ELSEVIER

NeuroImage

www.elsevier.com/locate/ynimg
NeuroImage 35 (2007) 1238–1246

Regulation of anterior insular cortex activity using real-time fMRI

Andrea Caria,[a,b,*] Ralf Veit,[a,e] Ranganatha Sitaram,[a] Martin Lotze,[a] Nikolaus Weiskopf,[c] Wolfgang Grodd,[d] and Niels Birbaumer[a,f]

[a]*Institute of Medical Psychology and Behavioral Neurobiology, Eberhard-Karls-University of Tübingen, Tübingen, Germany*
[b]*Department of Cognitive Science and Education, University of Trento, Trento, Italy*
[c]*Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, London, UK*
[d]*Section of Experimental MR of the CNS, Department of Neuroradiology, University of Tübingen, Tübingen, Germany*
[e]*Max Planck Institute for Biological Cybernetics, Tuebingen, Germany*
[f]*National Institute of Health (NIH), NINDS, Human Cortical Physiology, Bethesda, MD, USA*

Received 17 October 2006; revised 12 January 2007; accepted 12 January 2007
Available online 31 January 2007

Recent advances in functional magnetic resonance imaging (fMRI) data acquisition and processing techniques have made real-time fMRI (rtfMRI) of localized brain areas feasible, reliable and less susceptible to artefacts. Previous studies have shown that healthy subjects learn to control local brain activity with operant training by using rtfMRI-based neurofeedback. In the present study, we investigated whether healthy subjects could voluntarily gain control over right anterior insular activity. Subjects were provided with continuously updated information of the target ROI's level of activation by visual feedback. All participants were able to successfully regulate BOLD-magnitude in the right anterior insular cortex within three sessions of 4 min each. Training resulted in a significantly increased activation cluster in the anterior portion of the right insula across sessions. An increased activity was also found in the left anterior insula but the percent signal change was lower than in the target ROI. Two different control conditions intended to assess the effects of non-specific feedback and mental imagery demonstrated that the training effect was not due to unspecific activations or non feedback-related cognitive strategies. Both control groups showed no enhanced activation across the sessions, which confirmed our main hypothesis that rtfMRI feedback is area-specific. The increased activity in the right anterior insula during training demonstrates that the effects observed are anatomically specific and self-regulation of right anterior insula only is achievable. This is the first group study investigating the volitional control of emotionally relevant brain region by using rtfMRI training and confirms that self-regulation of local brain activity with rtfMRI is possible.

Keywords: Self-regulation; Physiological regulation; Real-time fMRI; Brain–computer interface; Neurofeedback; Blood oxygen level-dependent; Insula

* Corresponding author. Institute of Medical Psychology and Behavioural Neurobiology, Eberhard-Karls-University of Tübingen, Gartenstr. 29, D-72074 Tuebingen, Germany. Fax: +49 7071 295956.
E-mail address: andrea.caria@uni-tuebingen.de (A. Caria).
Available online on ScienceDirect (www.sciencedirect.com).

1053-8119/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2007.01.018

Introduction

Studies on physiological self-regulation of brain activity, mostly using electroencephalography (EEG) demonstrated that, with appropriate training, individuals can learn to control brain processes. Learned regulation of slow cortical potentials was used to allow communication in severely paralyzed patients (Birbaumer et al., 1999; Kübler et al., 2001) and to suppress epileptic activity (Kotchoubey et al., 2001). By using self-regulation of oscillatory EEG activity patients with motor impairments were able to control a hand prosthesis (Pfurtscheller et al., 2000; Neuper et al., 2003).

The mechanisms of these changes in EEG (SCP, mu and alpha rhythms) are neuroanatomically specific and reflect activity in complex brain networks (Hinterberger et al., 2005). Due to poor spatial resolution, highly localized and subcortical brain regions are difficult to regulate with EEG-neurofeedback.

Recent advances in functional magnetic resonance imaging (fMRI) data acquisition and processing techniques have made rtfMRI of localized brain areas feasible, reliable and less susceptible to artefacts. rtfMRI allows on-line analysis of functional brain activity and feedback of the Blood Oxygen Level-Dependent (BOLD) signal from a targeted region of interest. In addition, fMRI-based techniques in comparison to all the other human brain mapping techniques, represents the only non-invasive method allowing feedback regulation of deep subcortical brain regions such as the limbic and paralimbic areas.

Previous studies (for a complete review see Weiskopf et al., 2004b) showed that healthy subjects can learn to control local brain activity by operant training with rtfMRI-based neurofeedback.

These studies focused on different cortical and subcortical areas: the sensorimotor cortex (deCharms et al., 2004; Yoo and Jolesz, 2002), the supplementary motor area (SMA; Weiskopf et al., 2004a), the parahippocampal place area (PPA; Weiskopf et al., 2004a), the anterior cingulate cortex (ACC) (Weiskopf et al., 2003), and the amygdala (Posse et al., 2003). In a recent study

deCharms et al. (2005) demonstrated that subjects were able to learn to control activation in the rostral anterior cingulate cortex (rACC), a region implicated in mediating the perception of pain. Furthermore, this study showed that control of up- and down-regulation of rACC activation was associated with changes in pain perception induced by noxious thermal stimulation. Chronic pain patients were also trained to control activation in rACC and reported reduction of the level of chronic pain after training. All these studies provided significant evidence that individuals can learn to voluntarily self-regulate brain activity by using feedback training based on rtfMRI and that changes in behaviour might occur as a direct consequence.

Among the few studies conducted so far the study by deCharms et al. (2004, 2005) and Weiskopf et al. (2003, 2004a,b) provided visual rtfMRI feedback to the subjects. Only long delayed rtfMRI was used in the pilot study from Yoo and Jolesz (2002): information about fMRI data was delayed about 20 s. Posse et al. (2003) provided verbal feedback of the BOLD-activity in the amygdala with a delay of 60 s.

In addition, very few studies have explored the application of rtfMRI for training subjects to self-regulate activity in emotionally relevant areas. In the study made by Posse and colleagues (2003) the feedback was based on the experimenter's rating and it was not possible to disentangle whether regulation was achieved by feedback or mood induction. While Weiskopf et al. (2003) did indeed use rtfMRI for training to self-regulate BOLD signal of the affective division of the anterior cingulate cortex, only one subject was tested and hence the results may not be readily generalized.

In the present group study we investigated whether healthy subjects can voluntarily gain control over right anterior insular activity by using rtfMRI.

Cortical representation of smell and taste (Francis et al., 1999; Rolls, 1996, 2004), viscerosensation (Craig, 2002), and pain perception (Davis et al., 1998; Coghill et al., 1999; Peyron et al., 2000) converge in the insula and surrounding operculum. The activity of the insulae correlates with the subjective perception of emotional states (Craig, 2002, 2003). Studies on emotional perception showed that insula activity is correlating with the aversive valence of stimuli (Anders et al., 2004). A review of PET and fMRI studies investigating the neuroanatomy of emotion (Phan et al., 2002) revealed that the anterior cingulate and insula were recruited during induction by emotional recall/imagery and during emotional tasks with cognitive demand.

Awareness of salient emotionally stimuli increases right insula cortex activity (Critchley et al., 2004) suggesting that this area is critical for the representation of bodily responses and interoception (Adam, 1998).

Therefore, the possibility of volitional modulation of insula activity may be a valuable tool to study emotion regulation. Modulation of the insular activity with rtfMRI training might be relevant for the development of novel approaches for clinical treatment of social phobia or antisocial behavior which have shown overactivity and hypoactivity, respectively, in the insular region (Veit et al., 2002; Birbaumer et al., 2005).

Methods

Participants

Fifteen healthy right-handed subjects (9 women and 6 men; age range 22–38 years; mean age 25.13 years) participated in this study. Nine of them were trained to voluntarily control the local BOLD signal of the right anterior insular cortex using the rtfMRI information. The remaining six subjects participated in two different control conditions (see below). All participants were students of the Medical School and had no history of neurological or psychiatric disorders including substance abuse/dependence and psychotropic medications. All were naive to neurofeedback and fMRI experiments. Written instructions were provided to all participants and informed written consent was obtained. Subjects were carefully instructed not to move, relaxing and breathing regularly in order to avoid potential BOLD artefacts due to manipulation of internal state. This study was approved by the local ethics committee of the Faculty of Medicine of the University of Tübingen.

fMRI data acquisition

Functional images were acquired on 3.0 T whole body scanner, with standard 12-channel head coil (Siemens Magnetom Trio Tim, Siemens, Erlangen, Germany). A standard echo-planar imaging sequence was used (EPI; TR = 1.5 s, matrix size = 64 × 64, effective echo time TE = 30 ms, flip angle $\alpha = 70°$, bandwidth = 1.954 kHz/pixel). Sixteen slices (voxel size = 3.3 × 3.3 × 5.0 mm^3, slice gap = 1 mm), AC/PC 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 subject (MPRAGE, matrix size = 256 × 256, 160 partitions, 1 mm^3 isotropic voxels, TR = 2300 ms, TE = 3.93 ms, TI = 1100 ms, $\alpha = 8°$).

In order to reduce movements two foam cushions immobilized the participant's head.

Real-time fMRI data processing

The fMRI setup used for real-time data processing is based on Turbo-BrainVoyager (Brain Innovation, Maastricht, The Netherlands; Goebel, 2001) as previously described by Weiskopf et al. (2003).

Data were analyzed in real-time with Turbo-BrainVoyager software performing on-line incremental 3D motion detection and correction, and drift removal. The software is capable of incrementally computing statistical maps based on the General Linear Model (GLM) and event-related averages.

The selection of ROI1 – the right anterior insula– was anatomically based on the high resolution T1 structural scan. This ROI was a rectangular area encompassing 4 × 5 voxels (~15 × 20 mm) on a single slice (5 mm). The reference ROI2 was a large background region of interest selected from a reference slice positioned distant from ROI1 encompassing the whole brain with the intent to cancel global effects and to average out any unspecific activation. During training, the mean BOLD signal from the regions of interest ROI1 and ROI2 was extracted. The first ten volumes of each session were excluded from statistical analysis to account for T1 equilibration effects. For the feedback presentation the difference between the two ROI time-courses was calculated and normalized to the baseline.

The feedback signal was computed as $(BOLD_{reg} - BOLD_{rest})_{ROI1} - (BOLD_{reg} - BOLD_{rest})_{ROI2}$, $BOLD_{reg}$ and $BOLD_{rest}$ constitute the respective BOLD-signal during regulation and rest period. The subjects were provided visually with continuously updated information of the ROI1 level of activation. The visual feedback

of brain activity was calculated using Matlab 6.5 (The MathWorks, Natick, MA) software running on a separate personal computer connected via LAN to the scanner and to the Turbo-BrainVoyager. The feedback consisted of a graduated thermometer displaying changes of BOLD-activity with increasing or decreasing number of bars (see Fig. 1). The number of available bars was limited. Bars above the baseline level of activation were colored in red while those below the baseline level in blue. Thermometer bars were constantly updated and new fMRI information was available with a delay of about 1.5 s.

Experimental protocol

The training consisted of four feedback sessions followed by a 'transfer' session performed in 1 day. One feedback session consisted of four regulation blocks (22.5 s each) during which the subjects had to learn to increase insula activity alternating with five baseline blocks (22.5 s each) during which they had to return the activity to the baseline level. Each session lasted about 4 min and was repeated five times including the transfer session. During the feedback session the normalized average BOLD signal from the right anterior insula was presented to the subjects by means of thermometer bars. The thermometer display was present both during regulation and during rest period. The regulation blocks were cued with a red arrow at the thermometer display while during rest blocks a cross hair was presented in the same position (see Fig. 1).

During the transfer session, subjects were instructed to perform the same task as during feedback but fMRI information was not provided and bars were not shown. The transfer session was performed to verify the efficacy of the feedback and to check whether training effects might persist beyond the experimental situation.

Pilot experiments showed that learning without any guidelines for mental strategies was not achievable in a short training period and led to a drop of motivation especially in the uncomfortable environment of an MRI scanner (Sitaram et al., 2005; deCharms et al., 2005). For this purpose subjects were instructed to use cognitive strategies that potentially would help to learn to control the activity of the target ROI. Specifically, strategies for regulation blocks were focused on emotion induction by recall of personal and affectively relevant events. No cues for aversive or pleasant imagery was given. During baseline subjects were required to count back from 100. At the end of each session subjects reported mental strategies they had used during regulation blocks. Subjects were also informed of the data processing delay of about 1.5 s and of the intrinsic physiological hemodynamic response delay of about 6 s.

Additionally, two different control experiments were conducted to verify that the effects of the self-regulation of the insular activity were due to fMRI feedback. Three subjects participated to each of the control experiments. The first control condition aimed to verify the specificity of the feedback information; the control group performed three sessions of the same experimental paradigm but received sham feedback. This sham feedback was not specific to any particular brain area but consisted of information from a large background ROI from the same subjects not encompassing the anterior insulae. Feedback was comparable in terms of signal magnitude and variability. The second control condition assessed the effects of repetitive use of mental imagery. Subjects were provided with the same instructions and same general strategies as before, the thermometer frame was present but no rtfMRI information was available (no bars were shown). Subjects performed three consecutive sessions during which they were asked to recall and evoke memories and imagery of personally relevant affective events.

Off-line data analysis

Off-line image post-processing and data analysis were performed using SPM2 statistical parametric mapping software package (Wellcome Department of Imaging Neuroscience, London), while MarsBar toolbox (the Marseille region of interest toolbox for SPM2) and BrainVoyager QX were used for ROI analysis.

Before whole brain statistical analysis, functional EPI volumes were realigned spatially, normalized into Montreal Neurological Institute (MNI) space, and smoothed spatially (9-mm Gaussian kernel) and temporally (0.0088 Hz, 2.5 times the duration of the activation and baseline block) to remove high-frequency artefacts. Hemodynamic response amplitudes were estimated using standard regressors, constructed by convolving a boxcar function, representing the block duration, with a canonical hemodynamic response function using standard SPM2 parameters. Motion parameters were also included into the general linear model (GLM) as covariates to take into account artefacts caused by head motion.

Signal increase during regulation with respect to the baseline was evaluated by SPM2. Areas showing training related changes

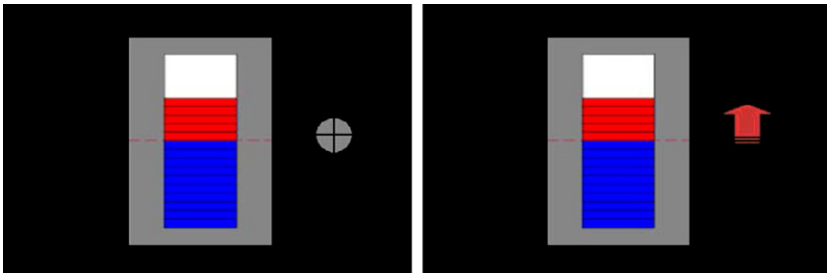

Fig. 1. Real-time feedback video projected to the subjects. The feedback consisted of a graduated thermometer displaying percentage change of BOLD-activity by showing an increasing or decreasing number of bars. The number of bars available was limited and fixed to a value of 20. Bars over the baseline level of activation were colored in red while those under the baseline level in blue. Thermometer bars were constantly updated and new fMRI information was available with a delay of about 1.5 s. The regulation blocks were cued with a red arrow (right) beside the thermometer display while during the rest blocks a cross hair (left) was presented in the same position.

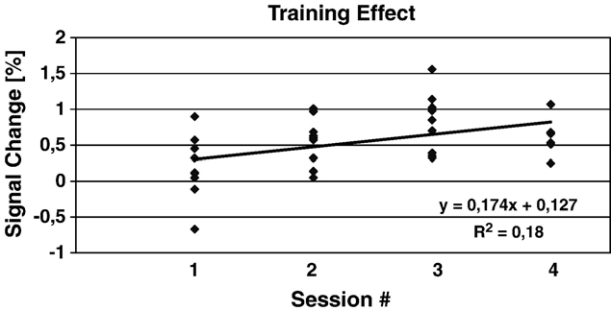

Fig. 2. BOLD percent single change computed on the individually selected region of interest across feedback sessions on single subjects. A significant BOLD increase in the target area was observed across sessions.

were analysed by performing *t*-test comparisons of increased BOLD-effect magnitude over sessions. Group analysis was performed session by session using a random effects analysis. Statistical significance of activation maps were based on a *t* test with a voxel-wise threshold of $P<0.01$.

Hypothesis-driven ROI analysis was performed using the ROI previously selected for each subject during the training. ROI time series underwent the same preprocessing and GLM used for whole brain analysis. The percent signal change during regulation blocks with respect to the baseline blocks was calculated for each session separately and then averaged across subjects.

ROI analysis was also performed on a comparable contralateral region of the same extension positioned at the left anterior insular region. The training effect was evaluated by computing an ANOVA for repeated measures on all subjects of percent signal changes in the specific ROI session by session. Furthermore, all significantly activated clusters from statistical maps other than the left and right anterior insular region were checked with MarsBar toolbox for potential increase across sessions. Activation maps produced by offline analysis matched and validated activations maps produced in real-time by Turbo-BrainVoyager. Additionally a lateralization index (LI) was calculated based on the normalized difference between percent signal change extracted from the target ROI (%R) and from the contralateral ROI (%L) as follows: (%R−%L)/(%R+%L). The LI calculation intended to assess laterality effects during training and it was calculated for each subject and then compared with control experiments.

Results

All participants were able to successfully regulate BOLD-magnitude in the right anterior insular cortex. Training resulted in a significantly increased activation cluster in the anterior portion of the right insula across sessions. Subjects reported the use of both positive and negative mental imagery. Positive strategies were focused on recalling themselves playing music, playing with daughter, engaging in sport activities, recall of holidays; while negative strategies were focused mostly on bringing back themselves in dangerous situations, anger states and while taking examinations.

Linear regression across all sessions performed on the individually selected region of interest showed significant increase of activity in the target area [$y=0.174+0.127$, $P<0.012$] (see Fig. 2). Three subjects did not complete the fourth training session hence we conducted the group analysis considering the first three sessions. The success of training is clearly visible by comparing the time course of the selected area during the last session (see Fig. 3, lower image) with the first session (see Fig. 3, upper image). Percent signal change calculated in the ROI as difference between task and rest for each subject and then averaged across all the participants resulted in a clear monotonic increase across the first three sessions [repeated measures ANOVA, $F(2,7)=10.32$, $P=0.001$] (see Fig. 4).

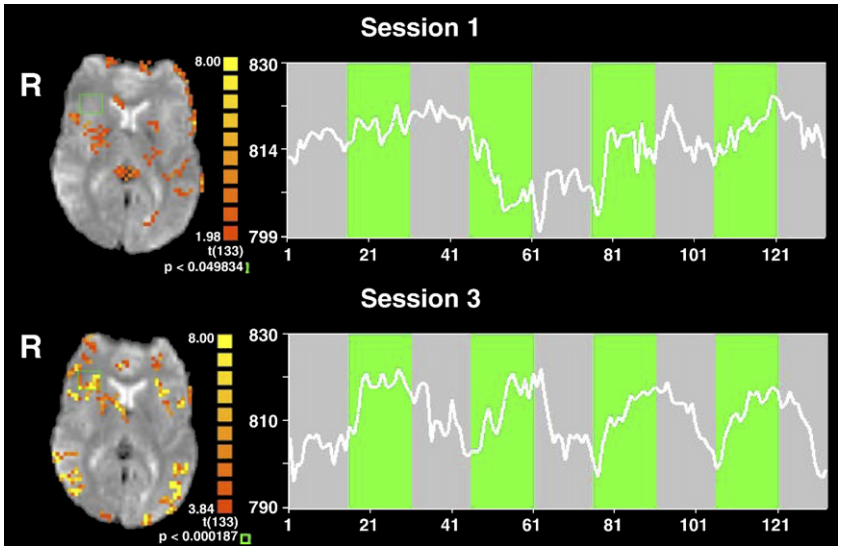

Fig. 3. Single subject statistical maps (left) and BOLD time-courses (right) of the right anterior insula in the first (top) and in the last session (bottom). The selected region of interest is delineated by the green box. Functional images are in the radiological convention and are not normalized. Statistical significance was based on *t* test comparing activation on each voxel during the regulation blocks with respect to the baseline blocks, with a threshold of $P<0.05$ false discovery rate (FDR) corrected for multiple comparisons (Genovese et al., 2002). The time course of the BOLD activity (white line) is related to the ROI selected and is showing the progress during the regulation blocks (green) and the baseline blocks (gray). Number of volumes is in the *x* axis and magnitude signal in the *y* axis; these values are the raw output from the scanner.

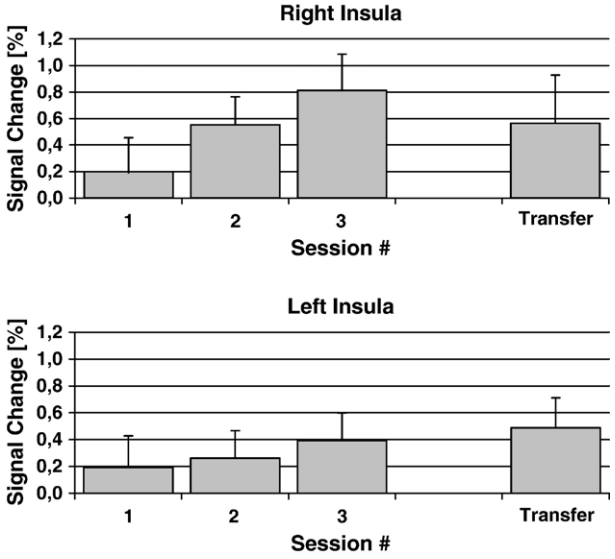

Fig. 4. Group analysis of percent signal change during training in the right (target ROI) (top) and left (bottom) anterior insula. Percent signal change, calculated in the ROI as difference between task and rest on each subject and then averaged across all the participants, reported clear monotonic increase across the three sessions. Percent signal change calculated in the corresponding contralateral area of the target ROI did not show a significant monotonic increase even though a significant increase was measured between session3 and session1. Transfer session (no rtfMRI information provided to the subjects) showed an increase of BOLD-magnitude in the right and left anterior insula but only the left insula showed a significant BOLD-magnitude increase in comparison with the first session.

Table 1
Comparison of significant signal increase during activation blocks in the last session

Brain regions	Brodmann area (BA)	Cluster size (voxels)	*t* value	MNI coordinates (*x*, *y*, *z*)
R insula	BA 47	**119**	10.23	**36, 26, 6**
R frontal inferior triangularis	**BA 45**		9.29	49, 20, 5
R premotor cortex	BA 6	**50**	8.24	**49, −3, 55**
			7.27	49, 0, 35
			6.26	36, −3, 35
R angular gyrus	BA 7	**30**	7.28	**26, −63, 50**
R inferior parietal gyrus			5.52	30 −49 50
R superior frontal gyrus	BA 6	**28**	6.82	**30, 3, 65**
			6.30	26, −7, 55
			5.81	30, 3, 55
R middle temporal gyrus	BA 37	**24**	6.78	**46, −69, 0**
			6.07	43, −53, 5
L premotor cortex	BA 6	**13**	6.53	**−53, −3, 50**
			5.34	−40, −3, 45
R supplementary motor area	BA 6	**24**	6.47	**10, 10, 60**
L insula	BA 47	**62**	5.63	**−33, 20, 0**

Clusters of significant signal increase during activation blocks from random effects analysis in the last session. Bold numbers correspond to highest peak of the cluster. Clusters exceeding the threshold of $P<0.01$ uncorrected and with a spatial extent large than 10 voxels were considered. Coordinates are in MNI stereotaxic space (Collins et al., 1994) and labelled anatomically according to Tzourio-Mazoyer et al. (2002).

Percent signal change was also calculated in the corresponding contralateral area of the target ROI (see Fig. 4). No significant monotonic increase was found in the left anterior insula [repeated measures ANOVA, $F(2,7)=1.94$, $P=0.177$] even though a small significant increase was found between session3 and session1 [paired samples *t* test, $t_{(8)}=2.92$, $P=0.019$]. Activation during training was lateralized to the right with a mean lateralization index of 0.38 ± 0.16 in the last session [one-sample *t* test, $t_{(8)}=2.20$, $P=0.029$] (see Fig. 8).

Random effects analysis on the experimental group confirmed an increased BOLD-magnitude in the right anterior insular cortex over time (see Fig. 5). Analysis of the single sessions revealed no significant activation during the first session in the target area; a significant activation cluster [$t=4.50$; $P=0.001$] during the second session (MNI coordinates: 39, 33, 0); and a highly significant activation cluster [$t=10.23$; $P<0.001$] during the third session (MNI coordinates: 36, 26, 6). Fixed effect analysis was also performed on the six subjects who completed the fourth session reporting a higher significant cluster [$t=12.47$, $P<0.001$, FWE corrected, MNI coordinates: 36, 23, 5].

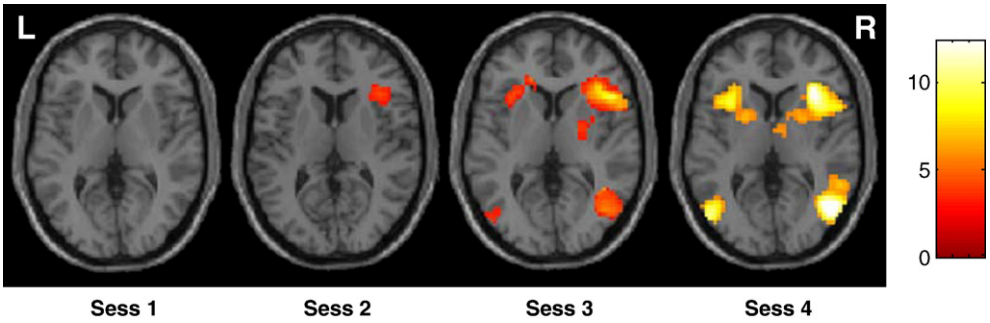

Fig. 5. Random effects analysis on the experimental group confirmed an increased BOLD–magnitude in the right anterior insular cortex over time course. SPM2 of the single sessions showed no significant activation during the first session in the target area; a significant activation cluster ($t=4.50$; $P=0.001$, uncorrected) during the second session (MNI coordinates: 39, 33, 0); and a highly significant activation cluster ($t=10.23$; $P<0.001$, uncorrected) during the third session located (MNI coordinates: 36, 26, 6). Fixed effect analysis was also performed on the six subjects who completed the fourth session reporting a higher significant cluster ($t=12.47$, $P<0.001$, FEW-corrected; MNI coordinates: 36, 23, 5). All activation maps are projected on a single-subject T1 template at the coordinate $z=5$.

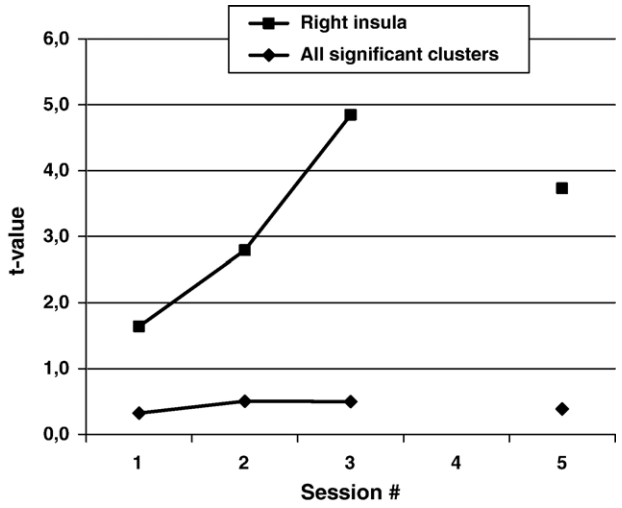

Fig. 6. All significantly activated clusters from statistical maps, besides the left and right anterior insular region, were checked with MarsBar toolbox for potential increase across sessions correlated with the feedback task due to effects of general arousal. No significant increase was observed in areas other than the target ROI during the feedback training.

Group analysis showed additional brain activations during the last session, specifically in the left and right premotor cortex, right angular gyrus, right superior frontal gyrus, right middle temporal gyrus, right supplementary motor area and left insula (see Table 1). Further ROI analysis of all additional activation clusters in the last session was also performed with MarsBar in order to estimate increase of signal change across sessions. No significant increase was observed in areas other than the target ROI during the feedback training (see Fig. 6).

The results of the transfer session showed an increase of BOLD-magnitude in the target ROI but in comparison to the first session t test it was not significant [$t_{(8)}$=4.86, P=0.06] because of the high variance between subjects. A significant increase of activity was found in the left anterior insula in comparison to the first session [paired samples t test, $t_{(8)}$=2.72, P=0.030]. Both control conditions showed no increase in BOLD-magnitude in the right anterior insula (see Fig. 7) and did not show any significant lateralization.

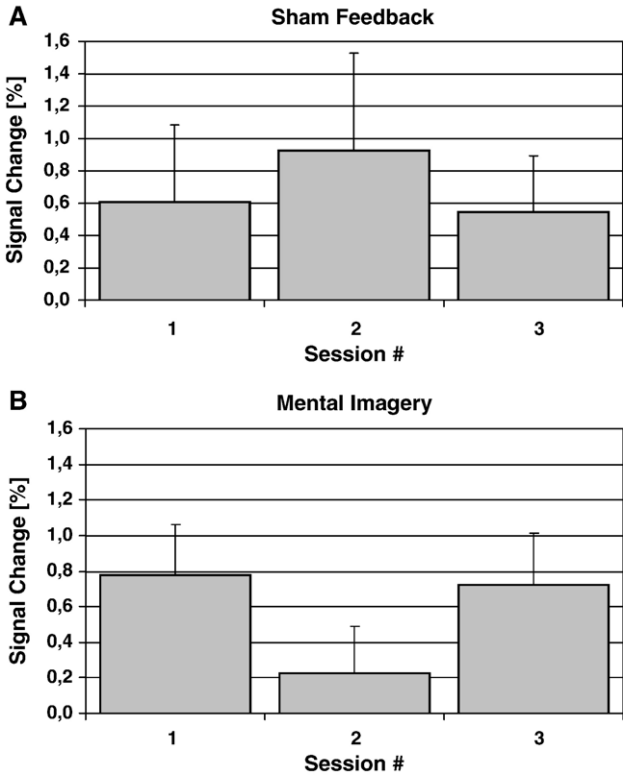

Fig. 7. Group analysis of percent signal change during control experiments in the right anterior insula. (A) Percent signal change averaged over the group during sham training. (B) Task-driven activation during mental imagery performance. Both control groups showed no increase in BOLD-magnitude in the right anterior insula.

Discussion

With rtfMRI feedback a specific modulation of the right anterior insula is possible. This was achieved after a short training time. BOLD signal in the target ROI increased with the number of feedback sessions, indicating training effects and learning. Previous studies from deCharms et al. (2005) and Weiskopf et al. (2004a,b) also showed that one single-day training with rtfMRI feedback is enough to achieve learning.

Areas which showed activation during the last training session did not show a training effect. They may, however promote the self-control of the specific target ROI. The left anterior insula showed increased activity in the last session with respect to the first session but the percent signal change was much lower than in the target ROI and no significant monotonic increase was found. Furthermore, the lateralization index confirmed a stronger effect in the right anterior insula during training (Fig. 8). This demonstrate that, even though emotional tasks often involve both insulae, specific regulation of the right anterior insula only is achievable. deCharms et al. (2004) did not report a significant increase in activation of the ROI placed in a comparable position to the target ROI but ipsilateral to the motor task being performed; in this study, subjects were instructed that during the task blocks they had to

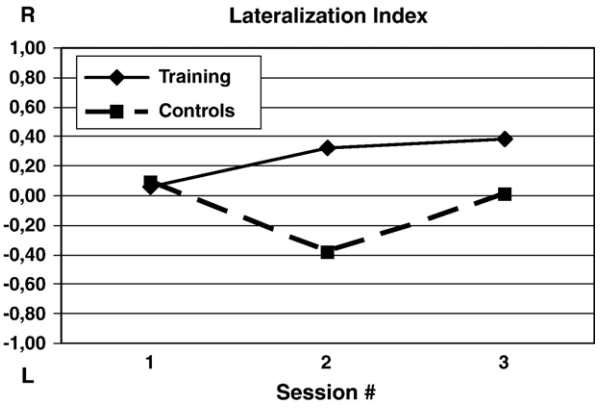

Fig. 8. Lateralization Index (R=+÷L=−). It was calculated based on the normalized difference between percent signal change extracted from the target ROI and from the contralateral ROI on each single subject and then averaged. Activation during training was lateralized to the right with a mean lateralization index of 0.38±0.16 in the last session. Control experiments did not show a similar result.

imagine moving their dominant (right) hand so as to increase the level of activation in the ROI of the contralateral somatomotor cortex.

The group analysis of the transfer session showed increase of activity in both right and left anterior insula. This result further underscores the specificity of the rtfMRI feedback in the target ROI. During feedback, the activation in the right anterior insula is larger than in the left anterior insula, but the activation are comparable during the transfer session (no rtfMRI information provided). One could speculate that the requirement for specific regulation of the target ROI reduces activity in the contralateral area. A previous study (deCharms et al., 2004) reported that after a longer training it was possible to continue regulation of the BOLD signal without feedback.

Two different control conditions demonstrated that the training effect was not due to global and unspecific activations. Both control conditions and activation of other brain areas showed no enhanced activation across sessions confirming our hypothesis. These findings are in line with the studies of deCharms et al. (2004, 2005) which reported no effect in several control conditions and confirmed feedback as an essential means to achieve self-regulation.

The subjects reported that the experiment was hard but challenging. In the previous rtfMRI studies (Weiskopf et al., 2003, 2004a,b; deCharms et al., 2004, 2005), subjects were presented with continuously updated time-course plot of the target ROI and they were also provided with the time-course of a background ROI and the difference between the two (deCharms et al., 2004), on-line motion correction (Weiskopf et al., 2003), or video images (deCharms et al., 2005). In this study only information of the target ROI activation was provided and no graph was displayed to the subjects; we assert that that subject's attention was focused more on the task by this display.

To our knowledge, this is the first group study reporting volitional control over emotionally relevant brain region with rtfMRI training. This provides further confirmation of self-regulation of local brain activity with rtfMRI and extends previous findings to the area of the anterior insula.

An important point to mention in order to properly interpret the reported results is the question whether insula cortex activity in humans represents or controls visceral and cardiorespiratory functions which still remains controversial. The experiments conducted so far (Penfield and Faulk, 1955; Oppenheimer et al., 1992; Sander and Klingelhöfer, 1995; Saper, 2002) indicate that the human insular cortex does contain autonomic control sites, however these results were obtained from patients and using poor anatomical localization. Moreover a study from King et al. (1999) indicated different sub-regions in the anterior insular cortex, a ventral part responding to gustatory stimulation and a more superior part responding to cardiopulmonary stimulation. Wager and Feldman Barrett (2004) suggest that the ventral anterior agranular insula is activated consistently by neuroimaging studies involved in aurally and recall-generated emotion induction. Furthermore Rainville et al. (2006) indicates that the feeling of basic emotions is inherently associated with distinct patterns of cardiorespiratory activity. However Nagai et al. (2004) reported no right anterior insula activity during biofeedback regulation of skin conductance level and in a rtfMRI study from Posse et al. (2003) subjects performing a sad mood induction task did not show changes in respiration rate and end-expiratory pco_2 ($Petco_2$) strong enough to alter global fMRI contrast. Yet, a study from Critchley et al. (2004) showed right anterior insula activity enhanced by interoceptive awareness in the absence of physiological changes indicating a major role of this region for feelings perception.

The increase in the right insula activity during the training may represent an increased attention to internal body sensations generated during subjective affective experience as the right insula contributes to subjective emotional responses (Critchley et al., 2004). This increased activation may even result in an increased sensitivity to emotionally relevant stimuli, a hypothesis not tested by the present study.

Previous studies on cognitive control of emotions (Ochsner et al., 2004; Ochsner and Gross, 2005) demonstrated that controlling arousing stimuli depend upon interactions between cortical and subcortical (e.g. insula, amygdala) emotional systems.

Furthermore, a recent hypothesis is that the anterior insula is involved in representing one's own and others' affective states. Activations in the insula bilaterally were reported both when feeling ones own pain and when observing a loved one experience pain (Singer et al., 2004).

In addition, fMRI studies investigating the hypothesis that underactivity of the frontolimbic fear circuitry underlies psychopathic behaviour revealed differential activation in the prefrontal–limbic circuit (orbitofrontal cortex, insula, anterior cingulate, amygdala) in the healthy subjects while psychopaths displayed brief amygdala, but no further brain activation (Birbaumer et al., 1998, 2005; Davidson et al., 2000; Raine, 2000; Blair, 2003; Veit et al., 2002). This prefrontal–limbic circuit mediates anticipatory avoidance and emotion regulation and adjustment, particularly in social contexts. The modulation of insular activity by using rtfMRI-based training may be particularly relevant for the development of novel approaches for the treatment of anxiety disorders and antisocial behaviour. Testing whether these patients are able to modulate the insula activity and whether its learned modulation may lead to behavioural changes is particularly intriguing.

Insula hyperactivity seems to be a common feature in persons with elevated trait anxiety (Simmons et al., 2006; Stein et al., 2007). Recently an interesting hypothesis to explain anxiety proneness has been proposed by Paulus and Stein (2006) indicating the anterior insula as the key region for integration of affective and cognitive processing. Anxiety-prone individuals would have altered interoception correlated with increased activity in the anterior insula as a basis of the trigger of the internal anxiety state which in turn would modify cognitive and behavioural components.

A potential and interesting application of the rtfMRI technique in such a clinical setting aiming to assess the effects of down regulation of anterior insular cortex in anxiety-prone subjects is of great interest and it would also extend the knowledge of the role of the anterior insular cortex in anxiety.

Finally, the functional interaction of different brain areas (e.g. insula, ACC, amygdala) may play a significant role in the local activation, the connectivity between brain areas may become an important physiological target for rtfMRI feedback. Selection of more than one brain region of interest or patterns of distributed network brain activity might be a fascinating target for future research.

Acknowledgments

This work was supported by grants from Deutsche Forschungsgemeinschaft (SFB 437/F1). Andrea Caria is supported by a Marie Curie Host Fellowship for Early Stage Researchers Training. We

are indebted to M. Erb for technical assistance in data acquisition and helpful discussions.

References

Adam, G., 1998. Visceral Perception: Understanding Internal Cognition. Plenum Press, New York.

Anders, S., Lotze, M., Erb, M., Grodd, W., Birbaumer, N., 2004. Brain activity underlying emotional valence and arousal: a response-related fMRI study. Hum. Brain Mapp. 23, 200–209.

Birbaumer, N., Grodd, W., Diedrich, O., Klose, U., Erb, M., Schneider, F., Weiss, U., Flor, H., 1998. fMRI reveals amygdala activation to human faces in social phobics. NeuroReport 9, 1223–1226.

Birbaumer, N., Ghanayim, N., Hinterberger, T., Iversen, I., Kotchoubey, B., Kübler, A., Perelmouter, J., Taub, E., Flor, H., 1999. A spelling device for the paralysed. Nature 398, 297–298.

Birbaumer, N., Veit, R., Lotze, M., Erb, M., Hermann, C., Grodd, W., Flor, H., 2005. Deficient fear conditioning in psychopathy. Arch. Gen. Psychiatry 62, 799–805.

Blair, R.J.R., 2003. Neurobiological basis of psychopathy. Br. J. Psychiatry 182, 5–7.

Coghill, R.C., Sang, C.N., Maisog, J.M., Iadarola, M.J., 1999. Pain intensity processing within the human brain: a bilateral, distributed mechanism. J. Neurophysiol. 82 (4), 1934–1943.

Collins, D.L., Neelin, P., Peters, T.M., Evans, A.C., 1994. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J. Comput. Assist. Tomogr. 18, 192–205.

Craig, A.D., 2002. How do you feel? Interoception: the sense of the physiological condition of the body. Nat. Rev., Neurosci. 3 (8), 655–666.

Craig, A.D., 2003. Interoception: the sense of the physiological condition of the body. Curr. Opin. Neurobiol. 13 (4), 500–505.

Critchley, H.D., Wiens, S., Rotshtein, P., Ohman, A., Dolan, R.J., 2004. Neural systems supporting interoceptive awareness. Nat. Neurosci. 7 (2), 189–195.

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, 591–594.

Davis, K.D., Kwan, C.L., Crawley, A.P., Mikulis, D.J., 1998. Functional MRI study of thalamic and cortical activations evoked by cutaneous heat, cold, and tactile stimuli. J. Neurophysiol. 80 (3), 1533–1546.

deCharms, R.C., Christoff, K., Glover, G.H., Pauly, J.M., Whitfield, S., Gabrieli, J.D.E., 2004. Learned regulation of spatially localized brain activation using real-time fMRI. NeuroImage 21, 436–443.

deCharms, R.C., Maeda, F., Glover, G.H., Ludlow, D., Pauly, J.M., Soneji, D., Gabrieli, J.D.E., Mackey, S.C., 2005. Control over brain activation and pain learned by using real-time functional MRI. Proc. Natl. Acad. Sci. 102 (51), 18626–18631.

Francis, S., Rolls, E.T., Bowtell, R., McGlone, F., O'Doherty, J., Browning, A., 1999. The representation of pleasant touch in the brain and its relationship with taste and olfactory areas. NeuroReport 10 (3), 453–459.

Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. NeuroImage 15 (4), 870–878.

Goebel, R., 2001. Cortex-based real-time fMRI. Neuroimage 13, S129.

Hinterberger, T., Veit, R., Wilhelm, B., Weiskopf, N., Vatine, J.J., Birbaumer, N., 2005. Neuronal mechanisms underlying control of a brain–computer interface. Eur. J. Neurosci. 21 (11), 3169–3181.

King, A.B., Menon, R.S., Hachinski, V., Cechetto, D.F., 1999. Human forebrain activation by visceral stimuli. J. Comp. Neurol. 413, 572–582.

Kotchoubey, B., Strehl, U., Uhlmann, C., Holzapfel, S., Konig, M., Froscher, W., Blankenhorn, V., Birbaumer, N., 2001. Modification of slow cortical potentials in patients with refractory epilepsy: a controlled outcome study. Epilepsia 42, 406–416.

Kübler, A., Kotchoubey, B., Kaiser, J., Wolpaw, J.R., Birbaumer, N., 2001. Brain–computer communication: unlocking the locked in. Psychol. Bull. 127, 358–375.

Nagai, Y., Critchley, H.D., Featherstone, E., Trimble, M.R., Dolan, R.J., 2004. Activity in ventromedial prefrontal cortex covaries with sympathetic skin conductance level: a physiological account of a "default mode" of brain function. NeuroImage 22, 243–251.

Neuper, C., Müller, G.R., Kübler, A., Birbaumer, N., Pfurtscheller, G., 2003. Clinical application of an EEG based brain–computer interface: a case study in a patient with severe motor impairment. Clin. Neurophysiol. 114, 399–409.

Ochsner, K.N., Gross, J.J., 2005. The cognitive control of emotion. Trends Cogn. Sci. 9, 242–249.

Ochsner, K.N., Ray, R.D., Cooper, J.C., Robertson, E.R., Chopra, S., Gabrieli, J.D.E., Gross, J.J., 2004. For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. NeuroImage 23, 483–499.

Oppenheimer, S.M., Gelb, A., Girvin, J.P., Hachinski, V.C., 1992. Cardiovascular effects of human insular cortex stimulation. Neurology 42, 1727–1732.

Paulus, M.P., Stein, M.B., 2006. An insular view of anxiety. Biol. Psychiatry 60, 383–387.

Penfield, W., Faulk Jr., M.E., 1955. The insula: further observations on its function. Brain 78, 445–470.

Peyron, R., Laurent, B., Garcia-Larrea, L., 2000. Functional imaging of brain responses to pain. A review and meta-analysis. Clin. Neurophysiol. 30, 263–288.

Pfurtscheller, G., Guger, C., Müller, G., Krausz, G., Neuper, C., 2000. Brain oscillations control hand orthosis in a tetraplegic. Neurosci. Lett. 292, 211–214.

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, 331–348.

Posse, S., Fitzgerald, D., Gao, K., Habel, U., Rosenberg, D., Moore, G.J., Schneider, F., 2003. Real-time fMRI of temporolimbic regions detects amygdala activation during single-trial self-induced sadness. NeuroImage 18, 760–768.

Raine, A., 2000. Autonomic nervous system factors underlying disinhibited, antisocial, and violent behavior. Ann. N. Y. Acad. Sci. 794, 46–59.

Rainville, P., Bechara, A., Naqvi, N., Damasio, A.R., 2006. Basic emotions are associated with distinct patterns of cardiorespiratory activity. Int. J. Psychophysiol. 61, 5–18.

Rolls, E.T., 1996. The orbitofrontal cortex. Philos. Trans. R. Soc. Lond., B Biol. Sci. 351 (1346), 1433–1443.

Rolls, E.T., 2004. The functions of the orbitofrontal cortex. Brain Cogn. 55 (1), 11–29.

Sander, D., Klingelhöfer, J., 1995. Changes of circadian blood pressure patterns and cardiovascular parameters indicate lateralization of sympathetic activation following hemispheric brain infarction. J. Neurol. 242, 313–318.

Saper, C.B., 2002. The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. Annu. Rev. Neurosci. 25, 433–469.

Simmons, A., Strigo, I., Matthews, S.C., Paulus, M.P., Stein, M.B., 2006. Anticipation of aversive visual stimuli is associated with increased insula activation in anxiety-prone subjects. Biol. Psychiatry 60, 402–409.

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R.J., Frith, C.D., 2004. Empathy for pain involves the affective but not sensory components of pain. Science 303 (5661), 1157–1162.

Sitaram, R., Caria, A., Veit, R., Kübler, A., Gaber, T., Birbaumer, N., 2005. Real-time fMRI based brain–computer interface enhanced by interactive virtual worlds. 45th Annual Meeting Society for Psychophysiological Research. Lisbon, Portugal.

Stein, M.B., Simmons, A., Feinstein, J.S., Paulus, M.P., 2007. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. Am. J. Psychiatry 164 (2), 318–327.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard,

O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 15, 273–289.

Veit, R., Flor, H., Erb, M., Hermann, C., Lotze, M., Grodd, W., Birbaumer, N., 2002. Brain circuits involved in emotional learning in antisocial behavior and social phobia in humans. Neurosci. Lett. 328, 233–236.

Wager, T.D., Feldman Barrett, L., 2004. From affect to control: functional specialization of the insula in motivation and regulation. Published online at PsycExtra.

Weiskopf, N., Veit, R., Erb, M., Mathiak, K., Grodd, W., Goebel, R., Birbaumer, N., 2003. Physiological self-regulation of regional brain activity using real-time functional magnetic resonance imaging (fMRI): methodology and exemplary data. NeuroImage 19, 577–586.

Weiskopf, N., Mathiak, K., Bock, S.W., Scharnowski, F., Veit, R., Grodd, W., Goebel, R., Birbaumer, N., 2004a. Principles of a brain–computer interface (BCI) based on real-time functional magnetic resonance imaging (fMRI). IEEE Trans. Biomed. Eng. 51 (6), 966–970.

Weiskopf, N., Scharnowski, F., Veit, R., Goebel, R., Birbaumer, N., Mathiak, K., 2004b. Self-regulation of local brain activity using real-time functional magnetic resonance imaging (fMRI). J. Physiol. (Paris 98 (4–6), 357–373.

Yoo, S.S., Jolesz, F.A., 2002. Functional MRI for neurofeedback: feasibility study on a hand motor task. NeuroReport 13, 1377–1381.