

“Listen to your heart”: A novel interoceptive strategy for real-time fMRI neurofeedback training of anterior insula activity

Yuan Zhang^{a,b}, Qiong Zhang^{a,b}, Jiayuan Wang^{a,b}, Menghan Zhou^{a,b}, Yanan Qing^{a,b}, Haochen Zou^{a,b}, Jianfu Li^b, Chenghui Yang^a, Benjamin Becker^{c,d}, Keith M. Kendrick^{a,b}, Shuxia Yao^{a,b,*}

^a The Center of Psychosomatic Medicine, Sichuan Provincial Center for Mental Health, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu 611731, China

^b The MOE Key Laboratory for Neuroinformation, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 611731, China

^c The State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China

^d Department of Psychology, The University of Hong Kong, Pokfulam, Hong Kong, China

ARTICLE INFO

Keywords:

Real-time fMRI
Neurofeedback
Anterior insula
Regulation strategy
Interoception

ABSTRACT

Real-time fMRI (rt-fMRI) neurofeedback (NF) training is a novel non-invasive technique for volitional brain modulation. Given the important role of the anterior insula (AI) in human cognitive and affective processes, it has become one of the most investigated regions in rt-fMRI studies. Most rt-fMRI insula studies employed emotional recall/imagery as the regulation strategy, which may be less effective for psychiatric disorders characterized by altered emotional processing. The present study thus aimed to examine the feasibility of a novel interoceptive strategy based on heartbeat detection in rt-fMRI guided AI regulation and its associated behavioral changes using a randomized double-blind, sham feedback-controlled between-subject design. 66 participants were recruited and randomly assigned to receive either NF from the left AI (LAI) or sham feedback from a control region while using the interoceptive strategy. $N = 57$ participants were included in the final data analyses. Empathic and interoceptive pre-post training changes were collected as behavioral measures of NF training effects. Results showed that participants in the NF group exhibited stronger LAI activity than the control group with LAI activity being positively correlated with interoceptive accuracy following NF training, although there were no significant increases of LAI activity over training sessions. Importantly, ability of LAI regulation could be maintained in a transfer session without feedback. Successful LAI regulation was associated with strengthened functional connectivity of the LAI with cognitive control, memory and learning, and salience/interoceptive networks. The present study demonstrated for the first time the efficacy of a novel regulation strategy based on interoceptive processing in up-regulating LAI activity. Our findings also provide proof of concept for the translational potential of this strategy in rt-fMRI AI regulation of psychiatric disorders characterized by altered emotional processing.

1. Introduction

Real-time fMRI (rt-fMRI) neurofeedback (NF) is a novel non-invasive technique that enables individuals to self-regulate brain activity or connectivity (Kvamme et al., 2022; Linhartova et al., 2019) and its translational therapeutic potential has been demonstrated in different clinical populations (Dudek and Dodell-Feder, 2021; Pindi et al., 2022).

Given the critical role of the insula in an entire array of cognitive, affective and salience processes (Lamm and Singer, 2010; Menon and Uddin, 2010; Saper, 2002) and that its dysfunction is closely associated with various psychiatric and neurological disorders (Droutman et al., 2015; Uddin and Menon, 2009; Wylie and Tregellas, 2010), the insula, particularly the anterior insula (AI), has therefore become one of the most investigated regions in previous rt-fMRI studies (Fede et al., 2020).

* Corresponding author at: School of Life Science and Technology, University of Electronic Science and Technology of China, No.2006, Xiyuan Ave., West Hi-Tech Zone, Chengdu, Sichuan 611731, China.

E-mail address: yaoshuxia@uestc.edu.cn (S. Yao).

<https://doi.org/10.1016/j.neuroimage.2023.120455>

Received 4 September 2023; Received in revised form 8 November 2023; Accepted 9 November 2023

Available online 10 November 2023

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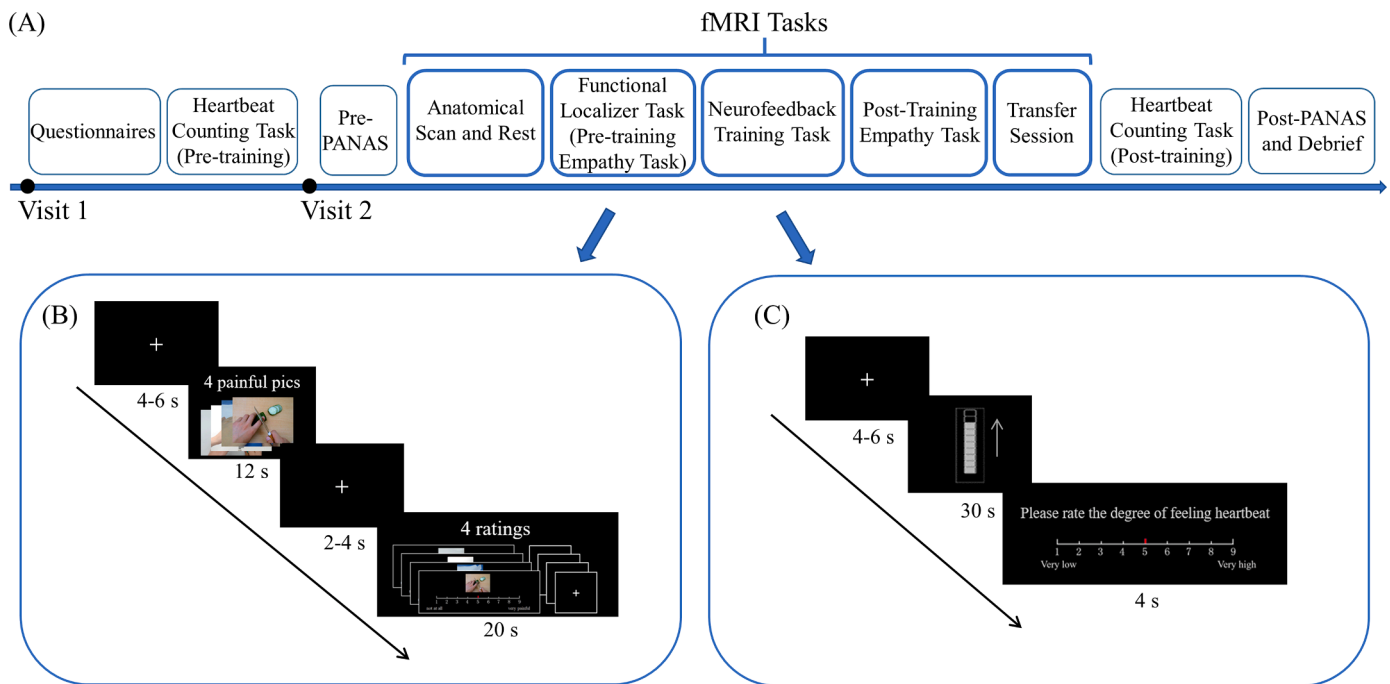


Fig. 1. (A) Experimental protocol. (B) Experimental paradigms for the functional localizer task (pain empathy task) and (C) the neurofeedback training task.

In healthy populations, previous studies have shown that rt-fMRI NF training is effective in regulating insula activity and that successful insula regulation is associated with behavioral changes in valence, empathic and pain-induced responses (Caria et al., 2010; Emmert et al., 2014; Rance et al., 2014; Yao et al., 2016). In clinical populations, rt-fMRI NF training can not only allow patients to volitionally self-regulate insula activity (Karch et al., 2015; Linden et al., 2012; Ruiz et al., 2013; Tinaz et al., 2018; Zilverstand et al., 2015), but also can improve symptom severity in schizophrenia, depression, and phobia disorders (Karch et al., 2015; Linden et al., 2012; Zilverstand et al., 2015). These findings suggest that rt-fMRI NF is an effective approach in normalizing abnormal activity of the insula and associated behavioral alterations.

Of note, a key factor that affects NF training efficacy is the selection of regulation strategies. Most previous rt-fMRI insula studies employed the regulation strategy of mental imagery in which subjects were instructed to imagine or recall emotional experiences and memories (see Zhang et al., 2022 for an overview). This strategy normally involves explicit past emotional experience, which works well for healthy individuals but may be less effective for those with psychiatric disorders, especially emotional disorders characterized by altered emotional processing and negative emotional bias such as depression and anxiety (DSM-V; APA, 2022) and when long-term NF training is required. However, there is a lack of alternative regulation strategies, which may restrict translational application of the rt-fMRI insula NF in clinical populations. Thus development of novel regulation strategies is of key importance and enables more flexibility for selecting regulation strategies depending on research purposes in future studies.

Based on functional relevance of the AI, interoceptive processing may represent a promising alternative for developing new AI regulation strategies. Interoception refers to perception of one's own internal bodily sensations and is closely associated with the AI (Adolfi et al., 2017; Craig, 2002, 2009). Compared with the middle and posterior insula (PI), which are two core primary interoceptive sensory nodes more closely associated with processing of primary sensory components of interoceptive signals (Chang et al., 2013; Uddin et al., 2014), the AI is a key node integrating these interoceptive sensory information for generating allostatic predictions and interoceptive awareness (Barrett

and Simmons, 2015; Craig, 2002; Khalsa et al., 2018). Previous studies mainly employed heartbeat counting or detection tasks to investigate neural substrates of interoception and also found that AI activation during heartbeat interoception was positively correlated with interoceptive accuracy (IaC) (Craig, 2009; Critchley et al., 2004). However, it is still unclear to date whether interoception can be used as a valid strategy for AI regulation.

Against this background, the present study aimed to examine the feasibility of an interoceptive strategy in AI regulation using a randomized double-blind, sham feedback-controlled design (see Fig. 1A). In accordance with our previous rt-fMRI AI study (Yao et al., 2016) and previous findings showing no obvious lateralization of AI activation during a heartbeat detection task (Yao et al., 2018), the left AI (LAI) was chosen as the target region of interest (ROI). Based on previous interoception studies (Craig, 2009; Critchley et al., 2004), participants were instructed to apply an interoceptive strategy of heartbeat detection to regulate their LAI activity. The LAI regulation success was assessed during NF training and in a transfer session. The transfer session was included to examine whether NF training effects could be maintained when no feedback was provided in line with previous studies and indicative of further translational potential (Tinaz et al., 2018; Yao et al., 2016). We hypothesized that participants in the NF group would exhibit stronger LAI activity than the control group if the interoceptive strategy was effective. Furthermore, interoceptive processing is closely associated with emotional experience including empathy (Ernst et al., 2014; Li et al., 2019) and emotion recognition (Critchley and Garfinkel, 2017; Uddin et al., 2014). Thus, employment of an interoceptive strategy may further facilitate a regulation of these emotional experiences. We therefore used pre-post training changes in empathy and IaC/interoceptive sensibility (IS) as behavioral indices of NF training.

2. Materials and methods

2.1. Participants

66 healthy participants (32 males, mean age = 21.83 years, SD = 2.11) were recruited from the University of Electronic Science and Technology of China (UESTC). Based on a priori power analysis using

Table 1
Statistics of ages and questionnaire scores in the NF and control groups (mean \pm SD).

Measurements	NF	control	t-value	p-value
Age (years)	22.172 \pm 2.188	21.107 \pm 1.969	1.930	0.059
PANAS – Negative (Pre)	13.621 \pm 3.678	14.071 \pm 4.776	-0.400	0.691
PANAS - Positive (Pre)	24.483 \pm 5.823	26.250 \pm 4.766	-1.251	0.216
PANAS – Negative (Post)	12.862 \pm 3.573	12.393 \pm 5.223	0.397	0.693
PANAS – Positive (Post)	21.967 \pm 6.138	21.607 \pm 5.756	0.227	0.821
STAI - State	38.724 \pm 7.289	38.643 \pm 7.713	0.041	0.968
STAI - Trait	40.860 \pm 7.735	40.930 \pm 8.580	-0.031	0.976
BDI	7.620 \pm 7.409	8.070 \pm 8.393	-0.215	0.830
EQ	36.970 \pm 9.686	37.710 \pm 12.024	-0.259	0.796
ASQ	20.550 \pm 5.767	21.140 \pm 4.680	-0.424	0.673
TAS	48.690 \pm 10.275	48.571 \pm 10.083	0.041	0.968
BVAQ	105.483 \pm 13.886	96.214 \pm 25.806	1.680	0.101
ICQ	51.210 \pm 9.868	48.640 \pm 9.600	0.994	0.325
MAIA	22.103 \pm 4.662	21.679 \pm 5.292	0.322	0.749

PANAS: Positive and Negative Affect Schedule; STAI: State-Trait Anxiety Inventory; BDI: Bermond-Vorst Alexithymia Questionnaire; EQ: Empathy Quotient; ASQ: Autism Spectrum Quotient; TAS: Toronto Alexithymia Scale; BVAQ: Bermond-Vorst Alexithymia Questionnaire; ICQ: Interoceptive Confusion Questionnaire; MAIA: Multidimensional Assessment of Interoceptive Awareness.

the G*Power v.3.7 toolbox with a power $>$ 0.8 (effect size = 0.25, α = 0.05) for a mixed ANOVA and sample sizes used in previous rt-fMRI studies (Linhartova et al., 2019; Zhang et al., 2022), this sample size was adequate to detect reliable NF training effects at both the behavioral and neural levels. In a randomized double-blind, sham feedback-controlled design, 33 participants (16 males) were randomly assigned to the NF group receiving NF from the LAI and 33 participants (16 males) were assigned to the control group receiving sham NF from a control region of the middle temporal gyrus (MTG; see *Definition of the sham control region for the control group*). Nine participants were excluded due to not completing the whole experiment (3 participants), excessive head movement (3 participants), failure of feeling heartbeat during the heartbeat counting task (HCT) and quitted the study (1 participant), or technical problems during NF training (2 participants). Consequentially, 29 participants (16 males, mean age = 22.17 years, SD = 2.19) in the NF group and 28 participants (15 males, mean age = 21.11 years, SD = 1.97) in the control group were included in the final data analyses (see supplementary Fig. S1 for the consort flow diagram).

Another independent group of 33 participants was recruited to examine whether a conventionally used control region encompassing a whole top slice distant from the AI (see supplementary Fig. S2B) in previous rt-fMRI insula studies was appropriate for AI regulation when an interoceptive strategy was applied (Berman et al., 2013; Caria et al., 2007; Ruiz et al., 2013; Yao et al., 2016). Prior to the experiment all participants gave informed consent and reported no psychiatric or neurological disorders. All procedures of the current study were approved by the local ethical committee at UESTC and followed the latest version of the Declaration of Helsinki. This study was preregistered on clinicaltrials.gov (<https://clinicaltrials.gov/ct2/show/NC T05260749>). Given confounding effects on NF training shown in the independent group (see *Definition of the sham control region for the control group* and Supplementary Materials), in contrast to the preregistration

the present study mainly focused on comparison of the NF group with the control group receiving sham NF from the MTG. A checklist following the consensus on the reporting and experimental design of clinical and cognitive-behavioral NF studies (CRED-NF checklist) (Ros et al., 2020) was also provided in the Supplementary Materials.

2.2. Experimental protocol

2.2.1. Questionnaires

To rule out potential confounding effects, all participants were instructed to complete validated Chinese versions of psychometric questionnaires in Visit 1, including the Autism Spectrum Quotient (Baron-Cohen et al., 2001), Beck Depression Inventory-II (Beck et al., 1996), State-Trait Anxiety Inventory (Spielberger et al., 1971), Empathy Quotient (Baron-Cohen et al., 2003), Toronto Alexithymia Scale (Taylor et al., 1992), Bermond-Vorst Alexithymia Questionnaire (Vorst and Bermond, 2001), Multidimensional Assessment of Interoceptive Awareness (Mehling et al., 2012) and Interoceptive Confusion Questionnaire (Brewer et al., 2016). For mood states, subjects were asked to complete the Positive and Negative Affect Schedule (Watson et al., 1988) before and after NF training in Visit 2.

2.2.2. Heartbeat counting task

To estimate changes of IAC and IS induced by NF training, the HCT was firstly conducted in Visit 1 as baseline and secondly after NF training. Similar to previous studies (Azevedo et al., 2016; Schandry, 1981), in the HCT participants were instructed to count the number of heartbeats they were aware of during different time windows (25 s, 35 s, 40 s, and 45 s, respectively) while undergoing electrocardiographic recording (i.e., actual heartbeats) using the BIOPAC MP150 system (BIOPAC Systems, Inc.). To minimize confounding effects of time estimation and prior knowledge of heartbeat, optimized instruction was used in line with previous studies (Desmedt et al., 2018, 2020). After each time window participants were asked to report their heartbeat counts (i.e., reported heartbeats) and rate the degree of confidence to their counts (i.e., interoceptive sensibility) (Forkmann et al., 2016) on a 9-point Likert scale (1-not confident at all; 9-very confident). IAC was computed using the following formula (Desmedt et al., 2018; Schandry, 1981):

$$1/N \Sigma (1 - (|\text{actual heartbeats} - \text{reported heartbeats}|) / \text{actual heartbeats}).$$

N is the number of time windows in the HCT ($N = 4$ in the present study).

2.2.3. Functional localizer task

Based on previous findings that the pain empathy task was effective in localizing the LAI (Caria et al., 2007; Veit et al., 2012; Yao et al., 2016), the LAI was defined by a functional localizer task of pain empathy (Fig. 1B). The functional localizer task of pain empathy consisted of 5 blocks of painful stimuli alternated with 5 blocks of neutral stimuli. Each block presented 4 painful/neutral pictures in a pseudo-random order with each picture being presented for 3 s. Pictures of painful situations (Valence: mean \pm SD = 2.90 \pm 1.15; Arousal: mean \pm SD = 5.07 \pm 1.03) were from Meng et al. (2012) and neutral pictures were selected from the International Affective Picture System (Lang et al., 2005) (Valence: mean \pm SD = 4.95 \pm 0.15; Arousal: mean \pm SD = 2.77 \pm 0.44) and all of them have been validated in our previous rt-fMRI AI study (Yao et al., 2016). Participants were instructed to imagine how painful the individual in the situation depicted in the pictures felt or just to passively watch the neutral pictures. After each block, participants were asked to rate the degree of pain empathy for each picture on a 9-point Likert scale (1-not painful at all; 9-very painful) within 4 s alternated with a 1 s interval. In addition, the pain empathy task also served as a baseline measurement of empathy before NF training. The same task was conducted again after NF training with stimuli being presented with a new pseudorandom order. Comparison between the

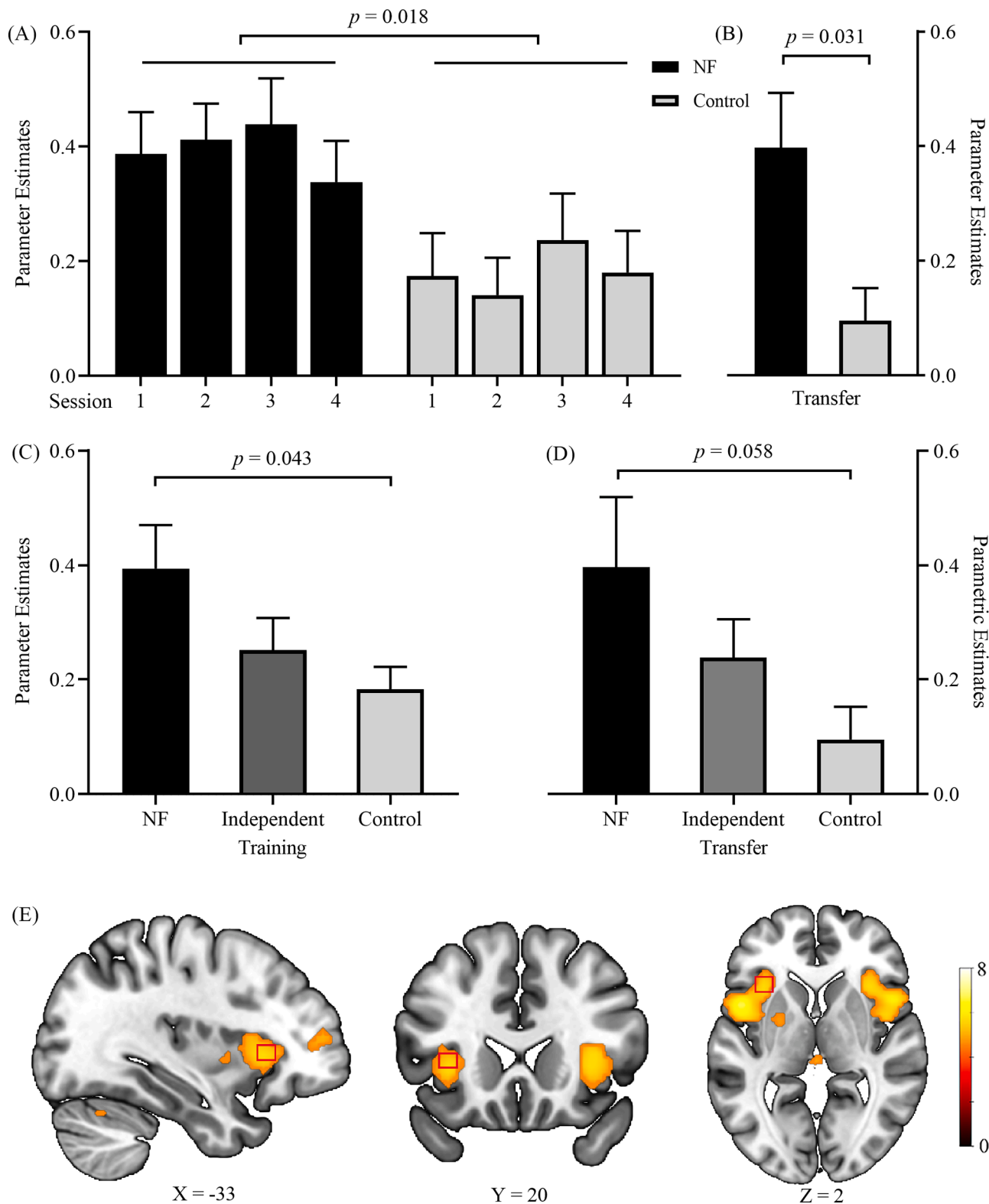


Fig. 2. (A) Left anterior insula (LAI) activity during regulation relative to baseline blocks over the 4 neurofeedback training sessions and (B) in the transfer session. (C) LAI activity averaged across 4 training sessions in the NF, the control (middle temporal gyrus), and the independent (whole top slice) groups during training sessions and (D) the transfer session. Error bars represent standard error. (E) LAI activity in the whole-brain level analysis across the 4 training sessions in the NF group ($p_{FDR} < 0.001$).

pre- and post-training empathy tasks allowed us to examine NF training effects on empathic responses.

2.2.4. Neurofeedback training task

Regulation success was assessed during NF training and in a transfer session. The NF training task comprised 4 training sessions and each session included 5 regulation blocks alternated with 5 baseline blocks.

Table 2

Brain regions showing stronger activity in regulation relative to baseline blocks across training sessions in the NF and control groups respectively (MNI coordinates).

Brain Region	BA	No. Voxels	Peak t-value	X	Y	Z
NF group: Regulation > Baseline						
L. Supplementary Motor Area	6/32/24	522	8.02	-9	2	62
Supplementary Motor Area			7.41	6	-1	62
Middle Cingulate Cortex			4.78	12	8	35
R. Inferior Frontal Gyrus	44/13/47	365	7.87	63	8	17
Inferior Frontal Operculum			6.11	48	11	2
Anterior Insula			6.00	36	17	8
L. Precentral Gyrus	6/13/44	642	7.02	-51	-1	47
Precentral Gyrus			6.93	-51	2	8
Anterior Insula			5.73	-33	20	2
Globus Pallidus			5.00	-21	-4	-4
R. Precentral Gyrus	6/8	62	6.59	51	2	47
L. Inferior Parietal Lobule	40/13	218	6.30	-54	-40	23
R. Superior Temporal Gyrus	40/42	96	6.02	63	-34	20
L. Middle Frontal Gyrus	10	51	5.97	-36	50	11
L. Middle Frontal Gyrus	10	34	5.83	-39	41	29
L. Cerebellum Posterior Lobe	6	14	5.44	-27	-61	-28
L. Midbrain		26	5.36	0	-31	-4
NF group: Baseline > Regulation						
None						
CTR group: Regulation > Baseline						
L. Superior Frontal Gyrus	6	22	7.56	-9	8	59
CTR group: Baseline > Regulation						
L. Superior Frontal Gyrus	10	27	4.30	-9	59	23

All regions are reported with a $p_{FDR} < 0.001$ threshold at the whole-brain level. L indicates left; R indicates right. CTR: control.

Conditions of blocks were indicated by the direction of an arrow, with an upward arrow indicating a regulation block and a downward one indicating a baseline block (Fig. 1C). NF was presented using a thermometer where more bars filled indicate a stronger AI activity. In regulation blocks, participants were instructed to up-regulate the activity of the LAI/control region as much as possible by feeling their own heartbeat in a relaxed state (the interoceptive regulation strategy). They were not allowed to intentionally hold their breath, change normal respiratory rate in a different way from usual (e.g., breathing more quickly or slowly in a short time), or use other unusual methods (e.g., by feeling pulse of the wrist or neck) as further aids for heartbeat perception. In baseline blocks, participants were asked to keep relaxed and rest to return the activity back to baseline. They were recommended to use these regulation strategies throughout the NF training task and informed that voluntary regulation of brain activity was a gradual learning process and not to get worried if they found the strategy did not work immediately. Participants were also informed of the delay of feedback display due to image data processing and nature of the intrinsic hemodynamic response. Each block lasted for 30 s and was followed by a rating task for regulation strategy check. After each regulation block participants were asked to rate the degree to which they could feel their own heartbeat, whereas after each baseline block they were asked to rate the degree of relaxation on a 9-point Likert scale (1-very low; 9-very high) within 4 s. Participants did not receive any information about which group they were assigned to.

After the 4 training sessions, there was a transfer session to examine whether participants could maintain their ability to regulate the LAI without NF. The transfer session was identical to the training session except that no feedback was provided and an empty thermometer was presented instead. After the MRI scanning, participants were debriefed on (I) whether they kept using the interoceptive regulation strategy during regulation blocks, and (II) whether they thought the interoceptive strategy was helpful for regulating the LAI activity (highly, moderate, or not at all).

2.2.5. Definition of the sham control region for the control group

Based on recommendations in Sorger et al. (2019) and our previous study (Yao et al., 2016), feedback from an alternative brain signal was used as a control condition in the present study. This type of sham control condition is one of the control conditions allowing to control for the most confounding factors, including motivation, placebo, and global effects (Sorger et al., 2019). Participants in the sham control group received NF from the MTG (see supplementary Fig. S2A), which is mainly engaged in language processing (Friederici, 2011; Xu et al., 2015), but not interoceptive processing. This control region was determined based on our previous study of interoception (Yao et al., 2018) and pilot results from an independent sample ($N = 33$) examining the feasibility of using a whole top slice as the sham control region (see Supplementary Materials).

2.3. Image data acquisition

Images were collected using a 3T, GE Discovery MR750 scanner (General Electric Medical System, Milwaukee, WI, USA). High-resolution whole-brain volume T1*-weighted images were firstly collected with a 3D spoiled gradient echo pulse sequence (repetition time, 6 ms; echo time, minimum; flip angle, 12°; field of view = 256 × 256 mm; acquisition matrix, 256 × 256; thickness, 1 mm; number of excitations, 2; 160 slices). Functional images were acquired using a T2*-weighted echo-planar imaging pulse sequence (repetition time, 2000 ms; echo time, 30 ms; slices, 32; thickness, 3.4 mm; gap, 0.6 mm; field of view, 220 × 220 mm; resolution, 64 × 64; flip angle, 90°).

2.4. Data analyses

2.4.1. Online real-time fMRI NF analyses

The rt-fMRI setup of the present study was identical to our previous study (Yao et al., 2016). Briefly, functional images were processed online using the Turbo Brain Voyager (TBV) 3.2 toolbox (Brain Innovation, Maastricht, The Netherlands). Functional brain images were transmitted from the MRI scanner to the local disk of the TBV-installed computer in real-time. The TBV toolbox preprocessed brain images immediately including 3D motion correction and drift removal. Individualized target ROI was defined functionally in the localizer task centered on the maximally activated voxel within the LAI (contrast of 'painful > neutral' for LAI localization) for each participant. The ROI was a 4 × 4 voxel square and extended 3 axial slices (see also Kanel et al., 2019; Yao et al., 2016). The blood oxygen level-dependent signal was then extracted from the target/control ROI via the TBV software to produce feedback for NF training. Participants were informed that the delay of NF was around 4–6 s due to data processing and the intrinsic nature of the hemodynamic response.

2.4.2. Offline imaging data analyses

SPM12 software (Wellcome Department of Cognitive Neurology, London, UK, <http://www.fil.ion.ucl.ac.uk/spm>) was used for offline image processing. After removing the first 5 vol, images were preprocessed including head-motion correction, co-registration of the mean functional image and the T1 image, normalization to the standard Montreal Neurological Institute (MNI) space, and smoothing with a Gaussian kernel (8 mm full-width at half maximum). For the pre- and the

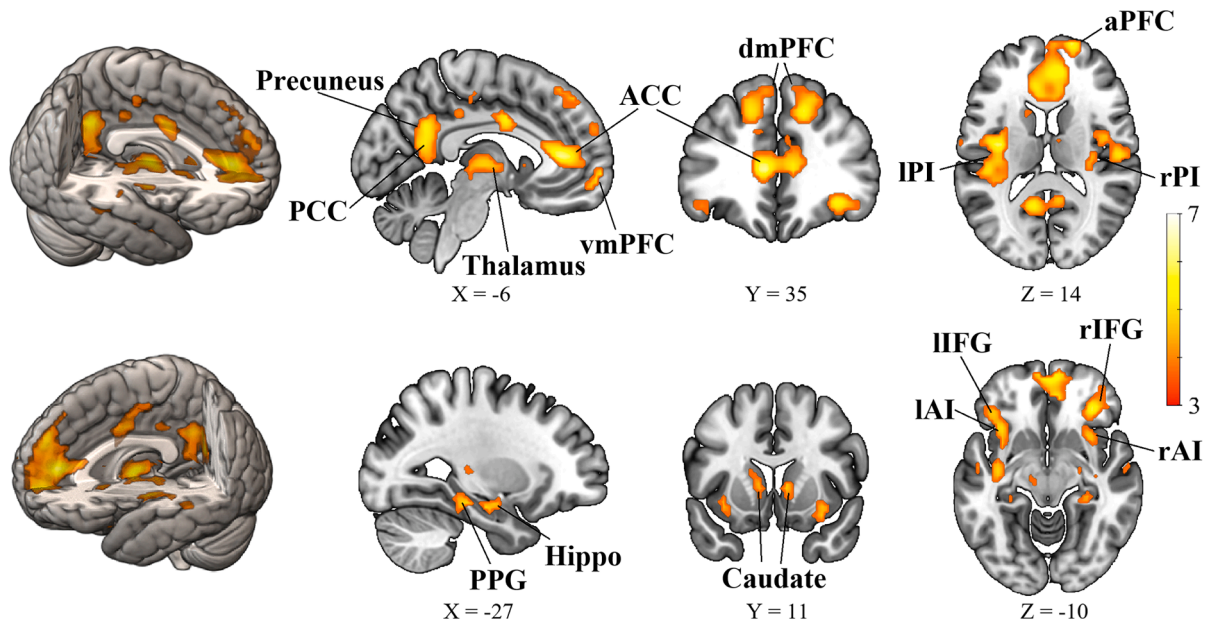


Fig. 3. Regions showing increased functional connectivity with the LAI during NF training (contrast: ‘regulation > baseline’; $p_{FDR} < 0.005$). ACC: anterior cingulate cortex; AI: anterior insula; aPFC: anterior prefrontal cortex; dmPFC: dorsal medial prefrontal cortex; hippo: hippocampus; IFG: inferior frontal gyrus; PCC: posterior cingulate cortex; PI: posterior insula; PPG: parahippocampal gyrus; vmPFC: ventral medial prefrontal cortex.

post-training empathy tasks, the first-level design matrix included 4 regressors (painful, painful rating, neutral, neutral rating) and the 6 head motion parameters convolved with the canonical hemodynamic response function (HRF). Contrast images between painful and neutral pictures in each of the empathy tasks (‘painful > neutral’) and between the two tasks (‘post-training_{painful>neutral} > pre-training_{painful>neutral}’) were created for each subject. For the NF training task, the first-level design matrix included 4 regressors (baseline, baseline rating, regulation, regulation rating) and the 6 head motion parameters convolved with the canonical HRF. Contrast images between regulation and baseline blocks (‘regulation > baseline’) were created for each training session, the transfer session, and across training sessions for each subject.

For the second level analysis of the pre- and the post-training empathy tasks, a one-sample *t*-test was first conducted to determine whether painful stimuli, relative to neutral stimuli (‘painful > neutral’), effectively induced significant neural responses in the core pain empathy network. A two-sample *t*-test on the contrast ‘post-training_{painful>neutral} > pre-training_{painful>neutral}’ was applied to examine the NF-induced empathic neural changes in the post- relative to the pre-training empathy tasks. For the second level analysis of the NF training task, an ROI analysis was first conducted to examine the NF training effect by extracting parameter estimates from the LAI (contrast ‘regulation > baseline’) of each training session and the transfer session. Consistent with previous studies (e.g., Berman et al., 2013; Yao et al., 2016; Zilverstand et al., 2015), parameter estimates were extracted using MarsBar (Brett et al., 2002) from the LAI (MNI coordinates: $X = -33$, $Y = 20$, $Z = 8$; 6-mm sphere; see supplementary Table S2) determined in the functional localizer task (contrast: ‘painful > neutral’) across the NF and control groups to ensure that the extracted activity was comparable between the 2 groups. A repeated-measures ANOVA was performed on these parametric estimates with session as within-subject factor (session 1–4) and group (NF vs. control) as between-subject factor using SPSS (version 22, SPSS Inc., Chicago, IL, USA). For the ROI analysis in the transfer session, a two-sample *t*-test was conducted to examine differences of the maintenance of the regulation ability between the two groups. Multiple comparisons in post-hoc analyses disentangling significant main and interaction effects were Bonferroni corrected. For training effects out of the target ROI, a

one-sample *t*-test was applied at the whole-brain level to examine the training effect (contrast: ‘regulation > baseline’) for the NF and control group separately. Group differences were also explored using a two-sample test. At the whole-brain level, results were corrected using the False Discovery Rate peak-level correction ($p_{FDR} < 0.001$) and only clusters larger than 10 voxels were reported.

2.4.3. Functional connectivity analyses

To further examine changes in functional connectivity (FC) between the target ROI and other brain regions during NF training, in line with previous studies (Berman et al., 2013; Yao et al., 2016; Zotev et al., 2011) we conducted a psychophysiological interaction (PPI) analysis using the gPPI toolbox (McClaren et al., 2012) based on the contrast of ‘regulation > baseline’ in the NF group using a one-sample *t*-test. The seed region was also defined as a 6-mm sphere centered on the same coordinates in the ROI analysis of the LAI. A threshold of $p_{FDR} < 0.005$ peak-level correction was used and only clusters larger than 10 voxels were reported. For completeness and transparency, group differences between changes in FC were also explored using a two-sample *t*-test.

2.4.4. Statistical analyses

Group differences of demographics and questionnaire scores of personality traits and mood were tested using two-sample *t*-tests. Repeated-measures ANOVAs were conducted to analyze behavioral NF training effects on IAC, IS, pain empathy rating scores, and rating scores of regulation strategy check. Bonferroni correction was applied for multiple comparisons in post-hoc analyses disentangling significant main or interaction effects. In addition, we used the Chi-square test of independence to examine the group difference of subjective estimation on effectiveness (highly, moderate, or not at all) of the interoceptive regulation strategy for LAI regulation (McHugh, 2013). Brain-behavior correlations were tested using Pearson correlations given normal distribution of the data. FDR corrections were used for multiple comparisons to account for the number of brain regions in correlation analyses. In all cases $p < 0.05$ (two-tailed) was considered significant.

Table 3

Regions which showed increased functional connectivity with LAI in the NF group during effective regulation (MNI coordinates).

Brain Region	BA	No. Voxels	Peak t-value	X	Y	Z
L. Anterior Cingulate Cortex	10/32/9	1168	6.40	-6	35	8
Ventral Medial Prefrontal Cortex			6.15	6	56	-13
Anterior Prefrontal Cortex			6.08	18	65	14
Anterior Cingulate Cortex			5.99	6	44	11
L. Posterior Insula	13/47	1155	6.13	-36	-10	14
Inferior Frontal Gyrus			5.33	-39	29	-13
Hippocampus			5.32	-30	-19	-19
R. Inferior Frontal Gyrus	47/13	163	5.93	33	32	-10
Anterior Insula			4.76	30	14	-10
L. Posterior Cingulate Cortex	31/23	344	5.76	-9	-55	17
Precuneus			5.65	-3	-55	29
L. Middle Cingulate Cortex	24/31	156	5.57	-6	-1	32
Middle Cingulate Cortex			4.78	9	2	32
R. Posterior Insula	2/13/4	534	5.38	39	-4	8
Precentral Gyrus			5.27	48	-7	23
Postcentral Gyrus			5.16	54	-16	14
R. Middle Temporal Gyrus	21	32	5.02	51	-1	-25
R. Parahippocampal Gyrus		44	4.77	33	-25	-19
Hippocampus			4.39	30	-31	-10
L. Caudate		41	4.76	-9	14	5
R. Amygdala		38	4.66	27	-4	-22
L. Middle Temporal Gyrus		11	4.66	-51	-10	-10
L. Superior Temporal Gyrus	22	55	4.36	-60	-40	5
Middle Temporal Gyrus			4.04	-66	-28	-1
L. Middle Cingulate Cortex	31	22	4.17	-3	-31	35
R. Middle Temporal Gyrus		18	4.01	57	-13	-16

All with a $p_{FDR} < 0.005$ corrected threshold. L indicates left; R indicates right.

3. Results

3.1. Demographics and questionnaires

Two-sample t -tests on demographics and questionnaire scores revealed no significant differences between the NF and control groups ($ps \geq 0.059$; see Table 1).

3.2. NF training task

3.2.1. ROI analyses of the LAI

A repeated-measures ANOVA on extracted parameter estimates of the LAI from the NF group and the MTG control group showed a significant main effect of group ($F(1,55) = 5.917, p = 0.018, \eta_p^2 = 0.097$), with stronger activation of the LAI in the NF than in the control group (Fig. 2A). However, there was no significant main effect of session ($F(3165) = 1.117, p = 0.340, \eta_p^2 = 0.020$) or group \times session interaction ($F(3165) = 0.515, p = 0.672, \eta_p^2 = 0.009$). Furthermore, a two-sample t -test on extracted parameter estimates in the transfer session also revealed significantly stronger LAI activity in the NF than in the control group ($t = 2.236, p = 0.031, \text{Cohen's } d = 0.589$; Fig. 2B).

For completeness and transparency, we also conducted a repeated-measures ANOVA on extracted parameter estimates of the LAI from

the NF, the control (MTG), and the independent (whole top slice) groups. Results showed a significant main effect of group ($F(2,81) = 3.264, p = 0.043, \eta_p^2 = 0.075$) (Fig. 2C), with the LAI activity being significantly stronger in the NF group than in the control group ($p = 0.043$), but not in the independent group ($p = 0.294$). However, there was no significant main effect of session ($F(3243) = 1.187, p = 0.315, \eta_p^2 = 0.014$) or group \times session interaction ($F(6243) = 0.344, p = 0.913, \eta_p^2 = 0.008$). Furthermore, a one-way ANOVA on extracted parameter estimates in the transfer session revealed a marginal main effect of group ($F(2,81) = 2.950, p = 0.058, \eta_p^2 = 0.068$) (Fig. 2D), with a trend towards stronger LAI activity in the NF than in the control group ($p = 0.052$), but not in the independent group ($p = 0.632$) (see also Supplementary Materials for a direct comparison between the NF and the independent groups).

3.2.2. Whole-brain analysis

A one-sample t -test on contrast 'regulation > baseline' across training sessions showed significant activity in the LAI (Fig. 2E), and other regions including the right AI, supplementary motor area (SMA), middle cingulate gyrus, inferior frontal gyrus (IFG) in the NF group ($p_{FDR} < 0.001$; Table 2). However, significant activity was only found in the superior frontal gyrus across sessions in the control group ($p_{FDR} < 0.001$; Table 2). No significant activity was found on contrast 'baseline > regulation' in the NF group and only the superior frontal gyrus was found in the control group ($p_{FDR} < 0.001$; Table 2). Two-sample t -tests on contrasts 'regulation > baseline' and 'baseline > regulation' between the two groups showed no significant effects at the whole brain level either during NF training or in the transfer session ($p_{FDR} < 0.001$).

3.2.3. NF-induced changes in functional connectivity of the LAI

To examine changes of FC between the target ROI and other brain regions during NF training, we conducted a PPI analysis based on the contrast of 'regulation > baseline' in the NF group in line with previous studies (Berman et al., 2013; Caria et al., 2007; Yao et al., 2016). Results showed strengthened FC in regulation compared to baseline blocks between the LAI and key regions of the cognitive control and executive networks including the bilateral anterior cingulate cortex (ACC), the right ventral medial prefrontal cortex (vmPFC) and the anterior prefrontal cortex (apPFC) extending to the bilateral dorsal medial prefrontal cortex (dmPFC); the salience/interoceptive network including the bilateral IFG, AI (with the right one extending from the left PI) and PI, and the right thalamus (extending from the right PI); the default mode network (DMN) including the posterior cingulate cortex (PCC) and precuneus; and the learning and memory networks, including the bilateral caudate (with the right one extending from the left PI) and hippocampus and the right parahippocampal gyrus and so on during regulation relative to baseline blocks ($p_{FDR} < 0.005$; details see Fig. 3 and Table 3). However, no significant differences of FC changes were found between the NF and control groups ($p_{FDR} < 0.005$).

3.2.4. Regulation strategy check

A repeated-measures ANOVA with session and block condition (regulation vs. baseline) as within-subject factors and group as between-subject factor on rating scores revealed a marginal effect of group ($F(1,55) = 3.949, p = 0.052, \eta_p^2 = 0.067$) and a significant main effect of condition ($F(1,55) = 9.916, p = 0.003, \eta_p^2 = 0.153$). Importantly, there was a significant group \times session \times condition interaction ($F(3165) = 2.855, p = 0.039, \eta_p^2 = 0.049$). Post-hoc analyses found significantly higher rating scores for degrees of heartbeat perception in regulation blocks in the NF than the control group in session 1 ($p = 0.035$; Fig. 4A) and higher rating scores for degrees of relaxation in baseline blocks in the NF than the control group in session 3 ($p = 0.025$; Fig. 4B). There were no other significant effects ($ps \geq 0.498$). For the transfer session, there was only a marginal main effect of condition ($F(1,55) = 3.885, p =$

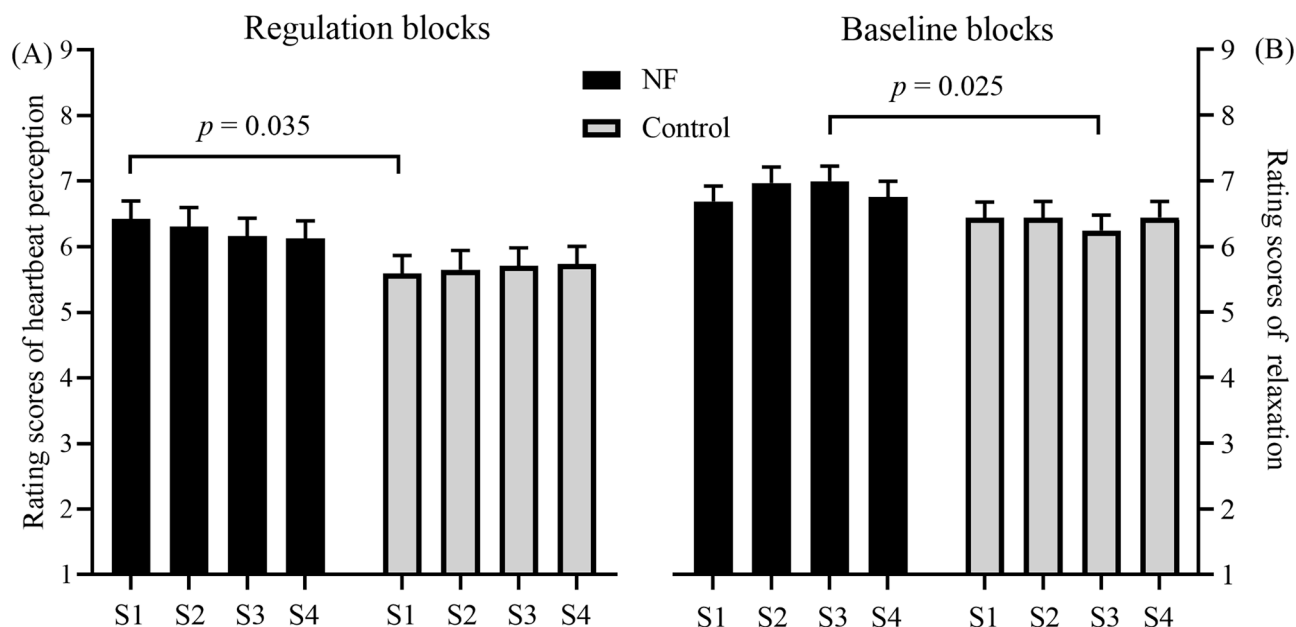


Fig. 4. Rating scores of regulation strategy check following regulation (A) and baseline blocks (B) from session 1 (S1) to session 4 (S4) in the NF and control groups. Error bars represent standard error.

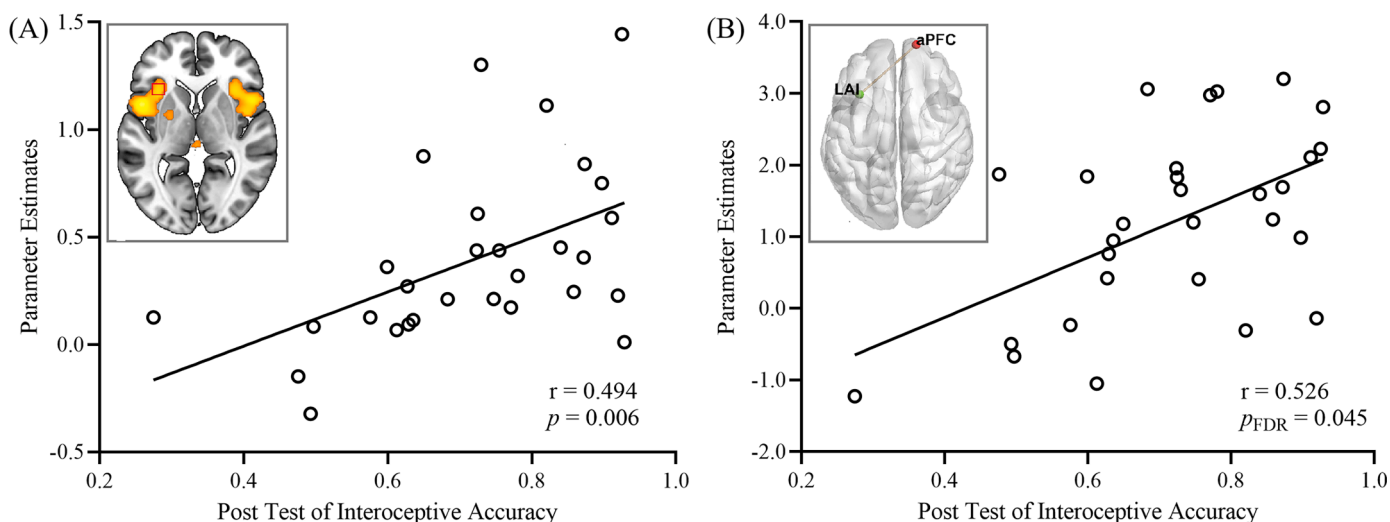


Fig. 5. Positive correlations between interoceptive accuracy in the post-training heartbeat counting task and left anterior insula (LAI) activity (A) and functional connectivity of the LAI with the anterior prefrontal cortex during NF training in the NF group (B).

0.054, $\eta_p^2 = 0.066$) but no significant effects related to NF training ($p_s \geq 0.180$).

In the debrief after the experiment, all participants reported that they felt their heartbeats during NF training and that they kept trying to employ the interoceptive strategy to modulate the LAI activity. A Chi-square test of independence showed a significant association ($\chi^2 = 6.222$, $p = 0.043$) between group and the number of participants reporting different options of regulation strategy effectiveness (highly, moderate, or not at all). Comparison of the observed count with the expected count between the two groups suggested that more participants in the NF group perceived the interoceptive strategy as helpful than in the control group (supplementary Table S3).

3.3. NF training effects on interoception and empathy

To estimate changes in IAC/IS and empathy induced by NF training, pre-post training performance in the HCT and pain empathy task was

analyzed using repeated-measures ANOVAs. For the HCT, a repeated-measures ANOVA with timepoint (pre- vs. post-training) as within-subject factor and group as between-subject factor showed no significant effects on either IAC (all $p_s \geq 0.121$) or IS (all $p_s \geq 0.179$) (details see Supplementary Materials). For the pain empathy task, there were no significant effects related to NF training either on the behavioral (all $p_s > 0.116$) or the neural level ($p_{FDR} < 0.001$).

3.4. Associations between NF training success and IAC

Pearson correlation analyses were conducted to examine whether participants' IAC was associated with NF training success. Results showed a significant positive correlation between IAC in the post-training HCT and LAI activity in the NF (Pearson $r = 0.494$, $p = 0.006$; Fig. 5A) but not in the control group (Pearson $r = 0.275$, $p = 0.156$). However, there were no significant correlations between LAI activity and IAC in the pre-training HCT (Pearson $r = 0.020$, $p = 0.918$)

and the difference in IAC between the post- and pre-training HCTs (Pearson $r = 0.348$, $p = 0.064$) in the NF group.

We also examined correlations between IAC and FC strengths of the LAI with core regions of the aforementioned neural networks. Significant positive correlations were found between IAC in the post-training HCT and FC strengths of the LAI with the aPFC (LAI-aPFC) (MNI: $X = 18$, $Y = 65$, $Z = 14$; Pearson $r = 0.526$, $p = 0.003$), the PI (MNI: $X = -36$, $Y = -10$, $Z = 14$; Pearson $r = 0.526$, $p = 0.040$), and the ACC (MNI: $X = 6$, $Y = 44$, $Z = 11$; Pearson $r = 0.399$, $p = 0.032$) in the NF group. However, only the correlation with the LAI-aPFC connectivity survived after multiple comparison corrections (Pearson $r = 0.526$, $p_{FDR} = 0.045$; Fig. 5B).

4. Discussion

The present study examined whether a novel interoceptive strategy could be used for AI regulation via rt-fMRI NF training. Results showed that participants in the NF group exhibited significantly stronger LAI activity than in the control group and that successful self-regulation of LAI activity could be maintained in a transfer session without feedback. A PPI analysis revealed increased LAI FC with cognitive control, memory and learning, and salience/interoceptive networks. Furthermore, stronger activity of the LAI was positively correlated with higher IAC in the post-training HCT.

More specifically, ROI analyses showed significantly stronger LAI activity across training sessions in the NF than the control group, which was further confirmed by significant LAI activity at the whole-brain level. These findings suggest that the interoceptive strategy is effective in aiding individuals to acquire voluntary control of LAI activity via brief NF-guided training. Of note, participants already performed well in session 1 and consequently we did not find significant increases of LAI activity from session 1 to session 3/4. This finding may suggest that the interoceptive-based strategy is efficient and participants can acquire LAI self-regulation in one single training session, although we cannot exclude the possibility that inclusion of a pre-training session of regulation strategy employment without feedback (i.e., the pre-training HCT) may be also beneficial for training performance (Haugg et al., 2021). In line with previous studies (Caria et al., 2007; Tinaz et al., 2018; Young et al., 2014; Zotev et al., 2011), stronger LAI activity was also found in the transfer session, suggesting that the LAI regulation ability based on interoceptive strategy can be maintained when no feedback is available, which is of importance in terms of translational potential. Thus in addition to the widely used emotional imagery or recall strategy, the present study provides an effective alternative regulation strategy for AI regulation.

Furthermore, the PPI analysis showed that successful LAI regulation was accompanied by increased LAI FC with prefrontal regions encompassing the bilateral dmPFC, vmPFC, ACC, and aPFC, a series of regions involved in cognitive and executive control that enable behavioral adaption and cognitive flexibility (Bush et al., 2000; Friedman and Robbins, 2022; Miller, 2000). More specifically, the mPFC is closely associated with performance monitoring/action-outcome evaluation (Alexander and Brown, 2011; Van Noordt and Segalowitz, 2012), with the vmPFC being engaged in information integration guiding goal-directed behaviors (Hiser and Koenigs, 2018; O'Doherty, 2011) and the dmPFC playing an important role in error detection/prediction (Modirrousta and Fellows, 2008) and action selection (Rushworth et al., 2007). Similarly, the ACC is closely associated with performance monitoring for errors and representation of reinforcement history (Bush et al., 2000; Rushworth et al., 2007) and the aPFC is implicated in intention maintenance (Ramnani and Owen, 2004). Strengthened connectivity between the target region and these prefrontal regions has also been found in previous rt-fMRI studies on up-regulation of the insula (Berman et al., 2013; Yao et al., 2016) and amygdala (Young et al., 2018; Zotev et al., 2011). These convergent findings indicate that these strengthened connectivities between the target region and prefrontal

regions may underlie performance monitoring of NF training, possibly by evaluating contingency of regulation strategy application with NF information, and is in accordance with a recently proposed conceptualization that NF training is a special form of reinforcement learning (Lubianiker et al., 2022). In support of this assumption, we also found increased LAI FC with core regions of learning and memory networks including the caudate and the hippocampal and parahippocampal gyri in line with previous rt-fMRI studies (Young et al., 2018; Zotev et al., 2011). Furthermore, increased FC of the target region was additionally found in the AI, PI, IFG, and thalamus of the salience/interoceptive network and the PCC and precuneus in the DMN, which may reflect integration of self-oriented/interoceptive signals and external salient information such as feedback (Craig, 2002, 2009; Menon and Uddin, 2010). Given that active neuromodulation based on rt-fMRI NF is closely associated with sustained attention performance (Pamplona et al., 2020) and attentional engagement can modulate sensitivity to interoceptive signals encoded in the insula (Zhang et al., 2020), training success based on the interoceptive strategy may also involve attentional switching between external feedback and interoceptive signals. Similar FC changes with regions in the DMN have also been reported in a previous rt-fMRI amygdala study using a self-oriented, although not interoceptive, regulation strategy of retrieving autobiographical memories (Zotev et al., 2011). These regions also correspond with a cingulo-opercular top-down control network providing stable 'set-maintenance' over an entire task epoch (Dosenbach et al., 2008). Extensive brain network reconfigurations including the insula during NF training have also been reported in a previous study (Haller et al., 2013). Thus, these findings suggest that NF training success may be supported by FC of the target region with some shared brain networks, particularly the prefrontal cognitive control, memory and learning networks, and the salience/interoceptive network, across different target regions.

On the behavioral level, there was a marginal main effect of group with higher rating scores of regulation strategy check in the NF than in the control group. Importantly, the three-way interaction was significant and post-hoc analyses found significantly higher rating scores for regulation blocks in session 1 and for baseline blocks in session 3 in the NF compared to the control group. These findings suggest that participants in the NF group tend to believe they performed better in implementing regulation strategies across sessions, although this effect was more robust in session 1 for implementing the interoceptive strategy and in session 3 for keeping relaxed. This finding was also consistent with participants' training performance of LAI regulation and their reports during post-training debriefing such that more participants in the NF group reported finding the interoceptive strategy helpful than the control group. Furthermore, while we did not find any significant IAC/IS changes between pre- and post-HCTs, we found a significant positive correlation of LAI activity with IAC in the post-training HCT only in the NF group. However, we did not observe such correlations with pre-training IAC and pre-post training differences of IAC, indicating that individual differences of AI regulation may be more closely associated with an ultimate state of IAC induced by NF training but may not critically depend on the inherent interoceptive ability at baseline. In accordance with findings of FC changes, an evaluative integration of regulation strategy implementation and NF information may be more important for NF training success when the interoceptive strategy was used, which can be further supported by a significantly positive correlation between the IAC in the post-HCT and FC strengths of the LAI-aPFC pathway during NF training.

In addition, inconsistent with our previous rt-fMRI study of the LAI (Yao et al., 2016), we did not find any training effects on increasing empathic responses. This discrepancy could be due to differences between the two studies. First, while the present study applied the interoceptive strategy, a negative mental recall/imagery strategy was used in Yao et al. (2016), suggesting that rt-fMRI NF with different regulation strategies may train different functions even for the same target region. The use of the same pain empathy localizer task in the two studies with

different regulation strategies may also contribute to the discrepancy. Compared with the pain empathy localizer task, an interoceptively focused localizer task may be more straightforward for the present study using an interoceptive strategy, although there is still no direct evidence of whether consistency between regulation strategies and the localizer task will affect training performance. Second, the experimental design was different, with empathic responses being measured by pre-post training differences in the present study but by immediate ratings following each regulation block in the previous one. Immediate measurements following regulation blocks that are more temporally associated with the LAI activity may be more sensitive for detecting effects of brief NF training, although no studies have directly compared the difference between these two types of measurements.

While previous rt-fMRI insula studies conventionally used a sham control region encompassing a whole top slice (Caria et al., 2007; 2010; Kanel et al., 2019; Veit et al., 2012; Yao et al., 2016), we chose the MTG as the sham control region based on findings from an independent sample. The whole top slice control region included some part of the SMA which is also involved in interoceptive processing (Critchley et al., 2004; Pollatos et al., 2007) and thus may confound NF training effects. This consideration was confirmed by significant activity in both the SMA and LAI, but not in the MTG, in the independent sample where participants received NF from the whole top slice using the interoceptive strategy (see Supplementary Materials). Given this confounding effect on NF training, in contrast to the preregistration the present study therefore mainly focused on comparison between the NF and the MTG control group. Further comparisons of NF training effects on LAI activity across the 3 groups also confirmed that the LAI activity in the NF group was significantly greater than the MTG control group but not the independent group. These findings together thus underline the importance of proper selection of the sham control region depending on specific regulation strategies used for NF training.

The present study has some limitations. First, there were no significant behavioral training effects in the transfer session in line with previous studies (Kanel et al., 2019; Yao et al., 2016; Young et al., 2014), which may indicate a need for optimizing protocols in future studies. Second, given a lack of optimal paradigm, the HCT was used to measure IAC in the present study, which can be confounded by factors such as time estimation and prior knowledge of heartbeat (Desmedt et al., 2018; Ferentzi et al., 2022). To minimize, although not completely exclude, possible confounding effects from those factors, optimized instruction following previous studies (Desmedt et al., 2018; 2020) was used for the HCT in the present study. Third, physiological signals (e.g., heartbeat or respiratory rate) were not recorded and controlled for in data analyses. Given that there was evidence showing the influence of physiological signals on BOLD responses (Chang et al., 2009; Chang and Glover, 2009; Windischberger et al., 2002; but see Lipp et al., 2014), this may confound training effects on findings on the neural level. In addition, the current study did not include a pre-training session without NF to exclude the potential confounding effects of pre-training differences between the two groups, which should be considered in future studies.

In summary, the present study provides the first evidence for the efficacy of a novel regulation strategy based on interoceptive processing in up-regulating the LAI activity, with the degree of NF-induced increases of LAI activity being associated with increased IAC following NF training and the regulation ability being maintained to a transfer session without NF. Furthermore, we showed that successful up-regulation of LAI activity was associated with a strengthened LAI FC with cognitive control, memory and learning, and salience/interoceptive networks. Given close associations of interoception with a variety of processes such as emotion, perception, decision-making, and mental health (Critchley and Garfinkel, 2017; Dunn et al., 2010; Khalsa et al., 2018; Tallon-Baudry, 2023), these findings provide proof of concept for the translational potential of this novel interoceptive strategy in rt-fMRI AI regulation of these processes and related psychiatric disorders, although more future studies are needed to investigate how to optimize training

protocol for reliable NF-induced behavioral changes and long-term effects before practical clinical translation.

Author contributions

Author contributions are as follows: experimental design: S.Y., Y.Z.; technical support: S.Y., Y.Z., and J.L.; data collection: Y.Z., Q.Z., J.W., M.Z., Y.Q., H.Z., C.Y., and H.Y.; statistical analyses: Y.Z., S.Y., and B.B.; paper drafting and revising: Y.Z., S.Y., B.B., and K.M.K.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are openly available via the Open Science Framework (OSF) Repository (https://osf.io/bkxna/?view_only=5bca806e6e3f4ceb919b25d6657ca2f5) and its supplementary material files.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (Grant No. 2018YFA0701400; B.B. and S.Y.) and the Natural Science Foundation of Sichuan Province (Grant No. 2023NSFSC1187; S.Y.). These funding sources played no role in the preparation of data or the writing or submission of the paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.neuroimage.2023.120455](https://doi.org/10.1016/j.neuroimage.2023.120455).

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