Changes in emotional processing following interoceptive network stimulation with rTMS

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Abstract—Theories of emotion suggest a close relation of interoception and emotion. However, knowledge of underlying neuronal networks is still sparse. Repetitive transcranial magnetic stimulation (rTMS) is one neurostimulation method allowing causal conclusions between functions and brain regions via stimulation or inhibition of underlying brain structures. In this study, rTMS with a continuous theta burst stimulation (cTBS) protocol was used aiming for inhibition of important interoceptive network structures (frontotemporal insular network and right somatosensory cortices). Stimulation effects were investigated on interoceptive accuracy (IAc), emotional evaluation and neuronal correlates of emotional picture processing in 18 male participants. The main findings were an emotional flattening in subjective valences for affective stimuli after inhibition of the frontotemporal anterior insular network and of somatosensory cortices, being mirrored in visual evoked potentials as increased N2/decreased P3, indicating an initial orientation reaction followed by decreased attentional processing of positive stimuli. Moreover, cardiac and respiratory IAc were positively associated with P3 amplitudes and negatively related to positive valence ratings. Positive associations of decreases of cardiac/respiratory IAc with decreases of arousal ratings and decreases of P3 amplitudes for negative stimuli after inhibition of the frontotemporal insular network and after inhibition of somatosensory cortices allow the conclusion of a causal relationship between reduced activity in interoceptive network structures and blunted emotional processing of visual stimuli. Our results suggest that both arousal, and valence aspects of emotional processing are disturbed after inhibition of interoceptive network structures, confirming core assumptions of peripheral theories of emotions and models of interoceptive predictive coding. © 2019 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: transcranial magnetic stimulation, interoception, emotions, insular cortex, somatosensory cortices, visual evoked potentials.

INTRODUCTION

Investigation of emotion processing has increased significantly in recent years, especially considering the field of cognitive neurosciences. The perception of signals of the internal state of the body (interoception) (Vaitl, 1996; Cameron, 2001) is assumed to be related to different components of emotion processing (Garfinkel et al., 2017). First brain imaging studies suggest an overlap of the interoceptive and the emotional neural network, including pons, somatosensory cortices, insula, caudate nucleus as well as anterior and posterior cingulate cortices (Critchley et al., 2001; Critchley et al., 2004; Harrison et al., 2010; Terasawa et al., 2013). These studies indicate the relevance of the (right) anterior insula as a structure of central representation of bodily states providing a neural substrate of feelings (Critchley et al., 2004; Harrison et al., 2010). A primary representation of bodily states in the insula is postulated being accessible to consciousness as subjective feelings (Critchley et al., 2004).

Interoception can be measured via different modalities, e.g. the cardiac, the respiratory or the gastrointestinal system (Critchley et al., 2004; Pollatos et al., 2005a; Herbert et al., 2012; Vlmincx et al., 2015; Schrojen et al., 2016). Moreover, different dimensions of interoception exist, one of which is interoceptive accuracy (IAc) as an objective measure assessable via behavioral tasks, for example the heartbeat perception task (Garfinkel and Critchley, 2013).
One theory describing the interaction of emotion and interoception is Damasio’s ‘Somatic Marker Theory’ (Damasio et al., 1996; Damasio, 1999; Damasio et al., 2000) postulating that physiological changes arise as a response to emotional events. According to this theory, somatic markers control emotion processing as well as decision making (Damasio, 1998; Blair and Cipolotti, 2000; Bechara, 2004; Bechara and Naqvi, 2004; Bechara et al., 2005). Damasio and colleagues (Damasio, 1999; Bechara et al., 2000; Bechara, 2004) postulate that the insular or somatosensory cortices map visceral states and events, which are related to emotions in the form of neural patterns. Those brain regions are part of an interoceptive neural network (Pollatos et al., 2007; Craig, 2009). Feelings are postulated to be evoked as a result of an interplay of first-order structures (insula, somatosensory cortices), mapping the organism’s visceral reactions, and second-order structures (cingulate, thalamus, hypothalamus, forebrain, brainstem nuclei), representing the pattern of the organism (Damasio et al., 2000). Several studies describe a positive relation between interoceptive accuracy/sensibility and the experienced intensity of emotions (arousal), being mirrored in physiological indicators of emotion processing (Wiens et al., 2000; Pollatos et al., 2005b; Herbert et al., 2007; Pollatos et al., 2007; Dunn et al., 2010; Terasawa et al., 2012). Also, methods of neuroimaging showed an activity of somatosensory and insular structures in processing emotion (Damasio et al., 2000; Craig, 2002; Rolls et al., 2003).

Neuronal models such as the multi-hierarchical model of embodied emotion, proposed by Smith and Lane (Smith and Lane, 2015) further support the idea of an association of interoception and emotion. The authors describe a body perception hierarchy, which can be contemplated as an emotion perception hierarchy. Physiological changes are conveyed as ‘moment-to-moment body state changes’ in afferent signals through different brain regions (Smith and Lane 2015). Representations of ‘whole-body patterns’ can be interpreted as bodily feelings when they are conscious (Critchley et al., 2004; Craig, 2009; Medford and Critchley, 2010). Not least, predictive coding models (e.g. Seth et al., 2012) illustrate the close relation between interoception and emotion. Predictive coding is a favored theory for explaining neuronal messaging among different cortical levels referring to the comparison of top-down predictions with bottom-up predicted error signals (Friston, 2010; Seth et al., 2012). Minimization of prediction errors is associated with efficient perception, cognition and behavior (Seth et al., 2012). Concerning interoception, predictive coding suggests that predictions about interoceptive bodily states determine emotion perception (Seth et al., 2012; Seth, 2013). The brainstem, the anterior insular cortex (AIC) and the anterior cingulate cortex (ACC) are described as relevant brain regions for interoceptive inference, and they are also involved in emotion processing (Seth et al., 2012).

Only a few studies examined the role of neural interoceptive networks in the processing of emotional stimuli. The human brain is susceptible to emotional stimuli. Several studies using electroencephalography (EEG) showed increased attention processes to emotional stimuli indicated by altered event-related potentials (ERPs; e.g. LPP, P1, P2, P3) (Delplanque et al., 2004; Delplanque et al., 2005; Delplanque et al., 2006). Moreover, in response to affective stimuli a higher arousal of the autonomic nervous system can be expected, being observable as an increased cardiac reactivity (Herbert et al., 2010), an enhanced skin conductance (Hayes and Northoff, 2011) or an enlargement of the pupils (Partala and Surakka, 2003). As studies with imaging techniques revealed, processing of affective stimuli is often related to increased activation in brain regions such as the amygdala, the insular cortex or the secondary somatosensory cortex, being regarded as crucial areas of interoceptive mechanisms (Phan et al., 2002; Anders et al., 2004; Becker et al., 2009; Straube and Mittner, 2011; van der Laan et al., 2011). Straube and Mittner (2011) suggest that these brain areas are associated with a person’s awareness of his/her own body by having shown that confrontation with emotional pictures led to an enhanced activation in these regions, in particular when the pictures were supposed to be evaluated regarding personal emotional involvement. Craig (2011) postulated the insula as part of an ascending pathway of bodily sensations whose activation is associated with subjective sensations of the body and with emotional experience. Simmons and colleagues (2004) revealed that even an anticipation of an emotional aversive stimulus led to an enhanced activation of the right insula.

Taking these aspects into consideration previous research showed that anatomic structures of the interoceptive neural network are involved in emotion processing. However, there is no study known to the authors so far that has investigated whether transcranial magnetic stimulation (TMS) might reveal the connection of critical neural structures being both involved in interoceptive and emotion processing. TMS is a neurostimulation technique allowing causal conclusions between functions and brain structures (Sack, 2006; Wagner et al., 2009; Casula et al., 2013). The non-invasive tool allows manipulating brain activity by a transitory disruption of stimulated brain areas (Sack, 2006). In the current study, we examined whether the inhibition of interoceptive networks by TMS would lead to a different perception of affective stimuli. We applied cTBS which has been shown to inhibit activity in the stimulated cortical area (Huang et al., 2005; Huang and Mouraux, 2015). As a neuroimaging technique, we assessed ERPs to reveal concomitant temporal processes of evaluation and attention (Casula et al., 2013). We investigated the N2, a negative deflection with a peak of 240ms, as an index of an orienting response towards novel or deviant stimuli (Campanella et al., 2002; Carretié et al., 2004; Gramann et al., 2007). The P3, showing highest peaks between 300 and 500ms, is sensitive to emotional stimuli, being modulated by valence, with higher amplitudes towards both unpleasant and pleasant stimuli, as compared to neutral stimuli (Lifshitz, 1966; Johnston et al., 1986; Mini et al., 1996) and being affected by arousal with enhanced positivity towards pleasant and unpleasant stimuli (Keil et al., 2002). It is assumed to underlie processes of attentional allocation (Polich, 2007). With regard to interoception, previous studies using emotional pictures showed a higher P3 for individuals with high IAc for positive and negative stimuli (Herbert et al., 2007; Pollatos et al., 2007) as well as a more intense processing (higher arousal) of affective...
stimuli, whereas no effects were shown for valences (Herbert et al., 2007; Pollatos et al., 2007).

In the first part of this study, our research group showed that cardiac and respiratory interoceptive accuracy, perception confidence of interoception as well as a neural correlate of interoception (heartbeat-evoked potential, HEP) were reduced following cTBS over the anterior insula and over somatosensory cortices, suggesting cTBS as a useful tool to investigate the interoceptive neural network (Pollatos et al., 2016). In addition, the study detected effects on emotional evaluation, as subjects stated increased levels of anxiety after cTBS of the insula (Pollatos et al., 2016). The current study reports results from the second part of the study from Pollatos and colleagues (Pollatos et al., 2016), including 18 healthy male participants who received either cTBS over the frontotemporal right anterior insular network or at right somatosensory cortices and who were sham stimulated over occipital areas. Stimulation was followed by an interoceptive and an emotional task, requiring subjective evaluation of an IAPS picture set (Lang et al., 1999). Based on results of Pollatos and co-workers (Pollatos et al., 2016) and in the light of the postulated association between interoceptive and emotional brain networks (Critchley et al., 2001; Critchley et al., 2004; Harrison et al., 2010) we assumed that inhibition of interoceptive networks using cTBS would affect emotion processing. We hypothesized that cTBS over somatosensory cortices and at the frontotemporal insular network would disturb emotional evaluation, which should result in more positive subjective valence ratings and lower arousal ratings of negative stimuli after stimulation of the frontotemporal insular network/somatosensory cortices compared to the occipital control region (hypothesis Ia). Moreover, emotional attenuation should be reflected in more negative valence ratings and lower arousal ratings of positive stimuli after stimulation of the frontotemporal insular network/somatosensory cortices compared to the occipital control region (hypothesis Ib). In addition, we expected this emotional network disturbance to be mirrored on a neuronal level by an orienting reaction compared to the occipital control region (hypothesis Ib). In the postulated association between interoception and emotion, we expected decreases of cardiac/respiratory IAc to be positively associated with more negative valences and lower arousal scores for positive stimuli. Also, decreases of cardiac/respiratory IAc should be associated with more positive valences and decreases of arousal for negative stimuli – at the frontotemporal insular network/somatosensory cortices (hypothesis IVa). Besides, we expected that decreases of cardiac/respiratory IAc would be positively associated with decreases of P3 amplitudes for both emotional categories at the frontotemporal insular network/somatosensory cortices (hypothesis IVb).

**EXPERIMENTAL PROCEDURES**

The method part of the current study is described by Pollatos and colleagues (Pollatos et al., 2016) in a first publication of the study. We refer the reader to this publication for further details.

**Participants**

We recruited eighteen healthy male students by advertisement at Ulm University. Their mean age was 23.6 years (SD = 2.8). Subjects gave written informed consent previous to study participation and received a recompense of €60. We determined the following exclusion criteria for the study: (history of) brain injury, history of/current psychiatric or neurological disorders, physical illness or medication, left-handedness. We conducted the study in agreement with the Declaration of Helsinki with the consent, approved by the ethics committee of Ulm University.

**Stimuli**

We created three full picture sets, each consisting of 10 positive, 20 neutral and 20 negative IAPS pictures (Lang et al., 1999) as well as of 10 high caloric food pictures (Blechert et al., 2014) so that in total 180 stimuli were shown to each participant. The category of positive pictures was composed of food pictures and of pleasant IAPS to represent a higher variety of positive images. Positive and negative IAPS involved humans, animals, objects and scenes, e.g., pictures of babies, butterflies; scenes of poverty, mugging; and food pics involved high caloric food and drinks, e.g. beer and burger. All picture sets were created to be equivalent in color and complexity. Moreover each category was matched for the three picture sets for valence and arousal considering available norms (Lang et al., 1999; Blechert et al., 2014).

**Procedure**

In a screening, the experimenters informed the participants about the study. They were inspected for criteria of exclusion and were advised about side effects of cTBS. 18 out of 22 screened participants took part in the experiment. The main experiment consisted of three sessions which all took place in a sound-protected booth, which allowed to record EEG (32 passive electrodes), ECG, respiration (belt), electromyography (EMG, first dorsal intersosseous) and EOG. We assessed the individual resting motor threshold only at the...
first session by stimulation of the left primary motor cortex (Power Mag 100 stimulator, Mag & More, Munich, Germany). We determined individual stimulation intensity for the main experiment at 80% of the resting motor threshold (cf. Vallesi et al., 2006). All three sessions were equal (see Fig. 1 for the experimental procedure), only the stimulation area for cTBS was varied (right frontotemporal insular network, right somatosensory cortices, central occipital cortex as a sham condition). We assigned participants in random order to the stimulation conditions, whereby every participant was stimulated at all three locations and evaluated all three picture sets, using a repeated measurement design.

Coil positions for stimulation were located using the EEG electrode positions of the 10/20 system. Ideal coil positions were derived once by TMS neuronavigation (Power Mag View) using a canonical structural MRI scan of one individual. The right frontotemporal insular network was targeted with a coil position over frontotemporal regions, building a 3-sided figure of the EEG positions FT10, F8, FC6, pointing at FT8 (F6/AF8). We targeted primary and secondary somatosensory cortices (chest/trunk location) with the coil building a triangle of CPZ, C2, and CP4, pointing to CZ. We targeted the central occipital cortex (V2/V3) by placing the coil over OZ, oriented to the horizontal plane (see Fig. 2).

At the screening session participants filled in a demographic questionnaire, the Edinburgh Handedness Inventory (Oldfield, 1971), the Beck Depression Inventory, BDI-II (Beck et al., 1996), the Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger, 2010) and the FFKA to assess daily physical activity (Frey et al., 1999). The procedure of the experiment was the following: At the beginning of each session participants filled in the state version of the STAI. Moreover, they were asked to fill in the short version of the Profile of Mood States (Bullinger et al., 1990) as an index of current feelings and mood during the last 24 hours. In the meantime, EEG and ECG electrodes were placed. Subsequently, the participants were stimulated for a duration of 40s with 600 cTBS pulses (one burst was comprised of three pulses at 50 Hz with a frequency of 5 Hz/one pulse every 200ms) at one of the three randomly selected locations. Following stimulation, participants first took part in the interoceptive tests (Pollatos et al., 2016). For assessment of cardiac IAc a heartbeat detection task comprising four counting phases (Schan dry, 1981; Pollatos and Schandry, 2004) was performed, during which ECG and EEG were recorded. We assessed respiratory IAc with a respiratory load estimation task where respiratory effort was manipulated through variance of respiratory resistances. Participants had to judge respiratory effort (Petersen et al., 2014; Petersen et al., 2015; Pollatos et al., 2016). Afterwards, participants watched one of three randomly selected picture sets. All images within one picture set were shown in random order to the participant at the computer screen. Every picture appeared for 1s on the screen. Afterwards, participants were asked to indicate their subjective valence and arousal ratings using 9-point SAM scales (Bradley and Lang, 1994) with the anchors ‘very unpleasant’ and ‘very pleasant’ for valence and ‘not aroused at all’ as well as ‘very aroused’ for arousal. The first session had a duration of two hours in total, whereas the second and third sessions lasted 90 minutes.

### Psychophysiological recording

During emotional picture viewing EEG activity was continuously recorded from 32 leads (FP1, FPZ, FP2, AF3, AFZ, AF4, F7, F5, Fz, F6, F8, FC5, FCZ, FC6, C5, C1, CZ, C2, C6, CP5, CP1, CPZ, CP2, CP6, P5, PZ, P6, PO3, PO4, O1, OZ, O2) with the Easy-Cap electrode system (Brain Products, Germany), using non-polarizable passive Ag/AgCl electrodes being placed at equidistant positions. The reference electrode was Cz, horizontal and vertical EOG was recorded. Impedance was controlled to be below five kΩ. We used an active amplifier system (Brain Products, Germany). We digitized signals with a sampling rate of 1000 Hz. Electrodes were mounted after TMS threshold measurement and kept for the whole experiment. We placed the TMS coil on top of the electrode cap.

### Data analysis

Cardiac IAc was calculated using the following transformation: \( \frac{1}{4} \sum [1 - (\text{recorded heartbeats} - \text{counted heartbeats})] / \text{recorded heartbeats} \). Scores typically range between 0 and 1, whereas higher scores...
indicate better IAc (Pollatos et al., 2016). Respiratory IAc was calculated using the following transformation: $1 - \frac{\sum_{k=1}^{e} |\text{estimated resistance} - \text{objective resistance}|}{2625}$ (mean difference with random distribution of errors)]. EEG data were analyzed with Brain Vision Analyzer 2.1 (Brain Products, Germany). Following visual inspection, EEG data were filtered (0.05-20 Hz) and examined for artifacts. As a criterion for automatic artifact rejection, the exceeding of voltage levels of ± 50 μVms⁻¹ was defined. Data were segmented for each emotional category building segments with lengths of 1000ms. Segments ranged from 200ms before the emotional trigger to 800ms after the trigger. Data were baseline corrected (200 ms pre emotional stimulus as a baseline), and averages of all segments were calculated for each emotional category. The selection of time frames referred to previous literature (Fein et al., 1995; Gramann et al., 2007), and to visual inspection, whereby a time window of 150-215 ms post-stimulus for the N2 and a time frame of 230-330 ms for the P3 were selected. For further analysis the following electrode clusters were built based on the criteria of previous research (Polich and Comerchero, 2003; Donkers and Van Boxtel, 2004; Polich, 2007) and resting upon visual inspection of highest amplitudes: Frontotemporal (FPz, FP2, F8, AF3), frntocentral (FCz, FC5) and centroparietal (C2, CPz, CP2).

We conducted statistical analysis with SPSS (v. 24). Demographic and questionnaire data were analyzed descriptively. t-Tests were performed to calculate differences between mean questionnaire scales. For the subjective ratings of valence and arousal separate repeated-measures ANOVAs were calculated with the factor location (frontotemporal insular network, somatosensory cortices, occipital cortex) and emotion category (positive, negative, neutral). For the EEG data of N2 and P3 mean amplitudes we performed separate repeated-measures ANOVAs for negative, positive and neutral pictures with the factors location (frontotemporal insular network, somatosensory cortices, occipital cortex) and cluster (frontoparietal, frontocentral, centro-parietal). Separate repeated measures ANOVAs were also calculated for cardiac/respiratory IAc with the factor location (frontotemporal insular network, somatosensory cortices, occipital cortex). The normal distribution of the data was investigated using the Kolmogorov-Smirnov test (Field, 2009). We calculated changes of IAc/valence/arousal and N2/P3 amplitudes as deltas of the occipital condition and each experimental condition (frontotemporal insular network, somatosensory cortices). Separate correlation analyses reporting Pearson’s r or Spearman-Rho correlation coefficients ($r_s$) (Field, 2009) were conducted to explore associations between $\Delta$ of IAc and $\Delta$ of emotional ratings/ERP amplitudes. $\Delta$ were calculated as the absolute values of occipital – somatosensory or occipital – frontotemporal insular network stimulation. Reported statistical levels of significance correspond to $P$ values smaller than .05, .01 and .001. In the results, we report uncorrected F-values and Greenhouse-Geisser epsilon values as well as corrected degrees of freedom. For the execution of multiple comparisons, we adjusted $\alpha$ levels, and we reported Bonferroni corrected values.

RESULTS

Demographic and questionnaire data

Table 1 illustrates demographic variables as well as questionnaire data. Due to uncompleted questionnaires we had some data loss in the questionnaire data. The sample did not show clinically relevant values for depression [BDI < 13, Beck et al., 1996], but a heightened score of trait anxiety [STAI trait P67, Laux, 1981]. The BMI was within a normal range [BMI < 25, World Health Organization, 2000]. Referring to the values and percentiles of a representative sample for the POMS (Grulke et al., 2006) our sample showed high scores on all scales. The fitness level of our sample was within a high range (Frey and Berg, 2002). Questionnaire scores of state anxiety (STAI) and mood (POMS) did not differ between the three stimulation conditions ($P > .05$).

Interoceptive accuracy

The following results are a repeated presentation of the results of Pollatos and colleagues, reported in their article ‘Changes in interoceptive processes following brain stimulation’ (Pollatos et al., 2016), they are not a replication of the previous findings: A significant main effect for cardiac IAc was observed for location $[F(2, 34) = 6.67, P < .01, \eta^2_p = .30]$. Post hoc tests revealed significantly lower cardiac IAc following stimulation.
was assessed \([F(4, 68) = 5.54, P = .001, \eta^2_p = .246]\). With regard to positive images, post hoc tests revealed significantly more negative valences for the frontotemporal insular network compared to occipital stimulation \([P = .016, d = .50]\) as well as more negative valences for the frontotemporal insular network compared to somatosensory stimulation \([P = .008, d = .50]\). Regarding negative images, post hoc tests revealed more positive valences for the frontotemporal insular network compared to occipital stimulation \([P = .020, d = .53]\) and more positive valences for somatosensory stimulation compared to occipital stimulation \([P = .043, d = .44]\). For neutral images, post hoc tests revealed no significant differences for valences between stimulation locations. The ANOVA for arousal ratings showed a significant main effect for emotion category \([F(2, 34) = 32.57, P < .000, \eta^2_p = .66]\). Post hoc tests revealed that negative images were rated with higher arousal compared to positive images \([P = .006, d = .82]\). Furthermore, both positive and negative images were rated with higher arousal compared to neutral images \([P < .000, d = 1.44\); neg. vs. neut.: \(P < .000, d = 1.93\)]

No other effects were detected for arousal ratings. In summary, effects of more negative valences for positive pictures and more positive valences for negative pictures were detected at both stimulation locations, at the frontotemporal insular network and at the somatosensory cortices, whereas no effects for arousal ratings appeared.

**ERP data**

Due to poor data quality, EEG segments had to be excluded from further analyses, with remaining EEG data sets from 16 participants for the calculation of grand averages for each stimulation location (see Table 2). Fig. 5 illustrates mean ERPs comparing the frontotemporal insular network, somatosensory cortices and the occipital cortex for affective stimuli.

**N2**

The ANOVA for N2 averages for positive pictures revealed a significant main effect for the factor location \([F(2,22) = 4.97, P = .017, \eta^2_p = .31]\). Post hoc tests revealed higher mean negative amplitudes at stimulation of somatosensory cortices as compared to stimulation of the frontotemporal insular network \([P = .003, d = .60]\). Significant higher negative amplitudes were also detected at somatosensory cortices compared to the occipital cortex \([P = .027, d = .54]\). Moreover, a significant main effect for cluster appeared \([F(2,22) = 8.05, P = .002, \eta^2_p = .42]\). Post hoc tests showed that N2 had significantly higher negative amplitudes for the frontocentral cluster compared to the frontoparietal cluster \([P = .001, d = 1.35]\). In addition, we assessed higher negative amplitudes over the centroparietal cluster compared to the frontoparietal cluster \([P = .003, d = 1.53]\).

The ANOVA for N2 for negative pictures showed a significant main effect for cluster \([F(2,22) = 8.89, P = .001, \eta^2_p = .45]\). Post hoc tests revealed higher negative N2 amplitudes over frontocentral compared to frontoparietal electrodes \([P = .007, d = 1.17]\). Moreover, higher negative amplitudes were found over the centroparietal cluster compared to the frontoparietal cluster \([P = .002, d = 1.66]\).

### Table 1. Demographic and questionnaire data of the sample. M = Mean. SD = Standard deviation. P = Percentile. FFKA = Freiburger Fragebogen zur Körperlichen Aktivität-Kurzform/short version of questionnaire for physical activity. BDI = Beck Depression Inventory-2. STAI = State-Trait anxiety inventory. POMS = Profile of mood states.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>M (SD)</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>Demographic Data</strong></td>
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<tr>
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<tr>
<td>BDI-2</td>
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<tr>
<td>State</td>
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<tr>
<td>Occipital Cortex State</td>
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<tr>
<td><strong>Frontotemporal insular network</strong></td>
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<tr>
<td>State</td>
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<td>34.73 (7.91)</td>
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<td><strong>Screening</strong></td>
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<td><strong>POMS</strong></td>
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<tr>
<td>Depression</td>
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<td>20.87 (5.93)</td>
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<tr>
<td>Vigor</td>
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<td>30.20 (7.99)</td>
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<tr>
<td>Fatigue</td>
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<td>16.50 (7.52)</td>
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<tr>
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<td>Anger</td>
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The ANOVA for N2 for neutral pictures also revealed a significant main effect for cluster \( F(2,22) = 8.80, P = .002, \eta^2_p = .44 \). Post hoc tests showed higher negative amplitudes over centroparietal compared to frontoparietal locations \( P = .001, d = 1.87 \).

Neither the ANOVA for N2 for negative pictures nor the ANOVA for neutral pictures did show any significant main effect for location \( P > .05 \).

P3

The ANOVA for P3 for positive pictures revealed a significant main effect for location \( F(2,22) = 8.94, P = .001, \eta^2_p = .45 \). Post hoc tests showed higher positive amplitudes for stimulation of the frontotemporal insular network compared to somatosensory cortices \( P < .000, d = 1.02 \). Moreover, we assessed higher positive amplitudes at stimulation of the occipital cortex compared to stimulation of somatosensory cortices \( P = .013, d = .92 \).

The ANOVA for negative pictures did not show any significant effects \( P > .05 \).

The ANOVA for neutral pictures showed a significant main effect for cluster \( F(1.30, 14.30) = 7.42, P = .012, \eta^2_p = .40 \). Post hoc tests revealed higher P3 amplitudes over frontoparietal compared to centroparietal electrodes \( P = .001, d = 1.44 \). Moreover, higher P3 amplitudes were assessed over frontocentral compared to centroparietal locations \( P = .026, d = 1.14 \).

In summary, ERP data showed heightened N2 amplitudes and reduced P3 amplitudes for positive images at the stimulation location somatosensory cortices.

Association between measures of IAc and behavioral and neuronal measures of emotion processing

At stimulation of the frontotemporal anterior insular network respiratory IAc was correlated negatively with valence ratings for positive stimuli \( r_S = -.502, P = .034 \). For stimulation of the frontotemporal anterior insular, network respiratory IAc was positively correlated with P3 amplitudes for negative \( r_S = .534, P = .027 \) and positive stimuli \( r_S = .573, P = .016 \) at centroparietal electrodes. No other correlations were detected \( P > .05 \).

For stimulation of somatosensory cortices, no correlations between IAc and emotional ratings were revealed \( P > .05 \). However, cardiac IAc was positively associated with P3 amplitudes for negative \( r = .536, P = .032 \) and positive stimuli \( r = .599, P = .014 \) at centroparietal electrodes.

To sum up, IAc was negatively correlated with valences for positive stimuli and positively correlated with P3 amplitudes for negative and positive stimuli.

Association between changes of measures of IAc and changes in measures of behavioral and neuronal emotion processing

Decreases in cardiac IAc for the frontotemporal anterior insular network compared to occipital stimulation were positively associated with decreases in arousal ratings for negative pictures \( r_S = .056, P = .32 \). We revealed
no correlations between changes in cardiac/respiratory IAc and changes in P3 amplitudes. For stimulation of somatosensory cortices, no associations were revealed between changes of IAc and changes of valences/arousal ($P > .05$). However, decreases in cardiac IAc were associated with decreased amplitudes of P3 for negative stimuli ($r_S = .565, P = .044$).

To sum up, decreases in IAc were positively associated with decreases in arousal/P3 amplitudes for negative pictures.

### DISCUSSION

This study aimed to demonstrate the possibility to affect emotional processing via inhibition of interoceptive networks (frontotemporal anterior insular network, somatosensory cortices), using rTMS with a cTBS protocol. Our findings indicate disturbances of emotional evaluation following cTBS, being observable as emotional flattening in subjective valences at the frontotemporal insular network and somatosensory cortices and being detectable in EEG data as an initial orientation reaction followed by decreased attentional processing of positive stimuli, appearing only at somatosensory cortices. Moreover, cardiac and respiratory IAc were positively associated with P3 amplitudes and negatively related to positive valence ratings. As decreases of IAc were positively associated with decreases of arousal ratings and decreases of P3 amplitudes for negative stimuli, we conclude a causal relationship between inhibition of interoceptive network activity, IAc, and emotional processing.

### Study Aim I: Investigation of the influence of cTBS to interoceptive network on subjective evaluation of emotional stimuli

Following hypothesis Ia, participants indicated more positive valence ratings for negative pictures at stimulation of the frontotemporal anterior insular network and somatosensory cortices compared to stimulation of the occipital cortex. Moreover, as assumed in hypothesis Ib more negative subjective valence ratings were indicated for positive stimuli at stimulation of the frontotemporal anterior insular network compared to somatosensory/occipital stimulation. Regarding somatosensory cortices (hypothesis Ib), more negative valences could not be confirmed for positive stimuli. Subjective arousal was not different between stimulation conditions for neither negative nor for positive stimuli, refusing hypothesis Ia and Ib for effects of arousal. We interpret our results regarding a disturbance of emotion processing, manifested in emotional flattening for both positive and negative stimuli, which are all evaluated as more neutral. Those effects of

#### Table 2. Mean averaged ERPs (N2/P3) related to emotional categories and stimulation conditions for $N = 16$ participants. The indicated unit for ERPs is $\mu V$.

<table>
<thead>
<tr>
<th></th>
<th>Occipital M (SD)</th>
<th>Somatosensory M (SD)</th>
<th>Frontotemporal insular network M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontoparietal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0.31 (1.07)</td>
<td>-0.22 (0.96)</td>
<td>0.02 (0.57)</td>
</tr>
<tr>
<td>Neutral</td>
<td>0.03 (0.65)</td>
<td>-0.23 (1.13)</td>
<td>0.15 (0.73)</td>
</tr>
<tr>
<td>Negative</td>
<td>0.17 (0.77)</td>
<td>0.02 (0.57)</td>
<td>0.05 (0.54)</td>
</tr>
<tr>
<td>Frontocentral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>-2.98 (4.02)</td>
<td>-5.15 (4.32)</td>
<td>-2.79 (4.29)</td>
</tr>
<tr>
<td>Neutral</td>
<td>-1.34 (5.11)</td>
<td>-2.76 (3.33)</td>
<td>-2.14 (2.95)</td>
</tr>
<tr>
<td>Negative</td>
<td>-1.80 (5.23)</td>
<td>-2.73 (3.04)</td>
<td>-2.66 (3.50)</td>
</tr>
<tr>
<td>Centroparietal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>-3.76 (4.58)</td>
<td>-5.98 (5.45)</td>
<td>-3.94 (3.13)</td>
</tr>
<tr>
<td>Neutral</td>
<td>-3.66 (3.59)</td>
<td>-4.85 (3.90)</td>
<td>-3.64 (4.81)</td>
</tr>
<tr>
<td>Negative</td>
<td>-2.94 (4.09)</td>
<td>-4.40 (4.51)</td>
<td>-3.86 (3.50)</td>
</tr>
<tr>
<td><strong>P3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontoparietal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1.36 (1.74)</td>
<td>0.36 (1.78)</td>
<td>1.22 (0.74)</td>
</tr>
<tr>
<td>Neutral</td>
<td>1.09 (1.21)</td>
<td>0.68 (0.86)</td>
<td>1.24 (0.74)</td>
</tr>
<tr>
<td>Negative</td>
<td>1.47 (1.23)</td>
<td>0.59 (1.14)</td>
<td>1.13 (0.85)</td>
</tr>
<tr>
<td>Frontocentral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0.43 (5.15)</td>
<td>-4.90 (5.45)</td>
<td>0.02 (6.38)</td>
</tr>
<tr>
<td>Neutral</td>
<td>1.17 (3.91)</td>
<td>0.39 (4.70)</td>
<td>-0.47 (4.46)</td>
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<tr>
<td>Negative</td>
<td>1.57 (4.74)</td>
<td>-1.01 (5.09)</td>
<td>0.12 (5.45)</td>
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<tr>
<td>Centroparietal</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>-0.24 (5.51)</td>
<td>-4.20 (6.26)</td>
<td>-1.70 (3.51)</td>
</tr>
<tr>
<td>Neutral</td>
<td>-1.86 (5.14)</td>
<td>-3.32 (3.31)</td>
<td>-2.20 (3.82)</td>
</tr>
<tr>
<td>Negative</td>
<td>-0.07 (3.97)</td>
<td>-2.82 (4.98)</td>
<td>-1.09 (4.04)</td>
</tr>
</tbody>
</table>

#### Fig. 5. Grand averages of visual evoked potentials in $\mu V$ ($N = 16$) at the frontocentral electrode cluster for positive, neutral and negative pictures for the occipital location, the frontotemporal insular network and for somatosensory cortices.

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\(\mu V\) positive

\(\mu V\) neutral

\(\mu V\) negative

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Occipital cortex

Frontotemporal insular network

Somatosensory cortices
emotional flattening were detectable at stimulation of the frontotemporal insular network for both emotional valences, whereas at somatosensory cortices an attenuation was only observable for negative valences. The emotional neutralization might be a consequence of inhibition/disturbance of typical functions of the insular cortex, e.g., evaluation of an emotional reaction (Phan et al., 2002; Anders et al., 2004; Caria et al., 2010; Straube and Miltnner, 2011). Referring to the theory of Smith and Lane (2015) one can assume a disturbed integration of ‘whole body patterns’ within the insula which might have activated different emotion categories and might, therefore, explain contrary emotional evaluations towards a more neutral direction. In the light of interoceptive predictive coding models (Seth et al., 2012), one might assume that cTBS of the frontotemporal anterior insular network might have disturbed the process of integration of top-down interoceptive predictions with bottom-up interoceptive prediction errors, and thereby might have affected the perception of emotions (Seth, 2013). Due to decreased IAC in our study, one might assume that reduced interoceptive input from bodily signals provoked by cTBS at the frontotemporal anterior insular network, elicited interoceptive prediction errors, which might have induced a conflict between predicted and current interoceptive signals. This process might have reduced salience of emotional stimuli and might have resulted in reduced subjective ratings of emotional stimuli (Seth, 2013).

As described by Seth and colleagues (Seth, 2013), the anterior insula is a crucial structure being responsive to interoceptive prediction errors by directing signals to visceromotor processes and by guiding awareness to emotional states. Several MRI studies support the processing of anticipatory responses and prediction error signals in the anterior insula (Seth, 2013), being activated by e.g. anticipation and prediction of pain and other unpleasant sensations (Ploghaus et al., 1999; Seymour et al., 2004; Holle et al., 2012). Gray and colleagues (2007) observed an interoceptive network disturbance by showing enhanced arousal ratings of initially neutral stimuli following false cardiac feedback, whereby enhanced insula activity during wrong feedback correlated with the heightened emotional intensity of neutral stimuli. These findings would be in keeping with our findings of opposite emotional reactions after inhibition of the frontotemporal insular network with cTBS.

For somatosensory cortices we had to reject hypothesis Ib, as no emotional attenuation appeared for positive valences, however flattening of negative valences was observed. Different emotion theories postulate the involvement of somatosensory areas in the processing of affective stimuli (James, 1884; Damasio, 1999). Our findings are in line with previous fMRI studies showing heightened activity at interoceptive structures (somatosensory cortices, insula) for aversive as compared to neutral stimuli (Straube and Miltnner, 2011). Activity specifically increased in primary and secondary somatosensory cortices and in the insula with a heightened attentional focus of participants on their own emotions (Straube and Miltnner, 2011). Our picture rating task also asked participants to evaluate the emotional stimuli related to their subjective emotional involvement, as we instructed ‘How negative or positive did you feel while watching the picture’ or ‘How aroused have you been while watching the picture?’. One explanation of our findings at somatosensory cortices might be that this brain region responds very specifically to aversive stimuli, especially to pain-related stimuli (Kakigi et al., 1995; Ploner et al., 2000; Bornhövd et al., 2002; Ogino et al., 2006). Ogino and colleagues (2006) showed a somatosensory activation for pain related images, whereas we detected no increased activity for aversive stimuli inducing different emotions than pain (e.g., fear). As we did not differentiate between pain- and fear-related stimuli in the current study, we were not able to investigate such specific somatosensory responses. Seth and colleagues (2013) also applied predictive coding theories to somatosensory cortices as they are assumed to be involved in processes of bodily perception (mainly body ownership) (Schaefer et al., 2006; Tsakiris et al., 2006). Our findings of emotional flattening at somatosensory cortices would also be explainable through difficulties in processes of multisensory integration (Seth, 2013).

For arousal, we did not find any effect for any stimulation condition for the emotion categories. First of all, this might represent a general effect of emotional flattening in all conditions. Another explanation might be a general lower arousal perception specifically related to the nature of the stimulus material. As the arousal norms for the IAPS are rather old (Lang et al., 1999), it might be possible that the images did induce a subjective lower arousal in our sample due to higher sensory overload and media use, as suggested by Grühn and Scheibe (2008) who also found lower arousal ratings for IAPS pictures as compared to the norms. Moreover, the strong tiredness of our sample might have been a relevant factor, which might have been augmented by the dimmed light in the test cabin. Furthermore, studies showed that negative IAPS pictures do not necessarily induce aversive emotions, as some of them also evoke positive emotions, which can also lower arousal scores (Mikels et al., 2005). From a neuroanatomical perspective, it has to be pointed out that different brain regions underlie valence and arousal (Anders et al., 2004; Cunningham et al., 2004; Lang and Davis, 2006; Caria et al., 2010). Whereas the insula is a more important structure for emotional valence, the ACC is more important for arousal (Viinikainen et al., 2010). In the current study, it would also have been interesting to include the ACC as one more stimulation site in order to verify the potential effects of arousal. Furthermore, it would be interesting to add more objective measures, e.g., physiological measures (e.g., skin conductance) to the self-report data, to investigate effects on arousal on multiple levels.

**Study Aim II: Investigation of the influence of cTBS to interoceptive network on neural correlates of emotion processing**

Due to the high number of results in this study, we will not further discuss the effects of the cluster, as they are not of relevance for the current research questions. We could only confirm hypothesis IIa and IIb for positive stimuli at somatosensory cortices. The expected effect of an initial orienting complex could only be confirmed for the N2 for positive
stimuli, only at somatosensory cortices compared to the occipital control region and compared to the frontotemporal insular network. This effect can be interpreted as an orienting response toward deviant stimuli (Campanella et al., 2002; Carretié et al., 2004; Gramann et al., 2007). As the behavioral data indicated, cTBS might have provoked a disturbance in the interoceptive system, followed by deficits in emotional processing, as indexed by flattened subjective reports of valences. This emotional attenuation might be visible in lowered P3 responses at somatosensory cortices, which we could again only confirm for positive stimuli. The specific effect on the neuronal level for the somatosensory cortices underlines the relevance of somatosensory processes for emotional processing. Referring to Damasio’s theory of emotion, somatosensory cortices are a first-order structure, mapping bodily reactions towards emotional stimuli in the form of neuronal patterns (Damasio et al., 2000). The heightened N2 amplitude might express a higher difficulty in encoding bodily reactions associated with emotional stimuli following cTBS stimulation. Considering this difficulty further, lowered P3 amplitudes might indicate impairments of deeper processing of emotional stimuli. However, it was unexpected that effects occurred for one specific emotion category (pleasant) and for one specific stimulation location (somatosensory cortices). This finding does not fit in with the behavioral results showing effects of emotion flattening at the frontotemporal insular network for all emotional pictures and behavioral effects at somatosensory cortices for unpleasant pictures only. One explanation for the specific neuronal effects at somatosensory cortices might be that our selected somatosensory stimulation cluster was closer to the ‘real’ location of this brain structure as compared to the frontotemporal insular network. Moreover, the specific effect for positive stimuli at somatosensory cortices only, might be due to the small sample size. If one looks at the ERP curves (see Fig. 5), the same pattern of higher N2/lower P3 could also be observed at stimulation of somatosensory cortices for negative stimuli, failing significance. Moreover, this pattern is observable after cTBS of the frontotemporal anterior insular network, more prominent for negative than for positive stimuli compared to the control condition, also failing levels of significance. Our findings would also be in keeping with previous studies showing neuronal responses of the insula and of somatosensory cortices for both pleasant and unpleasant stimuli (Rolls et al., 2003; Rudrauf et al., 2009) and with Damasio’s theory describing the insula together with somatosensory cortices as important first-order structures for perceiving emotion through bodily processes (Damasio et al., 2000). Interestingly, the pattern of enhanced N2/reduced P3 can also be observed for neutral stimuli and is more prominent after cTBS of somatosensory cortices than after cTBS of the frontotemporal anterior insular network, compared to the occipital condition. This observation might indicate that the neutral stimuli might also have been processed like emotional stimuli and might have even induced increased motivational salience. Another explanation might be that the occipital location was not an adequate control condition, as it is also involved in the process of emotional picture viewing (Lang et al., 1998a; Lang et al., 1998b). We selected that region mainly as a control region for the interoceptive paradigm which we will discuss in the limitations section.

**Study Aim III: Investigation of the association of IAc and behavioral and neuronal emotion processing following brain stimulation**

Our data reveals that levels of IAc are associated with the behavioral and neuronal processing of emotional stimuli at each stimulation region, pointing out the role of interoception in emotion processing. We had to reject hypothesis IIIa and IIIb, as higher IAc was not associated with more intense emotion processing. However, the inverse effect was found as higher cardiac IAc was associated with more negative valence ratings for positive stimuli at the frontotemporal insular network. This finding might indicate that individuals with higher IAc might underlie a stronger effect of emotional flattening under cTBS stimulation. However, it is surprising that IAc under cTBS was related to valences as previous studies showed only positive associations of cardiac IAc with arousal levels (Montoya and Schandry, 1994; Ferguson and Katkin, 1996; Wiens et al., 2000; Barrett et al., 2004; Herbert et al., 2007; Pollatos et al., 2007; Dunn et al., 2010). Missing associations with arousal in the current study might be explainable through an effect of stimulus material, as our stimuli did not evoke the expected levels of arousal.

Hypothesis IIIc was partly confirmed for the different locations as cardiac or respiratory IAc were associated with higher P3 amplitudes for positive and negative stimuli following cTBS stimulation of the frontotemporal anterior insular network or stimulation of somatosensory cortices. This finding might indicate deeper processing of affective stimuli in individuals with higher IAc, as described by previous studies showing a higher P3 for positive and negative stimuli in individuals with high IAc (Herbert et al., 2007; Pollatos et al., 2007). It is noteworthy that the positive association of IAc and P3 in the current study is still prominent following inhibition of the frontotemporal insular network/somatosensory cortices via cTBS and is prominent despite the observed reduction of positive valences associated with high IAc. One might suppose that individuals with high IAc processed emotional stimuli after cTBS stimulation deeper because they might have been more sensitive to the detection of affective ‘aberrant’ stimuli.

**Study Aim IV: Investigation of the association of changes of IAc and changes in behavioral and neuronal emotion processing as a causal consequence of cTBS stimulation**

We confirmed hypothesis IVa partly as decreases of cardiac IAc induced by cTBS were positively associated with decreases of arousal ratings for negative pictures induced by cTBS at the frontotemporal anterior insular network. We confirmed hypothesis IVb partly as decreases of cardiac IAc induced by cTBS at somatosensory cortices were positively associated with decreases of P3 amplitudes for negative stimuli. These results indicate a causal relationship between the frontotemporal anterior insular network, somatosensory...
cortices and interoception/emotion, as an impairment of interoceptive performance caused by cTBS at the frontotemporal anterior insular network/somatosensory cortices was causally associated with a cTBS induced attenuation in emotion processing for negative stimuli on a behavioral and on a neuronal level. Our results are in line with theories of emotion pointing out a close connection of interoception and emotion, with the insula and somatosensory cortices as relevant brain structures (Damasio et al., 2000; Craig, 2009; Seth et al., 2012; Smith and Lane, 2015). Moreover, our findings of decreased arousal ratings could also be explainable by theories of predictive coding (e.g. Seth, 2013), assuming an interoceptive network disturbance. Effects of decreased arousal ratings in the current study associated with decreases in cardiac IAC would also be in keeping with findings of Gray and colleagues (Gray et al., 2007), pointing to a similar effect of inverse emotional perception.

Limitations

One crucial limitation refers to the fact that we started the emotional picture rating task with a latency period of 30 min following cTBS stimulation, as the participants had to perform interoceptive tasks directly after stimulation. Due to the number of involved tasks, we had to tolerate more extended latency periods for one of the tasks. However, as previous studies described lasting effects of rTMS for 20-30 minutes following stimulation (Nyffeler et al., 2006; Huang et al., 2007), we cannot make sure that inhibition was still successful with all participants. In this context, we can also not exclude that other factors, such as the tiredness of the participants or reduced attention due to the length of the paradigm also influenced our observed effect of emotional flattening. Future studies should randomize the order of tasks through the participants to reduce the effects of order. Also, the inclusion of a short attention task would have been possible.

A second limitation refers to criticism of Coll and colleagues Coll et al. (2017) regarding missing evidence that we targeted the insular cortices directly by stimulation. We responded to that critique referring to results of a field analysis showing that the upper part of the anterior insula was directly reached by a field strength of about 0.5, meaning about a quarter of the maximum norm E-field (Pollatos and Kammer, 2017). However, we still cannot exclude either effects of trans-synaptic activation of the anterior insula induced by the prefrontal cortex, being close to the skull, nor direct contribution of areas subjected to influence of the coil (cf. Pollatos and Kammer, 2017). As it is very likely that we stimulated a whole network instead of only the insula, we described the target area as ‘frontotemporal anterior insular network.’

Another critical limitation refers to the fact that we did not apply neuronavigation to each participant. Instead, we used a structural MRI scan of one individual as a template for neuronavigation to figure out stimulation locations. We subsequently fixed the target locations via electrode positions on the EEG cap. Other research groups used the same technique of assessment of stimulation locations that we applied (Harmer et al., 2001; Schönfeldt-Lecuona et al., 2012). TMS literature describes the use of the 10-20 EEG system without neuronavigation for positioning of TMS with middle accuracy (Herwig et al., 2003; Schönfeldt-Lecuona et al., 2012), a technique which was used in many studies (Schutter and van Honk, 2006; Balconi and Bortolotti, 2012; Schönfeldt-Lecuona et al., 2012). However, stimulation in this study would have been more precise with individual neuronavigation based on structural MRI scans for each subject (Leyman et al., 2011; Brückner et al., 2013).

Another issue refers to the low arousal ratings of our sample. On the one hand, this effect is explainable by the relatively high fatigue of our sample, which was assessable with the POMS questionnaire at the beginning of each session. Possibly, fatigue was also promoted by the long duration of the experimental procedure. Probably, the selected stimulus material caused the effects of low arousal. Although we tried to select emotional pictures with high levels of arousal from the IAPS database (Lang et al., 1999), specific stimuli were excluded due to ethical aspects, e.g., erotic stimuli and very cruel stimuli (e.g., mutilation) which are both highly arousing (Lang et al., 1999). However, this could also be an effect of the suitability of the IAPS themselves, as first studies indicate that some negative IAPS also induce positive emotions which might have lowered arousal effects for unpleasant stimuli (Mikels et al., 2005). Moreover, Bradley and colleagues (2001) pointed to the fact that the categories fear/sadness/anger induce more negative emotional reactions and stronger physiological reactions compared to disgust. An exclusion of ‘disgust’ images might have provoked stronger effects.

Regarding the selected food stimuli, we did not check several confounding variables (e.g., current satiety, eating behavior) (Uher et al., 2006; Coletta et al., 2009). Only fitness level and BMI were assessed. In future studies, the listed variables should be considered.

Another critical point is the selection of the sham condition. As it is a conventional method to choose a control area in the brain which is not of relevance for the investigated process (Baudewig and Bestmann, 2007), we chose the occipital cortex which is not related to the interoceptive network. However, we did not consider that the picture viewing paradigm very likely activated the occipital cortex (Kober et al., 2008). Moreover, results of a meta-analysis of imaging studies showed evidence for the involvement of the occipital cortex specifically in emotion processing as occipital cortex activation was increased for emotional compared to neutral stimuli (Kober et al., 2008). As anatomical, neuroimaging and ERP studies indicate projections from the limbic system to areas of the visual occipital cortex (V1) (Vuilleumier et al., 2004; Freese and Amaral, 2005; Stolarova et al., 2005), we also have to assume direct activations of the occipital cortex by affective stimuli in the current study. Accordingly, we cannot denote the occipital cortex as an adequate control condition for the current study, but it rather constitutes another projection area of the emotional network. The use of a magnetically shielded sham coil system to apply an ineffective stimulation over the same location, with the same frequency and with similar auditory effects as in the experimental condition (Duecker and Sack, 2015) was no alternative option for sham stimulation in the current study, as this option is missing.
stimulation effects of peripheral nerves (Duecker and Sack, 2015). As the frontotemporal insular network stimulation in the current study activated facial peripheral nerves and muscles, participants would have recognized differences between the experimental and the sham condition.

The selection of stimulation sites according to the 10-20-EEG system can also be considered as critical. However, this stimulation setting is described to have middle accuracy in conjunction with coregistration (Herwig et al., 2003; Herwig and Schönfeld-Lecuona, 2007), as used in the present study. Nevertheless, as the EEG is only superficial, we cannot make sure that deeper brain structures such as the insula were reached (cf. Coll et al., 2017), and we cannot ensure that our electrode distribution was adequate to stimulate the insula.

Another limitation of the study is the exclusion of women, restricting the generalizability of the results. Due to the mood swing of women during the menstrual cycle (Großheinrich et al., 2007) and due to potential pregnancy (Paulus and Siebner, 2007), women were excluded. However, as women show different emotional reaction patterns, e.g. more intensive reactions towards aversive images (Damasio et al., 2000; Wager et al., 2003; Schienle et al., 2005) or different reactions to food images (Wansink et al., 2003), it would be interesting to investigate female samples in future studies. It would be possible to conduct pregnancy tests and to control menstrual cycle (e.g. examination of women at definite time frames based on their cycle).

The current study shows the close relationship between two interoceptive structures (the frontotemporal anterior insular network and somatosensory cortices), interoception and emotion. Following inhibition of interoceptive networks via cTBS, we could observe a reaction of emotional flattening, being assessable in attenuated self-reported valences for all affective stimuli and being observable in heightened/reduced ERPs towards positive stimuli, which indicates an orientation reaction. Moreover, we could show associations of IAc with emotional flattening, showing on the one hand higher IAc to be related to stronger attenuation of positive stimuli. On the other hand, we showed that IAc was associated with the deeper processing of both stimuli categories. Finally, the study could give evidence for causal effects of cTBS at the frontotemporal anterior insular network or over somatosensory cortices on decreases of valences and decreases of ERPs for negative stimuli, pointing out the causal role of the frontotemporal anterior insular network and of somatosensory cortices for processing of interoception and emotion. The described pattern of emotional flattening was observable for both locations, the frontotemporal anterior insular network and somatosensory cortices, however more prominent at somatosensory cortices, the structure which was better reachable by stimulation. We explain those effects through mechanisms of aggravated access to bodily processes, resulting in attenuation of emotional reactions, mirrored by an N2/P3 orienting complex towards deviant stimuli. Missing effects of arousal might be related to the selected stimuli material or fatigue of participants. Our findings support peripheral theories of emotions (James, 1884; Schachter and Singer, 1962; Damasio et al., 1996; Damasio, 1999), the multi-hierarchical model of embodied emotion (Smith and Lane, 2015) as well as predictive coding theories (Friston, 2010; Seth et al., 2012; Seth, 2013). Future studies to replicate our paradigm would be interesting in different samples, such as women or psychiatric samples with dysfunctional interoceptive and emotional processing. Also, studies including further brain structures of the interoceptive network would be of interest.

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**REFERENCES**


tion. PPM-Psychotherapie Psychosomatik Medizinische Psycholo
gie. 56. e.DOI: https://10.1056/s-2006-940129


tional 10-20 EEG system for positioning of transcranial magnetic sti


tion. PPmP-Psychotherapie Psychosomatik Medizinische Psycholo


lagen und Handanweisung, 1981.


Mikels JA, Fredrickson BL, Larkin GR, Lindberg CM, Maglio SJ, Reuter-
Lorenza PA. (2005) Emotional category data on images from the Interna

ssing and visual evoked brain potentials. Percept Mot Skills 83:143-


cence of pain: imagination of pain while viewing images showing pain


Partaia T, Surakka V. (2003) Pupil size variation as an indication of affec


cal issues regarding the use of transcranial magnetic stimulation to