

Using real-time fMRI to learn voluntary regulation of the anterior insula in the presence of threat-related stimuli

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Previous studies have shown that healthy participants learn to control local brain activity with operant training by using real-time functional magnetic resonance imaging (rt-fMRI). Very little data exist, however, on the dynamics of interaction between critical brain regions during rt-fMRI-based training. Here, we examined self-regulation of stimulus-elicited insula activation and performed a psychophysiological interaction (PPI) analysis of real-time self-regulation data. During voluntary up-regulation of the left anterior insula in the presence of threat-related pictures, differential activations were observed in the ventrolateral prefrontal cortex, the frontal operculum, the middle cingulate cortex and the right insula. Down-regulation in comparison to no-regulation revealed additional activations in right superior temporal cortex, right inferior parietal cortex and right middle frontal cortex. There was a significant learning effect over sessions during up-regulation, documented by a significant improvement of anterior insula control over time. Connectivity analysis revealed that successful up-regulation of the activity in left anterior insula while viewing aversive pictures was directly modulated by dorsomedial and ventrolateral prefrontal cortex. Down-regulation of activity was more difficult to achieve and no learning effect was observed. More extensive training might be necessary for successful down-regulation. These findings illustrate the functional interactions between different brain areas during regulation of anterior insula activity in the presence of threat-related stimuli.

Keywords: emotion; real-time fMRI; psychophysiological interaction; insula; prefrontal cortex

INTRODUCTION

Recently, physiological self-regulation of circumscribed brain regions and networks has become feasible using real-time functional magnetic resonance imaging (rt-fMRI) (Weiskopf *et al.*, 2007; Sitaram *et al.*, 2008, 2009; Caria *et al.*, 2011). Several studies have investigated learned modulation of neural activity in areas primarily implicated in emotional processes such as the amygdala (Posse *et al.*, 2003; Johnston *et al.*, 2010), the rostral anterior cingulate cortex (rACC; Weiskopf *et al.*, 2003), the subgenual ACC (Hamilton *et al.*, 2011), the anterior insula (Caria *et al.*, 2007, 2010; Johnston *et al.*, 2010) and it has been shown that support vector machine (SVM) classification of spatial patterns of activation in emotional networks can be used for real-time feedback (Sitaram *et al.*, 2010). It is also increasingly being recognized that the ability to regulate

activity of localized cortical areas can be useful in the treatment of various disorders including depression (Hamilton *et al.*, 2011), chronic pain (deCharms *et al.*, 2005), tinnitus (Haller *et al.*, 2010) and schizophrenia (Ruiz *et al.*, 2008, 2011), and for movement rehabilitation after stroke (Sitaram *et al.*, 2011).

The anterior insula is a key structure in the emotional circuitry and its activity has been shown to correlate with subjective feelings of emotional states (Craig, 2002, 2003, 2009). Studies on emotion perception have revealed that insula activity correlates with the aversive valence of stimuli (Anders *et al.*, 2004), sadness (Lane *et al.*, 1997), fear (Morris *et al.*, 1996) and disgust (Calder *et al.*, 2001). Positively valenced responses were also reported to correlate with activations of left anterior insula (Craig, 2009). A review of PET (positron emission tomography) and fMRI studies (Phan *et al.*, 2002) demonstrated that both the ACC and insula were recruited during emotion induction using emotional recall/imagery and during performance of emotional tasks with concurrent cognitive demands. By playing a critical role in mediating self-awareness, body integrity (Craig, 2009), and in the influence of peripheral autonomic arousal on consciously experienced emotional states (Critchley *et al.*, 2002, 2004), the insula serves as a strategic neural node in the

Received 30 September 2010; Accepted 30 August 2011

Advance Access publication 7 October 2011

The Deutsche Forschungsgemeinschaft (DFG) (BI 195/56-1, BI 195/59-1 and KU 1453/3-1). The research was supported by grants from the The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation; grant BI 195/56-1, BI 195/59-1 and KU 1453/3-1) and the European Union (grant EU-FP7, CEEDS #258749).

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appraisal of emotional responses (Craig, 2009). Using rt-fMRI, our group previously demonstrated that untrained participants can learn to up-regulate activity of the anterior insula within three training sessions (Caria *et al.*, 2007) and that the amount of up-regulation correlates with subsequent valence ratings of aversive pictures (Caria *et al.*, 2010). A follow-up investigation of the same data (Lee *et al.*, 2011) with multivariate pattern classification and Granger causality modelling (GCM) revealed that self-regulation training of the anterior insula caused an initial increase and subsequent pruning of the network density and a strengthening of the insula's connections with other regions involved in emotional processing (amygdala, medial prefrontal cortex). In the present study, we sought to extend these findings by examining the larger networks engaged during the self-regulation of insula activity.

Neuroimaging research on neural correlates of affect regulation often comprises either emotion suppression or reappraisal of the evocative stimuli, with the aim of reducing negative affect or increasing positive affect (Ochsner and Gross, 2005; Quirk and Beer, 2006; Goldin *et al.*, 2008; Mak *et al.*, 2009). Functional brain imaging of both these regulation strategies has shown that they engage specific frontal brain regions such as the orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC) and ACC (Beauregard *et al.*, 2001; Ochsner *et al.*, 2002, 2004; Phan *et al.*, 2005; Urry *et al.*, 2006; Eippert *et al.*, 2007; Mak *et al.*, 2009; Johnston *et al.*, 2010). The importance of these frontal areas in emotion regulation is underscored by studies showing that the initial appraisal of negative emotional stimuli similarly engages VLPFC, DLPFC and dorsomedial prefrontal cortex (DMPFC) (Hariri *et al.*, 2000, 2003; Taylor *et al.*, 2003). Moreover, ACC and VLPFC are activated during inhibition of cognitive-emotional interference (Whalen *et al.*, 1998; Bush *et al.*, 2000; Etkin *et al.*, 2006; Shafritz *et al.*, 2006) and when participants divert their attention away from threatening and/or painful stimuli (Bantick *et al.*, 2002; Tracey *et al.*, 2002; Bishop *et al.*, 2004).

Here, we investigate rt-fMRI-supported self-regulation of activity in the left anterior insula while subjects viewed threat-related pictures. To examine which brain areas are functionally connected during successful regulation of the targeted area, we employ psychophysiological interaction (PPI) analysis. We hypothesize that successful modulation of activity of the anterior insula is mediated by prefrontal areas that have previously been shown to subserve emotion regulation, as reviewed above. The present study thus aims to extend the understanding of the neural circuitry involved in rt-fMRI based self-regulation.

MATERIALS AND METHODS

Participants

Eleven healthy volunteers (aged 21–28 years, 8 females, 3 males) participated in the study. All participants were

right-handed as assessed by the Edinburgh handedness inventory and had normal or corrected-to-normal vision. None of the participants had a history of psychiatric, medical or neurological illness. They were given written instructions, and informed consent was obtained from each. The study was approved by the ethics committee of the Faculty of Medicine of the University of Tübingen.

fMRI data acquisition

Functional images were acquired on a 1.5-T whole-body scanner with a standard 8-channel head coil (Siemens Magnetom Trio Tim, Siemens, Erlangen, Germany). A standard echo-planar imaging sequence (EPI), adapted for real-time image reconstruction to generate images at the end of each volume, was used [TR (repetition time) = 1.5 s, matrix size = 64×64 , effective TE (echo time) = 40 ms, flip angle $\alpha = 70^\circ$, bandwidth = 1.954 kHz/pixel]. Sixteen slices (voxel size = $3.3 \times 3.3 \times 5.0 \text{ mm}^3$, slice gap = 1 mm), AC/PC (anterior commissure/posterior commissure) aligned in axial orientation were acquired. For superposition of functional maps upon brain anatomy, a high resolution T1-weighted structural scan of the whole brain was collected from each participant (MPRAGE, matrix size = 256×256 , 160 partitions, 1 mm^3 isotropic voxels, TR = 2300 ms, TE = 3.93 ms, T1 = 1100 ms, $\alpha = 8^\circ$).

The rt-fMRI system is based on Turbo-BrainVoyager 1.1 software (Brain Innovation, Maastricht, The Netherlands) in combination with in-house written scripts running on Matlab 6.5 (The Math Works, Natick, MA, USA) as previously described by Weiskopf *et al.* (2003).

Localizer session

Before feedback training, a localizer session consisting of 70 scans in total was performed to functionally identify the left anterior insula. After a 10-s baseline period, a set of highly aversive pictures from the International Affective Picture System (IAPS) (mutilation and burn victims) were presented in two blocks (22.5 s) alternating with resting periods of the same length. To mark the anterior insula region of interest (ROI) (ROI1), the final *t*-map of the activations during the localizer session was used to draw a rectangular box comprising 4×5 voxels centred on the voxel showing highest activation within the anterior part of the insula (Figure 2). The reference ROI (ROI2) was a slightly larger square (6×6 voxels). The placement of ROI2 was superior to ROI1, with at least two intervening slices between the two ROIs. We specifically ensured that no activation was present in the reference ROI during the localizer session. The purpose of using a reference ROI was to cancel out changes in BOLD (Blood Oxygen Level Dependent) signal due to global effects of movement and other task-unspecific changes.

rt-fMRI task procedure and feedback calculation

The experimental paradigm required participants to actively regulate BOLD activity in the left anterior insula while

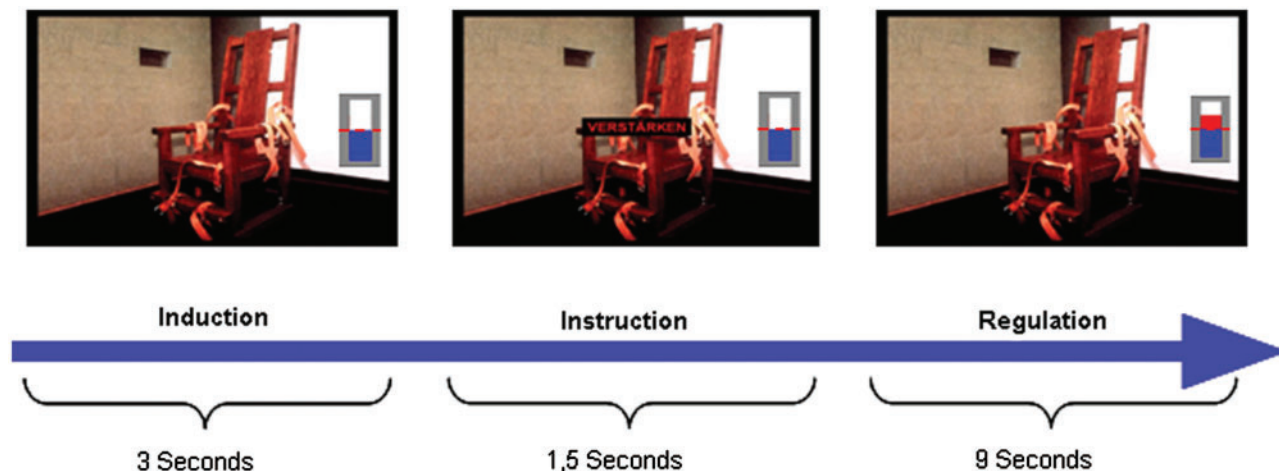


Fig. 1 Schematic overview of the experimental design. Pictures were shown to the participants for 3 s while in the MR scanner (emotion induction). According to task instructions (1.5 s), the participants had to regulate (increase or decrease) signal in the left anterior insula in response to the image shown for additional 9 s (with passive viewing as control condition). BOLD response from left anterior insula was fed back in the form of a graphical thermometer.

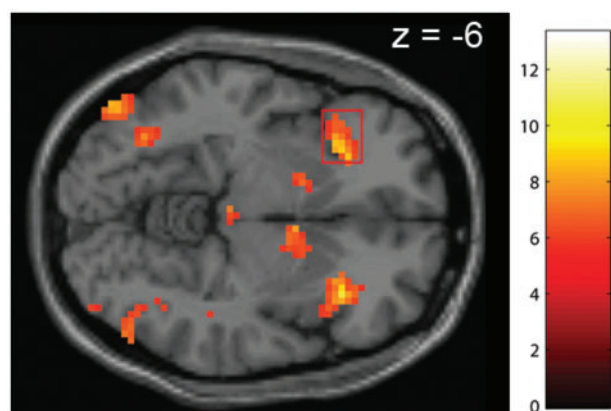


Fig. 2 Illustration of ROI definition in the left anterior insula. The red rectangular box delineates the ROI chosen for the rt-fMRI neurofeedback.

viewing emotional stimuli. The conceptual scheme of the experiment and the picture set used were adapted from Eippert *et al.* (2007). The stimulus set consisted of 18 aversive (average arousal: 6.22 s.d. 0.59; average valence: 2.73 s.d. 0.61) and six neutral pictures based on IAPS (Lang *et al.*, 1999). Moderately aversive or threatening pictures were chosen to avoid ceiling effects and allow differential regulation.

The protocol consisted of three sessions. Each session was 8 min 9 s long and comprised the following conditions: emotion induction, up-regulation, down-regulation and no-regulation. The trials started with a 3-s induction period and were followed by a 1.5-s task instruction superimposed on the pictures using the single words ‘increase’ for up-regulation, ‘decrease’ for down-regulation and ‘view’ for no-regulation. Each regulation block lasted 9 s (Figure 1) and was followed by a 7.5-s resting period. Feedback was provided in the form of a graphical thermometer whose bars increased or decreased in number from the baseline value (represented by the dashed red line in the middle of the

thermometer, see Figure 1), in proportion to the differential BOLD signal in the target (ROI1) and reference (ROI2) ROIs. The baseline value was calculated from the last two scans of the resting period at the end of the preceding trial. Feedback was computed from a temporally smoothed BOLD signal. Temporal smoothing was achieved by averaging the BOLD signal within a moving window that included the BOLD value for the current TR and values from the past three TRs. Thus, the BOLD signal during the induction and instruction period plus the first scan of the regulation period formed the basis of the first feedback signal. More specifically, the number of bars was computed using the following equation (equation 1):

$$\text{Number of bars} = (\text{BOLD}_{\text{reg}} - \text{BOLD}_{\text{base}})_{\text{ROI1}} - (\text{BOLD}_{\text{reg}} - \text{BOLD}_{\text{base}})_{\text{ROI2}} \quad (1)$$

If the computed number of bars was positive, the bars of the thermometer were shown in red colour above the baseline, and if the value was negative, the bars were shown in blue colour below the baseline. In addition, we implemented a real-time artefact correction that detected and ignored sudden changes in BOLD signal due to swallowing or movement of the tongue (Sitaram *et al.*, 2010). Such movements can cause signal increases five times higher than the normal BOLD increase in a single TR. The thermometer display with increasing and decreasing bars representing feedback of activation in the target region was presented during all conditions, except the no-regulation condition where a thermometer with static bars was presented. Participants were informed about the data processing delay of 1.5 s and the intrinsic physiological haemodynamic response delay of ~4–6 s.

To extend classical emotion regulation paradigms with the advantages of rt-fMRI-based neurofeedback, participants were instructed to use cognitive strategies that would help them learn to control the activity of the target ROI.

Specifically, for up-regulation, participants were instructed to imagine themselves being personally involved in the situation depicted in the picture. For down-regulation, participants were required to cognitively and emotionally distance themselves from the situation displayed in the image. During the no-regulation condition, participants were required to passively view the images presented. After each session, they were also required to rate their success in regulation on a scale from 1 to 8 (1 = very good; 8 = very bad).

rt-fMRI analysis of feedback regulation

The difference of the baseline-adjusted signal changes in ROI1 and ROI2 averaged over the regulation period (equation 1) was computed individually for each subject on a trial-by-trial basis. The mean signal changes and corresponding standard deviations in each regulation task and session were calculated. The extracted signal changes were further analysed using SPSS 13.0. Paired *t*-test was performed to analyse the signal changes between the first and last training session for each task. Furthermore, a linear regression analysis using the individual trial-specific signal changes as dependent variable and time as independent variable was conducted to evaluate possible learning effects over time. Results were considered significant at $P < 0.05$ (one tailed).

Time-series analysis

Peri-stimulus time histogram (PSTH) plots were extracted using the NOD Lab toolbox NERT4SPM (<http://www.hih-tuebingen.de/en/sensorimotor-lab/nod-lab/>). Based on the individual GLM analysis, a spherical ROI of 6 mm centred on the maximally activated voxel in the left anterior insula during aversive induction was used. This ROI definition differs slightly from the rectangular ROI used for the neurofeedback training, but has the advantage that only grey matter voxels showing significant activity are used for the time-course extraction. The average time course across all voxels in the ROI was calculated and normalized to percent signal change separately for each regulation condition and session. To test for signal changes during the regulation periods, the time course was scaled to 1.5 s pre-stimulus baseline (time zero represents the time-point after emotion induction). Repeated-measurement ANOVA with factors time (six time-points in the regulation condition) and session (three sessions) were performed for each regulation condition. Paired *t*-tests were used to compare session-wise differences between corresponding time-points separately for each regulation condition.

Whole-brain analysis of emotion induction and feedback regulation

The SPM5 statistical parametric mapping software package (Wellcome Department of Imaging Neuroscience, London) was used to perform off-line image pre-processing and data analysis. Before whole-brain statistical analysis, functional

EPI volumes were spatially re-aligned. The images were normalized to the Montreal Neurological Institute (MNI) space and spatially smoothed (9-mm Gaussian kernel). A temporal filter (0.0088 Hz) was applied to remove low-frequency artefacts. For each participant, a general linear model (GLM) with the conditions 'induction aversive', 'induction neutral', 'up-regulation aversive', 'down-regulation aversive', 'no-regulation aversive' and 'no-regulation neutral' was created. All conditions were modelled with a canonical haemodynamic response function (HRF) using standard SPM5 settings. The duration of the regulation trials was set to 9 s. Movement regressors were included as confounds in the general linear model to account for possible head movement related variance. The following contrasts were computed: induction aversive *vs* induction neutral, aversive up-regulation *vs* aversive no-regulation and aversive down-regulation *vs* aversive no-regulation. Random-effects *t*-statistics across participants were calculated separately for the main contrasts. Effects were considered significant based on a whole-brain false discovery rate of $P < 0.05$ (FDR; Genovese *et al.*, 2002).

PPI analysis

PPI is a functional connectivity analysis method that describes activity in a ROI based on its interaction with other brain regions and a psychological factor (Friston *et al.*, 2003). Essentially, PPI implies that this interaction between brain regions is significantly modulated by the experimental or psychological context (e.g. attention *vs* no attention; up-regulation *vs* down-regulation). By integrating the physiological and experimental influences on regional responses, PPI allows one to confer a degree of functional specificity when making inferences about functional integration or interactions between cortical areas. Kim and Horwitz (2008) compared PPI analysis with correlation measures between fMRI signals for computing functional connectivity using simulated data reflecting synaptic activity. Their main finding was that PPI results better reflect interregional connections between areas compared to simple correlations between fMRI signals from two regions. Our aim was to examine how the left anterior insula interacts with other brain regions while participants regulate left anterior insula activity using rt-fMRI feedback.

In the first step, we extracted time courses for each training session using the first eigenvariate of the volume of interest, i.e. a spherical ROI of 6 mm centred on the maximally activated voxel in the left anterior insula, as described in the time-course analysis. The PPI toolbox of SPM5 was then employed to generate differential contrasts of both up-regulation *vs* no-regulation and down-regulation *vs* no-regulation. The GLM for each PPI consists of the interaction vector of the corresponding psychological or context factor (up-regulation or down-regulation in comparison to no-regulation), the time course of the BOLD signal in the target ROI using the first eigenvariate, and the

context-specific contrast vector (up-regulation *vs* no-regulation, down-regulation *vs* no-regulation). The motion parameters were used as confounds in the design matrix. A contrast vector weighting the interaction contrast with one and the other regressors with zero results in a statistical parametric map with voxels showing a positive coactivation or interaction with the seed ROI, whereas a contrast weight of minus one yields a statistical map with voxels revealing a negative covariation with the target ROI.

For second-level analysis, we used the contrast images obtained from the first-level PPI analysis representing the interaction of brain regions with the left anterior insula during up- and down-regulation. This was followed by separate *t*-tests for each condition and session. An uncorrected threshold of $P < 0.001$ was applied for the group PPI analyses.

Self-report analysis

Statistical analysis of behavioural data (i.e. success ratings for regulation) were computed using the statistical package SPSS 13.0 (SPSS Inc., Chicago, IL, USA). A repeated-measurements ANOVA with the factors session and condition was carried out. Significant effects were further analysed using paired *t*-tests. A *t*-value exceeding a threshold of $P < 0.05$ was considered significant.

RESULTS

ROI analysis of feedback regulation

The comparison between tasks showed that the BOLD signal changes were significantly increased during up-regulation in comparison to down-regulation [$t(10) = 2.02$ $P = 0.035$ one-tailed] and no-regulation [$t(10) = 5.83$ $P < 0.001$] in the last training session (Table 1 and Figure 3). The comparison of BOLD signal changes during up-regulation based on the difference of baseline-corrected individual activities in ROI1 and ROI2 used for rt-fMRI training revealed a marginally significant increase between the first and last session [session 1: 0.17 (s.d. 0.17), session 3: 0.32, (s.d. 0.26), $t(10) = 1.68$ $P = 0.07$, one tailed]. Similarly, during down-regulation there was a small but non-significant increase between sessions 1 and 3 [session 1: 0.05 (s.d. 0.20), session 3: 0.17 s.d. (0.19), $t(10) = 1.21$ $P = 0.25$]. In the no-regulation condition, there was a significant change between the first and last training session [session 1: -0.27 (s.d. 0.15), session 3: -0.05 (s.d. 0.18), $t(10) = 3.27$ $P = 0.008$].

A linear regression analysis using the signal changes of every single trial as dependent variable revealed a trend for a progressive increase in performance during up-regulation over the course of training ($y = 0.10 + 0.283x$, $t = 1.181$, $P = 0.257$). When excluding the first trial under the assumption that participants were initially unfamiliar with the feedback display and the delay of the haemodynamic response, a significant learning effect was observed for up-regulation ($y = -0.017 + 0.550x$, $t = 2.55$, $P = 0.022$). In contrast,

Table 1 Online analysis of the BOLD signal changes during the rt-fMRI training (mean \pm s.d.)

	Up-regulation	Down-regulation	No-regulation
Session 1	0.17 (0.16) ^a	0.05 (0.20) ^a	-0.20 (0.14) ^a
Trial 1	0.53 (0.69)	0.09 (0.54)	-0.08 (0.54)
Trial 2	-0.06 (0.25)	0.27 (0.21)	-0.08 (0.42)
Trial 3	0.00 (0.46)	0.11 (0.26)	-0.21 (0.42)
Trial 4	0.05 (0.65)	-0.03 (0.33)	-0.15 (0.34)
Trial 5	0.23 (0.64)	-0.16 (0.76)	-0.34 (0.54)
Trial 6	0.29 (0.39)	0.06 (0.48)	-0.36 (0.42)
Session 2	0.10 (0.28) ^b	0.09 (0.27) ^b	-0.05 (0.16) ^b
Trial 1	0.02 (0.79)	0.36 (0.42)	0.12 (0.26)
Trial 2	0.09 (0.54)	0.25 (0.30)	-0.01 (0.32)
Trial 3	-0.01 (0.58)	0.08 (0.56)	-0.11 (0.47)
Trial 4	0.24 (0.33)	0.26 (0.44)	-0.27 (0.44)
Trial 5	0.20 (0.47)	-0.36 (0.55)	0.01 (0.52)
Trial 6	0.03 (0.49)	-0.01 (0.74)	-0.05 (0.44)
Session 3	0.32 (0.26) ^c	0.17 (0.18) ^c	-0.05 (0.17) ^c
Trial 1	0.65 (0.64)	0.23 (0.38)	-0.17 (0.33)
Trial 2	0.26 (0.42)	0.29 (0.31)	0.09 (0.36)
Trial 3	0.22 (0.51)	0.39 (0.46)	-0.11 (0.38)
Trial 4	0.12 (0.42)	0.25 (0.29)	0.02 (0.67)
Trial 5	0.27 (0.44)	-0.22 (0.39)	0.17 (0.30)
Trial 6	0.41 (0.30)	0.06 (0.37)	-0.28 (0.33)

(ROI1reg – ROI1base) – (ROI2reg – ROI2base).

^aMean s1.

^bMean s2.

^cMean s3.

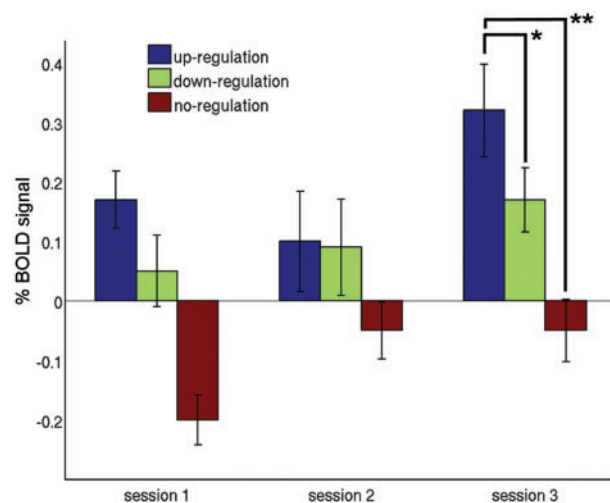


Fig. 3 Differential BOLD percent signal change (\pm s.e.m.) computed on the individual selected target and control ROI during the rt-fMRI training. A significant difference was found between up- and down-regulation as well as between up- and no-regulation in the last training session ($*P < 0.05$, $**P < 0.001$).

during down-regulation, no learning took place ($y = 0.103 + 0.016x$, $t = 0.06$, $P = 0.951$).

Overall, there was a large variability in BOLD signal changes across trials and subjects especially in the first two sessions (Table 1). However, during up-regulation in the last training session, we found consistent increases in all trials.

Interestingly, during down-regulation there was a tendency of improved down-regulation ability in the last compared to the first trials in each session.

Time-series analyses

The direct comparison of the time courses between tasks revealed that there was a significantly decreased activation in the anterior insula during down-regulation compared to up-regulation in the last training session at three consecutive time-points [paired *t*-tests: Second 3: $t(10) = 2.94$, $P = 0.015$, Second 4.5: $t(10) = 3.82$, $P = 0.003$, Second 6: $t(10) = 2.39$, $P = 0.037$; Figure 5]. Within task, repeated-measurements ANOVA of left anterior insula activity with time-bin and session as factors revealed a significant effect of time [$F(5,50) 5.15$, $P < 0.001$] during up-regulation. The session effect was not significant [$F(2,20) 1.69$, $P = 0.219$]. However, exploratory pair-wise comparisons showed higher BOLD activity in the third compared to the first session (mean BOLD amplitude session 1: 0.244; mean BOLD amplitude session 3: 0.407; $P = 0.037$, one sided). Post hoc paired *t*-tests between corresponding peri-stimulus time-points in Sessions 1 and 3 revealed significantly higher BOLD responses in Session 3 at time 4.5 s ($P = 0.034$), 6 s ($P = 0.022$) and 9 s ($P = 0.036$) (Figure 5).

During down-regulation, there was also a significant effect of time [$F(5,50) 3.712$, $P = 0.006$], but no session effect [$F(2,20) 0.322$, $P = 0.728$]. Pair-wise comparisons revealed no differences between Sessions 1 and 3. There was a tendency towards a difference in BOLD levels between sessions in the no-regulation condition [$F(2,20) 2.939$, $P = 0.104$]. Pair-wise comparisons showed a significant difference between Sessions 1 and 3 ($P = 0.018$). Post hoc paired *t*-tests yielded significantly reduced BOLD responses during no-regulation in the first session compared to the third session at time 1.5 s ($P = 0.036$), 3 s ($P = 0.023$) and marginally at 4.5 s ($P = 0.054$).

Whole-brain analysis of emotion induction and feedback regulation

The contrast 'induction-aversive' compared to 'induction-neutral' yielded activations in supplementary motor area (SMA), paracingulate cortex, anterior insula bilaterally extending into the frontal operculum, ACC, thalamus, left putamen, caudate nucleus bilaterally and left inferior parietal cortex, together suggesting successful emotion induction.

Up-regulation in comparison to no-regulation in the presence of threat-related stimuli revealed activation in the frontal operculum bilaterally, right anterior insula, left VLPFC, left ACC and right middle cingulate cortex (Table 2 and Figure 4A). In contrast to the no-regulation condition, down-regulation activated the right insula extending into the right VLPFC, right superior temporal cortex, right caudate nucleus, right inferior parietal cortex, right middle frontal cortex (MFC), right superior occipital

Table 2 Regions showing increased activation during up-regulation compared to no-regulation of the left anterior insula

Region (Brodmann's area)	<i>t</i> -value	MNI coordinates		
		<i>x</i>	<i>y</i>	<i>z</i>
Ventrolateral PFC/insula L (BA 47)	9.55	-48	18	-3
Frontal inferior opercularis L	6.16	-48	6	6
Insula R (BA 22)	7.56	48	12	-6
Frontal inferior opercularis R	4.81*	39	15	9
Middle cingulate cortex R (BA 32)	5.26	3	21	36
ACC L (BA 24)	4.33	-3	24	30

FDR $P < 0.05$ corrected for the amount of false positive activations of the whole brain.

* $P < 0.001$ (uncorrected for multiple comparisons).

L = left; R = right.

cortex, left VLPFC, right superior medial frontal cortex and right ACC (Table 3 and Figure 4B).

PPI analysis

PPI analysis for up-regulation vs no-regulation in the first session showed a right-lateralized connectivity pattern with left anterior insula activity in the lingual gyrus, anterior insula, VLPFC and frontal inferior operculum. In the second session, we found positive coactivation in the left inferior orbitofrontal, left middle frontal and left middle OFC. Analysis of the third training session revealed that successful up-regulation of the BOLD response in the left anterior insula was positively linked to activity in left DMPFC and bilateral VLPFC, presumably as an effect of the prolonged training (Table 4 and Figure 6). The parameter estimates of the PPI interaction analyses for all three sessions in these areas as displayed in Figure 6B confirm that a robust positive interaction was only found in the third training session. While a negative interaction was present in the first session, a significantly positive interaction was observed in the third session (the interaction in one representative subject is plotted in Figure 6A). Another area that showed significant interaction with left anterior insula was the bilateral inferior occipital cortex.

In the first two training sessions, there was no significant connectivity with the target ROI during down-regulation. Analysis of down-regulation data in the third session showed a positive covariation with activity in the left fusiform gyrus ($x = -39$, $y = -60$, $z = -15$, $t = 7.42$). However, we found no involvement of prefrontal areas.

Subjective ratings

There was a significant session effect [$F(2,22) = 4.184$, $P < 0.05$] in subjective ratings, indicating improved success to regulate over the course of the experiment. There was a tendency towards up-regulation being judged easier than down-regulation [success rating up-regulation: 4.47 ± 1.03 ;

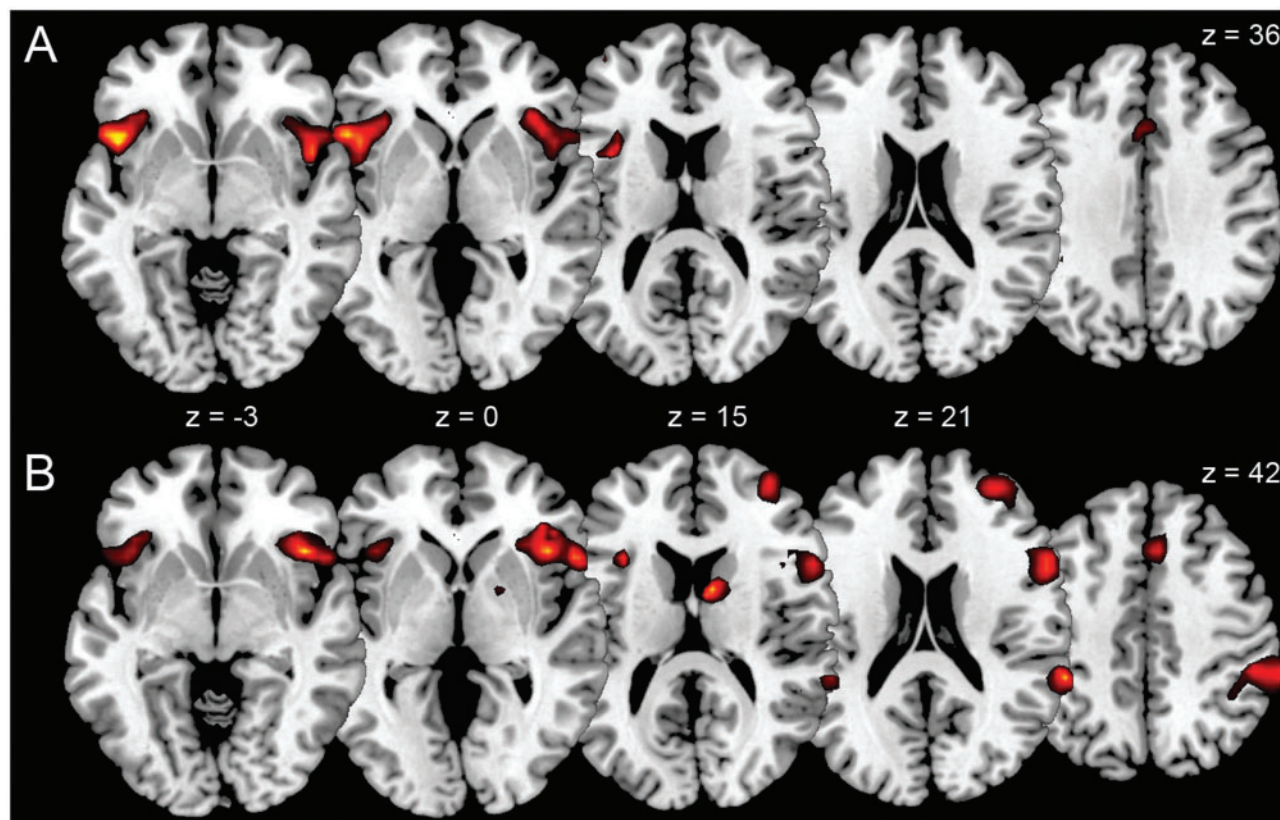


Fig. 4 Differential activation in the contrast up-regulation vs no-regulation (A) and in the contrast down-regulation vs no-regulation (B) rendered on a canonical single-subject brain ($p < 0.001$).

Table 3 Regions showing increased activation during down-regulation in comparison to no-regulation of the left anterior insula

Region (Brodmann's area)	t-value	MNI coordinates		
		x	y	z
Ventrolateral PFC/insula R (BA 47)	8.76	42	21	-9
Superior temporal R (BA 40)	8.44	66	-39	21
Nucleus caudate R	8.07	9	3	15
Inferior parietal R	7.75	51	-36	48
Frontal inferior opercularis L	6.68	-33	18	15
Frontal middle R (BA 10)	6.61	33	51	18
Frontal superior medial R (BA 32)	6.19	6	21	42
Frontal middle R	5.88	36	6	57
Superior occipital R (BA 19)	5.84	36	-81	42
Ventrolateral PFC L (BA 47)	5.57	-39	21	-3
ACC R (BA 32)	4.66	6	36	24

FDR $P < 0.05$ corrected for the amount of false positive activations of the whole brain. L = left; R = right.

success rating down-regulation: 4.81 ± 1.31 (mean \pm s.d.)], but the effect was not significant $F(1,11) = 1.609$, $P = 0.23$).

DISCUSSION

The present study aimed to explore the neural circuitry involved when healthy participants self-regulate BOLD

activity of the left anterior insula while viewing emotional pictures. Our findings corroborate and extend previous studies that trained participants to self-regulate anterior insula activity without emotion induction by external stimuli (Caria *et al.*, 2007, 2010). Whereas both emotion induction and self-regulation activated a number of predominantly frontal regions, PPI analysis uncovered a more focused network specifically involved in self-regulation of anterior insula activity.

Whole-brain analyses during up-regulation showed higher activity in the left insula, left VLPFC, right insula, left frontal operculum and anterior and middle cingulate cortex. Group analysis of the differential activation between the individual anterior insula and control ROI during up-regulation used for the neurofeedback training revealed that subjects learned to increase their BOLD activity from the first to the last training session. In fact, robust up-regulation ability over all trials was found in the last training session. Additional ROI time-course analysis showed enhanced BOLD activation in left anterior insula over training sessions, indicating a learning effect. This is in line with the studies of Caria *et al.* (2007, 2010), where feedback was supplied without preceding emotion induction by external stimuli. Our results thus support previous findings that self-regulation of localized brain areas can be learned within a few training sessions.

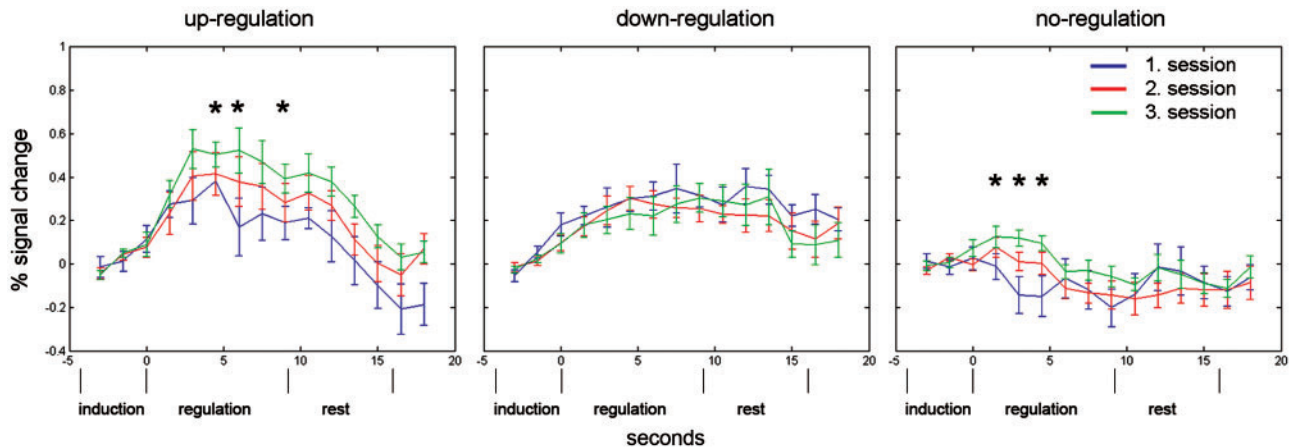


Fig. 5 PSTH plots of the BOLD response in the left anterior insula during up-regulation (left), down-regulation (middle) and no-regulation (right) in the first (blue lines), second (red lines) and third (green lines) training session averaged over participants (\pm s.e.m.). Each trial consisted of an induction/instruction period (4.5 s), a regulation period (starts at time 0 s and lasts 9 s) and a resting period (7.5 s). There was a significant learning effect during up-regulation over sessions. Significant differences between corresponding time-points in Sessions 1 and 3 are indicated with black stars.

Table 4 Regions showing increased functional connectivity during up-regulation of the left anterior insula during training

Region (Brodmann's area)	t-value	MNI coordinates		
		x	y	z
Session 1				
Lingual gyrus R (BA 18)	4.4	30	-93	-18
Insula R	4.09	42	9	-3
Ventrolateral PFC R (BA 47)	3.93	54	18	-6
Frontal inferior operculum R	3.89	42	27	-6
Session 2				
Inferior orbitofrontal L (BA 47)	6.95	-36	30	-18
Middle frontal L	3.91	-33	48	30
Middle orbitofrontal L (BA 11)	3.92	-27	48	-15
Session 3				
Occipital inferior R (BA 19)	9.48	45	-69	-18
Dorsal medial PFC L (BA 9)	8.62	-12	54	27
Occipital inferior L (BA 19)	4.74	-45	-78	-12
Ventrolateral PFC R (BA 47)	5.75	54	24	0
Ventrolateral PFC L (BA 47)	5.63	-45	33	3

All $P < 0.001$ (uncorrected for multiple comparisons).
L = left; R = right.

During down-regulation, we observed increased activation in specific frontal areas including left frontal inferior operculum, bilateral MFC, superior medial frontal cortex, bilateral VLPFC, right anterior insula and ACC. In addition to these frontal regions, we found activations in superior temporal cortex, in the caudate nucleus and in right inferior parietal cortex. However, no decrease of left anterior insula activity and no learning effect across training sessions were observed in this condition. Interestingly, a tendency of improved down-regulation ability in the last training trails of each session was observed. In contrast, during the no-regulation condition we consistently found no significant

BOLD signal increases, in line with other rt-fMRI studies using passive viewing as a baseline condition (Caria *et al.*, 2007, 2010). The relative signal increase from session 1 to sessions 2 and 3 during no-regulation cannot be attributed to differential activations in the control area across sessions, but rather—as suggested by time-series and rt-fMRI analyses—the effect of emotional induction on anterior insula activity varies over trials.

In up- and down-regulation conditions, we found increased activation in the dorsal part of the ACC and bilaterally in the anterior insula, the adjacent frontal inferior operculum and VLPFC (BA 47). The dorsal ACC activity can be interpreted in terms of ongoing monitoring of regulation performance, as previously reported in emotion regulation paradigms (Ochsner *et al.*, 2002, 2004). Conjoint activation of ACC and anterior insula is often reported in participants experiencing emotional feelings (Craig, 2009). VLPFC is specifically involved in the reappraisal of negative emotions (Ochsner and Gross, 2005), to support the selection and application of reappraisal strategies and to decrease, increase or maintain activity in appraisal systems such as the amygdala or insula in accordance with the goal of reappraisal (Beauregard *et al.*, 2001; Ochsner *et al.*, 2002; Schaefer *et al.*, 2002; Levesque *et al.*, 2003; Kim *et al.*, 2004; Ochsner *et al.*, 2004; Phan *et al.*, 2005). The present results thus corroborate previous research showing that increased frontal cortex activation supports the top-down processes required to exert control over emotion-related insula activity (Davidson *et al.* 2000).

In line with the studies of Caria *et al.* (2007, 2010), we found bilateral insula activation during volitional up-regulation of left anterior insula. However, we found strong right-lateralized anterior insula activation during down-regulation. Gray *et al.* (2007) reported right anterior insula activity when false physiological feedback was

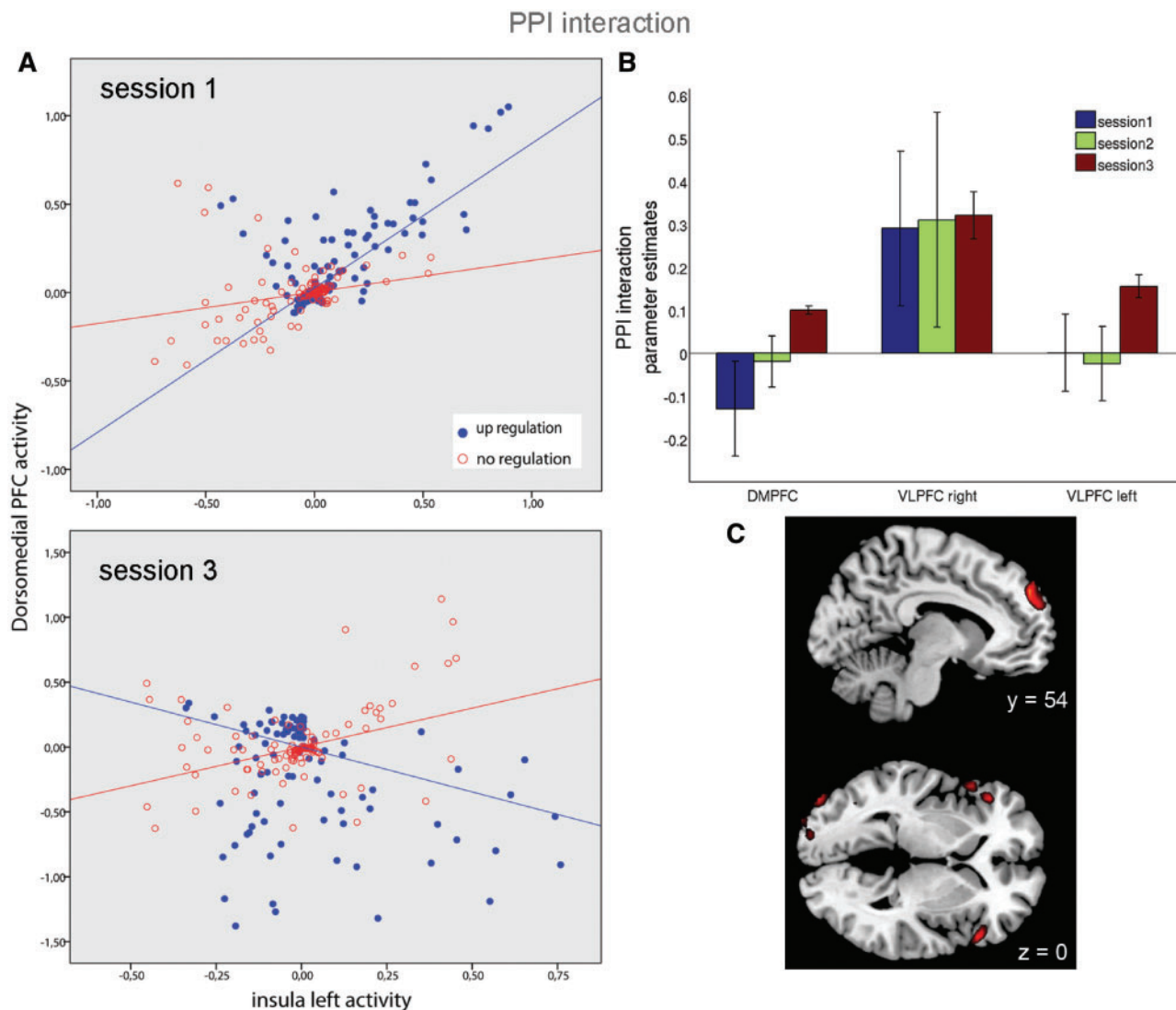


Fig. 6 (A) PPI interaction displayed as different regression slopes of DMPFC activity on left anterior insula activity during up-regulation compared to no-regulation in one representative subject. While a negative interaction was present in the first session, a significantly positive interaction was observed in the third session. (B) Parameter estimates reflecting the positive or negative connectivity between DMPFC/bilateral ventrolateral PFC and left anterior insula. (C) Regions showing effective connectivity with left anterior insula during emotional up-regulation while viewing threat-related pictures in the last training session, overlaid on a canonical single-subject brain. Significant positive modulations were found in the DMPFC and bilateral VLPFC ($p < 0.001$).

provided. They concluded that the right anterior insula acts as a superordinate appraisal system for bodily arousal. It should be noted that participants were not aware that they were regulating activity of the anterior insula, as they were simply informed about their general 'regulation success' by the increasing or decreasing thermometer bar. Similarly, Lee *et al.* (2006) have shown that incongruent emotional states (smiling while viewing sad movies) activate the right anterior insula. Therefore, one could speculate that particularly during the early regulation period where the BOLD signal is enhanced after emotion induction, a mismatch between one's own feelings and the increasing feedback signal could cause a conflict during down-regulation but not during up-regulation. The heightened right anterior insula activity

during down-regulation which we observed here could thus be interpreted as emotional conflict monitoring.

The multitude of additional activations in frontal, temporal and parietal areas during down-regulation may imply that more cognitive effort was involved during down-regulation. The greater difficulty to decrease negative emotions has been described previously (Ochsner *et al.*, 2004; Kim *et al.*, 2007). The middle frontal gyrus is involved in the selection and control of behavioural and emotional strategies during task performance and regulates selective attention (Garavan *et al.*, 2006). Koenigsberg *et al.* (2010) have shown that emotional distancing from negatively valenced pictures activates inferior parietal gyrus, as well as the middle and superior temporal gyri. We found activations in these

areas only during down-regulation and this supports the assumption that participants tried to distance themselves from the negative pictures.

A closer examination of activation time courses revealed different temporal dynamics during up- and down-regulation. While activity after up-regulation declined to baseline values during the resting period, activity after down-regulation kept increasing before the next trial. Interestingly, Goldin *et al.* (2008) reported a bilateral increase of insula activity when participants engaged in expressive suppression to regulate emotions. It is possible that different participants used different strategies for up- and down-regulation (i.e. reappraisal vs suppression) and this may partly explain our findings. It is also possible that the presence of threat-related images along with frustration about one's inability to down-regulate led to an unwanted increase in arousal and mental effort. Thus, while participants tried to reduce insula activity, they may have ended up being aroused by the self-regulation process, and their poor performance during down-regulation may have paradoxically activated the insula and other emotion-related areas. In this sense, down-regulation would become more difficult to achieve than up-regulation without previous training. In several studies, participants were successful at down-regulation of their emotional responses, but only after extensive training (Ochsner *et al.*, 2004; Eippert *et al.*, 2007). It can be assumed that without the conflicting feedback information, participants are better at controlling their emotional involvement. The fact that decreased anterior insula activity during down-regulation was observed in all sessions during the last trials supports the assumption that prolonged training periods might yield successful down-regulation.

The PPI analysis for the different training sessions during up-regulation revealed that in the first session, left anterior insula activity was functionally linked to right insula, right VLPFC and right frontal operculum, while in the second session, the left VLPFC and left middle OFC covaried with the seed region. The PPI analysis for up-regulation in the last training session, when most participants showed improved up-regulation of left anterior insula activity, revealed strengthened connectivity between left DMPFC and bilateral VLPFC and left anterior insula. Ochsner and Gross (2005) reported that top-down control of emotional responses via reappraisal activated the lateral and medial PFC and others have reported similar results (Quirk and Beer, 2006). Self-knowledge, i.e. the monitoring of one's own emotional state, has been associated with activation of MFC (Bush *et al.*, 2000; Phan *et al.*, 2002; Ochsner *et al.*, 2004; Steele *et al.*, 2004) and DMPFC. Pollatos *et al.* (2007) investigated brain areas involved in interoceptive awareness and demonstrated that the amount of interoceptive awareness correlated strongly with activity in DMPFC. A recent study using extended rt-fMRI training of the bilateral insula in schizophrenic patients showed that during successful up-regulation, the medial prefrontal cortex exhibited

strong effective connectivity (outflow) to the insula using GCM (Ruiz *et al.*, 2011). Based on these findings, our results suggest that the DMPFC plays a key role in the perception of feelings and feedback monitoring during up-regulation of the anterior insula.

A limitation of our study is that participants lacked training in self-regulation before the measurements were made. This prevents us from drawing firm conclusions about the effects of down-regulation, as no significant decrease in anterior insula activation was seen across subjects. Also, while rt-fMRI neurofeedback studies usually use regulation periods of 20–30 s, emotion regulation studies frequently apply regulation periods of only 11 s on average (Kalisch, 2009). It could be argued that a period of 9 s employed here was too short, because the BOLD signal is delayed relative to the onset of the task and the computed feedback signal during initial self-regulation is thus contaminated by late signals from the induction phase. It is possible that during the induction period, different pictures elicited stronger or weaker emotional responses and therefore modified the activation and hence the feedback signal. However, the BOLD signal during no-regulation trials reveals that the signal from the anterior insula can decrease even below baseline values in the presence of aversive pictures, making this confound appear less problematic. Nevertheless, optimized training protocols for rt-fMRI may be necessary when appraisal-relevant regions like the amygdala or anterior insula are the targets of self-regulation of stimulus-elicited activity. It is conceivable that longer regulation periods or the presentation of feedback signals only during the later parts of self-regulation periods are more suitable in these cases. The additional recording of peripheral data like breathing would further enhance the neural specificity of the feedback signal, although whole-brain and PPI analyses argue that respiratory effects do not account for the reported findings. Moreover, improved neurofeedback systems allowing the selection of ROIs solely in the grey matter over several slices, as well as advanced online artefact correction could increase the reliability of the feedback signal.

CONCLUSION

This study investigated the brain regions involved in the regulation of left anterior insula activity using rt-fMRI while subjects viewed aversive pictures. PPI connectivity analysis showed that during up-regulation, left anterior insula interacts positively with DMPFC and VLPFC. Our findings extend previous rt-fMRI work that focused on the targeted ROI, without addressing the question of how other regions may support or inhibit regulation success. Our results further demonstrate that rt-fMRI-based neurofeedback training may augment classical emotion regulation paradigms by providing direct feedback of activity in emotional brain networks and thus improving regulation success. Such an approach may prove especially useful in patients with

impaired emotional control mechanisms such as in schizophrenia and psychopathy.

Conflict of Interest

None declared.

REFERENCES

- Anders, S., Lotze, M., Erb, M., Grodd, W., Birbaumer, N. (2004). Brain activity underlying emotional valence and arousal: a response-related fMRI study. *Human Brain Mapping*, 23, 200–9.
- Bantick, S.J., Wise, R.G., Ploghaus, A., Clare, S., Smith, S.M., Tracey, I. (2002). Imaging how attention modulates pain in humans using functional MRI. *Brain*, 125, 310–9.
- Beauregard, M., Levesque, J., Bourgouin, P. (2001). Neural correlates of conscious self-regulation of emotion. *Journal of Neuroscience*, 21, RC165.
- Bishop, S., Duncan, J., Brett, M., Lawrence, A.D. (2004). Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. *Nature Neuroscience*, 7, 184–8.
- Bush, G., Luu, P., Posner, M.I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences*, 4, 215–22.
- Calder, A.J., Lawrence, A.D., Young, A.W. (2001). Neuropsychology of fear and loathing. *Nature Reviews Neuroscience*, 2, 352–63.
- Caria, A., Sitaram, R., Birabumer, N. (2011). Real-time fMRI: a tool for local brain regulation. *Neuroscientist*, 7 June 2011 [Epub ahead of print].
- Caria, A., Sitaram, R., Veit, R., Begliomini, C., Birbaumer, N. (2010). Volitional control of insula activity modulates the response to aversive stimuli. A real-time functional magnetic resonance study. *Biological Psychiatry*, 68, 425–32.
- Caria, A., Veit, R., Sitaram, R., et al. (2007). Regulation of anterior insular cortex activity using real-time fMRI. *Neuroimage*, 35, 1238–46.
- Craig, A.D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews Neuroscience*, 3, 655–66.
- Craig, A.D. (2003). Pain mechanisms: labeled lines versus convergence in central processing. *Annual Review of Neuroscience*, 26, 1–30.
- Craig, A.D. (2009). How do you feel - now? The anterior insula and human awareness. *Nature Reviews Neuroscience*, 10, 59–70.
- Critchley, H.D., Melmed, R.N., Featherstone, E., Mathias, C.J., Dolan, R.J. (2002). Volitional control of autonomic arousal: a functional magnetic resonance study. *Neuroimage*, 16, 909–19.
- Critchley, H.D., Wiens, S., Rotshtein, P., Ohman, A., Dolan, R.J. (2004). Neural systems supporting interoceptive awareness. *Nature Neuroscience*, 7, 189–95.
- Davidson, R.J., Putnam, K.M., Larson, C.L. (2000). Dysfunction in the neural circuitry of emotion regulation—a possible prelude to violence. *Science*, 289, 591–4.
- deCharms, R.C., Maeda, F., Glover, G.H., et al. (2005). Control over brain activation and pain learned by using real-time functional MRI. *Proceedings of the National Academy of Sciences USA*, 102, 18626–31.
- Eippert, F., Veit, R., Weiskopf, N., Erb, M., Birbaumer, N., Anders, S. (2007). Regulation of emotional responses elicited by threat-related stimuli. *Human Brain Mapping*, 28, 409–23.
- Etkin, A., Egner, T., Peraza, D.M., Kandel, E.R., Hirsch, J. (2006). Resolving emotional conflict: a role for the rostral anterior cingulate cortex in modulating activity in the amygdala. *Neuron*, 51, 871–82.
- Friston, K.J., Harrison, L., Penny, W. (2003). Dynamic causal modelling. *Neuroimage*, 19, 1273–302.
- Garavan, H., Hester, R., Murphy, K., Fassbender, C., Kelly, C. (2006). Individual differences in the functional neuroanatomy of inhibitory control. *Brain Research*, 1105, 130–42.
- Genovese, C.R., Lazar, N.A., Nichols, T. (2002). Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage*, 15, 870–8.
- Goldin, P.R., McRae, K., Ramel, W., Gross, J.J. (2008). The neural basis of emotion regulation: reappraisal and suppression of negative emotion. *Biological Psychiatry*, 63, 577–86.
- Gray, M.A., Harrison, N.A., Wiens, S., Critchley, H.D. (2007). Modulation of emotional appraisal by false physiological feedback during fMRI. *PLoS One*, 2, e546.
- Haller, S., Birbaumer, N., Veit, R. (2010). Real-time fMRI feedback training may improve chronic tinnitus. *European Radiology*, 20, 696–703.
- Hamilton, J.P., Glover, G.H., Hsu, J.-J., Johnson, R.F., Gotlib, I.H. (2011). Modulation of subgenual anterior cingulate cortex activity with real-time neurofeedback. *Human Brain Mapping*, 32, 22–31.
- Hariri, A.R., Bookheimer, S.Y., Mazziotta, J.C. (2000). Modulating emotional responses: effects of a neocortical network on the limbic system. *Neuroreport*, 11, 43–8.
- Hariri, A.R., Mattay, V.S., Tessitore, A., Fera, F., Weinberger, D.R. (2003). Neocortical modulation of the amygdala response to fearful stimuli. *Biological Psychiatry*, 53, 494–501.
- Johnston, S.J., Boehm, S.G., Healy, D., Goebel, R., Linden, D.E. (2010). Neurofeedback: a promising tool for the self-regulation of emotion networks. *Neuroimage*, 49, 1066–72.
- Kalisch, R. (2009). The functional neuroanatomy of reappraisal: time matters. *Neuroscience and Biobehavioral Reviews*, 33, 1215–26.
- Kim, J., Horwitz, B. (2008). Investigating the neural basis for fMRI-based functional connectivity in a blocked design: application to interregional correlations and psycho-physiological interactions. *Magnetic Resonance Imaging*, 26, 583–93.
- Kim, H., Somerville, L.H., Johnstone, T., et al. (2004). Contextual modulation of amygdala responsivity to surprised faces. *Journal of Cognitive Neuroscience*, 16, 1730–45.
- Koenigsberg, H.W., Fan, J., Ochsner, K.N., et al. (2010). Neural correlates of using distancing to regulate emotional responses to social situations. *Neuropsychologia*, 48, 1813–22.
- Lane, R.D., Reiman, E.M., Ahern, G.L., Schwartz, G.E., Davidson, R.J. (1997). Neuroanatomical correlates of happiness, sadness, and disgust. *American Journal of Psychiatry*, 154, 926–33.
- Lee, T.W., Josephs, O., Dolan, R.J., Critchley, H.D. (2006). Imitating expressions: emotion-specific neural substrates in facial mimicry. *Social Cognitive Affective Neuroscience*, 1, 122–35.
- Lee, S., Ruiz, S., Caria, S., Veit, R., Birbaumer, N., Sitaram, R. (2011). Detection of cerebral reorganization induced by real-time fMRI feedback training of insula activation. *Neurorehabilitation and Neural Repair*, 25, 259–67.
- Levesque, J., Eugene, F., Joanette, Y., et al. (2003). Neural circuitry underlying voluntary suppression of sadness. *Biological Psychiatry*, 53, 502–10.
- Mak, A.K., Hu, Z.G., Zhang, J.X., Xiao, Z.W., Lee, T.M. (2009). Neural correlates of regulation of positive and negative emotions: an fMRI study. *Neuroscience letters*, 457, 101–6.
- Morris, J.S., Frith, C.D., Perrett, D.I., et al. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature*, 383(6603), 812–5.
- Ochsner, K.N., Bunge, S.A., Gross, J.J., Gabrieli, J.D. (2002). Rethinking feelings: an FMRI study of the cognitive regulation of emotion. *Journal of Cognitive Neuroscience*, 14, 1215–29.
- Ochsner, K.N., Gross, J.J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences*, 9, 242–9.
- Ochsner, K.N., Ray, R.D., Cooper, J.C., et al. (2004). For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. *Neuroimage*, 23, 483–99.
- Phan, K.L., Fitzgerald, D.A., Nathan, P.J., Moore, G.J., Uhde, T.W., Tancer, M.E. (2005). Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. *Biological Psychiatry*, 57, 210–9.
- Phan, K.L., Wager, T., Taylor, S.F., Liberzon, I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*, 16, 331–48.

- Pollatos, O., Gramann, K., Schandry, R. (2007). Neural systems connecting interoceptive awareness and feelings. *Human Brain Mapping*, 28, 9–18.
- Posse, S., Fitzgerald, D., Gao, K., et al. (2003). Real-time fMRI of temporolimbic regions detects amygdala activation during single-trial self-induced sadness. *Neuroimage*, 18, 760–8.
- Quirk, G.J., Beer, J.S. (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Current Opinion in Neurobiology*, 16, 723–7.
- Ruiz, S., Lee, S., Soekader, S., et al. (2011). Acquired self-control of insula cortex modulates emotion recognition and brain network connectivity in schizophrenia. *Human Brain Mapping*, in press.
- Ruiz, S., Sitaram, R., Lee, S., et al. (2008). Learned control of insular activity and functional connectivity changes using a fMRI Brain Computer Interface in Schizophrenia. *38th annual meeting of the Society for Neuroscience*, Washington. November [Abstract].
- Schaefer, S.M., Jackson, D.C., Davidson, R.J., Aguirre, G.K., Kimberg, D.Y., Thompson-Schill, S.L. (2002). Modulation of amygdalar activity by the conscious regulation of negative emotion. *Journal of Cognitive Neuroscience*, 14, 913–21.
- Shafritz, K.M., Collins, S.H., Blumberg, H.P. (2006). The interaction of emotional and cognitive neural systems in emotionally guided response inhibition. *Neuroimage*, 31, 468–75.
- Sitaram, R., Caria, A., Birbaumer, N. (2009). Hemodynamic brain-computer interfaces for communication and rehabilitation. *Neural Networks*, 22, 1320–8.
- Sitaram, R., Lee, S., Ruiz, S., Rana, M., Veit, R., Birbaumer, N. (2010). Real-time support vector classification and feedback of multiple emotional brain states. *Neuroimage*, 56, 753–65.
- Sitaram, R., Veit, R., Steven, B., et al. (2011). Acquired control of ventral premotor cortex activity by feedback training: an exploratory real-time fMRI and TMS study. *Neurorehabilitation and Neural Repair*. 8 September 2011 (Epub ahead of print; doi:10.1177/1545968311418345).
- Sitaram, R., Weiskopf, N., Caria, A., Veit, R., Erb, M., Birbaumer, N. (2008). fMRI Brain-Computer Interfaces. *IEEE Signal Processing*, 25, 95–106.
- Steele, J.D., Lawrie, S.M. (2004). Segregation of cognitive and emotional function in the prefrontal cortex: a stereotactic meta-analysis. *Neuroimage*, 21, 868–75.
- Taylor, S.F., Phan, K.L., Decker, L.R., Liberzon, I. (2003). Subjective rating of emotionally salient stimuli modulates neural activity. *Neuroimage*, 18, 650–9.
- Tracey, I., Ploghaus, A., Gati, J.S., et al. (2002). Imaging attentional modulation of pain in the periaqueductal gray in humans. *Journal of Neuroscience*, 22, 2748–52.
- Urry, H.L., van Reekum, C.M., Johnstone, T., et al. (2006). Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *Journal of Neuroscience*, 26, 4415–25.
- Weiskopf, N., Sitaram, R., Josephs, O., et al. (2007). Real-time functional magnetic resonance imaging: methods and applications. *Magnetic Resonance Imaging*, 25, 989–1003.
- Weiskopf, N., Veit, R., Erb, M., et al. (2003). Physiological self-regulation of regional brain activity using real-time functional magnetic resonance imaging (fMRI): methodology and exemplary data. *Neuroimage*, 19, 577–86.
- Whalen, P.J., Bush, G., McNally, R.J., et al. (1998). The emotional counting Stroop paradigm: a functional magnetic resonance imaging probe of the anterior cingulate affective division. *Biological Psychiatry*, 44, 1219–28.