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Real-Time Functional Connectivity-Informed Neurofeedback of Amygdala-Frontal Pathways Reduces Anxiety

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Keywords

Emotion regulation · Real-time neurofeedback · Anxiety · Amygdala · Connectivity · Anxiolytic treatment · Prefrontal cortex · Treatment · Functional magnetic resonance imaging · Network

Abstract

Background: Deficient emotion regulation and exaggerated anxiety represent a major transdiagnostic psychopathological marker. On the neural level these deficits have been closely linked to impaired, yet treatment-sensitive, prefrontal regulatory control over the amygdala. Gaining direct control over these pathways could therefore provide an innovative and promising intervention to regulate exaggerated anxiety. To this end the current proof-of-concept study evaluated the feasibility, functional relevance and maintenance of a novel connectivity-informed real-time fMRI neurofeedback training. **Methods:** In a randomized crossover sham-controlled design, 26 healthy subjects with high anxiety underwent real-time fMRI-guided neurofeedback training to enhance connectivity between the ventrolateral prefrontal

cortex (vLPFC) and the amygdala (target pathway) during threat exposure. Maintenance of regulatory control was assessed after 3 days and in the absence of feedback. Training-induced changes in functional connectivity of the target pathway and anxiety ratings served as primary outcomes. **Results:** Training of the target, yet not the sham control, pathway significantly increased amygdala-vLPFC connectivity and decreased levels of anxiety. Stronger connectivity increases were significantly associated with higher anxiety reduction on the group level. At the follow-up, volitional control over the target pathway was maintained in the absence of feedback. **Conclusions:** The present results demonstrate for the first time that successful self-regulation of amygdala-prefrontal top-down regulatory circuits may represent a novel intervention to control anxiety. As such, the present findings underscore both the critical contribution of amygdala-prefrontal circuits to emotion regulation and the therapeutic potential of connectivity-informed real-time neurofeedback.

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Introduction

Successful regulation of negative affect is crucial for mental health and well-being [1, 2]. Deficient emotion regulation (ER) and exaggerated anxiety represent transdiagnostic markers across major psychiatric disorders, including the most prevalent axis I disorders such as anxiety and addiction as well as axis II disorders [3–5; see also recent meta-analysis in 6].

The functional interplay between and clinical relevance of ER and anxiety mirrors across different levels of observation. In healthy subjects, ER capability prospectively predicts anxiety levels for periods up to 5 years [7–9]. The clinical relevance is further emphasized by randomized trials evaluating the efficacy of cognitive-behavioral therapy indicating that improved ER predicts symptom reduction in anxiety disorders [10–12]. On the neural level efficient regulation of threat and anxiety is neurally underpinned by top-down governance of the amygdala, which is critically engaged in threat responsivity [13], via prefrontal regulatory regions [14, 15]. Within these regulatory circuits the ventrolateral (vlPFC) and dorsomedial prefrontal cortex are considered to specifically support explicit/volitional control of threat via downregulation of the amygdala [5, 15, 16]. Deficits in this top-down regulatory mechanism have been identified across major psychiatric disorders [17], with disorders characterized by exaggerated anxiety exhibiting decreased recruitment of the prefrontal cortex and concomitantly exaggerated amygdala activity in the context of attenuated functional interplay between these regions [17–19]. The therapeutic relevance of these pathways is further emphasized by studies reporting that anxiety reduction following behavioral and pharmacological interventions is accompanied by normalization of deficient amygdala-prefrontal coupling [20–22].

Despite the important contribution of neuroimaging research to identifying altered amygdala-prefrontal interaction and its normalization as a potential pathological and treatment-sensitive neural marker for neuropsychiatric disorders characterized by emotional dysregulations, it has yet to directly have a therapeutic impact [23]. Given that the currently available therapeutic interventions for anxiety reduction are generally characterized by moderate response rates and potential negative side effects [24–26], innovative treatments that directly target the identified brain markers are needed [27]. Within this context, the emergence of real-time functional magnetic resonance imaging neurofeedback (rt-fMRI NF) training (NFT) approaches that allow subjects to gain volitional

control over regional brain activity have been considered as a putatively promising strategy [28–30]. Importantly, previous studies have confirmed this potential of rt-fMRI NF by demonstrating that training success in terms of control over regional activity can be maintained beyond the training session [31–34], and that training-induced neural activity changes can modulate emotional experience in healthy subjects [31] and patients with major depression [32–35].

Initial studies have begun to evaluate the therapeutic potential of rt-fMRI NF in clinical populations and demonstrated that upregulating activity in primary emotion-processing regions such as the insula and amygdala can successfully decrease symptoms in patients with major depression [32–34]. Given the critical role of the amygdala in anxiety and consistently observed hyperresponsivity in this region in anxiety-related disorders [18, 36, 37], previous rt-fMRI NF studies trained subjects to downregulate neural activity in this region and demonstrated that this strategy has the potential to enhance ER and attenuate anxious arousal [38–41]. In line with current neurocircuitry models of ER, successful downregulation of the amygdala was accompanied by increased functional connectivity between the amygdala and prefrontal regulatory regions in both, healthy subjects [39, 42] as well as patient populations with exaggerated anxiety [40, 43].

Summarizing, the current literature suggests that (a) successful ER relies on top-down regulation of the amygdala via prefrontal regions and that (b) rt-fMRI NF-assisted modulation of these regions has the potential to modulate ER and anxious arousal. In the context of recent circuit level models of ER (for circuit level deficits in psychiatric disorders, see Insel and Wang [44]) the present preregistered randomized sham-controlled crossover proof-of-concept study aimed at evaluating whether (1) rt-fMRI NF has the potential to directly allow regulatory control of the strengths of functional connectivity in the amygdala-prefrontal regulatory pathways, (2) successful regulatory control decreases levels of anxiety in individuals with high anxiety, and (3) volitional control can be maintained in the absence of feedback and over a period of 3 days.

Methods and Materials

Participants

To increase the clinical relevance of the present proof-of-concept study while controlling for potential confounding factors in clinical populations, including comorbidity or medication, 30 healthy subjects with high anxiety (trait anxiety scores >40, as-

sessed by STAI [45]) were recruited. Given that the main aim was to evaluate the feasibility and functional relevance of connectivity-informed rt-fMRI NFT potential confounding effects of menstrual cycle-related variation in ER [46], as well as sex differences in ER capacity [47], ER strategies [48] and associated connectivity in the target pathway [49] were controlled for by focusing on a male sample. Detailed eligibility criteria and sample characteristics are provided in the online supplementary materials (for all online suppl. material, see www.karger.com/doi/10.1159/000496057). All subjects provided written informed consent. The study had full ethical approval by the local ethics committee, adhered to the latest version of the Declaration of Helsinki and protocols were preregistered (NCT02692196, <http://clinicaltrials.gov/show/NCT02692196>). Please note that the registration refers to both sexes; however, in line with one of our recent ER studies [50] and the rationale above, we decided to focus on male subjects before the start of enrollment.

Protocols and Procedures

Participants were scheduled for 4 MRI sessions: rt-fMRI NFT of the amygdala-vIPFC target pathway (EXP) plus transfer/maintenance assessment after 2 days (M-EXP) and rt-fMRI NF active control (henceforward referred to as sham control, SHC) training plus transfer/maintenance assessment after 2 days (M-SHC). During the training sessions feedback was provided but not during the transfer/maintenance sessions. Training sessions used identical procedures including 4 subsequent NFT runs, and during the sham session participants received connectivity feedback from a pathway connecting regions not engaged in ER (bilateral motor cortices, M1 [15]). In line with recent recommendations for intervention trials [51], an active sham control condition was employed to control for unspecific effects of training, which together with the crossover design allowed a thorough control of potential confounders [51]. To control for carry-over effects the order of training sessions was counterbalanced (analysis of potential carry-over effects on the behavioral and neural level given in online suppl. materials). For randomized allocation of the order of trainings, a random number generator was used. Training sessions were separated by an interval of 2–3 weeks, and subjects were informed that they had to discover new strategies each time. Both training sessions were preceded by fMRI localizer paradigms to determine the pathways used for feedback during EXP and SHC (see the section “Localizer Paradigms”). To explore training-induced changes on amygdala activity the emotion localizer was repeated after each training session. Two days after each training session, participants underwent two transfer runs (M-EXP/M-SHC) during which they were required to perform regulation with the same strategy they had learned during the preceding training but without feedback being provided (for protocols, see online suppl. Fig. S1). To evaluate the functional relevance of training success, anxiety levels were assessed before and after each session and served as a primary behavioral outcome. To this end visual analog scales (VAS, anxiety levels from 0 to 100) were administered before and after each training session. VAS scales were chosen based on previous studies demonstrating a high sensitivity of these scales to capture the functional relevance of rtfMRI trainings [32, 52] and studies reporting a direct association between self-reported anxiety and amygdala-prefrontal crosstalk [53]. To control confounding effects of pre-training mood and anxiety states, these were assessed additionally immediately before each training and maintenance session.

Localizer Paradigms

Pathway-specific localizer paradigms were employed to localize the target emotion regulation nodes (EXP, right amygdala and right vIPFC, emotion localizer) and the bilateral motor cortices (SHC, bilateral M1, motor localizer) using Turbo-BrainVoyager v3.2 (Brain Innovation, Maastricht, The Netherlands). A similar activation-based localizer approach has been employed by a previous study evaluating functional connectivity feedback [54] (details in online suppl. materials). By employing a combined structure-function approach, regions of interest (ROIs) for the functional connectivity analysis were determined for each training session (details provided in online suppl. materials). To further control for movement effects on functional connectivity [55] and physiological artifacts such as respiration and noise from cardiac activity, a third ROI (identical size as the target ROIs) was placed in a right postcentral white matter tract (online suppl. materials).

NFT Protocols

During the NFT strong negative (threatening) stimuli were displayed with real-time NF displayed by thermometer bars on both sides (stimuli details see online suppl. material and Fig. S1). Each of the 4 NFT runs included 4 blocks of threatening pictures (6 pictures per block, interblock interval 30 s fixation, pictures presented for 5 s, size gradually increased from half to full size stepped by the repetition time (TR, 1.5 s) to increase threat). rt-fMRI connectivity between the ROIs was calculated as a partial correlation between the time series from the two pathway ROIs (amygdala-vIPFC or bilateral M1) while including the time series from the white matter ROI as a covariate [for details, see 54]. The functional connectivity thermometer was updated in real time (logged to the TR = 1.5 s). Participants were informed that the purpose of the training was to enhance their emotion regulation abilities to improve coping with negative emotional events in daily life and reduce stress. Participants were instructed to learn to control the threatening feelings evoked by the pictures while breathing normally. To increase their regulation ability, the neural emotion regulation success would be presented to them (thermometer bars corresponding to better success), and they should aim to develop a strategy to increase the bars. Based on previous rt-fMRI NF studies reporting increased amygdala-prefrontal connectivity following training-induced reductions in amygdala activity [40, 43, 56] and studies reporting an association between higher emotion regulation success and stronger amygdala-prefrontal connectivity [16, 57], subjects were trained to increase positive connectivity in the target pathway. As a specific strategy is not necessary for successful learning in NFT [58, 59], no explicit strategies for emotion regulation were introduced to the participants. Participants were instructed not to control the thermometers by physical means such as breathing or head/body motion but rather to discover an efficient emotional control strategy. Once they discovered an efficient strategy to increase the feedback bars they were asked to continue using it during the subsequent training and transfer sessions. Finally, subjects were informed that the feedback would be computed in real time but displayed with approximately 10 s delay (see evaluation of training success on the neural level).

MRI Data Acquisition, Online Preprocessing and Connectivity NF

Data were acquired by a 3-Tesla MRI system using evaluated sequences (online suppl. materials). Online data preprocessing

and real-time feedback were computed using Turbo-BrainVoyager v3.2. To increase the signal-to-noise ratio of the functional data during online processing, real-time preprocessing was applied including motion correction and spatial smoothing with a 4-mm full width at half maximum Gaussian kernel and temporal drift removal applied as confound predictor to the general linear model. Based on findings from a previous study [60] a sliding window approach with a length of 7.5 s (5 volumes per window, TR 1.5 s) was chosen to compute the real-time connectivity feedback. Feedback was thus provided as a partial correlation coefficient between the two ROI time series segmented in consecutive windows while controlling for the nuisance signal from the third ROI (online suppl. materials).

Offline Preprocessing and Analyses

Preprocessing for offline analysis was conducted using standard procedures in SPM12 (Statistical Parametric Mapping, <http://www.fil.ion.ucl.ac.uk/spm/>). To evaluate BOLD level changes during the localizers on the group level, first level general linear models were built. To increase the sensitivity of the offline connectivity analysis individual ROIs from the training sessions were exported (online suppl. Table S2, Fig. S2, S3). To evaluate whether NFT increased functional connectivity in the emotion regulation circuit, task-based functional connectivity was employed using a generalized form of context-dependent psychophysiological interaction [61] implemented as whole-brain connectivity models with the individualized amygdala (online suppl. Table S2) ROIs used during training as seeds. The generalized psychophysiological interaction models were built on the first level by adding the time series from the seed region as a new regressor into the general linear model design matrix. A previous study showed that a 12-s time window had comparable sensitivity in detecting task-relevant connectivity changes as a longer time window (26 s) for a finger tapping task [62]. Since valid online connectivity feedback started at approximately 12.5 s (5 s delay from the BOLD response, and 7.5 s length of the slide time window) after the start of a regulation block, analysis of NFT task-based functional connectivity focused on the second half of the regulation blocks (last 15 s of every training block – additional analyses using the entire block lengths fully confirmed the findings, online suppl. Material).

Primary Outcomes and Evaluation of Training Success

Training-induced changes in the amygdala-vlPFC target pathway served as primary outcome on the neural level. To this end, connectivity strengths per run were extracted from this pathway using beta-estimate maps generated in the generalized psychophysiological interaction analysis. Estimates were further analyzed using SPSS (ver. 22) by means of repeated-measure ANOVAs (analysis of variance) and Bonferroni-corrected post hoc tests. In line with previous studies evaluating the functional and clinical relevance of rt-fMRI NFTs [31, 52] training success was assessed by comparing changes in connectivity strengths between the early stages of learning (first 2 runs) and later stages of the training during which successful control has been implemented (run 3 + run 4 > run 1 + run 2). Training-induced changes in subjective anxiety levels as assessed by the VAS administered before and after each training session served as the primary behavioral outcome. In line with recommendations to apply nonparametric statistics to VAS data due to its ordinal nature [63, 64] rank-based statistics (ANOVA-type statistic in nparLD [65], post hoc tests using Wilcoxon

signed-rank test) were employed to evaluate training effects on the behavioral level.

Control of Potential Confounders

To further control for confounding effects of pretraining differences in mood and state anxiety between the sessions, corresponding indices were assessed by means of the PANAS (the Positive and Negative Affect Schedule [66]) and SAI (State Anxiety Inventory [45]) administered before each training and maintenance session. Behavioral measures were assessed outside of the scanner by an experimenter blinded to the training condition (EXP, SHC).

Results

Data Quality Assessment Protocols

While 26 out of the 30 participants completed the whole experiment procedure, 3 subjects did not display above-threshold activity in the vlPFC during the emotional localizer and their data were thus excluded from all analysis, resulting in $n = 23$ for the final analyses (participants' flow shown in Fig. 1). Five runs showed >2.5 mm or >2.5° head motion, data from these runs were consequently excluded. Head motion (mean frame-wise displacement [67]) did not differ between the experimental and the sham training (EXP vs. SHC, $t_{22} = 0.58$, $p = 0.568$, two-tailed), which together with further control analysis (online suppl. material) argues against confounding effects of movement on the present results.

Mood States and Anxiety

Mood and anxiety data for 1 participant were lost (pretraining assessment, EXP). Examination of the pretraining data from the remaining participants confirmed the recruitment of subjects with high anxiety (reflected in high state anxiety scores) and did not reveal differences in pretraining anxiety and mood between training sessions (Table 1, $p > 0.375$, paired t test, two-tailed).

BOLD Response during the Localizer and Training

Group-level analysis of the localizer tasks revealed that the emotional localizer reliably activated the emotional brain networks, including the amygdala and vlPFC (SPM one-sample t test, whole-brain, false discovery rate [68], corrected $p < 0.01$, online suppl. Fig. S2). As expected, the motor localizer reliably activated the motor networks including bilateral M1 (online suppl. Fig. S3). Importantly, emotion regulation (regulation – baseline) during both EXP and SHC training induced a similar activity pattern as in the emotion localizer tasks in the ER brain networks, including dorsomedial prefrontal cortex, vlPFC, amyg-

Fig. 1. CONSORT flow diagram of the clinical trial. vIPFC, ventrolateral prefrontal cortex.

dala, insula, and parietal regions [69]. Moreover, offline analysis of the emotion localizer paradigm revealed strong functional connectivity between the amygdala and vIPFC region as determined by the functional localizer, further validating the localizer approach (for details, see online suppl. materials and Fig. S4).

Functional Connectivity Changes in the Target Pathway over the Training Runs

Examining changes in functional connectivity between right amygdala and vIPFC across the 4 NFT runs with one-way ANOVA (repeated measures) revealed a significant difference between them ($F_{3, 66} = 3.33$, $p = 0.025$). Importantly, this pathway did not show significant changes across the training runs with sham feedback ($F_{3, 54} = 1.02$, $p = 0.393$) (online suppl. Fig. S5). Post hoc tests for the EXP training revealed that connectivity in the target pathway did not show changes between the early training runs (run 1 vs. run 2, $t_{22} = 1.22$, $p = 0.236$, paired t test, two-tailed) but increased significantly after the second NFT run (run 3 vs. run 2, $t_{22} = 2.95$, $p = 0.007$, Cohen's $d = 0.78$; run 4 vs. run 2, $t_{22} = 3.83$, $p = 0.001$, Cohen's $d = 1.04$, paired t tests, two-tailed, both significant after Bonferroni corrected $p = 0.05/6$) (online suppl. Fig. S5).

Evaluation of Training Success – Primary Neural Outcome

Two-way repeated-measures ANOVA with the factors training (EXP vs. SHC) and time (run 1 + run 2 vs.

Table 1. Mood states before both training sessions

	Before EXP training (n = 22)	Before SHC training (n = 23)
PANAS-P	24.95 (4.84)	22.22 (5.38)
PANAS-N	14.86 (4.02)	14.48 (4.23)
SAI	38.05 (7.06)	36.57 (7.06)

Mean scores of positive and negative mood and anxiety levels and their SDs (in parentheses) assessed by questionnaires before training. None of these control variables showed any difference between the two sessions ($p > 0.375$, paired t test, two-tailed). PANAS-P, Positive and Negative Affect Schedule – positive; PANAS-N, Positive and Negative Affect Schedule – negative; SAI, State-Trait Anxiety Inventory – state anxiety.

run 3 + run 4) and connectivity strengths of the target pathway as dependent variable [31, 52] revealed a significant training $\times$ time interaction effect ($F_{1, 21} = 4.47$, $p = 0.047$). Post hoc comparisons demonstrated that connectivity in the target pathway increased over the course of the EXP training (run 3 + run 4 > run 1 + run 2, $t_{22} = 2.81$, $p = 0.010$, Cohen's $d = 0.79$, paired t test, two-tailed). Concordant analysis of the sham training data did not yield significant changes in this pathway ($t_{21} = -0.52$, $p = 0.607$, paired t test, two-tailed). Additional control analysis confirmed the training success when data from the entire block lengths were used (see online suppl. materials).

Fig. 2. Self-reported anxiety level (VAS rating) decreased after EXP training (columns in white) but not after SHC training (columns in light gray). ANOVA-type nonparametric test revealed a marginal significant interaction effect on the primary behavioral outcome ($F_{1, \infty} = 3.55, p = 0.059$) between the factors treatment (experimental feedback vs. sham feedback) and time (before vs. after training). Post hoc test indicated that anxiety levels decreased after training of the amygdala-ventrolateral prefrontal emotion regulation pathway (before vs. after training, $Z = 2.16, p = 0.031$, Wilcoxon test, two-tailed, effect size = 0.46) but not after training of the sham pathway ($p = 0.948$).

Evaluation of Training Success – Primary Behavioral Outcome

Examining effects on the behavioral outcome using nonparametric ANOVA-type analyses with the factors training (EXP, SHC) and time (pretraining, posttraining) revealed a marginal significant interaction effect between training and time on self-reported anxiety levels ($F_{1, \infty} = 3.55, p = 0.059$) in the absence of main effects (both p values > 0.45). In concordance with the evaluation of the neural training success, post hoc comparisons revealed a significant decrease in self-reported anxiety following the EXP training (pre- vs. posttraining, $Z = 2.16, p = 0.031$, Wilcoxon test, two-tailed, effect size = 0.46; on a descriptive level a 42.9% mean decrease, pre – post/pre $\times 100\%$, Fig. 2), but no changes following the sham training (pre- vs. posttraining, $Z = 0.07, p = 0.948$, Wilcoxon test, two-tailed). Importantly, an additional control analysis revealed that pretraining anxiety levels did not differ significantly between the training sessions ($Z = 0.38, p = 0.702$, Wilcoxon test, two-tailed).

Association between Behavioral and Neural Training Success

A correlation analysis examined whether the behavioral (changes in anxious arousal) and neural (changes early vs. late connectivity) training success were associated. Results indicated that on the group level the EXP training-associated anxiety decrease associated positively with stronger training-induced increases in amygdala-vlPFC coupling ($r_{20} = 0.51, p = 0.016$, Spearman correlation).

Transfer and Maintenance of Training Success

The maintenance of the learned self-regulation in the current study was explored by comparing the mean amygdala-vlPFC connectivity between the training runs before successful learning on day 1 (run 1 and run 2) and the 2 transfer runs on day 3 (M-EXP). This analysis revealed significantly higher functional connectivity strength in the transfer runs than in the prelearning runs ($t_{22} = 2.50, p = 0.020$, paired t test, two-tailed) for the EXP training while concordant analysis for the SHC session revealed no significant change ($p = 0.995$, paired t test, two-tailed). Furthermore, for the EXP training a marginal significant correlation between amygdala-vlPFC connectivity during successful learning (run 3 and run 4 on day 1) and transfer runs ($r_{21} = 0.39, p = 0.068$, Pearson correlation) was observed, together suggesting that training success on the neural level in terms of regulatory control over the target pathway can be maintained independently of feedback and for a period of up to 3 days.

In line with the evaluation of the maintenance effect on the neural level, the maintenance effect on the behavioral level was explored by comparing the pretraining anxiety level on day 1 and the pretransfer anxiety level on day 3. This analysis revealed no significant differences between the initial training and the transfer session for neither the EXP ($p = 0.159$) nor the SHC condition ($p = 0.148$, Wilcoxon test, two-tailed).

Effects of Connectivity-Informed Training on Amygdala Activity

Training-induced changes in amygdala activity towards negative stimuli were explored by means of a repeated-measures ANOVA with the factors training (EXP vs. SHC) and time (pre- vs. posttraining on day 1) and extracted activity from the amygdala ROI during the emotion localizers as dependent variable. Results revealed a significant main effect of time ($F_{1, 22} = 10.40, p = 0.004$), with post hoc tests indicating that amygdala activity decreased after both training sessions (both p values

<0.025, paired *t* tests, two-tailed). Amygdala activity decreases were not associated with anxiety reduction (all *p* values >0.441, Spearman correlation).

Deteriorations after Treatment

In line with recent recommendations [70], deteriorations with respect to the primary behavioral outcome were explored. Visual inspection of self-reported anxiety measures before and after EXP training suggested that 5 out of 23 subjects displayed a numerical increase in anxiety while 9 subjects displayed a numerical anxiety increase following the SHC training. The proportion of deterioration did not differ between EXP and SHC training (Fisher exact test, *p* = 0.337, two-tailed).

Discussion

The present proof-of-concept study employed a randomized, sham-controlled, crossover design to evaluate the feasibility, functional relevance and maintenance of a novel connectivity-informed rt-fMRI NF approach as a strategy to strengthen emotion regulation and decrease anxiety. During training of the amygdala-vlPFC pathway, but not the sham control motor pathway, healthy participants with high anxiety gained regulatory control over this ER-relevant pathway in terms of successfully increasing functional connectivity strength over 4 subsequent training runs. On the behavioral level training of the target pathway – but not the sham pathway – was accompanied after training by decreased anxious arousal ratings. On the group level, the neural and behavioral indices of training success were significantly positively associated, further confirming the functional relevance of successful amygdala-vlPFC connectivity regulation. Finally, training success in terms of regulatory control over the amygdala-vlPFC pathway was maintained in the absence of feedback and for a period of 3 days. Importantly, no changes in the primary neural and behavioral outcomes were observed during the sham condition, arguing against unspecific effects of training.

The target amygdala-vlPFC pathway in the present study has previously been demonstrated to play an important role in successful ER [5, 15, 16] with rt-fMRI NF studies suggesting that successful downregulation over regional amygdala activity associates with both, increased connectivity in the amygdala-prefrontal pathways as well as enhanced ER [38, 39]. Moreover, previous clinical studies have emphasized the relevance of the amygdala-PFC circuits for treatment success, with changes in this pathway

predicting symptom reduction after cognitive behavioral therapy [20] and anxiolytic drug treatment [21, 22] in patients with exaggerated anxiety. In line with our hypothesis, successful training of the target pathway resulted in associated decreases in anxiety ratings thereby confirming both the important role of the amygdala-vlPFC pathway in the regulation of anxiety as well as the functional relevance of the training. To increase the clinical relevance of the present proof-of-concept study, healthy subjects with high anxiety were recruited, and the training-associated decrease in anxious arousal thus suggests that amygdala-vlPFC training may have the potential to normalize deficient prefrontal control of the amygdala and exaggerated levels of anxiety in clinical populations.

Plotting functional connectivity of the target pathway over the training runs revealed that learning occurred after the 2 initial runs with no further increase from run 3 to run 4. This indicates that learning during the training might not have followed a linear increase over the 4 runs but rather regulation ability changed qualitatively after the first 2 runs. Notably, a similar learning curve has been observed in a previous study evaluating amygdala-prefrontal connectivity training in the absence of an explicit regulation strategy [56]. Findings may reflect that subjects explored different regulation strategies during the initial 2 runs and maintained the successful strategies during the subsequent training runs. This difference to BOLD activity NFT studies may be explained by the fact that the functional connectivity feedback signal inherently comes at a longer delay and involves higher dimensionality [71, 72], which may lead to a higher difficulty for the subjects to discover successful regulation strategies. Future studies may increase the number of training runs and sessions to determine whether regulatory control on the neural level can be further increased.

Exploring changes in amygdala reactivity towards negative stimuli before and after training revealed that both, training of the target and the sham pathway was accompanied by decreased amygdala responses. The lack of differences between the training sessions may be explained in terms of rapid habituation of amygdala threat reactivity or the employment of successful emotion regulation strategies during both trainings. Importantly, training-associated amygdala response decreases were not associated with anxiety changes, which together with the EXP training-specific anxiety decreases suggests that successful regulation of the amygdala-vlPFC pathway specifically contributed to the anxiety reduction.

Of particular relevance for the application of NFT approaches in clinical practice [30, 73, 74] the present study

observed that subjects were able to maintain the control over the emotion regulation pathway in the absence of feedback and for a period of at least 3 days. These findings are in line with previous studies evaluating transfer and maintenance effects of rt-fMRI NF-assisted control over regional brain activity [31, 75], and additionally suggest that successful neuromodulatory control on the pathway level can last beyond the duration of the initial training and thus transfer to contexts outside of the MRI environment. However, despite maintenance of the neural training success, attenuation of anxiety levels following the training of the target pathway as observed on day 1 was not maintained over the follow-up period [for similar results, see 31].

Despite increasing interest in the application of rt-fMRI, only a few studies to date have directly evaluated effects of NFT on functional connectivity between brain regions [54, 56, 71, 76–79]. In line with the present findings, a previous study with a relatively small sample of healthy subjects revealed initial evidence for the feasibility of connectivity-informed NF which was associated with increased perception of positive valence stimuli. Importantly, the present study demonstrated the efficacy of this approach in decreasing anxious arousal, a transdiagnostic psychopathology marker [3], in subjects with high anxiety levels and thus may represent an important initial step towards the clinical application of connectivity-informed NF.

While these initially promising evaluations of functional connectivity-informed NFT approaches are encouraging, there is still considerable room for improvement to promote transfer into clinical practice. Future studies should explore whether improved training strategies [59] and more intense or longer training schedules may lead to more robust and enduring behavioral effects in the absence of NF. Moreover, a considerable interindividual variance in the neural and behavioral indices of training success was observed suggesting that some individuals are more likely to benefit from functional connectivity-informed training than others. Future studies are needed to identify optimal neural or behavioral predictors of training success allowing better selection of individuals who may benefit most from rt-fMRI NFT approaches. Recent findings suggest that baseline anxiety [56] or behavioral performance [80] may represent promising behavioral markers, although robust training success predictors on the neural level remain to be determined. A recent review emphasized the important contribution of expectancy (placebo) effects to the therapeutic effects of NFT [29]. The crossover randomized sham-controlled design of the present study allowed

to carefully control placebo effects, and the direct comparison of both treatment sessions revealed an advantage in terms of training success arguing against effects driven by treatment expectancies. Moreover, empirical evidence from a very recent study challenged the view of unspecific training effects as a primary mechanism of action of NF success by demonstrating decreased rather than increased training success following a sham booster (sham transcranial direct current stimulation) [81].

Although the crossover design with a comparably large sample (compared to previous rt-fMRI experiments, see overview [30]), inclusion of an active-control and the preregistration of the primary outcomes permitted a rigorous control for a number of potential confounds, the present findings still need to be considered in the context of some limitations. First, to allow an evaluation of the training independent of menstrual-cycle or gender effects on ER and associated neural activity [46, 47, 49], our proof-of-concept study focused on male participants. The question of whether training success generalizes to female subjects and potential sex differences therefore remains to be addressed. For clinical practice it will be important to compare both training and functional outcome success using activity- and connectivity-based feedback approaches. Connectivity-based NF comes at the cost of longer delay times and higher dimensionality [71] so that learning with this signal may be more demanding, possibly limiting efficacy in patients with cognitive impairments. In line with initial studies successfully evaluating the clinical potential of amygdala activity NFTs in patient populations [82], repeated training sessions may be necessary to alleviate symptoms in patient populations [30] or to maintain effects on the behavioral level. Finally, a small number of participants reported increased anxiety after the intervention. Importantly, the proportion did not differ between the EXP and the SHC training arguing against specific effects of amygdala-vlPFC connectivity training. The outcome of an intervention is influenced by multiple factors [70], including pretreatment individual differences. The identification of markers that predict treatment response [83] and the use of repeated training sessions may increase the number of participants that respond to rt-fMRI NFTs.

In summary, the present findings demonstrate that real-time functional connectivity-informed NFT is feasible, and targeting amygdala-prefrontal pathways with this training may represent a potential strategy to decrease anxiety in clinical populations. Importantly, neural training success was maintained in the absence of feedback guidance and for a period of at least 3 days.

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Disclosure Statement

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