Electrical Fingerprint of the Amygdala Guides Neurofeedback Training for Stress Resilience

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Abstract

Real-time functional magnetic resonance imaging (rt-fMRI) has revived the translational perspective of neurofeedback (NF). Particularly for stress management, targeting deeply located limbic areas involved in stress processing has paved new paths for brain-guided interventions. However, the high cost and immobility of fMRI constitute a challenging drawback for the scalability (accessibility and cost-effectiveness) of the approach, particularly for clinical purposes. The current study aimed to overcome the limited applicability of rt-fMRI by using an electroencephalography (EEG) model endowed with improved spatial resolution, derived from simultaneous EEG–fMRI, to target amygdala activity (termed amygdala electrical fingerprint (Amyg-EFP). Healthy individuals (n = 180) undergoing a stressful military training programme were randomly assigned to six Amyg-EFP-NF sessions or one of two controls (control-EEG-NF or NoNF), taking place at the military training base. The training results demonstrated specificity of NF learning to the targeted Amyg-EFP signal, which led to reduced alexithymia and faster emotional Stroop indicating better stress coping following Amyg-EFP-NF relative to controls. Neural target engagement was demonstrated in a follow-up fMRI-NF, showing greater amygdala blood-oxygen-level-dependent activity downregulation and amygdala–ventromedial prefrontal cortex functional connectivity following Amyg-EFP-NF relative to NoNF. Together, these results demonstrate limbic specificity and efficacy of Amyg-EFP-NF during a stressful period, pointing to a scalable non-pharmacological yet neuroscience-based training to prevent stress-induced psychopathology.

Introduction

The introduction of real-time functional magnetic resonance imaging (rt-fMRI) has revived the translational interest in volitional neuromodulation via neurofeedback (NF)[1]. The possibility of targeting deep-brain limbic areas such as the amygdala, known to be involved in emotional processes that are abnormal in psychopathology[2], has opened a new path for non-pharmacological brain guided treatment. In stress related psychopathologies in particular the down-regulation of amygdala activity via the pre-frontal- or anterior cingulate-cortex (PFC and ACC respectively) is considered a key mechanism in emotion regulation[3], and an essential feature for adaptive stress coping[4]. This pivotal role of the amygdala was recently demonstrated in a prospective study with a priori healthy soldiers[5] by showing that amygdala hyper-activation is a predisposing factor for military stress vulnerability. Therefore, learning to regulate one's own amygdala activity may diminish detrimental- and facilitate adaptive-stress coping mechanisms.

Indeed, initial results of amygdala targeted fMRI-NF studies favorably point to the translational potential of this approach by showing strengthened amygdala–ventro-medial PFC (vmPFC) functional connectivity[6–8], improved emotion regulation[9–11], and reduced symptoms of major depression following treatment[12]. Despite the apparent promise of fMRI-NF, it`s high cost, immobility and relatively low accessibility has been a challenging drawback in the scalability of this approach, especially for clinical purposes[13]. EEG on the other hand, is mobile and low cost but provides limited spatial specificity, particularly for deep-brain limbic areas such as the amygdala[14]. In a series of recent studies, we aimed to overcome the drawbacks of both imaging techniques by applying machine learning algorithms to simultaneously recorded EEG and fMRI data[15,16], yielding an EEG model of weighted coefficients

that could be used to probe localized Blood-Oxygen-Level-Dependent (BOLD) activity in the amygdala (hereby termed, "amygdala-Electrical Finger Print[17]"; Amyg-EFP; see Supplementary Figure 1). A follow-up study further validated that the Amyg-EFP can reliably probe amygdala BOLD activity, and that compared to sham-NF, Amyg-EFP-NF can lead to improved amygdala BOLD down-regulation capacities via fMRI-NF[11]. In the current study we aimed to test the efficacy of repeated Amyg-EFP-NF sessions on neural, cognitive and behavioral indices of emotion regulation, using a double blind randomized controlled trial (RCT) with a large sample (N=180) of a-priori healthy male soldiers experiencing a uniquely stressful life period; the first weeks of combat military training[18,19]. In order to demonstrate scalability in terms of mobility and applicability, the study took place at the soldiers' training base.

The project aimed to: (1) Demonstrate the target signal specificity of Amyg-EFP-NF relative to controls, (2) Examine the efficacy of Amyg-EFP-NF on amygdala related emotion regulation processes via anxiety[20] and alexithymia[21] self-reports and performance on an emotional Stroop task[22], and (3) Demonstrate target engagement of the amygdala and its cortical connections using a follow-up fMRI. To pursue the first and second aims, participants were randomly assigned to either Amyg-EFP-NF (n=90), or one of two control groups: Control-EEG-NF that probed Alpha/Theta ratio (control-NF; n=45), or No NF (NoNF; n=45). Assignment to Amyg-EFP-NF or control-NF was double blind. The Amyg-EFP-NF group underwent six NF sessions targeting Amyg-EFP down-regulation, within a period of four weeks (Figure 1A). To enable a distinction between the global effects of the NF procedure and the specific effects of Amyg-EFP regulation, we designed a control condition that would account for the key common processes that underlie NF[23] (see supplementary information for

more details) without targeting the neural circuit of interest (amygdala regulation and amygdala-mPFC connectivity). Therefore, similarly to the "different region" approach in fMRI-NF studies[9,12,24], our control-NF condition was guided by the Alpha/Theta ratio (reduced Alpha [8-12Hz] and increased Theta [4-7Hz]), a commonly used EEG-NF probe[25]. Moreover, since Theta and Alpha both contribute to the Amyg-EFP model (see Supplementary Figure 1) it was imperative to further demonstrate the specificity of the Amyg-EFP on limbic processing by not only using a correlative approach, as done previously[11], but also causally showing amygdala related behavioral changes following Amyg-EFP-NF as compared to A/T-EEG-NF alone. The control-NF group underwent the identical training protocol as the Amyg-EFP-NF group (Figure 1A) but learned to down-regulate A/T ratio. To further control for transient psychological changes that may take place during a stressful military period, we also compared the effect of Amyg-EFP-NF to a condition without NF training (NoNF). Importantly, during the study period participants of all three groups underwent the same mandatory military training program, which took place at the same military base.

To facilitate NF learning we used a multimodal animated NF interface (Figure 1B; Supplementary Video[26]) that has been shown to induce higher engagement and a more sustainable learning effect as compared to abstract visual feedback[26]. To test for learning sustainability, participants underwent a no-feedback trial following training sessions 4-6 with the animated scenario. To further test whether learned regulation of Amyg-EFP could be transferred to situations with additional cognitive demands, upon completion of session 5, we introduced a *cognitive interference trial* to test volitional regulation while conducting a memory task (see Supplementary Table 1 for NF trials conducted at each session). Before and after the training period all participants

conducted an emotional Stroop[22] (eStroop) task, testing implicit emotion regulation previously found to involve amygdala activation[27]. In addition, all participants completed anxiety[20] and alexithymia[21] self-report questionnaires. Alexithymia refers to difficulties in cognitively processing emotions and was found related to stress vulnerability[28,29].

We hypothesized that Amyg-EFP-NF would result in greater Amyg-EFP down regulation relative to control-NF, and that this learned regulation would be sustained in the absence of on-line feedback (no-feedback trial), and under the cognitive load of an irrelevant cognitive task (cognitive-interference trial). We further hypothesized that relative to control-NF and NoNF, Amyg-EFP-NF would lead to a larger improvement in emotion regulation, as indicated by performance on the eStroop task and a greater reduction in reported anxiety and alexithymia. To pursue the third aim of neural target engagement, one month following the completion of the in-base testing, 60 participants (30 Amyg-EFP-NF; 30 NoNF) arrived at the Tel-Aviv Medical Center and underwent amygdala targeted fMRI-NF. We hypothesized that relative to NoNF, Amyg-EFP-NF would result in greater down regulation of BOLD-amygdala via fMRI-NF. As previously shown[6,8,9], we further hypothesized that in addition to increased down regulation of amygdala BOLD activity, Amyg-EFP-NF would result in greater amygdala-vmPFC functional connectivity.

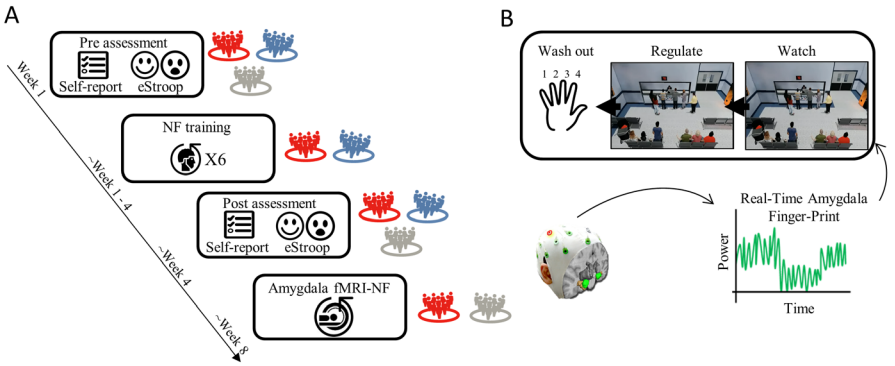

Figure 1: (A) Experimental time-line. NF training, and Pre- / Post-NF assessments took place in the military training base within a period of 4 weeks. The assessments included self-report of anxiety (STAI) and alexithymia (TAS-20) and the eStroop task. Upon completion of pre-NF assessments (week 1), participants were randomized into three groups; Amyg-EFP-NF (red; n=90), Control-NF (blue; n=45) or NoNF (grey; n=45). Amyg-EFP-NF and Control-NF conducted 6 NF session targeting down regulation of either the Amyg-EFP or a control signal (Alpha/Theta ratio) respectively, while NoNF underwent no intervention. Approximately one month following completion of NF training in the military base, a subset of 60 participants (30 Amyg-EFP-NF, 30 NoNF) underwent amygdala targeted fMRI-NF at the Sagol Brain Institute. **(B) EEG-NF session.** Success in down regulating the targeted signal (Amyg-EFP or Control) is reflected by audiovisual changes in the *unrest level* of a virtual 3D scenario (a typical hospital waiting room), manifested as the ratio between characters sitting down and those loudly protesting at the counter[26,48]. The NF paradigm consists of 3 consecutive conditions each repeating 5 times: *Watch* (60 sec.), *Regulate* (60 sec.) and *Washout* (30 sec.). During *Watch* the participant is instructed to passively view the virtual scenario while it is in a constant 75% *unrest level*. During *Regulate* the participant is instructed to find the mental strategy that will lead to an appeasement in the scenario unrest level. During *Washout* the participant taps his thumb to his fingers according to a 3-digit number that appears on the screen.

Results

Amyg-EFP-NF learning specificity

Amyg-EFP-NF success was measured as the delta of Amyg-EFP power during the active *regulate* condition relative to the passive *watch* condition (*regulate – watch*). The mean delta of each group in each session was subject to a 2X6 repeated measures ANOVA with *NF success* as the dependent variable and *group* (Amyg-EFP-NF vs control-NF) and *session* (1-6) as independent variables (See statistical analysis in the methods section for further details). As hypothesized, Amyg-EFP-NF resulted in larger Amyg-EFP down-regulation relative to control-NF (Figure 2A), demonstrating the signal specificity of Amyg-EFP-NF training (group effect: mean group

difference=-0.08, standard error (se)=0.02, *F(1,104)=16.73, p<0.001, η^2=0.14*, 90% Confidence Interval (CI) [0.05 , 0.24]). This specificity was also shown by a group-by-session interaction *(F(5,224)=2.39, p=0.038, η^2=0.05, 90% CI [0.00, 0.08]) means and sds of each session are reported in Supplementary Table 2A)*, affirming our hypothesis that Amyg-EFP-NF will lead to a larger improvement in Amyg-EFP down regulation as training progresses. The group differences reached significance at session 4 and were maintained through sessions 5 and 6 (see Supplementary Table 2B for means, SDs, between group *p* values, effect sizes and CIs for each session). Outlier removal (±1.5IQR; see Supplementary Figure 2 for box plots) did not alter these results (group effect: mean difference=-0.06, se=0.01, *F(1,69)=21.25, p<0.001, η^2=0.24, 90% CI [0.10, 0.36]* ; group by session: *F(5,154)=2.33, p=0.045, η^2=0.07, 90% CI [0.00, 0.12]; See Supplementary Table 5 for means and sds of each session*). To test which group drove the effect we conducted a post-hoc repeated measures ANOVA for each group separately, using session (S1-S6) as independent variable and Amyg-EFP-NF success *(regulate – watch)* as dependent variable (Figure 2B & 2C). A main effect of session for the Amyg-EFP-NF group *(F(5,168)=3.68, p=0.003, η^2=0.10, 90% CI [0.02, 0.15])* was found, with a significant linear trend (*F(1,87)=18.48, p<0.001, η^2=0.18, 90% CI [0.07, 0.29]*). Our analysis further indicated that a significant improvement relative to the first session was obtained by session 4 and was maintained throughout the last session (Figure 2B & Supplementary Table 3). No such effect was observed for the control-NF group (*Figure 2C; F(5,122)=0.79, p=0.562, η^2=0.01, 90% CI [0.00, 0.05])*, nor any significant trends. See Supplementary Table 3 for means, sds t statistics, effect size estimates and CIs of within group comparison between each session (2-6) and the first session. To verify that the control-NF group learned to down regulate the target signal

(A/T), we looked for A/T signal modulations (Supplementary Figure 3 & Supplementary Table 4). As hypothesized, a repeated measures ANOVA with NF success *(regulate – watch)* as the dependent variable and session (1-6) as independent variable revealed a significant effect of session (*F(5,156)=2.92, p=0.015, η^2=0.09, 90% CI [0.01, 0.14]*), with significant linear (*F(1,37)=6.26, p=0.017, η^2=0.14, 90% CI [0.01, 0.31]*) and quadratic trends *(F(1,37)=4.27, p=0.046, η^2=0.10, 90% CI [0.00, 0.26]*). Our analysis further indicated that a significant improvement relative to the first session was obtained by session 5 and maintained in session 6. See Supplementary Table 4 for means, sds, t statistics, effect size estimates and CIs of within group comparisons between each session (2-6) and the first session.

Amyg-EFP-NF learning sustainability: To evaluate learning sustainability we tested participant's capacity to volitionally regulate Amyg-EFP in the absence of online feedback[30] (i.e. no-feedback trial; see methods). To evaluate whether the learned skill of volitional regulation is transferable to real world on-task conditions, we also tested participants' ability to down-regulate the recorded signal while conducting a simultaneous memory task (i.e. cognitive-interference trial; see methods). Results of the no-feedback trial (Figure 2D) demonstrated that as hypothesized, volitional regulation of Amyg-EFP could be sustained in the absence of on-line feedback, as indicated by a larger reduction of Amyg-EFP signal (*regulate -watch*) following Amyg-EFP-NF relative to control-NF (*mean group difference=-1.06, se difference=0.14, t(124)=7.42, p(one tailed)<0.001, d=1.44, 95% CI [1.02, 1.86]; Amyg-EFP-NF=-1.34±1.24; control-NF=-0.28±0.35*). Similar results were also obtained during the cognitive-interference trial (Figure 2E), further indicating that the Amyg-EFP signal could be regulated while conducting a simultaneous cognitive task (*mean group difference=-0.09, se difference=0.03, t(124)=3.05, p(one tailed)=0.001,*

d=0.59, 95% CI [0.20, 0.98]; Amyg-EFP-NF=-0.13±0.23; control-NF=-0.03±0.10). This result suggests that Amyg-EFP-NF learning is maintained even in face of additional cognitive demands. To test whether volitional regulation during the no-feedback and cognitive-interference trials was successful in each group separately, we compared the power of the targeted signal during *regulate* relative to *watch* (A/T for control-NF and Amyg-EFP for Amyg-EFP-NF). During the no-feedback trial as expected, both groups showed a significant reduction in signal power during *regulate* relative to *watch* (Amyg-EFP-NF: *mean (regulate – watch) = -1.34±1.24, t(87)=10.15, p(one tailed)<0.001, d=1.08, 95% CI [0.82, 1.34]*, *watch=-0.12±0.14, regulate=-1.46±1.23*; control-NF: *mean (regulate – watch)=0.07±0.21, t(37)=2.19, p(one tailed)=0.014, d=0.36, 95% CI [0.02, 0.68], watch=1.41±0.41, regulate=1.34±0.43*). However, during cognitive-interference only down regulation of the Amyg-EFP was found to be feasible (Amyg-EFP-NF: *mean (regulate – watch)*=-0.13±0.23, *t(87)=5.03, p<0.001, d=0.54, 95% CI [0.31, 0.76], watch=-1.04±1.29, regulate=-1.17±1.35*; *control-NF: mean (regulate – watch)=-0.01±0.09, t(37)=0.51, p(one tailed)=0.305, d=0.08, 95% CI [-0.24, 0.40], watch=1.05±0.19, regulate=1.06±0.22*). Results of the memory task showed that on average participants answered *11.09±1.55* out of 13 questions correctly, with no group differences (*mean difference=0.17, se=0.32, t(102)=0.54, p=0.591; d=0.11, 95% CI [-0.30, 0.52], Amyg-EFP-NF:11.14±1.56; control-NF: 10.97±1.54*), possibly suggesting a ceiling effect for cognitive load.

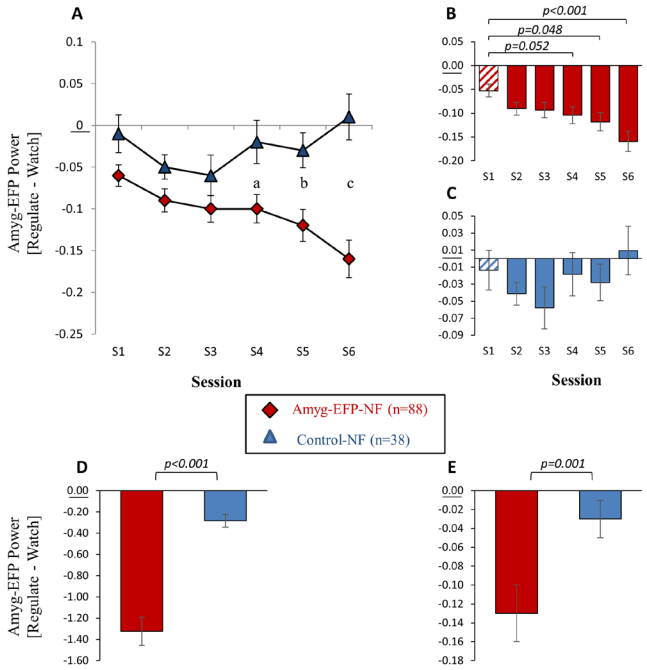

Figure 2: NF learning. (A) Group difference in Amyg-EFP signal modulation across the six NF sessions. Amyg-EFP NF (red, n=88) led to a larger reduction in Amyg-EFP signal power (*regulate – watch*; y-axis) relative to control-NF (blue, n=38) as indicated by a significant group effect (*mean difference=-0.08, se=0.02, F(1,104)=16.73, p<0.001, η^2=0.13, 90% CI [0.05 , 0.24]).* Furthermore, as indicated by a significant group by session interaction *(F(5,224)=2.39, p=0.038, η^2=0.05, 90% CI [0.00, 0.08])*, down-regulation of Amyg-EFP increased as the Amyg-EFP-NF training progressed, while control-NF had no such effect on the Amyg-EFP signal power. [a]*p=0.014*, [b]*p=0.020*, [c]*p<0.001*. See Supplementary Table 2B for means, sds, between group t statistics, *p* values, effect size estimates and CIs for each session. **(B)** Post-hoc analysis showing that the Amyg-EFP-NF group reached a significant improvement in Amyg-EFP down regulation relative to the first session, from session 4 onward. **(C)** Post-hoc analysis showing that Control-NF did not result in significant changes in Amyg-EFP down regulation throughout. See Supplementary Table 3 for means, sds, within group t statistics, *p* values, effect size estimates and CIs comparing each session (2-6) to the first session in each group separately. **(D-E) NF learning sustainability.** Averaged down regulation of Amyg-EFP (y-axis) during cycles with **(D)** the absence of online feedback in the No-Feedback condition, and when **(E)** conducting a simultaneous memory task in the Cognitive-Interference condition. Relative to the control-NF (blue, n=38), Amyg-EFP-NF (red, n=88) resulted in larger down regulation of amyg-EFP signal (y-axis) in both the No-Feedback condition (*mean difference=-1.06, se=0.14, t(124)=7.42, p(one tailed)<0.001, d=1.44, 95% CI [1.02, 1.86]*) and the Cognitive-Interference condition (*mean difference=-0.09, se=0.03, t(124)=3.05, p(one tailed)=0.001, d=0.59, 95% CI [0.20, 0.98]*). Error bars indicate standard error.

Amyg-EFP-NF training efficacy

To evaluate the efficacy of Amyg-EFP-NF in modifying emotion regulation we measured changes in performance on the eStroop task and in self-reports of anxiety and alexithymia (see methods).

In the eStroop task participants viewed fearful or happy facial expressions with superimposed congruent or incongruent words (‘‘happy’’\‘‘fear’’) and were asked to identify the emotional expression while ignoring the words that appeared. The emotional Stroop task provides a measure of *‘emotional conflict regulation'* indicated by the difference in response times between congruent and incongruent stimuli and of *'emotional conflict adaptation'* calculated as the difference in response times between two consecutive incongruent stimuli [ii] and incongruent stimulus following congruent stimulus [ci] (*adaptation* = [ii]-[ci])[22]. Comparing the post- vs pre-NF eStroop performance of each group revealed that as hypothesized, Amyg-EFP-NF led to a greater improvement in *'emotional conflict regulation'* (incongruent - congruent) relative to the control groups (Figure 3A). A group (Amyg-EFP-NF, control-NF, NoNF) by time (pre- vs post-training) interaction (*$F(2,164)=5.00$, $p=0.008$, $\eta^2=0.06$, 90% CI [0.01, 0.12], means and sds of each time point are reported in Supplementary Table 6*) revealed that while Amyg-EFP-NF led to improved *'emotional conflict regulation'* following training, control-NF had no effect and NoNF resulted in reduced conflict regulation post- vs pre-training (Amyg-EFP-NF*: mean (post-pre)*=-9.97±38.27, *$t(87)=2.45$, p(one tailed)$=0.008$, $d=0.26$, 95% CI [0.05, 0.47];* control-NF*: mean (post-pre)*=4.16±43.15*, $t(37)=0.59$, $p=0.553$, $d=0.10$, 95% CI [-0.22, 0.41];* NoNF*: mean (post-pre)*= 10.27±28.07*, $t(42)=2.40$, $p=0.017$, $d=0.37$, 95% CI [0.06, 0.67]).* No group effect was observed (*$F(2,164)=1.93$, $p=0.148$, $\eta^2=0.02$, 90% CI [0.00, 0.07])* and no a-priori differences in *emotional conflict regulation* were observed between the Amyg-EFP-NF group and the control-NF (*mean difference=5.92, se=6.12, $t(124)=0.97$, $p=0.333$, $d=0.19$, 95% CI [-0.19, 0.57]*) or NoNF (*mean difference=2.22, se=5.54, $t(129)=0.40$, $p=0.689$, $d=0.07$, 95% CI [-0.29, 0.44]*) groups. To test our main hypothesis, that Amyg-EFP-NF would lead to a

larger improvement in *emotional conflict regulation* relative to each of the control groups separately, we conducted a post-hoc analysis comparing the change in conflict regulation (post vs pre). As hypothesized, the improvement in *emotional conflict regulation* (Figure 3B) was larger following Amyg-EFP-NF compared to control-NF (*mean difference=-14.13, se=7.72, t(124)=1.83, p(one tailed)=0.034, d=0.36, 95% CI [-0.03, 0.74])* and NoNF (*mean difference=-20.24, se=6.57, t(129)=3.08*, *p(one tailed)=0.001, d=0.57, 95% CI [0.20, 0.94]*; Amyg-EFP-NF=-9.97±38.27; control-NF=4.16±43.15; NoNF=10.27±28.07). No correlations were found between improvement in *emotional conflict regulation* and Amyg-EFP (*Amyg-EFP-NF: r=0.04, p=0.742, 95% CI [-0.17, 0.25]; control-NF: r=-0.05, p=0.787, 95% CI [-0.36, 0.27]*) or A/T (*Amyg-EFP-NF: r=0.06, p=0.629, 95% CI [-0.15, 0.27]; control-NF: r=0.14, p=0.436*, *95% CI [-0.19, 0.44]*) signal reductions. Contrary to our hypothesis, no differences were found between the groups in '*Emotional Conflict Adaptation'* ([ci]-[ii]) post- vs pre-training, as shown by an insignificant group (Amyg-EFP-NF, control-NF, NoNF) by time (pre vs post) interaction (F(2,164)=0.90, p=0.410, η^2*=0.01, 90% CI [0.00, 0.04], means and sds of each time point are reported in Supplementary Table 6).*

The distribution of TAS-20 scores at baseline was consistent with previous reports of alexithymia prevalence among healthy populations[21,31–33] (mean=42.50±11.02). No alexithymia was exhibited by 72.8% of the sample (scores lower than 51[21]), 27.2% indicated moderate alexithymia (scores ≥ 51) and less than 5% showed high alexithymia (scores ≥61; see Supplementary Figure 4). Consistent with our hypothesis, Amyg-EFP-NF resulted in a larger reduction of alexithymia scores relative to controls (Figure 3C) as indicated by a group (Amyg-EFP-NF, control-NF, NoNF) by time (pre- vs post-training) interaction (F(2,164)=10.69, p<0.001, η^2*=0.12,*

90% CI [0.04, 0.19], means and sds of each time point are reported in Supplementary Table 6). Interestingly, while the control-NF group showed no differences following the training period, the NoNF group showed increased alexithymia (Amyg-EFP-NF*: mean (post-pre)=-3.37±9.19, t(87)=3.43 p(one tailed)<0.001, d=0.37, 95% CI [0.15, 0.58];* control-NF*: mean (post-pre)=*0.01±7.27*, t(37)=0.01 p=0.994, d=0.01, 95% CI [-0.07, 0.07];* NoNF*: mean (post-pre)=6.11±13.57, t(42)=2.96, p=0.003, d=0.45, 95% CI [0.13, 0.76]).* No group effect (*F(2,164)=1.64, p=0.198, η^2=0.02, 90% CI [0.00, 0.06])* or a-priori differences in alexithymia were observed between the Amyg-EFP-NF group and the control-NF group (*mean difference=0.96, se=2.16, t(124)=0.45, p=0.655, d=0.09, 95% CI [-0.29, 0.47]*) or NoNF group (*mean difference=0.95, se=2.07, t(129)=0.46, p=0.645, d=0.09, 95% CI [-0.28, 0.45]*). To test our main hypothesis, that Amyg-EFP-NF would lead to a larger reduction in alexithymia ratings relative to each of the control groups separately, we conducted a post-hoc analysis comparing the change in alexithymia scores (post – pre). As hypothesized, the reduction (post vs pre) was greater for the Amyg-EFP-NF group (Figure 3D) as compared to control-NF (*mean difference=-3.38, se=1.69, t(124)=2.00, p(one tailed)=0.023, d=0.39, 95% CI [0.00, 0.77])* and NoNF (*mean difference=-9.48, se=2.29, t(129)=4.14, p<0.001, d=0.77, 95% CI [0.39, 1.15]; Amyg-EFP-NF=-3.37±9.19; control-NF=0.01±7.27; NoNF=6.11±13.57*). A Pearson correlation further demonstrated the association between the changes in alexithymia scores and Amyg-EFP-NF training (Figure 3E), by showing that the change over time in alexithymia self-reports (post-NF – pre-NF) corresponded (*r=0.35, p=0.002*, 95% CI [0.15, 0.52]) with the participants best NF session (i.e. maximum Amyg-EFP reduction out of six sessions; see supplementary information). Importantly, we found this correlation only among participants who trained with Amyg-EFP-NF, and not

among control-NF (*r=0.09, p=0.644*, 95% CI [-0.24, 0.40]). Furthermore, learned regulation of A/T (control-NF) did not correlate with reduced alexithymia *(r=0.07, p=0.670*, 95% CI [-0.11, 0.24]), nor did oscillations in the Theta (*r=-0.07, p=0.441*, 95% CI [-0.24, 0.11]) or Alpha (*r=-0.10, p=0.288*, 95% CI [-0.27, 0.08]). A post-hoc analysis suggested that the differences between the groups in alexithymia reduction was driven by individuals who showed moderate-severe alexithymia at baseline (i.e. equal to or higher than a score of 51). This was tested by comparing between and within group differences in alexithymia reduction post- vs pre- NF, while excluding participants with a score lower than 51 pre-NF (*Amyg-EFP-NF n=24; control-NF n=12; NoNF n=10*). A paired samples t-test revealed a significant reduction in alexithymia scores, but only among those who underwent Amyg-EFP-NF (*Amyg-EFP-NF: mean (post-pre)=-10.75±11.73, t(23)=4.49, p<0.001, d=0.92, 95% CI [0.43, 1.39], pre-NF=57.29±5.75, post-NF=46.54±13.59; control-NF: mean (post-pre)=0.25±6.47, t(11)=0.13, p=0.893, d=0.04, 95% CI [-0.53, 0.60], pre-NF=55.50±4.60, , post-NF=55.75±8.17; NoNF: mean (post-pre)=0.56±4.43, t(9)=0.40, p=0.691, d=0.13, 95% CI [-0.50, 0.75], pre-NF=55.50±2.55, post-NF=54.94±5.72*). This analysis further revealed that this reduction in alexithymia following Amyg-EFP-NF was larger relative to both control-NF (*mean difference=-11.00, se=3.65, t(34)=3.01, p=0.003, d=1.06, 95% CI [0.32, 1.79])* and NoNF *(mean difference=-10.19, se=2.78, t(32)=3.67, p<0.001, d=1.38, 95% CI [0.56, 2.18]*).

Contrary to our hypothesis, an insignificant group (Amyg-EFP, control-NF, NoNF) by time (pre vs post NF) interaction (*F(2,152)=0.63, p=0.530, η^2=0.01, 90% CI [0.00, 0.04], means and sds of each time point are reported in Supplementary Table 6*) indicated no between group differences in post- vs pre-NF self-reports of state

anxiety. Interestingly however, a time effect (*mean (post-pre)=-2.04±9.80, F(1,150)=6.25, p=0.013, η^2=0.04, 95% CI [0.00, 0.10]; pre=32.58±9.41, post=30.54±8.11)* indicated a reduction in state anxiety that was significant only for the Amyg-EFP-NF and control-NF groups but not for the NoNF group, possibly pointing to a non-specific effect of NF training (Amyg-EFP-NF*: mean (post-pre)=-2.25±9.57, t(87)=2.21 p(one tailed)=0.014, d=0.24, 95% CI [0.02, 0.45];* control-NF*: mean (post-pre)=-3.25±8.40, t(37)=2.38 p=0.017, d=0.39, 95% CI [0.05, 0.71];* NoNF*: mean (post-pre)=-0.62±10.02, t(42)=0.40, p=0.687, d=0.06, 95% CI [-0.24, 0.36]).* No group effect (*F(2,162)=1.09, p=0.340, η^2=0.01, 90% CI [0.00, 0.05]; means and sds of each time point are reported in Supplementary Table 6*) nor a-priori differences in state anxiety were observed between the amyg-EFP group and the control-NF group (*mean difference=-2.01, se=1.89, t(124)=1.06, p=0.287, d=0.21, 95% CI [-0.18, 0.59]*) or the NoNF group (*mean difference=-1.35, se=1.73, t(129)=0.78, p=0.434, d=0.15, 95% CI [-0.22, 0.51]*). No correlations were found between reductions in state-anxiety and Amyg-EFP (*Amyg-EFP-NF: r=0.16, p=0.136, 95% CI [-0.05, 0.36]; Control-NF: r=-0.06, p=0.769, 95% CI [-0.37, 0.26]*) or A/T oscillations (*Amyg-EFP-NF: r=0.01, p=0.966, 95% CI [-0.20, 0.22]; Control-NF: r=0.01, p=0.950, 95% CI [-0.31, 0.33]*).

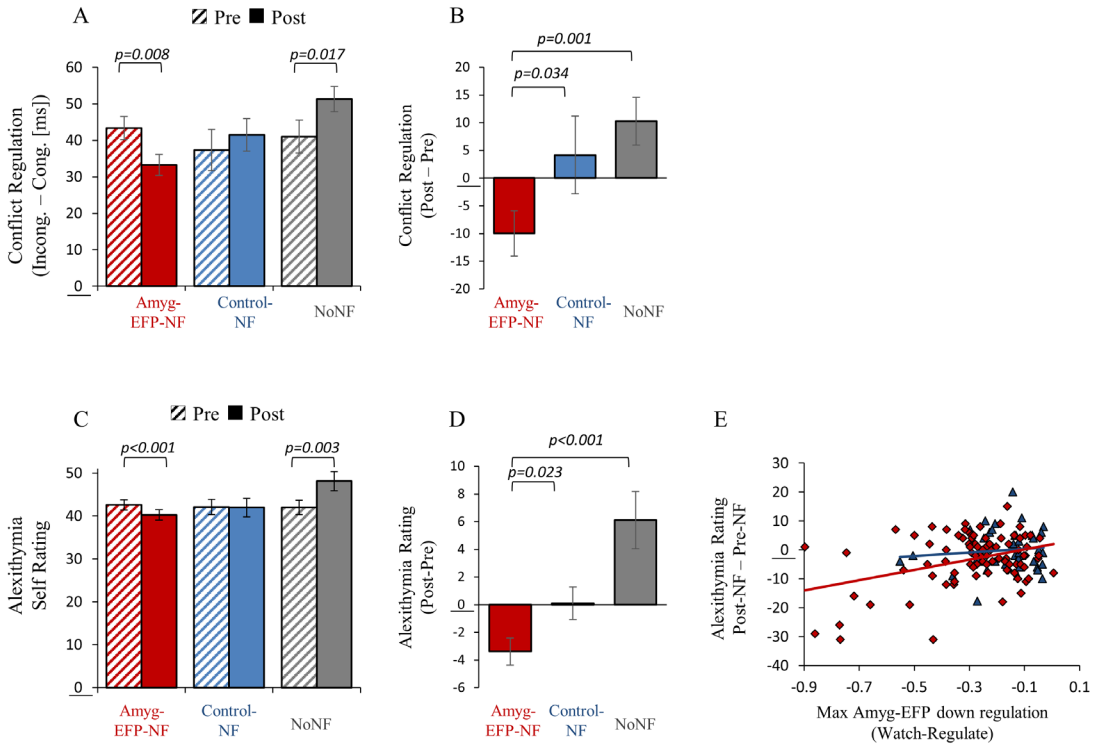

Figure 3: Outcomes of NF training per group. (A-B) eStroop performance change. (A) Group by time (Pre vs Post) interaction (*F(2,164)=5.00, p=0.008, η^2=0.06, 90% CI [0.01, 0.12]*) showing that Amyg-EFP-NF (red, n=88) resulted in improved eStroop performance (y-axis; *mean (post-pre)*=-9.97±38.27, *t(87)=2.45, p(one tailed)=0.008, d=0.26, 95% CI [0.05, 0.47])*, while the control groups (control-NF [blue, n=38], NoNF [gray, n=43]) showed the opposite pattern *mean (post-pre)*=4.16±43.15, *t(37)=0.59, p=0.553, d=0.10, 95% CI [-0.22, 0.41]; NoNF: mean (post-pre)=* 10.27±28.07, *t(42)=2.40, p=0.017, d=0.37, 95% CI [0.06, 0.67])*. **(B)** eStroop improvement (y-axis) was grater following Amyg-EFP-NF relative to Control-NF (*mean difference=-14.13, se=7.72, t(124)=1.83, p(one tailed)=0.034, d=0.36, 95% CI [-0.03, 0.74])* , as well as, NoNF (*mean difference=-20.24, se=6.57, t(129)=3.08, p(one tailed)=0.001, d=0.57, 95% CI [0.20, 0.94]*. **(C-E) Alexithymia rating changes. (C)** Group by time interaction (F(2,164)=10.69, p<0.001, *η^2=0.12, 90% CI [0.04, 0.19])* showing that Amyg-EFP-NF training (red, n=88) resulted in reduced alexithymia ratings (y-axis; *mean (post-pre)=-3.37±9.19, t(87)=3.43 p(one tailed)<0.001, d=0.37, 95% CI [0.15, 0.58]*), while the control groups showed no change (Control-NF (n=38): *mean (post-pre)*=0.01±7.27, *t(37)=0.01 p=0.994, d=0.01, 95% CI [-0.07, 0.07]*) or the opposite pattern (NoNF (n=43): *mean (post-pre)=6.11±13.57, t(42)=2.96, p=0.003, d=0.45, 95% CI [0.13, 0.76]*). **(D)** Alexithymia score changes with time (y-axis) showing that the reduction exhibited by the Amyg-EFP-NF group was grater compared both to control-NF (*mean difference=-3.38, se=1.69, t(124)=2.00, p(one tailed)=0.023, d=0.39, 95% CI [0.00, 0.77]*) and NoNF *(mean difference=-9.48, se=2.29, t(129)=4.14, p<0.001, d=0.77, 95% CI [0.39, 1.15])*. **(E)** Scatterplot showing that the best performance out of the six Amyg-EFP-NF training (x-axis) correlated (*r=0.35, p=0.002*, 95% CI [0.15, 0.52]) to the reduction in alexithymia ratings (y-axis) within the Amyg-EFP-NF group only. Error bars represent standard error.

Amyg-EFP-NF related target-engagement

To test engagement of the targeted brain mechanism we assessed participants' ability to volitionally regulate Amygdala-BOLD activity via fMRI-NF. One month following the training period 60 participants (30 Amyg-EFP-NF; 30 NoNF) underwent amygdala targeted fMRI-NF with a similar design to Amyg-EFP-NF but with a different NF interface (Supplementary Figure 6). Beta weighted activity of the targeted amygdala functional cluster during *regulate* relative to *watch* was subjected to a region of interest (ROI) analysis. Figure 4A shows that, as hypothesized, Amyg-EFP-NF resulted in better down regulation of amygdala BOLD activity, as indicated by a group (Amyg-EFP-NF vs NoNF) by condition (regulate vs watch) interaction (*F(1,54)=10.77, p=0.002;* η^2*=0.17, 90% CI [0.04, 0.31]*; *Amyg-EFP-NF: watch=0.03 ± 0.67, regulate=-0.08±0.67; NoNF: watch=0.17±0.69, regulate=0.28±0.73).* Also consistent with our hypothesis, down regulation of amygdala BOLD activity was successful only following Amyg-EFP-NF (*Amyg-EFP-NF: mean (regulate – watch)=-0.11±0.24, t(29)=2.55, p(one-tailed)=0.008; d=0.47, 95% CI [0.08, 0.84]; NoNF: mean (regulate – watch)=0.11±0.25, t(25)=2.11, p=0.045*, *d=0.41*, 95% CI *[0.01, 0.81]*). A Pearson correlation further revealed that participants' best performance during Amyg-EFP-NF training predicted amygdala BOLD down regulation (*regulate vs watch*) during fMRI-NF (*r=0.43, p=0.016, 95% CI [0.08, 0.68]; Figure 4B*). This correlation was shown to be specific to Amyg-EFP and was not observed for changes in Theta (*r=0.01, p=0.945, 95% CI [-0.35, 0.37]*), Alpha (*r=-0.01, p=0.996, 95% CI [-0.37, 0.35]*) or A/T ratio (*r=-0.02, p=0.911, 95% CI [-0.38, 0.34]*). To examine whether improved down-regulation of the amygdala during fMRI-NF could be explained by a reduction in state anxiety, as observed following both Amyg-EFP-NF and control-NF, we tested a correlation between

changes in anxiety ratings, amygdala-BOLD down regulation and learned control over A/T ratio within the group that conducted follow-up fMRI. The analysis showed no correlation between A/T regulation and anxiety reduction (*r=-0.02, p=0.885, 95% CI [-0.38, 0.34]*) nor between anxiety reduction and follow-up amygdala BOLD down-regulation (*r=0.03, p=0.883, 95% CI [-0.34, 0.39]*).

To examine our assertion regarding the neural mechanism of amygdala down regulation capacity we used the targeted amygdala cluster as a seed region in a whole brain Psycho-Physical Interaction (PPI) analysis with group (Amyg-EFP-NF vs NoNF) and condition (*regulate vs watch*) as independent variables. This analysis revealed that relative to NoNF, Amyg-EFP-NF led to higher amygdala—vmPFC functional connectivity (Figure 4C) during both the regulate and watch conditions (vmPFC *peak voxel: x=9, y=62, z=-2, p(FDR) < 0.05*).

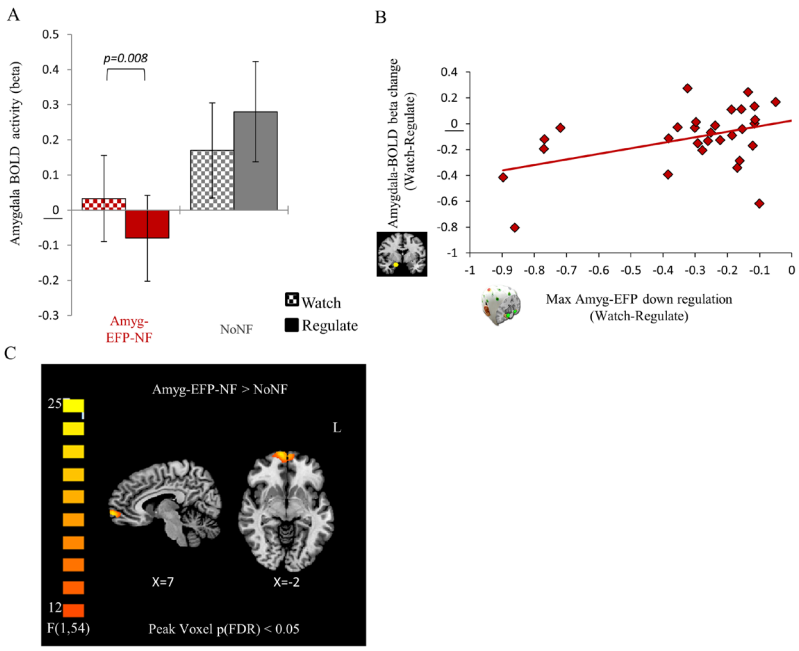

Figure 4: Amygdala-fMRI-NF, one month following Amyg-EFP-NF training. (A) Group by Condition interaction (*F(1,54)=10.77, p=0.002; η^2=0.17, 90% CI [0.04, 0.31]*) showing that relative to NoNF (grey, n=26), Amyg-EFP-NF (red, n=30) resulted in greater down regulation of Amygdala BOLD activity (y-axis) during fMRI-NF (watch vs regulate). Only the Amyg-EFP-NF group, exhibited reduced amygdala BOLD activity (y-axis) during regulate (solid filled bars) relative to watch (dashed filled bars) (*Amyg-EFP-NF: mean (regulate – watch)=-0.11±0.24, t(29)=2.55, p(one-tailed)=0.008; d=0.47, 95% CI [0.08,*

0.84]; NoNF: mean (regulate – watch)=0.11±0.25, t(25)=2.11, p=0.045, d=0.41, 95% CI *[0.01, 0.81]*). **(B)** Scatterplot showing that the maximum value of Amyg-EFP down-regulation across the six training sessions (x-axis) predicted (*r=0.43, p=0.016, 95% CI [0.08, 0.68]*) the ability to down regulate Amygdala-BOLD activity during fMRI-NF one month later (y-axis) **(C)** Whole brain PPI analysis with amygdala as a seed region, showing that Amyg-EFP-NF compared to NoNF, resulted in higher amygdala-vmPFC functional connectivity during *watch* and *regulate*. Error bars represent standard error.

Discussion

The current work conducted a multi-level investigation of a scalable (mobile, cost effective and applicable) NF method for the modulation of deeply located limbic activity, performed as an RCT among young healthy individuals during a particularly stressful life period. The Amyg-EFP computational approach for targeting limbic activity allowed us to conduct repeated NF sessions at the soldiers' base, using a large sample with multiple controls. Importantly, comparing Amyg-EFP-NF to active (control-NF) as well as NoNF controls provided careful differentiation between the specific and non-specific effects of the NF training. Relative to control-NF, Amyg-EFP-NF led to greater learning of Amyg-EFP signal reduction during training (Figure 2A – 2C), which was maintained in the absence of online feedback and when under cognitive interference (Figure 2D & 2E). We further tested the efficacy of Amyg-EFP-NF training with regards to emotion regulation and found greater improvement in emotional conflict regulation (Figure 3A & 3B), and in self-reports of alexithymia (Figure 3C & 3D) following Amyg-EFP-NF, relative to controls. Lastly, follow-up fMRI-NF performed on a subset of the sample, one month after completion of Amyg-EFP-NF training, demonstrated target engagement by showing that Amyg-EFP-NF resulted in a better ability to volitionally down regulate amygdala BOLD and stronger amygdala-vmPFC functional connectivity relative to NoNF (Figure 4). Together, our results confirm the specificity and efficacy of Amyg-EFP-NF training for emotional regulation modification under stressful life conditions.

Amyg-EFP-NF learning

Consistent with previous studies[12,34], an analysis of the NF performance across the six sessions positively demonstrated that volitional brain activity regulation is a learned skill that can improve as training progresses (Figure 2). Importantly, control-NF did not influence the Amyg-EFP signal, demonstrating training specificity. A closer look at the results in Figure 2A however, shows that Amyg-EFP-NF and control-NF showed a similar pattern of increased Amyg-EFP down regulation until the third session. The specificity of Amyg-EFP-NF is evident in sessions 4-6, demonstrating the importance of repeated NF sessions to achieve specificity. Also consistent with previous studies, we found that some degree of Amyg-EFP down regulation was already observable at the end of the first session[13]. Nevertheless, the current results show that NF improvement did not reach plateau, what may suggest that more sessions could allow for the full realization of individual learning potential. This assumption is supported by the finding that most individuals attained their best performance during the last session (Supplementary Figure 7). If one considers that the best performance predicted both a reduction in alexithymia and follow-up amygdala BOLD down-regulation (Figures 3E & 4B), additional sessions could presumably result in stronger correlations and a larger influence on the outcome measures. This might be critical when moving forward to clinical populations. Thus, future studies should make use of the enhanced applicability of the Amyg-EFP approach by testing dose effect in a systematic manner, while considering a longer training period and different session intervals[35].

Importantly, the learned ability to regulate the Amyg-EFP was sustainable in the absence of online feedback (no-feedback trial; Figure 2D) and transferred to situations with additional cognitive demands, as demonstrated by the cognitive-interference trial

(Figure 2E). However, while the learned regulation of the targeted control signal (A/T) following control-NF was sustained during the no-feedback trial (Supplementary Figure 3C), it was not transferable to the cognitive-interference trial (Supplementary Figure 3D). Given the nature of the targeted signal in control-NF (elevation of slow wave Theta power and lowering Alpha power), it is possible that the induction of fast wave activity via a memory task hampered volitional regulation of the A/T ratio. One might therefore argue that this difference in regulation during cognitive-interference hampers the comparisons that could be made between the groups. It should be noted however that cognitive-interference was introduced upon completion of the NF training (without cognitive-interference) at session 5 (see Supplementary Table 1). Considering that two sessions with significant groups differences were observable before the introduction of the cognitive-interference task (Figure 2A) and that volitional regulation during cognitive-interference did not correlate with training outcomes, it is unlikely that this difference could explain the other group differences found in the current study. Furthermore, because Theta and Alpha contribute to the Amyg-EFP model (Supplementary Figure 1) we found it important to show that Amyg-EFP could be transferred to on-task demands. Such transferability might be critical for clinical translation in stress related disorders, as well as for preventive applications prior to exposure in prone populations (e.g. soldiers, fire fighters and policemen).

Amyg-EFP-NF training outcome

Testing the effect of Amyg-EFP-NF on several domains and comparing this effect to control-NF and NoNF provides valuable insights into the current debate regarding the specificity of targeted signal modulation during NF to the targeted outcome[36]. Relative to both controls, Amyg-EFP-NF resulted in a reduction in self-reports of

alexithymia and performance improvements on an eStroop task (Figure 3), suggesting a change that is specific to Amyg-EFP-NF. This was particularly evident in alexithymia for which the reduction also correlated with Amyg-EFP signal regulation among Amyg-EFP-NF trainees only (Figure 3E). Demonstrating a reduction in alexithymia following Amyg-EFP-NF is particularly interesting in light of the alleviated alexithymia scores observed by the NoNF, possibly due to the relatively stressful period during the first few weeks of military training[37]. These results point to a possible stress inoculation effect of learning to down regulate an amygdala related neural signal. Considering previous research associating alexithymia with PTSD and combat related PTSD in particular[38], the current results may further indicate the clinical potential of Amyg-EFP-NF. This assertion is supported by the finding that only Amyg-EFP-NF led to reduced alexithymia among participants with moderate-severe baseline alexithymia (TAS-20 $\geq$ 51). Nevertheless, as expected from an a-priori healthy sample, less than a third exhibited moderate alexithymia and less than 5% exhibited severe alexithymia (TAS-20 $\geq$ 61). Further research with clinical populations exhibiting high alexithymia at baseline is needed to fully understand the relation between amygdala targeted NF and alexithymia, and whether such a relation interacts with the overall clinical prognosis.

In contrast to alexithymia, reduction in state-anxiety was observed following both Amyg-EFP-NF and control-NF with no correlations to either Amyg-EFP, A/T signal, nor to Amygdala-BOLD regulation in follow-up fMRI-NF. Together, these findings point to the reduction in state-anxiety as resulting from general NF training effects[23] that are not specific to Amyg-EFP signal reductions. Interestingly, while in the current work we demonstrated an effect of Amyg-EFP-NF on *emotional conflict regulation* in the eStroop task, in our previous work[11] we found an influence on emotional

adaptation ([ci]-[ii]). This discrepancy might be explained by the different designs and populations used in each study. In our previous work the pre- and post-NF measurements were conducted on the same day following a single session. It is possible that the relatively stressful period between the two measurements in the current study mediated the effect on emotional adaptation. Also, considering that no correlation was found between NF success and improved eStroop performance, future replication of this result is needed to corroborate this effect as an Amyg-EFP-NF specific process modification. Future studies should further assess the long-term sustainability of the effects demonstrated in the current study and whether Amyg-EFP-NF could reduce the likelihood of developing stress related psychopathology following traumatic exposure.

Amyg-EFP-NF target-engagement

Our final goal in the current work was to test target engagement in the amygdala and its associated cortical connections. To that end we conducted amygdala targeted fMRI-NF approximately one month following the completion of Amyg-EFP-NF training. As expected, relative to NoNF, Amyg-EFP-NF resulted in a better ability to down-regulate amygdala BOLD using fMRI-NF (Figure 4A). We recently obtained a similar result[11] showing that one session of Amyg-EFP-NF resulted in improved amygdala BOLD down regulation compared to sham-NF. By conducting multiple sessions, the current study further showed that the learned skill of amygdala regulation can be sustained (longer than one month), and that one's best performance during training importantly correlated with one's success on a follow-up fMRI-NF (Figure 4B). This demonstration of transferability between EEG based repeated training to fMRI guided volitional regulation holds great promise in making region targeted NF clinically applicable. From a mechanistic perspective the PPI analysis

showed that relative to NoNF, Amyg-EFP-NF resulted in higher amygdala-vmPFC functional connectivity (Figure 4C), possibly suggesting an adaptive plasticity of a major path in the emotion regulation circuit[39]. This result is consistent with converging evidence demonstrating that amygdala-vmPFC functional connectivity increases following amygdala targeted volitional regulation training[6–9,40]. Together these findings demonstrate not only the plausibility of the amygdala as a target of volitional regulation but more so the adaptive effect that such regulation training could have on neural circuits central to emotion regulation.

Comparing post training fMRI-NF performance following Amyg-EFP-NF to NoNF only, and not control-NF, is a main limitation of the current study. As a reduction in state anxiety was observed both following Amyg-EFP-NF and control-NF it could be suggested that merely learning to control a brain signal may lead to reduced anxiety and better control over amygdala activity in fMRI-NF. As stated above however, we found no correlation between A/T modulation and reductions in state anxiety, nor between reductions in state anxiety and follow-up fMRI. Together with similar previous results obtained with simultaneous EEG/fMRI, these point to anxiety reduction as an unspecific effect of training with no evidence of a relation to volitional regulation of amygdala during fMRI-NF[41]. Future demonstrations of target engagement relative to an active control is nonetheless important. It could also be contended that a pre-training fMRI scan is essential to assert causality between Amyg-EFP-NF and amygdala volitional regulation during fMRI-NF. However, it should be noted that the population of the current study was highly homogeneous, consisting only of healthy males aged 18-24, all undergoing the same military training with the same daily schedule and nutrition.

Conclusions

The current results suggest that learning to down regulate the amygdala via Amyg-EFP-NF strengthened amygdala-vmPFC connectivity and was specific to the cognitive processing of emotions (alexithymia and eStroop) but not necessarily to state anxiety. These findings are in line with recent perspectives of the amygdala as not only a 'fear center', as initially assumed[42–45], but as also involved in the integration of introspective and sensory information allowing for higher order emotional processing[2,46,47]. Demonstrating that this limbic mechanism can be modified via a scalable approach such as the EFP may facilitate clinical translation. Implementation of additional EFP models targeting different brain matrices, along with content specific interfaces, could further enhance the mechanistic specificity of the intervention, especially in context to specific disturbances such as PTSD, OCD or phobia.

Methods

NIH trial registration number: NCT02020265**.**

https://clinicaltrials.gov/ct2/show/NCT02020265

Participants: 180 healthy male Israeli Defense Forces (IDF) combat soldiers (aged 18-24) were recruited to the study during basic training and prior to operational deployment. Physiological, including neurological, health was pre-determined during military screening. Exclusion criteria consisted of an existing diagnosis of a mental disorder or use of psychoactive drugs, regarding which the participants were asked to report on prior to agreeing to enroll in the study. NF training and pre- and post-behavioral measurements took place at the military training base. Post-training fMRI scans were conducted at the Sagol Brain Institute, Wohl Institute for Advanced Imaging, Tel-Aviv Sourasky Medical Center. All participants gave written informed

consent. The study was approved both by the Sourasky Medical Center and the IDF ethics review boards.

Procedure: Participants were randomly assigned to one of three conditions: (1) Amyg-EFP-NF (n=90) (2) control-NF (n=45) or (3) No-NF (NoNF; n=45). The Amyg-EFP-NF group were trained in down-regulation of the Amyg-EFP signal. The control-NF group were trained in down-regulation of Alpha (8-12Hz) relative to Theta (4-7Hz) and the NoNF group underwent no NF training. The assignment to control-NF and Amyg-EFP-NF was double blind. The training protocol (Figure 1) included 6 NF sessions within a period of 4 weeks (~ 1-2 sessions per week). Before group assignment all participants answered the 20 item Toronto Alexithymia Scale (TAS-20), the State and Trait Anxiety Inventory (STAI) and conducted an emotional Stroop task. Four participants (1 Amyg-EFP; 3 control-NF) requested not to participate in the NF training and were excluded. Seven additional participants (1 Amyg-EFP; 4 control-NF; 1 NoNF) could not participate due to a change in their military posting and were thus also excluded. The final analysis included 168 participants (88 Amyg-EFP; 38 control-NF; 43 NoNF). One month following training 60 participants (30 Amyg-EFP-NF; 30 NoNF) underwent post-training fMRI-NF. Due to technical difficulties four participants of the NoNF group could not complete the fMRI-NF scan. The final fMRI analysis included 56 participants.

Randomization and Blinding: Participants were randomly assigned to either the Amyg-EFP-NF, Control-NF or NoNF groups at a 2:1:1 ratio respectively. Randomization took place following completion of the pre-assessment phase using a custom-made software. The software further allowed for blinding between Amyg-EFP-NF and Control-NF by providing on-line feedback without revealing the source signal. Both participants and experimenters were blind to NF group allocation.

NF Training: NF was guided by the animated scenario interface previously developed by Cavazza et al.[48] and validated by Cohen et al.[26]. The paradigm across the 6 sessions followed a similar block design, composed of 5 training cycles, each including 3 consecutive conditions: (a) *watch* (60 sec.), (b) *regulate* (60 sec.) and (c) *washout* (30 sec.). During *watch* participants were instructed to passively view the interface animation and were explained that at this time the animation was not influenced by their brain activity. During *regulate* participants were instructed to find the mental strategy that would cause the animated figures to sit down and lower their voices. Instructions were intentionally unspecific, allowing individuals to adopt the mental strategy that they subjectively found most efficient[49]. During washout blocks participants were instructed to tap their thumb to their fingers according to a 3-digit number that appeared on the screen. Sessions 1-3 included an additional *warmup* conducted before NeuroFeedback Training consisting of 2 cycles. NF success at each session was measured as mean difference in the targeted signal power (Amyg-EFP or A/T) between all *regulate* and *watch* conditions conducted at that session. To facilitate learning sustainability, following NF training in sessions 4-6 participants also underwent a no-feedback trial[26,30]. The no-feedback trial was introduced upon completion of the five NF cycles via the animated scenario, from session 4 onward. This trial consisted of one 60 sec. long *watch* block in which participants were instructed to passively view a fixation cross followed by 2 consecutives *regulate* blocks, on which participants were instructed to down regulate their targeted brain signal (either Amyg-EFP or A/T) while still viewing the same fixation cross. We instructed individuals to use the same mental strategies that were successful in modulating the target signal in previous sessions. To further test whether participants could down-regulate the targeted brain activity while engaged in an additional

cognitive task, upon completion of NF training in sessions 5-6 we conducted a "cognitive-interference" trial during which participants were instructed to down-regulate the relevant brain signal while conducting a simultaneous memory task. The interference task consisted of a single cycle, including one watch condition (60 sec) and one regulate condition (120 sec). While regulating the targeted signal participants were instructed to memorize as many details as possible from the animated scenario (positioning of different characters, clothing, objects etc.). After the completion of the NF trial (watch and regulation conditions) participants were asked to answer a 13-item multiple choice questionnaire.

The emotional Stroop task: Participants viewed fearful or happy facial expressions with superimposed congruent or incongruent words (‘‘happy’’\‘‘fear’’) and were asked to identify the emotional expression while ignoring the words that appeared. The emotional Stroop task provides a measure of *'general conflict regulation'* measured by the difference in response times between congruent and incongruent stimuli and of *'Emotional conflict adaptation'* measured by the difference in response times between two consecutive incongruent stimuli [ii] and incongruent stimulus following congruent stimulus [ci] (*adaptation* = [ii]-[ci])[22].

Self-report questionnaires: Alexithymia was measured using the Hebrew version of the 20 item Toronto Alexithymia Scale (TAS), previously tested for reliability and factorial validity[50]. TAS-20 measures difficulties in expressing and identifying emotions[21], a tendency previously demonstrated to correlate with stress vulnerability[28,29]. The overall alexithymia score comprises three sub-scores: (a) difficulty identifying feelings (IDF), (b) difficulty describing feelings (DDF) and (c) externally oriented thinking (EOT).

State anxiety was measured using the previously validated Hebrew version of the State Trait Anxiety Inventory (STAI)[51]. STAI[20] consists of two 20 item inventories measuring state and trait anxiety.

The Amyg-EFP model: The Amyg-EFP model was previously developed by our lab to enable the prediction of localized activity in the amygdala using EEG only[15,16]. This was done by applying machine learning algorithms on EEG data acquired simultaneously with fMRI. The procedure resulted in a *Time-Delay X Frequency X weight* coefficient matrix. EEG data recorded from electrode Pz at a given time-point are multiplied by the coefficient matrix to produce the predicted amygdala fMRI-BOLD activity. Keynan et al.,[11] validated the reliability of the Amyg-EFP in predicting amygdala BOLD activity by conducting simultaneous EEG-fMRI recordings using a new sample not originally used to develop the model.

EEG data acquisition and online processing: EEG data were acquired using the V-Amp™ EEG amplifier (Brain Products™, Munich Germany) and the BrainCap™ electrode cap with sintered Ag/AgCI ring electrodes providing 16 EEG channels, 1 ECG channel, and 1 EOG channel (Falk MinowServices™, Herrsching-Breitburnn, Germany). The electrodes were positioned according to the standard 10/20 system. The reference electrode was between Fz and Cz. Raw EEG was sampled at 250 Hz and recorded using the Brain Vision Recorder™ software (Brain Products).

On line calculation of Amyg-EFP and A/T power: Online EEG processing was conducted via the RecView software (Brain Products). RecView makes it possible to remove cardio-ballistic artifacts from the EEG data in real time using a built-in automated implementation of the average artifact subtraction method[52]. Amyg-EFP data were collected from electrode Pz and A/T ratio was extracted from electrodes O1, Oz and O2. RecView™ was custom modified to enable export of the corrected

EEG data in real time through a TCP/IP socket. Preprocessing algorithm and signal (Amyg-EFP or A/T) calculation models were compiled from Matlab R2009b™ to Microsoft .NET™ in order to be executed within the Brain Vision RecView™ EEG Recorder system. Data were then transferred to a MATLAB.NET compiled DLL that calculated the value of the targeted signal power every 3 seconds.

Animated Scenario Feedback Generation: The neurofeedback interface included a virtual hospital waiting room whose visual setting constitutes a metaphor for arousal within a realistic context. Characters waiting in the room exist in a resting state (waiting seated) or agitated state (protesting at the counter) and the overall level of agitation depends on the ratio between these two states. This mechanism ensures smooth visual transitions through an individual characters' change of state and as a result the room as a whole may become either more agitated or more relaxed by the user (Figure 1B; Supplementary Video[26]). The ratio between characters sitting down and protesting at the counter is considered to be a two-state Boltzmann distribution[48], whose evolution is driven by a "virtual temperature" whose value is derived from the momentary value of the targeted signal power (Amyg-EFP or A/T). The scenario uses the probability (*p* value) of a momentary signal value during *regulate* to be sampled under the previous *watch* distribution. This *p* value is used to determine the probability of virtual characters to be moving in the virtual room, with the character distribution updated accordingly. A matching soundtrack recorded inside a real hospital complements the system output. Three alternative soundtracks with different agitation levels were produced and switched according to the signal value. During the *watch* condition 75% of the characters congregate at the front desk while expressing their frustration through body and verbal language. The system is implemented using the Unreal Development Kit (UDK™) game engine, which controls relevant

animations (walking, sitting, standing, protesting), as well as their transitions for individual characters.

Statistical Analysis: Statistical analysis was conducted using IBM SPSS Statistics 20^{TM}, and MATLAB R2017b. NF Success in each session was measured as the mean difference in the targeted signal power (A/T or Amyg-EFP) during *regulate* relative to *watch*[11,26]. The mean result of each group was analyzed using a repeated measures ANOVA with session (1-6) and group (Amyg-EFP-NF vs control-NF) as factors. Behavioral measures were each assessed with a separate repeated measures ANOVA with group (Amyg-EFP-NF, control-NF and NoNF) and time (pre- vs post-training) as factors. Unless specified otherwise, all reported *p* values are two-tailed. One-tailed tests were used only when a one-sided a-priori hypothesis existed. Data distribution was assumed to be normal, but this was not formally tested. Box plots showing data distribution (individual data points) for all variables are available in the supplementary information. Sphericity assumptions were tested using Box's test of equality of covariance matrices and Levene's test for equality of variances. Where sphericity assumption was violated, corrected statistics and *p* values were used.

Missing Data: To control for bias[53], missing data were imputed using multiple data imputation (predictive mean matching) with 5 iterations and was treated as missing at random. To account for the added uncertainty a repeated measures ANOVA was conducted following van Ginkel & Kroonenberg[54] correcting variances and degrees of freedom**.** Between and within groups simple effects were tested using built in SPSS procedure for t-test on multiply imputed data, accounting for added uncertainty.

Power analysis: Sample size calculation was based on behavioral results (emotional Stroop) from Keynan et al.,[11]. The effect size of the group by time (pre- vs post-NF) interaction in Keynan et al., was relatively large *(η^2=0.19*). Power analysis suggested

that to allow detection (*alpha=0.05*) of a more conservative effect (η^2*=0.09*), with at least 80% power in a 3 by 2 design, a total sample of 150 participants is required. Considering our expectation of an 85% retention rate we recruited 180 participants.

Post-training fMRI-NF: To test for target engagement in the amygdala, one month following training participants came to the Sagol Brain Institute and underwent amygdala targeted fMRI-NF. To further allow for the testing of learning transferability between contexts, and to refute the possibility that observed group difference are merely a result of familiarity with the animated scenario, the fMRI-NF paradigm was of a similar block design as in the training period but utilized different and unfamiliar visual feedback[11]. This visual interface consisted of a 2D unimodal flash-based graphic interface with an animated figure standing on a skateboard, skating down a rural road. The participant`s goal was to lower the speed of the moving skateboard which is determined by amygdala beta (mean parameter estimates) weighted activity. During *watch* the skateboard moved at a constant pre-set speed of 90km/h. During *regulate* the skateboard's speed was set in accordance to the momentary amygdala beta weighted activity ranging between 50-130 km/h. To avoid new learning, the fMRI-NF paradigm consisted of 2 cycles[11].

Real-time calculation of amygdala activity and visual feedback generation: The visual feedback is generated in a mathematically identical manner to the animated scenario, only using amygdala beta weighted activity instead of Amyg-EFP power. Momentary beta weights of the pre-defined amygdala region of interest (ROI) were extracted on-line using Turbo Brain voyager 3.0^{TM} (Brain Innovation, Maastricht, Netherlands). The beta weights were then transferred to MATLAB^{TM} which in turn set the speed of the moving skate board. The amygdala ROI was defined according to

the Talairach coordinates of the amygdala functional cluster used for the initial Amyg-EFP model development[11] (x=20, y=-5, z=-17; 3mm Gaussian sphere).

fMRI data acquisition: Structural and functional scans were performed in a 3.0 Tesla Siemens MRI system (MAGNETOM Prisma, Germany) using a twenty-channel head coil. To allow high-resolution structural images a T1-weighted 3D Sagittal MPRAGE pulse sequence (TR/TE = 1860/2.74 ms, flip angle = 8º, pixel size = 1X1mm, FOV = 256×256 mm) was used. Functional whole-brain scans were performed in an interleaved top-to-bottom order, using a T2*-weighted gradient echo planar imaging pulse sequence (TR/TE=3000/35 ms, flip angle=90º, pixel size=1.56 mm, FOV=200×200 mm, slice thickness=3 mm, 44 slices per volume). A sample of 13 participants were scanned with a GE 3T Signa scanner using the same parameters only with 39 slices per volume. No differences were found between scanners on the measured ROIs.

fMRI data preprocessing: Preprocessing and statistical analysis were performed using BrainVoyager QX version 2.8 (Brain Innovation, Maastricht, Netherlands). Slice scan time correction was performed using cubic-spline interpolation. Head motions were corrected by rigid body transformations, using three translations and three rotation parameters and the first image served as a reference volume. Trilinear interpolation was applied to detect head motions and sinc interpolation was used to correct them. The temporal smoothing process included linear trend removal and usage of a high-pass filter of 1/128 Hz. Functional maps were manually co-registered to corresponding structural maps and together they were incorporated into three-dimensional datasets through trilinear interpolation. The complete dataset was transformed into Talairach space and spatially smoothed with an isotropic 8 mm full width at half maximum (FWHM) Gaussian kernel.

Amygdala region of interest (ROI) analysis: Using a random-effects general linear model (GLM), we extracted beta values for all the voxels in the amygdala ROI targeted during fMRI-NF. The model included 3 regressors for each condition (watch, regulate and washout). Regressors were convolved with a canonical hemodynamic response function. Additional nuisance regressors included the head-movement realignment parameters. A two-way repeated measures ANOVA was then conducted with the amygdala beta values as a dependent variable and group (Amyg-EFP-NF vs NoNF) and condition (watch vs regulate) as factors.

Amygdala whole brain psycho-physiological interaction (PPI): Group (Amyg-EFP-NF>NoNF) differences in functional connectivity during *watch* and *regulate* were examined using an in-house generalized psychophysiological interaction (PPI) analysis tool, previously implemented in our lab for Brainvoyager[55]. A whole-brain psycho-physiological interaction (PPI) random effects GLM analysis was conducted, using the psychological variables (the original regressors of the fMRI-NF paradigm) and the physiological variable (the activity time course of the seed amygdala ROI) as regressors.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code Availability

Code used to analyze the data that support the findings of this study are available from the corresponding author upon reasonable request.

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Competing Interest

Talma Hendler, Nathan Intrator and Yehudit Meir-Hasson are inventors of related patent applications entitled "Method and system for use in monitoring neural activity in a subject's brain" (US20140148657 A1, WO2012104853 A3, EP2670299 A2). This does not alter the authors' adherence to all nature human behaviour policies.

Author Contribution

TH Conceived the study. TH & JNK designed the study. JNK, AC, NGr & AD collected the data. GJ developed the online analysis techniques and the custom-made randomization, blinding and data management software. TH conceptualized the Amyg-EFP model. YMH & NI constructed the model. GR & MC developed the animated scenario interface. AD, EF & KG managed the contact with the IDF. GL

provided statistical advice. JNK analyzed the data. NGl assisted in data analysis, figure preparation, and proofing. JNK & TH wrote the paper.

Supplementary Information

Electrical Fingerprint of the Amygdala Guides Neurofeedback Training for Stress Resilience

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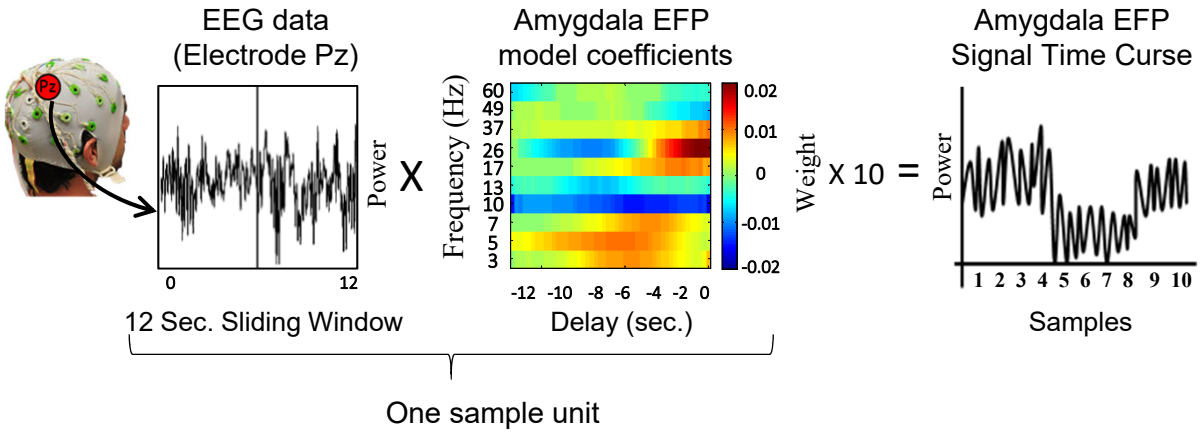
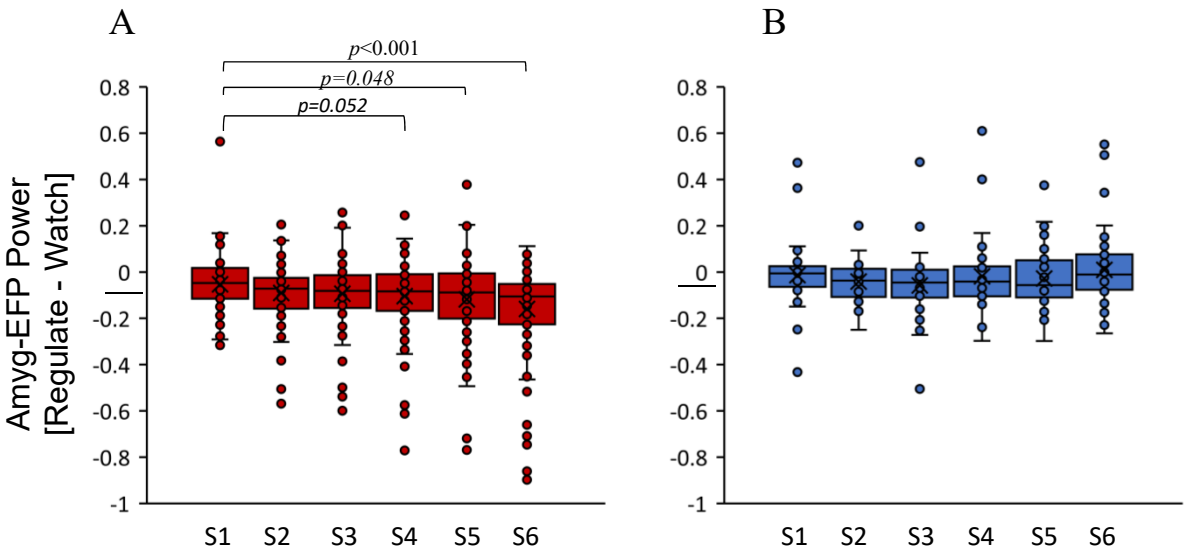
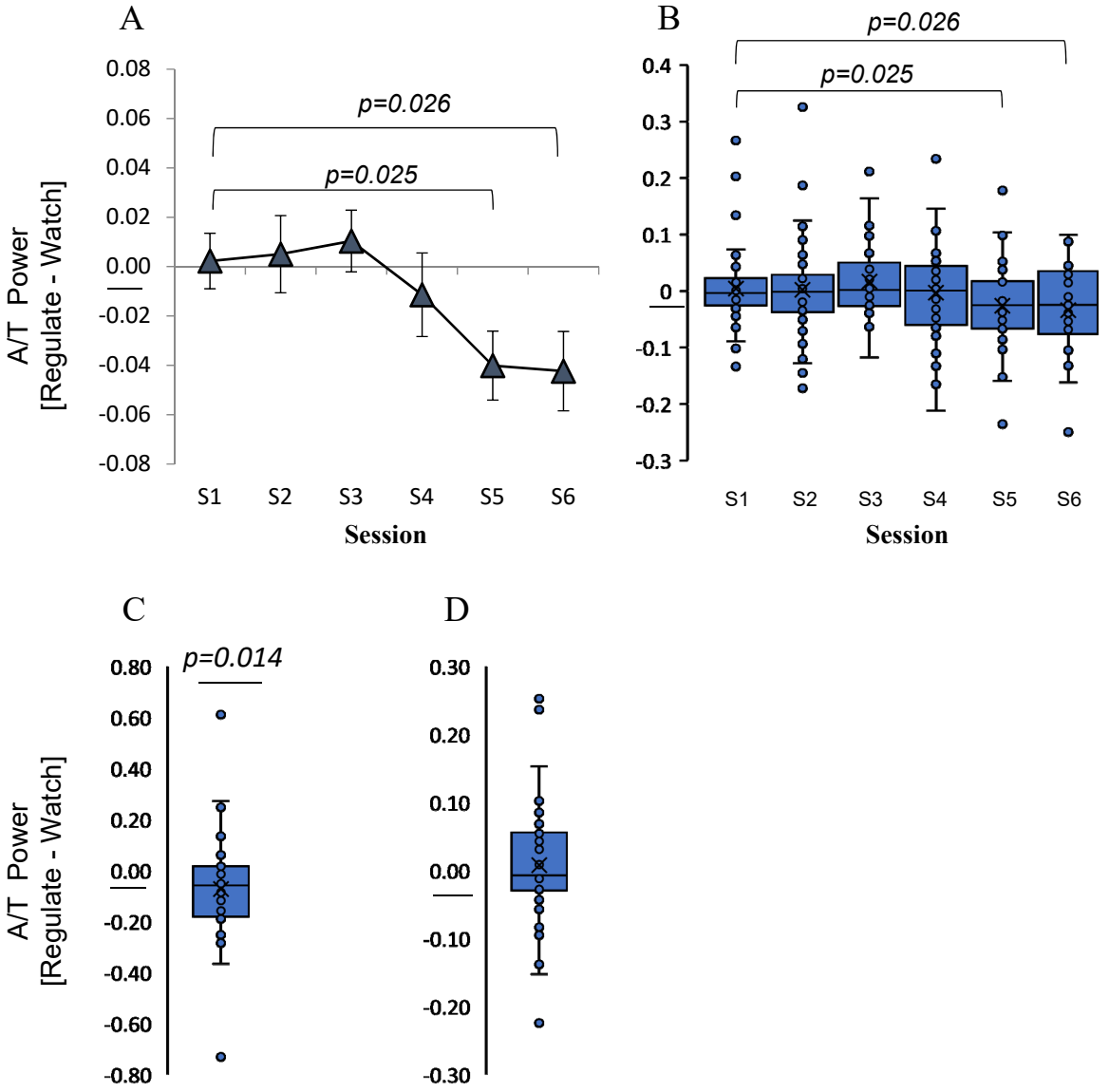
Supplementary Figures



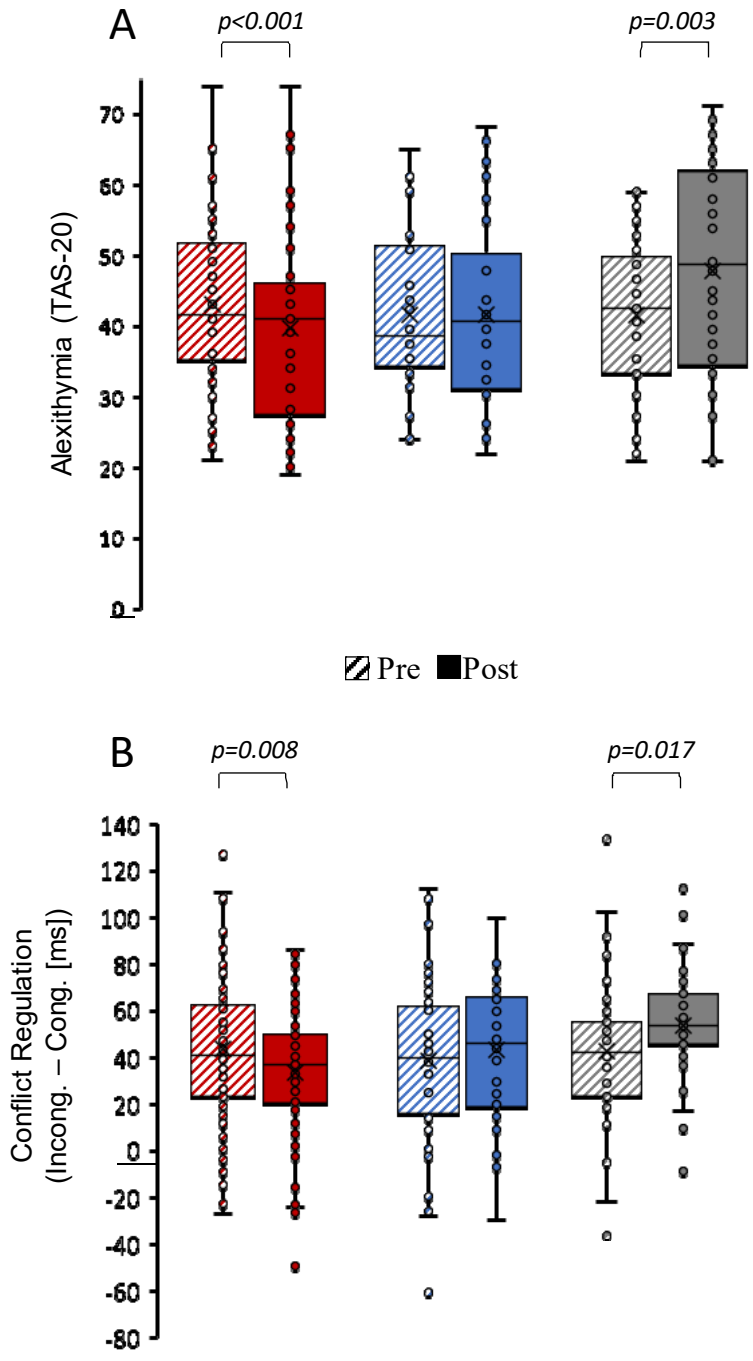
Supplementary Figure 1: The Amyg-EFP signal extraction. EEG data used for the model is a Time/Frequency matrix recorded from electrode Pz including all frequency bands in a sliding time window of 12 seconds. To obtain the amygdala BOLD predictor, the EEG data are multiplied by the EFP model coefficients matrix. The EFP model consists of a frequency by delay by weight matrix in which every frequency band is differently weighted in different time delays. One sampling unite, calculated every three seconds, contains weighted data from the last 12 seconds. While conventional EEG measures used for NF commonly calculate the amplitude of specific band-widths or the ratio between them, the Amyg-EFP takes into account the spectrum of 1-60Hz in a time window of 12 seconds



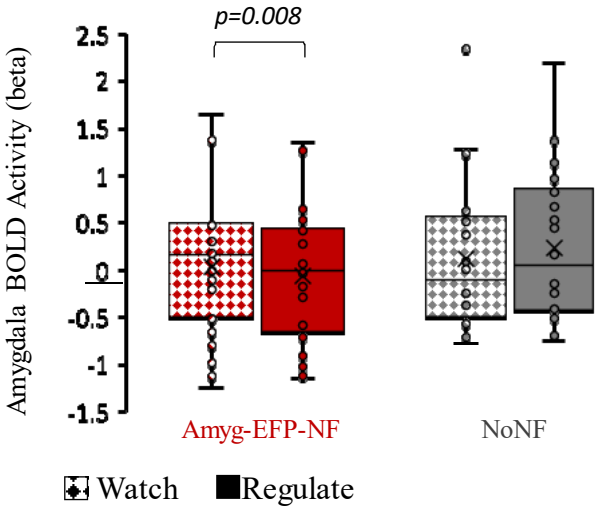
Supplementary Figure 2: Box plots showing the distribution of Amyg-EFP signal modulation (y-axis; *Regulate vs Watch*) across the six sessions (x-axis; S1-S6). (A) Results obtained for the Amyg-EFP-NF group**. (B)** Results obtained for the Control-NF group. The mean and median are marked respectively by an X and a line inside each box. Whisker lines represent 1.5X interquartile range. Detailed statistics of within group comparisons between sessions are reported in Supplementary Table 3.



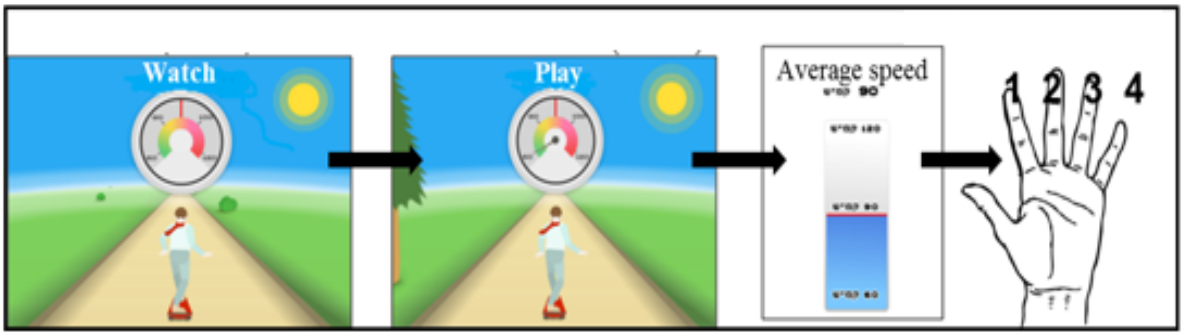
Supplementary Figure 3: NF learning of the control signal (A/T ratio) in the Control-NF group (n=38). (A) Average change (regulate vs watch) in A/T ratio per session (S1-6). Significant difference from session 1 is evident at sessions 5 and 6. See Supplementary Table 4 for detailed statistics. Error bars stand for standard error. **(B)** Box plots showing the distribution of A/T ratio signal modulation (y-axis; *Regulate vs Watch signal power change*) across the six sessions (x-axis; S1-S6). **(C-D) Box plots of control-NF learning sustainability. (C)** No-Feedback condition**.** A/T ratio down regulation was sustained in the absence of on-line feedback as indicate by a significant reduction in A/T signal (y-axis; *mean (regulate – watch)=0.07±0.21, t(37)=2.19, p(one tailed)=0.014, d=0.36, 95% CI [0.02, 0.68], watch=1.41±0.41, regulate=1.34±0.43*) **(D)** While conducting a simultaneous memory task (cognitive interference condition), A/T signal reduction (y-axis; *Regulate vs Watch*) was not significant (*mean (regulate – watch)=-0.01±0.09, t(37)=0.51, p(one tailed)=0.305, d=0.08, 95% CI [-0.24, 0.40], watch=1.05±0.19, regulate=1.06±0.22)***.** *p<.05 (regulate vs watch). The mean and median are marked respectively by an X and a line inside each box. Whisker lines represent 1.5X interquartile range.



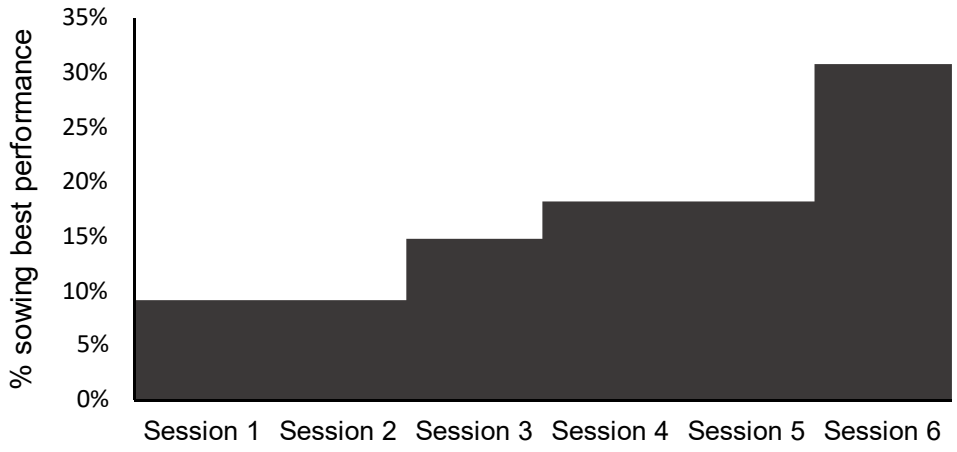
Supplementary Figure 4: Box plots showing the distribution of **(A)** alexithymia ratings and **(B)** eStroop performance before (dashed bars) and after (solid filled bars) NF training for each group [Amyg-EFP NF (red; n=88), Control-NF (blue; n=38), NoNF (grey; n=43)]. The mean and median are marked respectively by an X and a line inside each box. Whisker lines represent 1.5X interquartile range. Detailed statistics of within group comparisons between time points are reported in Figure 3.



Supplementary Figure 5: Box plots showing the distribution of amygdala BOLD activity (y-axis; beta weights) during the Watch (pattern filled bars) and Regulate (solid filled bars) conditions. **(A)** Amyg-EFP group (red, n=30). **(B)** NoNF (gray; n=26). The mean and median are marked respectively by an X and a line inside each box. Whisker lines represent 1.5X interquartile range. Detailed statistics of between and within group comparisons are reported in Figure 4.



Supplementary Figure 6: Amygdala-fMRI-NF paradigm. The fMRI-NF paradigm followed similar block design used during EEG-NF training, with an interface composed of a 3D animation of a character moving forward via skateboard on a road. Momentary BOLD beta weight (*Regulate vs Watch*) from the pre-defined right amygdala ROI was used to set the speed of the moving skateboard on the screen.



Supplementary Figure 7: Histogram showing the percentage of participants (y-axis) in the Amyg-EFP-NF group (n=88) that reached their best performance (minimum [*Regulate vs Watch*]) in each session (x-axis; S1-S6).

Supplementary Tables

	NeuroFeedback Training (5 Cycels, 12:30 min.)	No-Feedback (2 Cyces, 3 min.)	Cognitive-Interference (1 Cycle, 2 min.)
Session 1[i]	√		
Session 2[i]	√		
Session 3[i]	√		
Session 4	√	√	
Session 5	√	√	√
Session 6	√	√	√

Supplementary Table 1: Order and type of NF tasks conducted at each session. NF training included 5 cycles (Figure 2B) and was performed in all sessions. During the No-Feedback condition, participants were instructed to down regulate the recorded brain signal (Amyg-EFP or A/T ratio) in the absence of online feedback. In the cognitive-interference condition participants were instructed to down regulate the recorded brain signal while simultaneously memorizing details of the animated 3D scenario (see method). [i] Sessions 1-3 included an additional *warmup* conducted before NeuroFeedback Training consisting of 2 cycles.

A	**Amyg-EFP-NF**				**Control-NF**			
			Mean CI (95%)				**Mean CI (95%)**	
	Mean	**sd**	**Lower**	**Upper**	**Mean**	**sd**	**Lower**	**Upper**
Session 1	-0.05	0.13	-0.08	-0.03	-0.01	0.14	-0.06	0.03
Session 2	-0.09	0.13	-0.12	-0.07	-0.04	0.08	-0.08	-0.01
Session 3	-0.09	0.15	-0.13	-0.06	-0.06	0.15	-0.11	0.01
Session 4	-0.10	0.17	-0.14	-0.07	-0.02	0.16	-0.07	0.03
Session 5	-0.12	0.18	-0.15	-0.08	-0.03	0.13	-0.08	0.03
Session 6	-0.16	0.20	-0.20	-0.12	0.01	0.18	-0.05	0.07

B	**Between Group Comparison (Amyg-EFP-NF - Control-NF)**						
						Effect Size CI (95%)	
	Mean	**se**	**t(124)**	***p***	***d***	**Lower**	**Upper**
Session 1	-0.04	0.03	1.37	=0.173	0.27	-0.12	0.65
Session 2	-0.05	0.03	1.66	=0.107	0.32	-0.06	0.70
Session 3	-0.04	0.03	1.04	=0.298	0.20	-0.18	0.58
Session 4	-0.09	0.04	2.46	=0.014	0.48	0.09	0.86
Session 5	-0.09	0.04	2.36	=0.020	0.46	0.07	0.84
Session 6	-0.17	0.04	3.87	<0.001	0.75	0.36	1.14

Supplementary Table 2: **Amyg-EFP signal modulations (regulate-watch) of each group at each session.** **(A)** Means, Standard Deviations (sd), and CIs of Amyg-EFP signal down regulation (Regulate – Watch) of each group at each session. **(B)** Means, standard errors (se), t statistics, p values effect size estimations (Cohen's d) and 95% CIs of a between groups comparison conducted for each session. One can see that session 4-6 show significant group differences with enlarging effect sizes.

	Amyg-EFP-NF						
						Effect Size CI (95%)	
	Mean	sd	t(87)	*p*	*d*	Lower	Upper
Session 1 vs 2	-0.04	0.19	1.89	=0.058	0.20	-0.01	0.41
Session 1 vs 3	-0.04	0.23	1.63	=0.105	0.17	-0.04	0.38
Session 1 vs 4	-0.05	0.25	1.95	=0.052	0.21	0.00	0.42
Session 1 vs 5	-0.07	0.30	2.05	=0.047	0.22	0.01	0.43
Session 1 vs 6	-0.11	0.25	4.06	<0.001	0.43	0.21	0.65
	Control-NF						
						Effect Size CI (95%)	
	Mean	sd	t(37)	*p*	*d*	Lower	Upper
Session 1 vs 2	-0.03	0.24	0.70	=0.494	0.11	-0.21	0.43
Session 1 vs 3	-0.04	0.19	1.42	=0.156	0.23	-0.09	0.55
Session 1 vs 4	-0.01	0.22	0.14	=0.892	0.02	-0.30	0.34
Session 1 vs 5	-0.01	0.21	0.43	=0.671	0.07	-0.25	0.39
Session 1 vs 6	0.02	0.22	0.63	=0.527	0.10	-0.22	0.42

Supplementary Table 3: Improvement in Amyg-EFP signal modulations of each group relative to the first session. Mean, Sd, t statistic, p value, effect size estimate (Cohen's d) and 95% CI, of within group comparisons of Amyg-EFP signal modulation (regulate – watch) between each session (2-6) and the first session.

	Control-NF (A/T ratio)								
			Delta vs Session 1					Effect Size CI (95%)	
	Mean	Sd	Mean	Sd	t(37)	*p*	*d*	Lower	Upper
Session 1	0.002	0.07							
Session 2	0.005	0.10	0.003	0.09	0.19	0.853	0.03	-0.29	0.35
Session 3	0.010	0.08	0.008	0.09	0.55	0.586	0.09	-0.23	0.41
Session 4	-0.011	0.10	-0.014	0.13	0.67	0.505	0.11	-0.21	0.43
Session 5	-0.040	0.09	-0.042	0.12	2.25	0.025	0.36	0.03	0.69
Session 6	-0.043	0.10	-0.045	0.13	2.22	0.026	0.36	0.03	0.69

Supplementary Table 4: Control-NF A/T ratio signal modulation at each session and improvement relative to the first session. The left sided Means and Sds are of the average performance (regulate – watch) at each session. The following columns report, Mean, Sd, t statistic, p value, effect size estimate (Cohen's d) and 95% CI, of within group comparisons of A/T signal modulation (regulate – watch) between each session (2-6) and the first session.

	Amyg-EFP-NF				Control-NF			
			Mean CI (95%)				Mean CI (95%)	
	Mean	sd	Lower	Upper	Mean	sd	Lower	Upper
Session 1	-0.05	0.09	-0.07	-0.03	-0.04	0.10	-0.07	-0.01
Session 2	-0.08	0.09	-0.09	-0.06	-0.05	0.07	-0.07	-0.02
Session 3	-0.09	0.08	-0.10	-0.07	-0.04	0.09	-0.07	-0.02
Session 4	-0.09	0.12	-0.12	-0.06	-0.01	0.15	-0.05	0.04
Session 5	-0.11	0.14	-0.13	-0.08	-0.03	0.12	-0.08	0.01
Session 6	-0.12	0.14	-0.14	-0.09	0.01	0.12	-0.04	0.05

Supplementary Table 5: Statistics of Amyg-EFP signal modulations following outlier removal. The table reports means, sds and CIs of Amyg-EFP signal reductions (regulate – watch) of each group in each session.

	Pre-Training				Post-Training			
			Mean CI (95%)				Mean CI (95%)	
	Mean	sd	Lower	Upper	Mean	sd	Lower	Upper
	Amyg-EFP-NF							
e-Conflict Regulation (Incong. - Cong.)	43.23	29.92	36.73	49.73	33.26	27.11	27.76	38.75
e-Conflict Adaptation (ii - ci)	-2.37	45.74	-11.34	6.61	5.95	31.48	-1.21	13.11
Alexithymia (TAS-20)	42.95	11.22	40.62	45.29	39.58	11.63	36.87	42.29
State Anxiety (STAI)	31.46	9.55	29.48	33.45	29.20	7.76	27.51	30.90
	Control-NF							
e-Conflict Regulation (Incong. - Cong.)	37.31	35.01	27.41	47.20	41.47	27.61	33.10	49.83
e-Conflict Adaptation (ii - ci)	-0.97	44.35	-14.62	12.68	-6.77	38.92	-17.66	4.12
Alexithymia (TAS-20)	41.99	10.85	38.44	45.54	42.00	13.25	37.88	46.12
State Anxiety (STAI)	33.47	10.29	30.46	36.49	30.23	7.64	27.64	32.81
	NoNF							
e-Conflict Regulation (Incong. - Cong.)	41.01	29.38	37.71	50.31	51.28	22.89	43.42	59.15
e-Conflict Adaptation (ii - ci)	-4.06	33.35	-16.90	8.77	-1.02	34.39	-11.26	9.22
Alexithymia (TAS-20)	42.00	11.02	38.67	45.34	48.11	13.57	44.24	51.98
State Anxiety (STAI)	32.81	8.92	29.97	35.65	32.20	9.00	29.77	34.62

Supplementary Table 6: Behavioral outcome measures. The table reports means, sds and CIs of each group at each time point.

Supplementary Methods

Control condition justification: An optimal control condition should account for three of the global processes that are induced by NF without targeting the mechanism of interest. These main processes are (a) *reward*: a feedback cue indicating success or unsuccess; (b) *control*: control on a mental state and brain signal; and (c) *learning*: the consolidation of associations between an applied mental strategy and its outcome via operant learning. In fMRI-NF for example a control condition that deals with such general processes should consist of feedback from a different region[1–3]. A yoked sham control on the other hand, would account for the reward aspect but would not generate contingent learning. Indeed, in our previous study[4] we used a yoked sham control, in which participants received feedback derived from the Amyg-EFP signal of a different participant. Following training when given the opportunity to regulate via veritable feedback in a follow-up fMRI-NF session, participants who trained via sham-NF showed an impaired ability to volitionally regulate the amygdala. Thus, the yoked sham was actually an active control of incorrect learning that could bias the results. Similar results were obtained recently in a study testing the placebo control using NF in a systematic manner[5]. Furthermore, when conducting repeated sessions, as in the current study, participants may notice the lack of contingency between the feedback and their mental effort. Additional options could include random feedback that also lacks contingency, training regulation in the inverse direction (amygdala upregulation) that may have undesired influences and mental rehearsal without NF which disables blinding.

In the current study we chose to control for these general processes as much as possible and therefore applied a commonly used Alpha/Theta probe[6], which is the EEG equivalent of a "different region" approach. Moreover, since theta and alpha contribute to the Amyg-EFP model (see new Figure 1) we found it important to demonstrate the specificity of the Amyg-EFP to limbic processing; not only using a correlative approach as done previously[4] but by also

causally showing amygdala related behavioral changes following Amyg-EFP-NF in contrast to A/T-EEG-NF alone.

According to previous studies of A/T-EEG-NF (see Gruzlier et al.[6,7] for review) our underlying assumption was that A/T-EEG-NF mainly targets general arousal brain networks. An assumption also supported by our concurrent fMRI\EEG study, demonstrating the fMRI correlates of successful A/T modulation[8].

Selection of number of sessions: Successful amygdala volitional regulation was previously shown in fMRI-NF studies following relatively few sessions (up to 3)[2,9,10]. Our previous study similarly demonstrated improved amygdala BOLD regulation following a single sessions of Amyg-EFP-NF[4]. While conventional EEG studies commonly apply at-least 10 sessions, learning A/T regulation was observed with healthy participants after less than 6 sessions[11,12,8]. Considering the intensive military training our participants underwent, in addition to the reported feasibility of the effect following relatively few NF sessions, we aimed to administer 6 sessions. As the results show, learning to control the targeted signal was observed in Amyg-EFP-NF following 4 sessions (Figure 3) and following session 5 in the control-NF group (Figure S2). Nevertheless, as stated in the discussion (lines 340-350) the current findings suggest that learning was not exhausted after six session and that the optimal number of sessions should be systematically investigated in future studies.

Correlating NF success and outcome measures: To correlate individual NF success and training outcome, we aimed for an index that captures individual learning potential while taking in to account that different individuals show differently shaped learning curves[13]. The average performance across six sessions is influenced by the first session in which participants have yet to be trained. The delta between the first and last session assumes that each individual will reach the best performance at the last session. A coefficient of the slop also assumes a similarly

shaped learning curve between individuals. We there for used the best performance out of six sessions as index of learning potential making less a-priori assumptions.

Supplementary References

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