). Supporting a central role of the operculo-insular cortex in nociception and pain is the observation that more than 40% of the spinothalamic tract is relayed by thalamic neurons projecting to the insular cortex in primates (Dum *et al.*, 2009). Second, brain responses elicited by painful stimuli have been consistently observed in the human operculo-insular cortex using functional magnetic resonance imaging, positron emission tomography, and intracerebral electroencephalography (Peyron *et al.*, 2002; Mazzola *et al.*, 2012a). Interestingly, intracerebral recordings performed in patients have shown that nociceptive stimuli elicit early-latency responses in both the contralateral and ipsilateral operculo-insular cortex (Frot *et al.*, 1999; Peyron *et al.*, 2002). Several authors have proposed that the posterior part of the operculo-insular cortex could be more specifically involved in the processing of nociceptive inputs and in pain perception (Segerdahl *et al.*, 2015). Indeed, direct intracerebral electrical stimulation of the dorsal-posterior insular cortex can elicit painful sensations, especially when the posterior part of the insular cortex is stimulated (Ostrowsky *et al.*, 2002; Afif *et al.*, 2008; Mazzola *et al.*, 2009; Mazzola *et al.*, 2012b) but not when other brain areas activated by nociceptive stimuli were stimulated such as the primary somatosensory cortex (S1) or the anterior cingulate cortex (ACC) (Hutchison *et al.*, 1999; Mazzola *et al.*, 2006; Mazzola *et al.*, 2012b). However, it is important to note that painful sensations were reported in only 26/101 patients (Ostrowsky *et al.*, 2002; Mazzola *et al.*, 2006; Afif *et al.*, 2008; Mazzola *et al.*, 2009; Stephani *et al.*, 2011) and for only 10% of the stimuli delivered to this region (Ostrowsky *et al.*, 2002; Mazzola *et al.*, 2006; Afif *et al.*, 2008; Mazzola *et al.*, 2009). Finally, whether lesions of the posterior insula and adjacent parietal operculum impairs the ability to perceive thermal sensations and/or pain remains debated. Garcia-Larrea *et al.* (2010) retrospectively studied 270 patients suffering from somatosensory abnormalities after stroke. Five of these patients had a

selective impairment of thermonociception. All of these patients had lesions involving the posterior operculo-insular cortex. However, a later review of 24 patients with unilateral stroke lesions primarily affecting the insular cortex reported no changes in cold, heat or mechanical pain thresholds (Baier *et al.*, 2014). Furthermore, Starr *et al.* (2009) reported two patients with extensive lesions of the insular cortex, and unscathed abilities to perceive and evaluate pain (see also Feinstein *et al.* (2016)). In fact, these patients even exhibited increased pain ratings to noxious heat stimuli as compared to age-matched controls. The noxious stimuli also elicited stronger activity in primary and secondary somatosensory cortices (S1 and S2), suggesting a functional reorganization of nociceptive processing. Whether the perception of thermal and/or nociceptive stimuli depends on the function of the operculo-insular cortex in healthy individuals thus remains a very open question.

In the present study, we attempted to address this question by characterizing the after-effects of repetitive transcranial magnetic stimulation (rTMS) delivered over the posterior operculo-insular cortex on the perception elicited by a set of somatosensory stimuli selectively activating heat- or cold-sensitive afferents of the spinothalamic system, and mechano-sensitive afferents of the lemniscal system. Because of the deep location of the insula, the TMS pulses were delivered using a double-cone coil specifically designed to reach deep cortical targets such as the representation of the lower limb in the primary motor cortex (M1) (Stokic *et al.*, 1997; Terao *et al.*, 2000; Groppa *et al.*, 2012; Deng *et al.*, 2014). This procedure was recently proposed by Ciampi de Andrade *et al.* (2012), who also showed that repetitive TMS delivered at 10 Hz over the operculo-insular cortex using a double cone coil is safe and well tolerated. We chose to deliver repetitive TMS using a protocol referred to as continuous theta-burst stimulation (cTBS; Huang and Rothwell (2004)). Applied to the hand representation of the motor cortex, this protocol has been shown to have an inhibitory effect on motor evoked potentials (MEPs) lasting at least 20 minutes after the stimulation (Huang *et al.*, 2005).

In addition to testing whether cTBS delivered over the operculo-insular cortex differentially affects the ability to perceive thermal and/or nociceptive inputs conveyed by the spinothalamic system, we also examined whether the after-effects are restricted to the perception of sensory inputs originating from the hemibody contralateral to the operculo-

insular cortex targeted by cTBS, or whether they affect similarly the perception of inputs originating from both hemibodies. As mentioned above, numerous studies reported bilateral operculo-insular activation by nociceptive stimuli (Coghill *et al.*, 1999; Maihofner *et al.*, 2002; Peyron *et al.*, 2002; Garcia-Larrea *et al.*, 2003; Iannetti *et al.*, 2005; Mazzola *et al.*, 2009; Mazzola *et al.*, 2012a). Therefore, modulating the excitability of one insula might affect not only the processing of inputs originating from the contralateral hemibody but also from the ipsilateral hemibody. Finally, deep cTBS delivered using a double-cone coil can be expected to affect not only the targeted structure but also more superficial structures such as S2 (Valmunen *et al.*, 2009; Lockwood *et al.*, 2013). For this reason, we also compared the after-effects of deep cTBS delivered using a double-cone coil to the after-effects of superficial cTBS delivered using a conventional flat figure-of-eight coil.

Material and Methods

Ethical Approval

The experiments were conducted according to the latest revision of the Declaration of Helsinki, except for registration in a database. Approval for the experimental procedures was obtained from the local Ethics Committee (Commission d'Éthique Biomédicale Hospitalo-Facultaire) of the Université catholique de Louvain (UCL) (B403201316436). All participants were informed of the experimental procedures and provided a written informed consent form and were financially compensated for their participation.

Subjects

A sample size of 10 participants was planned in both experiments. A neurologist screened all participants for contra-indications to TMS (Rossi *et al.*, 2009). None of them had any history of psychiatric or neurological disorders including migraine and epilepsy or family history of seizure. None of the participants was allergic to lidocaine. All participants were right handed (Flinders Handedness Survey; Nicholls *et al.* (2013)). Eleven healthy volunteers (3 women/8 men; 29.2±5.3 years; range 23 – 41) took part in Experiment 1. Eight healthy volunteers (3 women/5 men; 23.3±1.7 years; range 20 - 26) took part in Experiment 2. In both

experiments, cTBS was delivered over the right operculo-insular cortex. In one participant of Experiment 1, cTBS triggered a partial seizure starting by a very transient euphoria followed by a dystonic attitude of the left hemibody and hemiface, anxiety, a feeling of thoracic oppression and dysarthria lasting less than one minute. Lateralization of the symptoms was difficult to confirm because the participant was positioned in left lateral decubitus when receiving cTBS. Subsequently, in one participant of Experiment 2, cTBS triggered similar symptoms with a dystonic attitude of the right hemibody and hemiface followed by a generalized tonicoclonic seizure lasting approximately three minutes, and acute post-ictal confusion. The two subjects fully recovered after the incident. The data of these two participants was excluded from the analyses, and decision was taken to end the study. These two seizures induced by deep cTBS of the operculo-insular cortex have been fully described elsewhere (Lenoir et *al.,* 2018).

Experimental design

In Experiment 1, we examined whether deep cTBS delivered using a double-cone coil over the right operculo-insular cortex differentially affects the perception of transient heat, cool and vibrotactile stimuli delivered to the contralateral hand by comparing detection thresholds, and intensity of perception before cTBS (T0), immediately after cTBS (T1), 10 minutes after cTBS (T2) and 20 minutes after cTBS (T3) (Figure 1). In Experiment 2, we examined – in two separate sessions whose order was counterbalanced across participants – whether deep cTBS delivered using a double-cone coil and superficial cTBS delivered using a flat figure-of-eight coil over the right operculo-insular cortex differentially affects the perception of transient heat and vibrotactile stimuli delivered to the contralateral and ipsilateral hands. Detection thresholds were determined before (T0), immediately after (T1) and 20 minutes after (T3) cTBS. Reaction times, intensity and quality of perception elicited by suprathreshold stimuli were assessed before (T0) and 10 minutes after cTBS (T2). In both experiments, all assessments were completed within 30 minutes after ending cTBS. Our experimental design did not include a sham condition, because our aim was to assess the differential effect of deep vs. supercificial cTBS delivered over the operculo-insular cortex. Furthermore, a control condition with sham cTBS would not have matched the strong

sensations associated with the delivery of cTBS over the lateral aspect of the skull resulting in part from the peripheral activation of the temporalis muscle.

Determination of the dorsal posterior operculo-insular target

The target of cTBS was determined using individual 3D T1-weighted structural MRI data of the whole head (1x1x1 mm; 3T Achieva; Philips Healthcare, The Netherlands), acquired before the experiment. The right insular cortex was identified on the 3D MRI image of each participant (Ciampi de Andrade *et al.*, 2012). A landmark was positioned over the dorsal-posterior region of the insular cortex, corresponding to the dorsal portions of the anterior and posterior long gyrus (Nieuwenhuys (2012); Figure 2). The Visor2 neuronavigation system (Visor 2.1 and Visor 2.3.3, Advanced Neuro Technologies, The Netherlands) was used to generate a 3D reconstruction of scalp and cortical volumes using the individual MRI data, to coregister 3D space with the reconstructed MRI space using landmark-based markers (nasion and tragi) followed by head-shape matching (Wang *et al.*, 1994; Gugino *et al.*, 2001), and to accurately position and monitor the target of the TMS coil relative to the defined MRI target.

Continuous theta-burst stimulation (cTBS)

To reduce the scalp discomfort associated with the delivery of cTBS, 45 minutes prior to the experiment, 2.5 g of lidocaine cream (EMLA cream; AstraZeneca, Belgium) was applied on the scalp of the participants where the coil would be positioned (Borckardt *et al.*, 2006). Furthermore, during cTBS, participants were provided with earplugs and a mouth guard to reduce discomfort due to the sound generated by the TMS pulses and teeth contact resulting from the peripheral activation of the temporalis muscle, respectively. During stimulation, the participants were comfortably positioned in left lateral decubitus. The stimulation consisted of trains of 3 biphasic pulses (280 μs) delivered at 50 Hz, repeated every 200 ms (i.e. 5 Hz; Huang and Rothwell (2004); Huang *et al.* (2009)) during 20 seconds (total number of pulses: 300; Di Lazzaro *et al.* (2005); (Huang *et al.*, 2005)). The TMS pulses were generated using a MagPro X100 magnetic stimulator (Magventure, Denmark). The direction of the current induced in the brain was set to the anterior-posterior posterior-anterior (AP-PA) direction. In both experiments, the TMS coil (double cone coil or figure-of-

eight coil) was positioned tangentially to the scalp on the right temporoparietal region with the handle pointing towards the back of the head, approximately parallel to the midline (Figure 2A).

In Experiments 1 and 2, *deep cTBS of the operculo-insular cortex* was delivered using an angled double-cone coil designed for deep stimulation (70 mm; D-B80 Butterfly Coil; MagVenture, Denmark). The intensity of the TMS pulses was set individually to 80% of the average of the resting motor thresholds (rMT) obtained for the left and right tibialis anterior (TA). The TA-rMT was determined as the minimum intensity required to elicit MEPs ≥ 50 µV (peak-to-peak amplitude between 20 and 50 ms after stimulus onset; Rossini *et al.* (2015)) in the contralateral TA in at least 5 out of 10 consecutive trials. This approach to determine stimulation intensity was used previously by Ciampi de Andrade *et al.* (2012), in a study aiming to modulate the insular cortex using a double-cone TMS coil, and is justified by the fact that the distance from the skull to the motor representation of the lower limbs in M1 is similar to the distance from the scalp to the insular cortex (respectively 47.1±4.8 and 48.8±4.2 mm; Ciampi de Andrade *et al.* (2012)). In Experiment 2, *superficial cTBS over the operculo-insular cortex* was delivered using a flat figure-of-eight coil (75 mm; C-B60 Butterfly Coil; MagVenture, Denmark). The intensity of the TMS pulses was set individually to 80% of the average of the rMTs of the right and left first dorsal interosseous (FDI). Deep cTBS was delivered such as in Experiment 1.

Skin temperature

Skin temperature of the contralateral hand dorsum in Experiment 1 and of both hand dorsums in Experiment 2 was measured before and after each time point measurement, using an infrared thermometer (Raytek MI3, Raytek Corporation, Santa Cruz, CA, USA).

Sensory stimulation

Transient heat stimuli consisted of radiant heat pulses (CO_2 laser; 100 ms duration; 6 mm diameter flat-top beam) delivered to the hand dorsum and generated by a temperature-controlled CO_2 laser (Laser Stimulation Device; SIFEC, Ferrières, Belgium). These stimuli could be expected to generate responses related to the selective activation of heat-sensitive Aδ- and/or C-fibers (Towell *et al.*, 1996; Mouraux *et al.*, 2003; Plaghki & Mouraux, 2003). To

avoid habituation and/or sensitization effects, the target of the laser was slightly displaced after each stimulus by approximately 2 cm. *Transient cool stimuli* consisted of fast cooling of the hand dorsum skin (200°C/s; 200 ms duration; 125 mm^2 probe surface) using a novel contact cold stimulator based on micro-Peltier elements (TCS; QST.Lab, Strasbourg, France). These stimuli would be expected to generate responses related to the selective activation of cool-sensitive Aδ-fibers (Simone & Kajander, 1997). Such as for laser stimuli, the location of the probe on the skin was slightly displaced after each stimulus by approximately 2 cm. *Transient vibrotactile stimuli* consisted of short lasting mechanical vibrations (300 Hz frequency; 100 ms duration; 20 mm diameter round-tipped probe) delivered to the index finger tip and generated by a piezo-electric actuator (VTS, Arsalis, UCL, Louvain-la-Neuve, Belgium). These stimuli could be expected to generate responses related to the selective activation of low-threshold Aβ-fiber mechanoreceptors.

Detection thresholds

Detection thresholds were determined using an adaptive staircase procedure based on the detection of the stimulus (Churyukanov *et al.*, 2012). Participants were requested to press a button held in the contralateral hand as soon as they detected the stimulus delivered on the other hand dorsum. The intensity of the next stimulus was decreased or increased by a fixed amount, depending on whether the previous stimulus was detected or undetected, respectively. The threshold was computed by averaging the intensities of the four stimuli at which a staircase reversal occurred (detected stimulus followed by undetected stimulus or the reverse). In Experiment 1, four different detection thresholds were assessed at the left hand dorsum using four randomly-intermingled staircases: C-fiber heat detection threshold, Aδ-fiber heat detection threshold, cool detection threshold and vibrotactile detection threshold (Figure 3). In Experiment 2, two different detection thresholds were assessed at both the left hand dorsum and the right hand dorsum: Aδ-fiber heat detection threshold and vibrotactile detection threshold. The stimuli of these two modalities were alternatively delivered to one of the two hands, followed by the same procedure on the other hand. In both experiments, the time-interval between two successive stimuli varied between 10 and 20 seconds. For each type of stimulus, the staircase was ended as soon as four reversals had occurred. Several previous studies have shown that, for transient heat stimuli applied onto

the skin, the detection threshold to C-fiber input is markedly lower than the detection threshold to Aδ-fiber input. Therefore, if detection of the stimulus is used as sole criterion, the estimated threshold will be related to the ability to detect input conveyed by heat-sensitive C-fibers. Conversely, if detection of the stimulus with a reaction time (RT) compatible with the conduction velocity of myelinated Aδ-fibers is used as criterion, the estimated threshold will be related to the ability to detect input conveyed by heat-sensitive Aδ-fibers (Towell *et al.*, 1996; Mouraux *et al.*, 2003; Plaghki & Mouraux, 2003). *C-fiber heat detection thresholds* were thus estimated with a staircase procedure using detection of the CO_2 laser stimulus as sole criterion. To reduce the number of steps required to estimate the threshold, the temperature of the first stimulus of the staircase was set close to the expected threshold (41°C;Meyer and Campbell (1981); Treede *et al.* (1995); Namer *et al.* (2009); Wooten *et al.* (2014)). *Aδ-fiber heat detection thresholds* were estimated with a staircase procedure using detection of the CO_2 laser stimulus with a RT<650 ms as criterion. The temperature of the first stimulus of the staircase was set to 46°C. For both staircases, step size was 1°C until the first reversal was obtained and then set to 0.5°C. Because transient innocuous cooling of the skin produces sensations strictly related to the activation of cool-sensitive Aδ-fibers (Mackenzie *et al.*, 1975; Campero & Bostock, 2010), *Aδ-fiber cool detection thresholds* were estimated with a staircase procedure using detection of the cool stimulus as sole criterion. The intensity of the first stimulus of the staircase was set to a temperature decrease of -1°C relative to baseline skin temperature. Step size was 0.5°C until the first reversal was obtained and then set to 0.25°C. *Aβ-fiber vibrotactile detection thresholds* were also estimated using detection as sole criterion. The intensity of the first stimulus of the staircase was set to 0.16 μm (detection threshold of 300 Hz vibration; Freeman and Johnson (1982); Bensmaia (2008)). Step size was 0.05 μm until the first reversal was obtained and then set to 0.02 μm. In both experiments, the modality of the first stimulus of the intermingled staircases was counterbalanced across participants. In Experiment 2, the hand which was tested first was also counterbalanced across participants. In both experiments, to reduce the number of steps required to estimate the threshold at T1 and T3, the intensity of the first stimulus of the staircase was set to the threshold obtained at the preceding time point.

Intensity of perception to suprathreshold stimuli

In Experiment 1, participants were asked to report verbally the intensity of perception elicited by supra-threshold high-intensity heat stimuli co-activating Aδ- and C-fiber heat-sensitive afferents, low-intensity heat stimuli preferentially activating low-threshold heat-sensitive C-fiber afferents, cool stimuli and vibrotactile stimuli. Ratings were provided using a numerical rating scale (NRS) ranging from 0 (no perception) to 100 (maximal conceivable intensity). For each of the four modalities, a block of 10 stimuli was delivered on the left hand dorsum of all participants. In Experiment 2, the same procedure was used to assess the intensity of the percept elicited by suprathreshold Aδ-heat and Aβ-vibrotactile stimuli delivered to the left and right hands. In both experiments, the order of the blocks was counterbalanced across participants. Furthermore, in Experiment 2, reaction-times to the suprathreshold stimuli were recorded, and participants were asked to describe the quality of the percept elicited by suprathreshold stimuli by selecting one item from a list of seven descriptors for Aδ-heat stimuli ("not perceived", "light touch", "touch", "tingling", "warm", "pricking" and "burning") and a list of five descriptors for Aβ-vibrotactile ("not perceived", "light touch", "touch", "flutter" and "vibration") (Ochoa & Torebjork, 1983; Nahra & Plaghki, 2003; Mouraux *et al.*, 2010). *Suprathreshold Aδ-heat stimuli* consisted of 60°C CO_2 laser pulses. *Suprathreshold C-heat stimuli* consisted of CO_2 laser pulses delivered at a target temperature corresponding, for each participant, to the mean of Aδ- and C-fiber heat detection thresholds estimated before cTBS (44±1.8°C), i.e. a temperature expected to be above the threshold of C-fibers, but below the threshold of Aδ-fibers. *Suprathreshold Aδ-cool stimuli* consisted of cooling of the skin down to 10°C. *Suprathreshold Aβ-vibrotactile stimuli* consisted of mechanical vibrations of 95 µm amplitude.

Statistics

Statistical analyses were conducted using SPSS 25 (SPSS, Chicago, IL, USA). Significance threshold was set at $p < .05$.

In Experiment 1, to assess the differential effects of cTBS on the detection thresholds to heat, cool and vibrotactile stimuli, a two-way repeated-measures ANOVA (RM-ANOVA) was conducted with the factor 'time' (T0: before cTBS, T1: immediately after cTBS, T3: 20

minutes after cTBS) and 'modality' (Aδ-heat, C-heat, Aδ-cool, Aβ-vibrotactile). The same design was used to assess changes in perceived intensity to suprathreshold stimuli, except for the fact that the factor 'time' had only two levels (T0: before cTBS, T2: 10 minutes after cTBS). With this model, an interaction between the factors 'time' and 'modality' would indicate a differential effect of cTBS on the responses to the different types of stimuli.

In Experiment 2, separate four-way RM-ANOVAs with the factors 'time', 'treatment' (deep vs. superficial cTBS), 'modality' (Aδ-heat vs. Aβ-vibrotactile) and 'side' (stimuli delivered to the contralateral vs. ipsilateral hand) were used to assess changes in detection thresholds, RTs and intensity of the perception elicited by suprathreshold stimuli. Because of the absence of any significant effect of 'side' (all $p>.114$), measures from both sides were merged and further analysed using three-way RM-ANOVAs with the factors 'time', 'treatment' and 'modality'. With this model, a two-way interaction between the factors 'time' and 'modality' would indicate that both deep and superficial cTBS exert, at both hands, a differential effect on the responses to the two types of stimuli, but that the effects of deep and superficial cTBS are similar. A three-way interaction between the factors 'time' × 'treatment' × 'modality' would indicate that deep and superficial cTBS differentially modulate the responses to the two types of stimuli.

When necessary a Greenhouse-Geisser correction was performed (denoted $F_{G\text{-}G}$). When justified, the effects were further assessed using univariate within-subjects contrasts and/or pairwise comparisons using paired-sample t-tests, Bonferroni-corrected for multiple comparisons.

To test if cTBS affected the quality of perception in Experiment 2, chi-square or Fisher tests were performed on the number of time each descriptor was used to qualify the sensation elicited by each stimulus. In addition, to examine whether the effects of cTBS on detection or perception of somatosensory stimuli was dependent on the intensity of the cTBS pulses, linear Pearson correlation were performed.

Results

M1 lower limb and insula distances from the scalp

The distance from the dorsal posterior insular cortex to the scalp, and from the lower limb representation in M1 to the scalp were measured in each individual MRI (n=16; scalp-insular cortex: 46±4 mm; scalp-M1 lower limb: 45±3 mm; Table 1). The mean difference between scalp-M1 lower limb and scalp-insula distances is -0.5±3.9 mm. No significant difference between the two measures ($t_{(15)}$= .519; p= .612) was observed.

Skin temperature

The skin temperature of the tested hand dorsum did not vary significantly over time, neither in Experiment 1 (at T0: 30.9±2.4, T1: 30.4±1.7, T2: 30.5±2°C), nor in Experiment 2 (deep cTBS condition at T0: 32.6±1.9, T1: 31.9±1.9, T2: 32.3±2.3°C; superficial cTBS condition at T0: 32.7±1.5, T1: 31.9±1.7, T2: 31.8±2.4°C). Indeed, the one-way RM-ANOVA did not reveal any significant difference in Experiment 1 (main effect of 'time'; $F_{(2,18)}$=1.95; p=.171; η^2=.178) and neither the two-way RM-ANOVA in Experiment 2 when considering the temperatures of the contralateral and ipsilateral hands (main effect of 'time': $F_{(2,12)}$=1.878; p=.195; η^2=.238; 'time' × 'treatment' interaction: $F_{(2,12)}$=1.247; p=.322; η^2=.172; 'time' × 'treatment' × 'side' interaction: $F_{G\text{-}G\ (1.137,6.821)}$=.911; p=.387; η^2=.132).

Experiment 1

Detection thresholds

Figure 4 shows the individual changes in detection threshold for the different types of stimuli, immediately after cTBS (T1 vs.T0) and 20 minutes after cTBS (T3 vs. T0). In almost all participants, the detection threshold to Aδ-heat stimuli delivered to the contralateral hand was increased both at T1 and at T3. In contrast, cTBS did not appear to induce any reproducible change in the detection threshold to C-heat stimuli, Aδ-cool stimuli and Aβ-vibrotactile stimuli. This was confirmed by the RM-ANOVA which revealed a significant a two-way 'time' × 'modality' interaction ($F_{G\text{-}G\ (2.04,18.37)}$=7.947; p=.003; η^2=.469) (Table 2). The univariate within-subjects contrasts showed a significant interaction for Aδ-heat vs. C-heat,

Aδ-cool and Aβ-vibrotactile stimuli at T1 vs. T0 (Aδ-heat vs. C-heat: $F_{(1,9)}$=7.321; p=.024; η^2=.449; Aδ-heat vs. Aδ-cool: $F_{(1,9)}$=17.806; p=.002; η^2=.664; Aδ-heat vs. Aβ-vibrotactile: $F_{(1,9)}$=7.742; p=.029; η^2=.428) and at T3 vs. T0 (Aδ-heat vs. C-heat: $F_{(1,9)}$=20.879; p=.001; η^2=.699; Aδ-heat vs. Aδ-cool: $F_{(1,9)}$=29.871; p=.0004; η^2=.768; Aδ-heat vs. Aβ-vibrotactile: $F_{(1,9)}$=13.399; p=.005; η^2=.598). Post-hoc paired t-tests showed that the increase in detection threshold for Aδ-heat stimuli was significant 20 minutes after cTBS (T3 vs. T0: $t_{(9)}$=-3.661; p=.009), but not immediately after cTBS (T1 vs. T0: $t_{(9)}$=-2.597; p=.057).

Intensity of perception

As shown in Figure 5, the intensity of the percept elicited by suprathreshold Aδ-heat stimuli was decreased 10 minutes after cTBS (T2) as compared to before cTBS (T0), in all but one participant. In contrast, cTBS did not appear to induce consistent changes in the intensity of the percept elicited by C-heat stimuli, Aδ-cool stimuli and Aβ-vibrotactile stimuli. The RM-ANOVA showed a significant main effect of 'time' ($F_{(3,27)}$=21.222; p=.001; η^2=.702), a significant main effect of 'modality' ($F_{(3,27)}$=22.284; p<.0001; η^2=.712), but no 'time' × 'modality' interaction ($F_{(3,27)}$=1.133; p=.354; η^2=.112) (Table 2). Paired sample t-tests comparing, for each modality, the ratings obtained at T2 vs. T0 showed a significant decrease of the perception elicited by Aδ-heat stimuli ($t_{(9)}$=2.74; p=.045), but not for C-heat stimuli ($t_{(9)}$=1.166; p=.274), Aδ-cool stimuli ($t_{(9)}$=2.138; p=.061) and Aβ-vibrotactile stimuli ($t_{(9)}$=1,699; p=.128).

Experiment 2

Detection thresholds

The three-way RM-ANOVA showed a significant three-way 'time' × 'treatment' × 'modality' interaction ($F_{(2,12)}$=10.662; p=.002; η^2=.640), indicating that deep and superficial cTBS did not induce the same after-effects and, such as in Experiment 1, that cTBS did not exert the same effect on Aδ-heat and Aβ-vibrotactile detection thresholds. The univariate within-subjects contrasts showed a significant interaction 'time' × 'treatment' × 'modality' immediately after cTBS (T1 vs. T0: $F_{(1,6)}$=8.890; p=.025; η^2=.597) and 20 minutes after cTBS (T3 vs. T0: $F_{(1,6)}$=14.919; p=.008; η^2=.713). Post-hoc pairwise comparisons showed that, such

as in Experiment 1, the detection threshold for Aδ-heat stimuli was significantly increased 20 minutes after deep cTBS (T3 vs. T0: p=.022). In contrast, the detection threshold for Aδ-heat stimuli was not significantly changed immediately after deep cTBS (T1 vs. T0: p=.144). No significant changes in Aδ-heat detection thresholds were observed after superficial cTBS (T3 vs. T0: p=.969; T1 vs. T0: p=.320). Finally, there was no significant change in Aβ-vibrotactile detection thresholds, both after deep cTBS (T3 vs. T0: p=1.0; T1 vs. T0: p=.685) and after superficial cTBS (T3 vs. T0: p=1.0; T1 vs. T0: p=1.0) (Figure 6).

Intensity of perception

The intensity of perception elicited by suprathreshold Aδ-heat and Aβ-vibrotactile stimuli were not significantly affected after either deep cTBS or superficial cTBS (Table 3 and Figure 7).

Reaction time

The RTs elicited by suprathreshold Aδ-heat and Aβ-vibrotactile stimuli were not significantly affected after either deep or superficial cTBS (Table 3 and Figure 8). The group-level average RT across conditions were 330 ± 50 ms for Aδ-heat stimuli and 227 ± 42 ms for Aβ-vibrotactile stimuli. During the threshold procedure, the RTs related to Aδ-heat stimuli detected within the time window criterion were similar before (at T0: 399±75 ms) and after (at T3: 401±35 ms) deep cTBS, and before (at T0: 452±61 ms) and after (at T3: 435±46 ms) superficial cTBS. This was also the case for the RTs related to Aβ-vibrotactile stimuli detected during the threshold procedure, before (at T0: 386±71 ms) and after (at T3: 375±125 ms) deep cTBS, and before (at T0: 369±67 ms) and after (at T3: 361±65 ms) superficial cTBS.

Quality of perception

The descriptors most often chosen to qualify the percept elicited by suprathreshold Aδ-heat stimuli and Aβ-vibrotactile stimuli before and after deep and superficial cTBS are shown in Figure 9. Aδ-heat stimuli were most often considered as painful and described as burning or pricking (75-88%). The relative proportion of the different descriptors remained similar both

after deep cTBS (Aδ-heat: p=.445; Aβ-vibrotactile: p=.071) and after superficial cTBS (Aδ-heat: p=.090; Aβ-vibrotactile: p=.321).

Influence of cTBS intensity

The average intensity of TMS to deliver deep cTBS was 44±8% of maximum stimulator output in Experiment 1 and 34±9% in Experiment 2. There was a significant correlation between the magnitude of the increase in detection threshold of Aδ-heat stimuli 20 minutes after deep cTBS and the intensity of TMS (Figure 10A). Specifically, taking the 16 participants of Experiments 1 and 2, there was a strong positive correlation between the increase in Aδ-heat thresholds at T3 vs. T0 and the intensity of cTBS pulses (r=.733; n=16; p=.001; Pearson correlation; one observation has a standardized residual greater than the cut-off of three standard deviations this participant was excluded for linear regression). When all the 17 participants of Experiments 1 and 2 were considered, there was still a significant positive correlation (r=.613; n=17; p=.009; Pearson correlation). This indicates that increasing cTBS intensity led to a larger increase in Aδ-heat detection thresholds. On the contrary, there was no significant correlation between the change in Aβ-vibrotactile detection threshold 20 minutes after deep cTBS and the intensity of TMS (r= .110; n= 17; p= .676; Pearson correlation; Figure 10B). Additionally, we estimated, for each participant, the intensity of cTBS pulses at the depth of the insular cortex using the attenuation coefficient for a batwing coil proposed by Cai *et al.* (2012) (Table 1). Taking into account coil-cortex distance did not improve the correlation between intensity of cTBS and the changes in Aδ-heat detection (r= .592; n=16; p= .016 vs. r=.733; n=16; p=.001; Pearson correlation; Figure 10C).

Discussion

Our results show that deep cTBS of the operculo-insular cortex selectively impairs the ability to perceive thermonociceptive input conveyed by Aδ-fiber thermonociceptors. Indeed, deep cTBS but not superficial cTBS of the right operculo-insular cortex induced a significant increase of Aδ-fiber heat detection thresholds, without concomitantly affecting the perception of thermal sensations conveyed by C-fibers, the perception of cold sensations conveyed by cool-sensitive Aδ-fibers, and the perception of vibrotactile input conveyed low-

threshold Aβ-fiber mechanoreceptors. The effect of operculo-insular cTBS on the perception of Aδ-fiber input was present at both the contralateral and the ipsilateral hand. Importantly, the magnitude of the increase in detection threshold was correlated with the intensity of the cTBS pulses, indicating that the change was truly due to a neuromodulatory effect of cTBS.

Deep operculo-insular cTBS selectively affects the perception of Aδ-fiber heat

Deep cTBS over the operculo-insular cortex induced a significant change in the ability to detect heat sensations related to the transient activation of Aδ-fiber thermonociceptors, without concomitantly affecting the detection of heat sensations conveyed by unmyelinated C-fibers, the detection of cold sensations conveyed by cool-sensitive Aδ-fibers, and the detection of vibrotactile sensations conveyed by large-diameter Aβ-fibers. The significant increase of Aδ-heat detection thresholds was present in both experiments after deep cTBS. Importantly, it was not observed in Experiment 2 after superficial cTBS, indicating that the increase in detection threshold after deep cTBS was not a consequence of response habituation (Greffrath *et al.*, 2007; May *et al.*, 2012) or decreased vigilance (Legrain *et al.*, 2002; Legrain *et al.*, 2012). Given that the criterion to determine Aδ-heat detection thresholds depended on whether participants detected the stimuli with a reaction time compatible with the conduction velocity of myelinated Aδ-fibers, whether the change in Aδ-heat detection thresholds could have been driven by an effect of cTBS on motor/sensorimotor processes should also be considered. This seems very unlikely as there was no significant difference in the average detection latency for Aδ-heat stimuli detected within the time window criterion during the threshold procedure, as well as in the detection latency for suprathreshold Aδ-heat stimuli. There was also no change in the detection latency of Aβ-vibrotactile stimuli. Finally, there was a clear relationship between the intensity of the TMS pulses used to deliver cTBS and its after-effect on Aδ-heat detection threshold. Although our results allow us to conclude a differential effect of deep cTBS on the different modalities (with a significant effect on Aδ-heat perception), they do not allow us to conclude an absence of effect on the other modalities. Unfortunately, to answer this question, increasing the sample size is not possible for safety and ethical reasons given the

induction of one suspected and one confirmed epileptic seizure in two participants after the deep cTBS protocol.

It has been proposed that cTBS induces cortical inhibition through a local increase of γ-aminobutyric acid (GABA) (Stagg *et al.*, 2009; Trippe *et al.*, 2009). Supporting this interpretation, Jasmin *et al.* (2003) demonstrated in rodents that increasing GABA concentration in the insular cortex reduces pain behaviour to noxious heat. Therefore, the modulation of thermonociception by cTBS observed in the present study could be explained, at least in part, by a GABAergic modulation of the operculo-insular cortex, as suggested by several authors (Enna & McCarson, 2006; Lefaucheur, 2006; Mylius *et al.*, 2012; Denis *et al.*, 2016; Moisset *et al.*, 2016).

Previous evidence suggesting a specific role of the operculo-insular cortex in thermonociception would predict that deep operculo-insular cTBS preferentially affects the perception of all inputs conveyed by the spinothalamic system; i.e. that deep operculo-insular cTBS would similarly affect the perception of heat and cold stimuli, as compared to vibrotactile stimuli. Other studies, suggesting that the processing of cold and heat may involve distinct operculo-insular subregions (Casey *et al.*, 1996; Davis *et al.*, 1998; Davis *et al.*, 1999; Craig *et al.*, 2000; Mano *et al.*, 2017), would predict that cTBS can differentially affect the detection of heat and cold stimuli, but would not predict a differential effect of cTBS on the detection of heat sensations conveyed by Aδ- and C-fibers.

Studies investigating the relationship between the activity of the operculo-insular cortex and heat perception have revealed different patterns of activation depending on whether the stimulus is perceived as painful. Bornhovd *et al.* (2002) reported that heat-evoked BOLD responses in the operculo-insular cortex show a linear relationship with pain ratings but not for stimulus intensities below pain threshold. Frot *et al.* (2007) showed using intracerebral EEG recordings that the responses in the secondary somatosensory cortex correlate with the intensity of stimulation below pain threshold and exhibit a ceiling effect for stimulation intensities above pain threshold. Conversely, responses recorded in the posterior insula were of similar magnitude for intensities below pain threshold, but increased when the intensity of stimulation entered the painful range. Such observations could be related to our

observation of a differential effect of cTBS on the ability to perceive sensations conveyed by heat-sensitive Aδ-fiber nociceptors having a high activation threshold vs. heat-sensitive C-fiber afferents and cool-sensitive Aδ-fiber afferents having low activation thresholds.

Deep cTBS over the right operculo-insular cortex similarly affects the perception of Aδ-fiber heat stimuli delivered to the left and right hands.

In Experiment 2, we found that 20 minutes after deep cTBS, Aδ-heat detection thresholds were increased similarly at the contralateral hand and at the ipsilateral hand. This bilateral effect of cTBS is in line with the work of Denis *et al.* (2016) who reported that high-frequency (150 Hz) intracerebral electrical stimulation of the insular cortex in epileptic patients increases heat pain thresholds bilaterally, without affecting cold and pressure pain thresholds. Currently, one can only speculate on the mechanism responsible for this bilateral effect. A first explanation could be that the operculo-insular cortex is involved in the processing of thermonociceptive inputs originating from both hemibodies. Supporting this view, it is well known that nociceptive stimuli elicit strong responses in both the contralateral and the ipsilateral operculo-insular cortex. For example, using intracerebral EEG, Frot *et al.* (1999) showed that nociceptive laser stimuli delivered to the hand dorsum elicit early-latency local field potentials in the left and right operculo-insular cortex. The latency of the response elicited in the ipsilateral operculo-insular cortex was, on average, delayed by 15 ms relative to the response elicited in the contralateral operculo-insular cortex, compatible with transcallosal interhemispheric conduction times. A second explanation could be that cTBS delivered over the operculo-insular cortex induces remote effects leading to a generalized modulation of thermonociception. For example, operculo-insular cTBS could activate descending projections involved in the modulation of nociceptive transmission at spinal level (Garcia-Larrea *et al.*, 1999; Garcia-Larrea & Peyron, 2007; Onesti *et al.*, 2013).

Deep vs. superficial operculo-insular cTBS and intensity of stimulation

In Experiment 2, we observed that, unlike deep operculo-insular cTBS delivered using a double-cone coil at 80% of the lower-limb resting motor threshold, superficial operculo-insular cTBS delivered using a flat figure-of-eight coil at 80% of the upper-limb resting motor

threshold had no effect on the ability to perceive Aδ-heat stimuli. This is an indication that the effects of deep operculo-insular cTBS could be due to neuromodulation of the deeply-located insular cortex rather than neuromodulation of more superficial opercular areas such as S2. The recent work of Koyama *et al.* (2017) who showed that applying bilaterally transcranial direct current stimulation over the opercular cortex did not affect pain perception is in line with our results. However, the differential effect of deep and superficial cTBS could also be due to the fact that the double-cone coil used to deliver deep cTBS generates a less focal magnetic field than the flat figure-of-eight coil used to deliver superficial cTBS. Hence, deep cTBS is likely to activate a larger area of both superficial and deep cortex (Deng *et al.*, 2014). The differential effect of deep and superficial cTBS could also be due to the fact that during deep cTBS, intervening superficial structures are probably exposed to higher magnetic fields than during superficial cTBS (Deng *et al.*, 2014; Lu & Ueno, 2017) which could lead to a differential modulatory effect on intracortical excitability (McAllister *et al.*, 2009).

Several other studies have reported that superficial TMS over the operculo-insular cortex can modulate thermonociception. Valmunen *et al.* (2009) found that, as compared to other targets (M1, S1, the occipital lobe), superficial 10 Hz repetitive TMS over the right S2 induces a long-lasting elevation of heat pain thresholds and a short-lasting impairment of the ability to discriminate different temperatures. Conversely, Ciampi de Andrade *et al.* (2012) did not observe any significant change in heat or pain detection thresholds after deep 10 Hz repetitive TMS of the operculo-insular cortex delivered using the same double-cone coil that was used in the present study. However, their feasibility study included a limited number of participants, and the after-effects of repetitive TMS were assessed at a relatively late time point, one hour after stimulation.

Deep operculo-insular cTBS could be associated with a higher risk of TMS-evoked seizures

Experiment 2 was stopped after the occurrence of a generalized TMS-induced seizure in one participant. Furthermore, in Experiment 1, deep cTBS triggered a short-lasting manifestation compatible with a partial TMS-induced seizure. Both TMS-induced adverse events were preceded by a sensation of mirth followed by a dystonic posture of the left or right

hemibody, which tented to become diffuse. Both participants presented a dysarthric speech and had breathing difficulties associated to laryngeal sensation and thoraco-abominal heaviness. These clinical manifestations were very similar to the clinical manifestations of insular lobe seizures (Isnard *et al.*, 2004; Wynford-Thomas & Powell, 2017). Therefore, in the present study, deep cTBS over the operculo-insular cortex may have triggered two epileptic seizures involving the insula.

This was highly unexpected. To our knowledge, there is only one case of TMS-induced seizures reported during cTBS. This case occurred while stimulating the hand representation of M1 using a flat figure-of eight coil (Oberman & Pascual-Leone, 2009). Furthermore, our study is not the first study attempting to modulate relatively deep brain structures using various repetitive TMS protocols delivered with a double-cone coil, including TBS (Bakker *et al.* (2015); for review Dunlop *et al.* (2015)), rTMS delivered at 1 Hz (Gerschlager *et al.*, 2002; Vanneste *et al.*, 2012; Vanneste & De Ridder, 2013; Nauczyciel *et al.*, 2014; Schuwerk *et al.*, 2014; Bradley, 2015; Modirrousta *et al.*, 2015), at 5 Hz (Vanneste *et al.*, 2011; Vanneste *et al.*, 2012; Garg *et al.*, 2016), at 10 Hz (Hayward *et al.*, 2007; Vanneste *et al.*, 2011; Ciampi de Andrade *et al.*, 2012; Vanneste *et al.*, 2012; Vanneste *et al.*, 2014; Bakker *et al.*, 2015; Dunlop *et al.*, 2015; Kreuzer *et al.*, 2015) and at 20 Hz (Rollnik *et al.*, 2002; Vanneste *et al.*, 2011; Benito *et al.*, 2012) with comparable or even higher intensities of TMS.

None of these studies, totalling 615 participants, reported any TMS-induced seizures. The main distinction between these studies and the present study appears to be the fact that we targeted the operculo-insular cortex, whereas the other studies targeted the dorso medial prefrontal cortex, the cingulate cortex, the cerebellum or the lower limb motor representation in M1. Only two studies including respectively five and seven participants also targeted the operculo-insular cortex (Ciampi de Andrade *et al.*, 2012; Bradley, 2015), using respectively 10 Hz and 1 Hz repetitive TMS.

Repetitive TMS delivered using a double-cone coil to target deep brain structures could be associated with a higher risk of TMS-induced seizures because the double-cone coil induces a less focal magnetic field than conventional flat-surface figure-of-eight coils and, therefore synchronously activates a larger brain volume. The intensity at which the brain structures

located between the coil and the target site are stimulated could also play a role (Rossi *et al.*, 2009; Oberman *et al.*, 2011; Deng *et al.*, 2013, 2014). Finally, as our study is the first to apply deep cTBS over the operculo-insular cortex, the possibility that stimulation of this specific brain structure could be associated with a higher risk of seizure should be considered. One possible reason could be that the insular cortex is highly connected with the operculum (Peyron *et al.*, 2002) and numerous other brain structures (Augustine, 1996; Moayedi, 2014).

Operculo-insular cortex as an alternative rTMS target for pain relief?

Most studies aiming at reducing pain with rTMS have targeted M1 (Poreisz *et al.*, 2008b; O'Connell *et al.*, 2011; Onesti *et al.*, 2013; Torta *et al.*, 2013; Lefaucheur *et al.*, 2014; O'Connell *et al.*, 2014; Rossini *et al.*, 2015). A few studies have examined the effects of applying rTMS to other targets, such as S1 (Poreisz *et al.*, 2008a; Antal & Paulus, 2010; Torta *et al.*, 2013), the dorso-lateral prefrontal cortex (DLPFC) (Nahmias *et al.*, 2009; Fierro *et al.*, 2010; Borckardt *et al.*, 2011; Brighina *et al.*, 2011; de Andrade *et al.*, 2011; Taylor *et al.*, 2012; Taylor *et al.*, 2013; Ciampi de Andrade *et al.*, 2014), ACC (Fan *et al.*, 2012; Tzabazis *et al.*, 2013), S2 (Valmunen *et al.*, 2009; Fregni *et al.*, 2011) and the insular cortex (Ciampi de Andrade *et al.*, 2012). The mechanism underlying the analgesic effect of rTMS over M1 remains largely unknown. Garcia-Larrea and Peyron (2007) suggested that it could be due to the activation of cortico-thalamic projections which, in turn, would activate the lateral thalamus leading to a cascade of modulations of remote areas such as the ACC, the insular cortex and the orbitofrontal cortex, finally leading to the activation of descending inhibitory pain mechanisms (Garcia-Larrea *et al.*, 1999). Because rTMS of M1 leads to changes in the activity of remote brain areas and because these same brain regions are implicated in the processing of nociceptive inputs and/or in pain perception (Treede *et al.*, 2000; Peyron *et al.*, 2002; Apkarian *et al.*, 2005), there is a growing interest to consider these non-motor areas as more direct targets for rTMS when it is applied to reduce pain. Future studies aiming to assess whether deep rTMS over the operculo-insular cortex may alleviate pain in chronic pain patients should consider the fact that deep cTBS delivered over that structure is associated with a higher risk of triggering a TMS-induced seizure.

Conclusion

Our study shows that neuromodulation of the operculo-insular cortex using deep cTBS induces a bilateral reduction of the ability to perceive transient Aδ-fiber heat pain, without concomitantly affecting the ability to perceive innocuous warm sensations conveyed by low-threshold heat-sensitive C-fibers, cold sensations conveyed by cool-sensitive Aδ-fibers and vibrotactile sensations conveyed by low-threshold Aβ-fiber mechanoreceptors.

Competing interests.

None declared.

Author contributions.

Both experiments of the present study were conducted in the laboratory of Prof. André Mouraux in the Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium. CL, MA and MA contributed to the design of the work. CL and MA acquired the data. All authors contributed to the analysis and interpretation of the data. All authors wrote and revised the present work.

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Table 1

	distance (mm)		cTBS intensity (% MSO)	
Experiment 1	**scalp – M1**	**scalp – insula**	**uncorrected**	**corrected**
Subject 1	47.5	46.1	39	41
Subject 2	45.9	48.3	46	44
Subject 3	42.6	44.8	50	48
Subject 4	42.1	39.8	32	35
Subject 5	41.1	44.1	50	47
Subject 6	49.7	48.1	37	39
Subject 7	48.2	44.9	60	64
Subject 8	48.0	47.5	36	37
Subject 9	42.7	48.3	47	41
Experiment 2				
Subject 1	49.2	50.6	39	31
Subject 2	43.7	46.6	34	27
Subject 3	44.7	41.3	37	30
Subject 4	47.0	41.3	38	30
Subject 5	43.2	41.2	27	22
Subject 6	43.3	53.2	33	26
Subject 7	43.7	44.4	33	26

Distance of the cortical targets to the scalp and intensities of cTBS pulses used during deep cTBS of the operculo-insular cortex. In the first two columns are reported the distances between the scalp and the lower-limb representation in M1 and between the scalp and the insular cortex for all the participants (Experiment 1 and 2). In the last two columns are reported the real intensities at which cTBS pulses were delivered during deep cTBS of the operculo-insular cortex and the estimated intensities corrected for the differences between the distances scalp – lower limb representation in M1 and scalp – insular cortex.

Table 2

	Experiment 1										
	main effect of 'time'				**main effect of 'modality'**				**interaction 'time' × 'modality'**		
	F value	p	η^2		F value	p	η^2		F value	p	η^2
threshold	$_{(G-G)}$2.85	.084	.241		$_{(G-G)}$943.4	**<.001***	.991		$_{(G-G)}$7.95	**.003***	.469
perception	22.22	**.001***	.702		22.28	**<.001***	.712		1.13	.354	.112

Two-way RM-ANOVAs in Experiment 1. Repeated-measures ANOVAs for thresholds and intensity of perception with the factors 'time' (T0: before cTBS, T1: immediately after cTBS, T3: 20 minutes after cTBS; for thresholds) and (T0: before cTBS, T2: 10 minutes after cTBS; for perception) and 'modality' (Aδ-heat, C-heat, Aδ-cool, Aβ-vibrotactile). * p <.050. (G-G) indicates Greenhouse-Geisser correction.

Table 3

	Experiment2										
	main effect of 'time'				**interaction 'time' × 'modality'**				**interaction 'time' × 'modality' × 'treatment'**		
	F value	p	η^2		F value	p	η^2		F value	p	η^2
threshold	6.05	**.015***	.502		6.06	**.015***	.503		10.66	**.002***	.640
perception	.798	.406	.117		.078	.789	.013		2.303	.180	.277
RTs	.566	.480	.086		.053	.826	.009		.670	.444	.100

Three-way RM-ANOVAs in Experiment 2. Repeated-measures ANOVAs with the factors 'time' (T0: before cTBS, T1: immediately after cTBS, T3: 20 minutes after cTBS; for thresholds) and (T0: before cTBS, T2: 10 minutes after cTBS; for perception and reaction time), 'modality' (Aδ-heat vs. Aβ-vibrotactile) and 'treatment' (deep vs. superficial cTBS).* p <.050.

Figures captions

Figure 1

Experimental design. Both experiments followed the same procedure. Detection thresholds were monitored before (T0), immediately after (T1) and 20 minutes after cTBS (T3). The perceived intensity elicited by suprathreshold stimuli was monitored before (T0) and 10 minutes after cTBS (T2). Note that the thresholds measurements at T0 and T1 and the perception evaluation at T0 and T2 were separated by approximately the same amount of time. In Experiment 1, thresholds and perception were assessed for four modalities: Aδ-heat, C-heat, Aδ-cool and Aβ-vibrotactile stimuli delivered on the contralateral hand (left) relative to the right insular cortex onto which deep cTBS was applied. In Experiment 2, sensory changes were assessed for two modalities: Aδ-heat and Aβ-vibrotactile stimuli delivered on the contralateral and ipsilateral hand at the same time points before and after deep cTBS of the operculo-insular cortex or superficial cTBS of the operculum. The experimental procedures were completed within 30 minutes following cTBS.

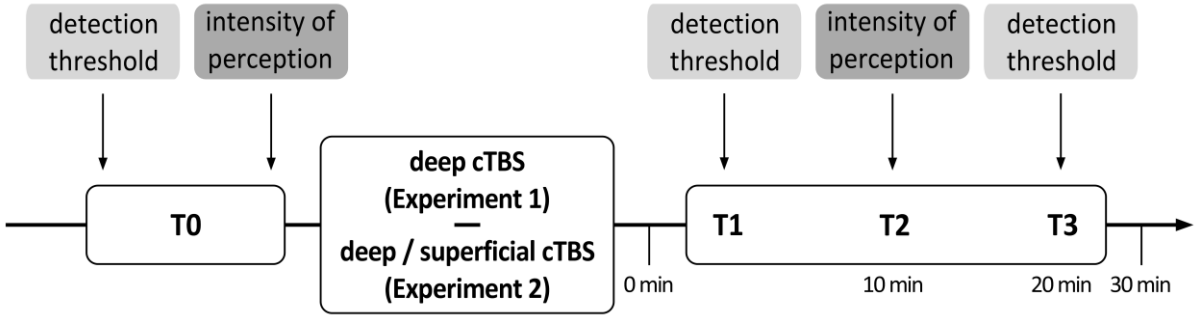


Figure 2

Localization of the target sites in the dorso-posterior insular cortex and the localization of the positions of the TMS coil. A. Position of the TMS double cone coil with the handle pointing backwards during the cTBS protocol, the participants were lying in left lateral decubitus position. B. Localization of the target sites in the dorso-posterior insular cortex (black dots; MNI coordinates x: [34 – 42], y: [-17 – -5], z: [4 – 14]) defined on individual 3D structural MRI image for the MRI-guided neuronavigation system. C. Projections on the cortical surface of the position of the centre of the TMS coil (white dots) at which cTBS was applied for all participants, in transparency are displayed the location of the insula (dark grey) and the corresponding target markers. The apparent discrepancy between the target markers and the position of the coil is due to the 2D visualisation of the different orientations of the TMS coil according to the individual curvature of the head (MNI Colin 27 brain reconstruction adapted from JuBrain (Mohlberg *et al.*, 2012)).

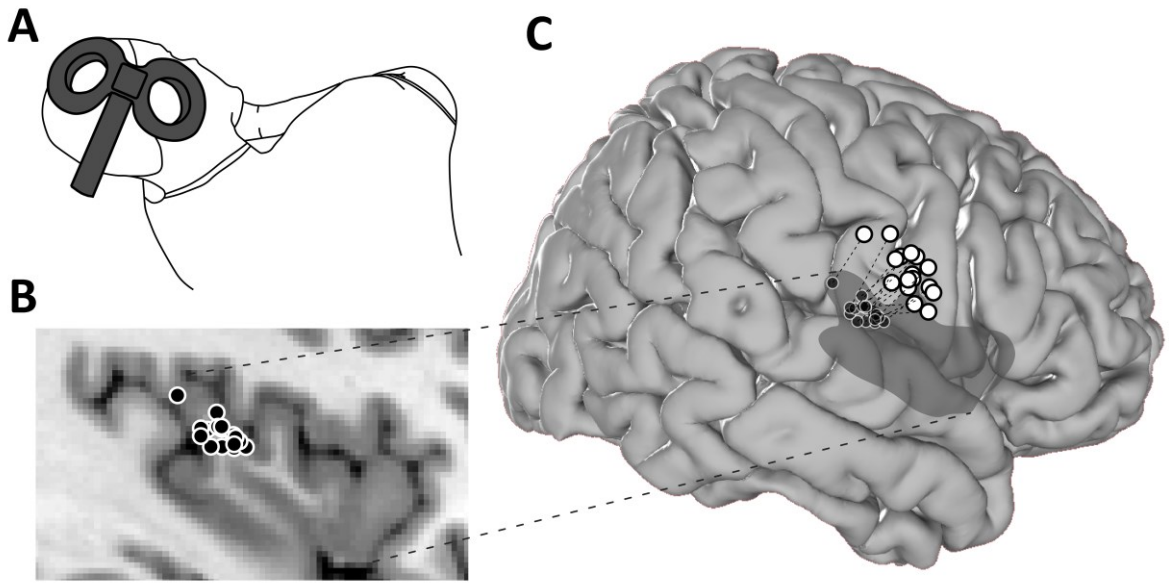


Figure 3

Example of the threshold intermingled staircase procedure for Experiment 1. In this representative participant, the first stimulus delivered was Aβ-vibrotactile (1; lower panel) followed by a C-heat stimulus (2) followed by a Aδ-cool stimulus (3) followed by an Aδ-heat stimulus (4; upper panel). The order is indicated by the arrows. This sequence was repeated until four reversals (open circles) were obtained for each modality. The order of the modalities was counterbalanced across participants. The different thresholds were computed within each modality by averaging stimulation intensities of the first four staircase reversals. In Experiment 2, the procedure was the same with alternative delivery of Aδ-heat and Aβ-vibrotactile stimuli. This procedure was conducted on one hand at a time. The order of the first tested hand was counterbalanced across participants.

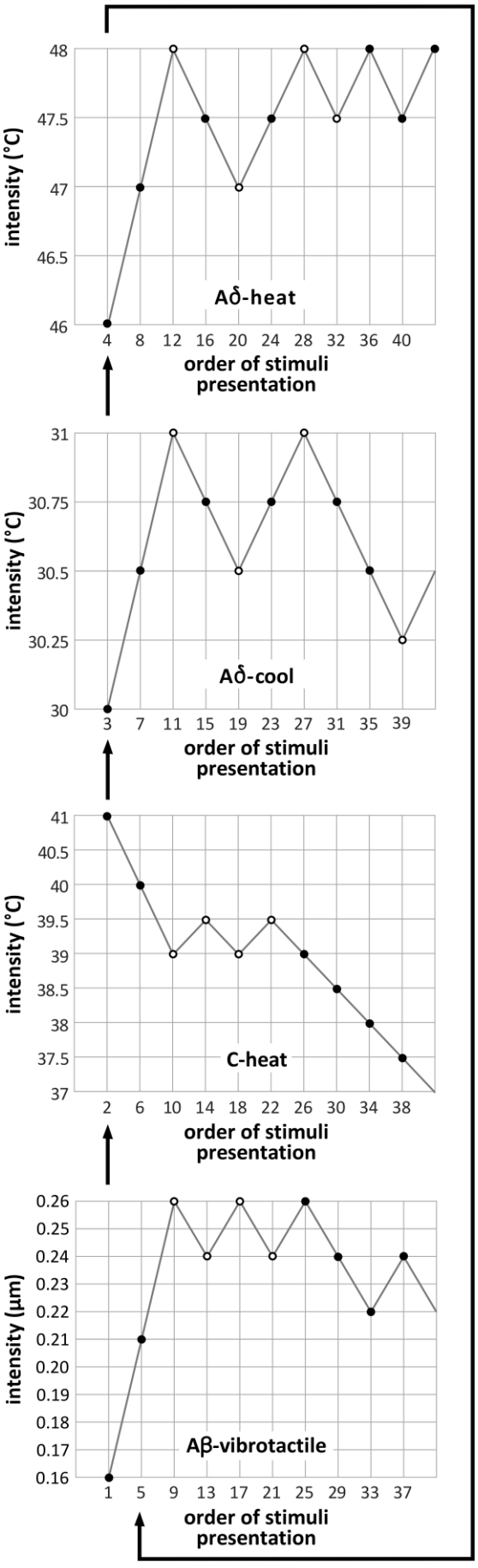


Figure 4

The effect of deep cTBS over the right operculo-insular cortex on detection thresholds in Experiment 1. Bar graphs represent the individual changes (increase in red, decrease in blue) in detection thresholds (n=10) immediately after cTBS (T1-T0) and 20 minutes after cTBS (T3-T0) for the four somatosensory modalities: Aδ-heat, C-heat, Aδ-cool and Aβ-vibrotactile delivered on the left contralateral hand. The lower graphs show the individual absolute thresholds at T0, T1 and T3, group-level average is displayed in green (mean±SD).

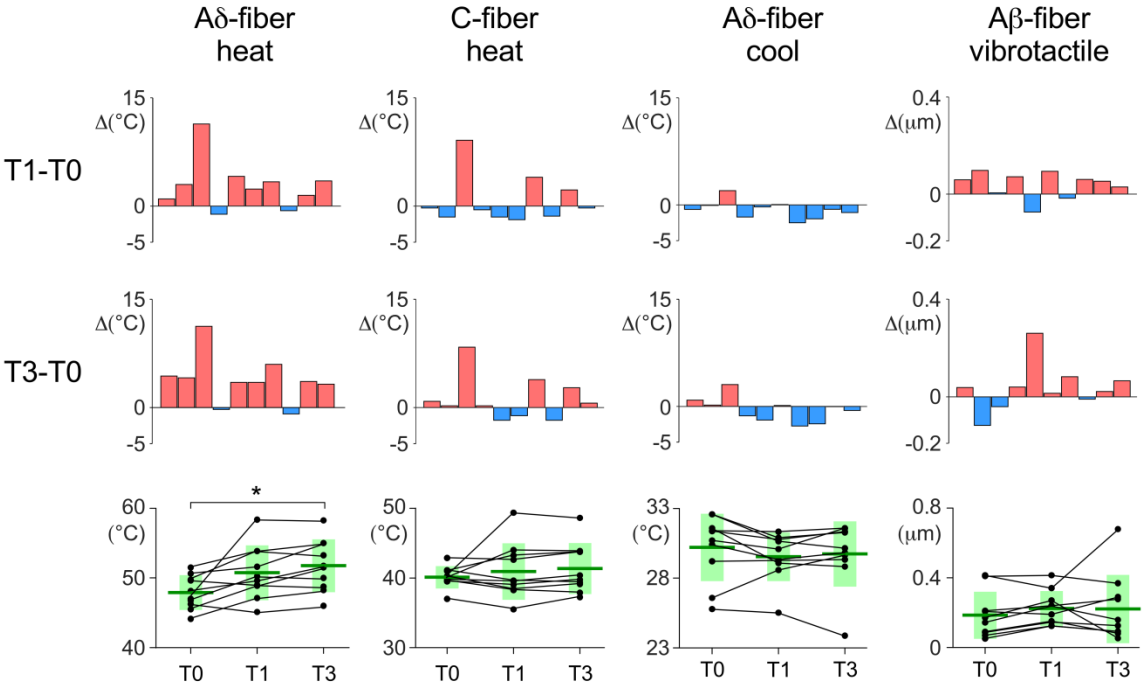


Figure 5

Effects of deep cTBS over the right operculo-insular cortex on perceived intensity in Experiment 1. Bar graphs represent the individual changes in perception (numerical rating scale, NRS; increase in red, decrease in blue; n=10 expect for Aβ-vibrotactile n=9 because of missing data) elicited by the four different suprathreshold stimuli delivered on the contralateral hand: Aδ-heat (60°C), C-heat (44°C), Aδ-cool (10°C) and Aβ-vibrotactile (95 μm) 10 minutes after cTBS (T2-T0). The lower graphs show the individual intensity of perception at T0 and T2, group-level average is displayed in green (mean±SD).

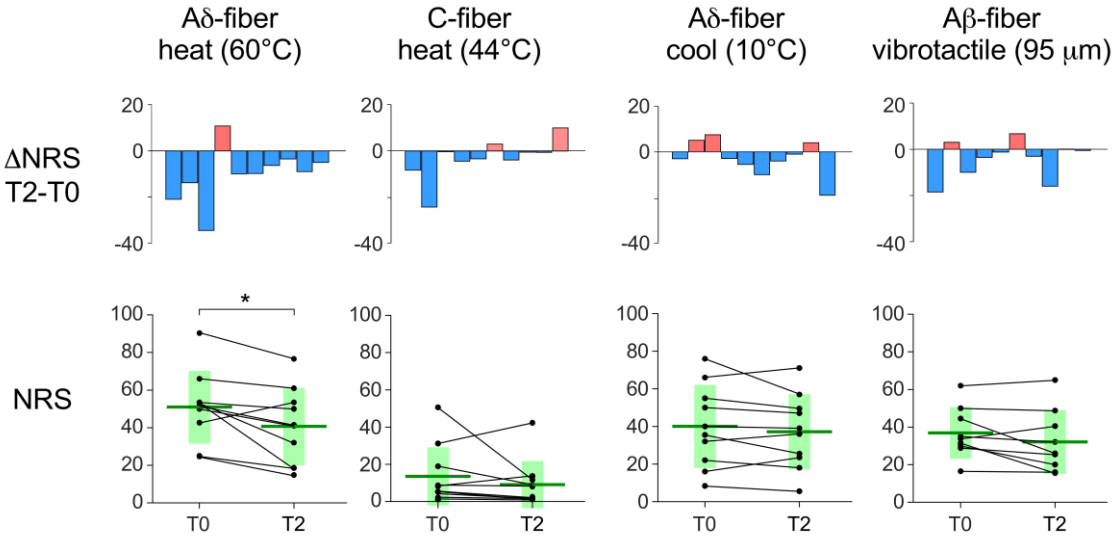


Figure 6

The effect of cTBS on detection thresholds in Experiment 2. Effects of deep and superficial cTBS of the operculo-insular cortex on Aδ-heat and Aβ-vibrotactile thresholds. Bar graphs represent the individual changes (increase in red, decrease in blue) in threshold (n=7) immediately after cTBS (T1-T0) and 20 minutes after cTBS (T3-T0). The lower graphs show the individual thresholds, group-level average is displayed in green (mean±SD).

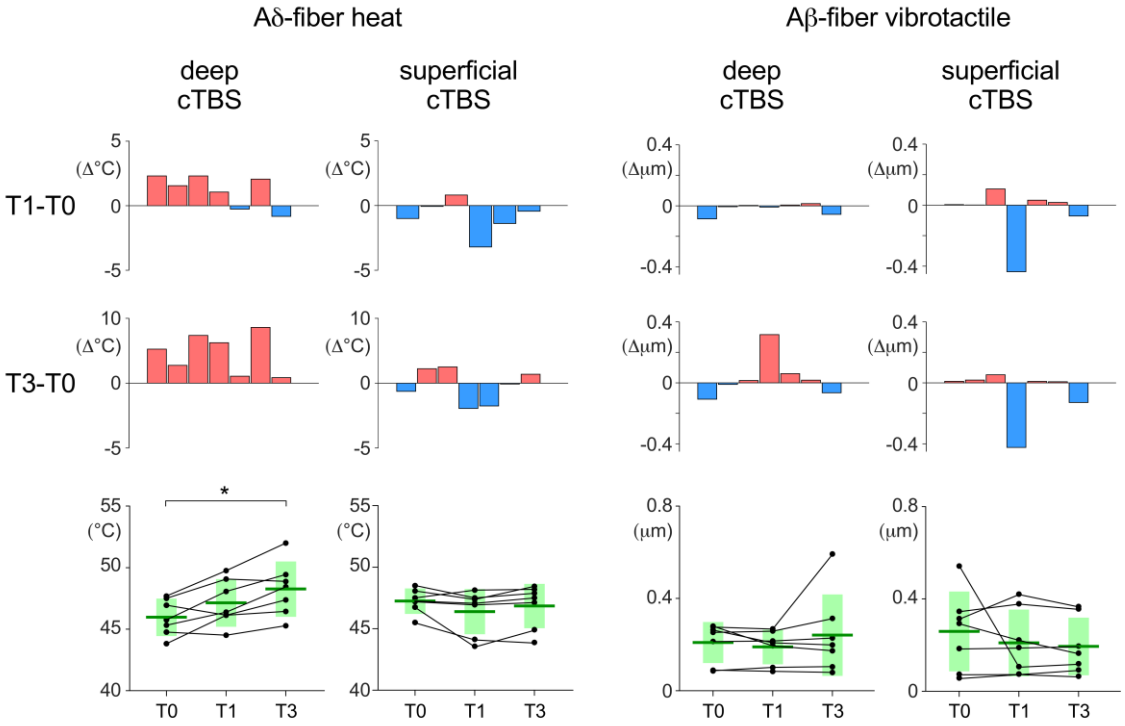


Figure 7

Effect of deep and superficial cTBS on intensity of perception in Experiment 2. Bar graphs indicate individual changes in perception (numerical rating scale, NRS; increase in red, decrease in blue; n=7) elicited by Aδ-heat (60°C) and Aβ-vibrotactile (95 µm) stimuli. The lower graphs show the individual perception, group-level average is displayed in green (mean±SD).

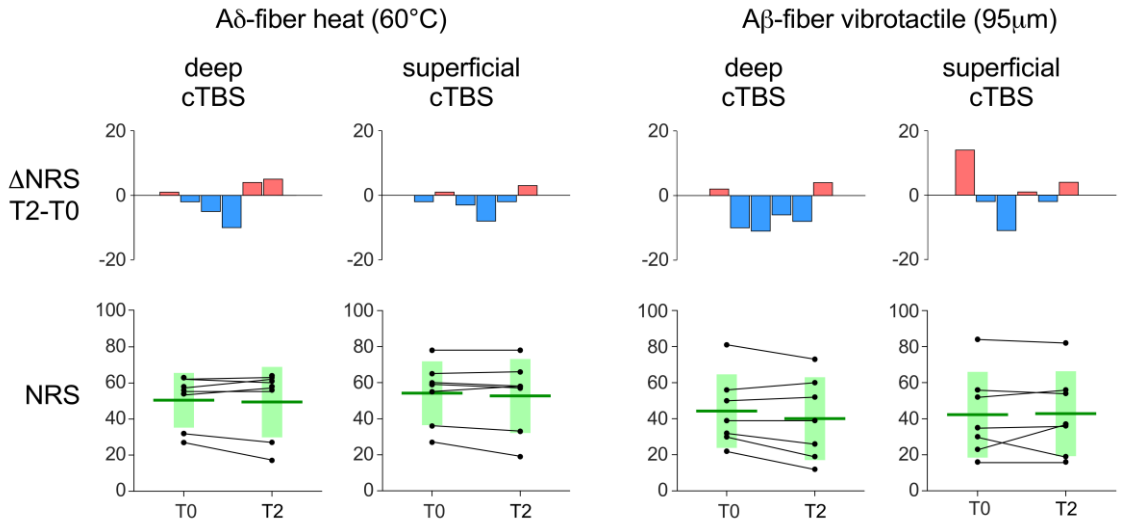


Figure 8

Relative frequency distribution of reaction times to suprathreshold Aδ-heat and Aβ-vibrotactile stimuli in Experiment 2. A. Effect of deep and superficial cTBS on reaction times to suprathreshold Aδ-heat stimuli (60°C). The relative frequency distribution of RTs are displayed before (T0) and 10 minutes after (T2) cTBS. B. Effect of deep and superficial cTBS on RTs to suprathreshold Aβ-vibrotactile stimuli (95 µm) at T0 and T2.

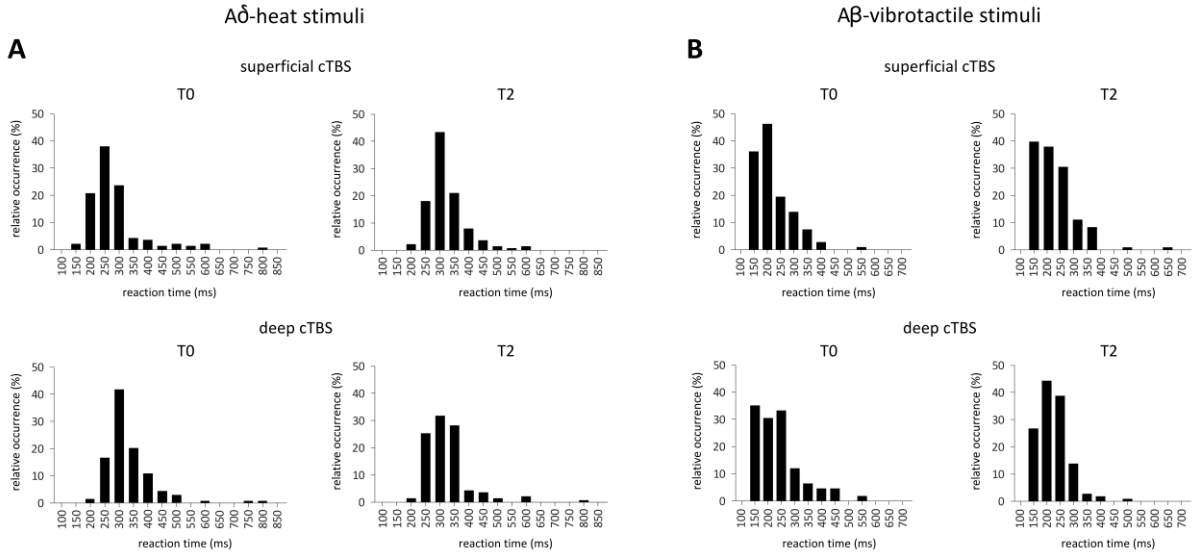


Figure 9

Quality of perception of suprathreshold stimuli in Experiment 2. A. Quality of perception of suprathreshold Aδ-heat stimuli. B. Quality of perception of suprathreshold Aβ-vibrotactile stimuli (95 µm). Pie charts represent the proportion of the use of each descriptor before (T0) and 10 minutes after (T2) cTBS. In both cases, all stimuli were perceived, in all conditions Aδ-heat stimuli were mainly perceived as painful (burning or pricking) before and after deep or superficial cTBS.

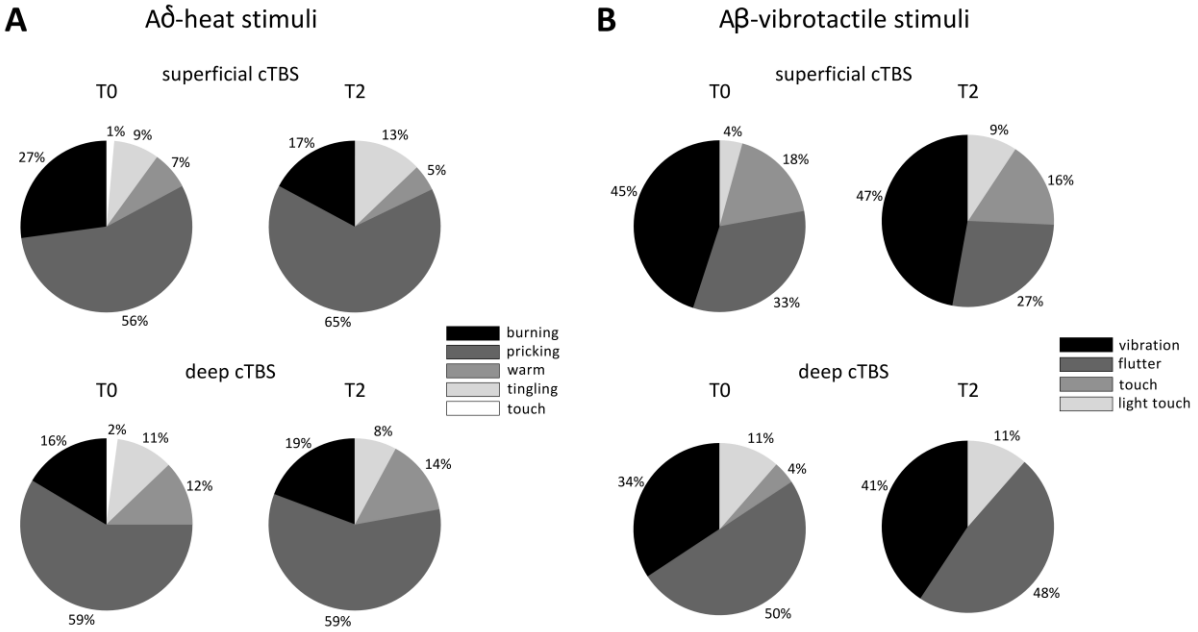
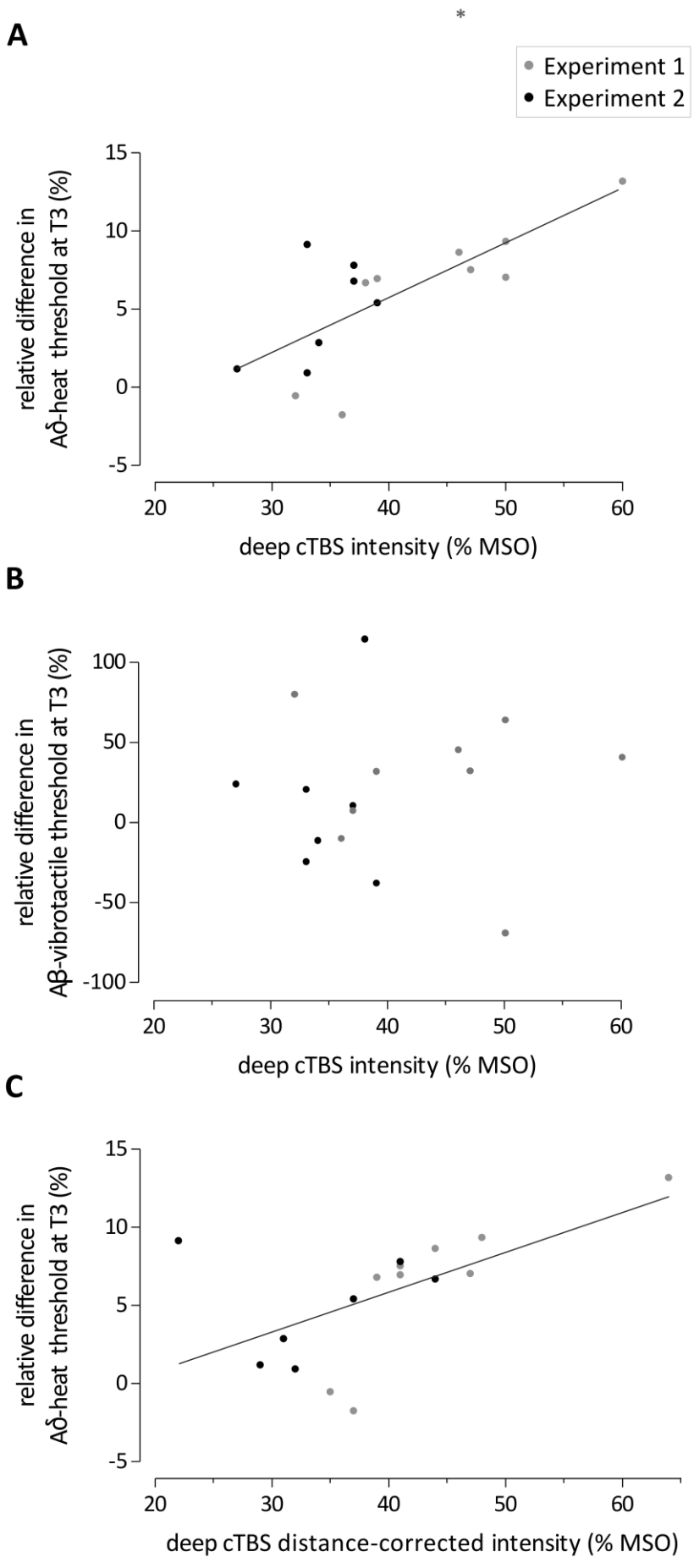


Figure 10

Relationship between the effect of deep cTBS on thresholds and intensity of cTBS pulses. **A**. Linear regression for all the participants (Experiment 1 and 2) between the intensity of cTBS pulses used during deep cTBS of the operculo-insular cortex and the relative difference in Aδ-heat threshold 20 minutes after cTBS (T3-T0). The increase of Aδ-heat threshold was significantly positively correlated with the intensity of cTBS pulses (r=.733; n=16; p=.001; one observation, indicated by *, was excluded due to a standardized residual greater than three standard deviations; when all participants were included: r=.613; n=17; p=.009). **B**. Linear regression for all the participants (Experiment 1 and 2) between the intensity of cTBS pulses used during deep cTBS of the operculo-insular cortex and the relative difference in Aβ-vibrotactile threshold 20 minutes after cTBS (T3-T0). There was no significant correlation (r= .110; n= 17; p= .676). **C**. Linear regression for all the participants (Experiment 1 and 2) between the intensity of deep cTBS pulses, corrected for the coil – cortical target distance, used during deep cTBS of the operculo-insular cortex and the relative difference in Aδ-heat threshold 20 minutes after cTBS (T3-T0). The increase of Aδ-heat threshold was significantly positively correlated with the intensity of cTBS pulses (r=.592; n=16; p=.016).



Cédric Lenoir and Maxime Algoet are both currently finalizing their PhD at the Institute of Neuroscience of UCLouvain, under the supervision of André Mouraux. The work of Cédric Lenoir focuses primarily on the central and peripheral mechanisms involved in human nociception in normal conditions and during sensitization. The research conducted by Maxime Algoet has aimed at characterising spinal and supraspinal pain-motor interactions in humans. Both are also studying the changes in cortical excitability and brain connectivity related to chronic pain by combining functional neuroimaging techniques with non-invasive transcranial magnetic stimulation in order to isolate pain-related changes in brain function.