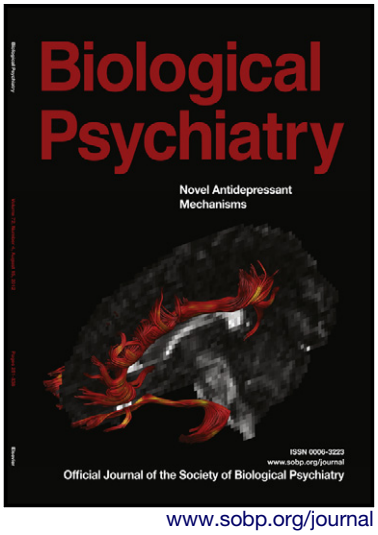

www.sobp.org/journal

Limbic activity modulation guided by fMRI-inspired EEG improves implicit emotion regulation

Jackob N. Keynan, Yehudit Meir-Hasson, Gadi Gilam, Avihay Cohen, Gilan Jackont, Sivan Kinreich, Limor Ikar, Ayelet Or-Borichev, Amit Etkin, Anett Gyurak, Ilana Klovatch, Nathan Intrator, Talma Hendler

PII: S0006-3223(16)00003-2
DOI: http://dx.doi.org/10.1016/j.biopsych.2015.12.024
Reference: BPS12777

To appear in: *Biological Psychiatry*

Cite this article as: Jackob N. Keynan, Yehudit Meir-Hasson, Gadi Gilam, Avihay Cohen, Gilan Jackont, Sivan Kinreich, Limor Ikar, Ayelet Or-Borichev, Amit Etkin, Anett Gyurak, Ilana Klovatch, Nathan Intrator, Talma Hendler, Limbic activity modulation guided by fMRI-inspired EEG improves implicit emotion regulation, *Biological Psychiatry,* http://dx.doi.org/10.1016/j.biopsych.2015.12.024

Limbic Activity Modulation Guided by fMRI-Inspired EEG Improves Implicit Emotion Regulation

**Jackob N. Keynan, [a,b¶] Yehudit Meir-Hasson, [c¶] Gadi Gilam,[a,b] Avihay Cohen,[a] Gilan Jackont,[a,b] Sivan Kinreich,[a,b] Limor Ikar,[a] Ayelet Or-Borichev,[a,b] Amit Etkin,[d,e] Anett Gyurak,[d,e] Ilana Klovatch,[a] Nathan Intrator[c,f], Talma Hendler[a,b,f,g]*

[a]Functional Brain Center, Wohl Institute for Advanced Imaging, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel.

[b]The School of Psychological Sciences, Tel-Aviv University, Tel-Aviv, Israel.

[c]Blavatnik School of Computer Science, Tel-Aviv University, Tel-Aviv, Israel.

[d]Dept. of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA.

[e]Sierra-Pacific Mental Illness Research, Education, and Clinical Center (MIRECC), Veterans Affairs Palo Alto Health Care System, Palo Alto, CA.

[f]Sagol School of Neuroscience, Tel-Aviv University, Tel-Aviv, Israel.

[g]Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

[¶]These authors contributed equally to this work.

*Corresponding Author. E-mail: hendlert@gmail.com Tel: 972-3 6973953

Short Title: fMRI Inspired EEG-NF for Improved Emotion Regulation

Keywords: Amygdala; Neurofeedback; fMRI; EEG; Emotion-Regulation; Stress

Abstract

The amygdala has a pivotal role in processing traumatic stress, hence gaining control over its activity could facilitate adaptive mechanism and recovery. To date, amygdala volitional regulation could be obtained only via real-time fMRI; a highly inaccessible procedure. The current paper presents high impact neurobehavioral implications of a novel imaging approach that enables bed-side monitoring of amygdala activity using fMRI-inspired EEG; hereby termed, ***"amygdala-EEG Finger Print"*** **(amyg-EFP**). Simultaneous EEG/fMRI indicated that the amyg-EFP reliably predicts amygdala-BOLD activity. Implementing the amyg-EFP in neurofeedback demonstrated that learned down-regulation of the amyg-EFP facilitated volitional down-regulation of amygdala-BOLD activity via real-time fMRI, and manifested as reduced amygdala reactivity to visual stimuli. Behavioral evidence further emphasized the therapeutic potential of this approach by showing improved implicit emotion regulation following amyg-EFP neurofeedback. Additional EFP models denoting different brain regions could provide a library of localized activity, for low-cost and highly accessible brain based diagnosis and treatment.

Introduction

Neurocognitive models of stress related psychopathologies point to down-regulation of amygdala activity as a key mechanism in emotion regulation (1); an essential feature in the effective recovery from traumatic-stress (2). Therefore, learning to regulate one's own amygdala activity may diminish detrimental- and facilitate adaptive-stress coping mechanisms (3). Obtaining volitional regulation of the amygdala was thus far possible only via online closed-loop training (i.e. neurofeedback; NF) guided by real-time functional magnetic resonance imaging (rt-fMRI) (4,5,6). Such learned regulation was indeed demonstrated to result in reduced stress (7) and depression (8) related symptoms. Despite these seemingly promising findings, the clinical utility of fMRI-based interventions is considerably limited due to immobility, high-cost and extensive physical requirements of the scanning procedure (9). Electroencephalography (EEG) on the other hand, is a relatively inexpensive and mobile brain imaging technique that can be easily implemented at any location. Albeit, the clinical benefit of EEG-NF, particularly for affective disturbances such as depression and post-traumatic stress disorder (PTSD), remains dubious (10,11); possibly due to poor spatial resolution, which hampers the targeting of deep limbic areas such as the amygdala (12). The current study conducted a first of its kind investigation of the neurobehavioral implications of a novel imaging approach that allows for the bed-side monitoring of amygdala activity using fMRI-inspired EEG; hereby termed, ***"amygdala-EEG Finger Print" (amyg-EFP)***.

The amyg-EFP was recently developed in our lab by applying advanced machine learning algorithms on EEG data acquired simultaneously with fMRI. This resulted in a new individually fitted EEG model of weighted coefficients that can be used to probe localized blood oxygen level dependent (BOLD) activity of a pre-defined region (13). However, this approach still required prior fMRI scanning for each subject, precluding its widespread use and easy application**.** The current work reached beyond the initial computational study and tested a new one-class model of the amyg-EFP that is valid across different individuals and thus could be used without prior fMRI (Figures 1 & S1). The current study

further aimed to portray the translational potential of this novel approach in treating human traumatic-stress while considering its underlying process. To this end, we performed three NF experiments comparing volitional regulation of the amyg-EFP to either EFP-Sham or no-treatment controls. Simultaneous EEG/fMRI recordings conducted in the first experiment, validated the amyg-EFP as a reliable predictor of amygdala fMRI-BOLD activity. In a second experiment we further examined the relation between the amyg-EFP and the amygdala-BOLD using a prospective design; conducting fMRI before and after training to down-regulate the amyg-EFP outside the scanner. This experiment demonstrated a causal link between learned down-regulation via amyg-EFP NF and a subsequent improved ability to regulate amygdala-BOLD activity via fMRI-NF. Moreover, this learned skill manifested as reduced reactivity of the amygdala in response to provoking visual stimuli, possibly indicating an adaptive acquired plasticity. Lastly, the third experiment pointed to the clinical potential of this novel approach by showing improved implicit emotion regulation following amyg-EFP NF.

Methods and Materials

All experiments and data analysis were conducted at the Functional Brain Center, Wohl Institute for Advanced Imaging, Tel-Aviv Sourasky Medical Center and were approved by the Sourasky ethics review board. Participants gave written informed consent, were healthy, and had normal hearing and normal or corrected-to-normal vision. Each group in each experiment contained different individuals (Total N=82).

Experiment 1

Twenty-four participants were randomly assigned either to the EFP-Test (*n=15; aged 22-29*) or EFP-Sham (*n=9; aged 23-29)* group in a single blind manner. Participants were simultaneously scanned by EEG/fMRI during five consecutive blocks, each lasting seven minutes. The EFP-Test group received continuous auditory feedback driven by their amyg-EFP amplitude changes, calculated on-line every three seconds (14). The EFP-Sham group received auditory feedback unrelated to their amyg-EFP signal. In the first Rest block participants were given no specific instructions and received no auditory

feedback. In the subsequent four NF blocks participants were instructed to lower the volume of an auditory stimulus by exercising mental strategies. Instructions were intentionally unspecific, allowing individuals to adopt the mental strategy that they subjectively found most efficient (15). See supplemental material for online calculation of amyg-EFP amplitude and feedback generation.

Experiment 2

Eighteen participants were randomly assigned either to the EFP-Test *(n=9; aged 23-29)* or EFP-Sham group (*n=9; aged: 22-29)* in a single blind manner. The prospective design of experiment 2 included three separate sessions (Pre-Training/Training/Post-Training) (Figure S2). Participants baseline ability to volitionally regulate amygdala-BOLD activity, was measured Pre-Training by conducting a two-block session (60 seconds each) of amygdala-targeted fMRI-NF (amyg-fMRI NF) (Figure S3). This two-block duration was previously found insufficient to facilitate fMRI-NF learning (16). At the Training session (5-7 days later) participants were randomly assigned to either EFP-Test or EFP-Sham. The amyg-EFP NF took place outside the MRI scanner and consisted of six blocks (Rest block and five NF blocks; 5 minutes each). Post-Training (24-48 hours after Training), participants underwent a full-length five-block session of amyg-fMRI NF. The amyg-fMRI NF followed a commonly used design (16) and was applied as a test to verify that by learning to regulate the amygdala, as measured by EEG alone (i.e. amyg-EFP), participants actually learned to regulate amygdala-BOLD activity, as measured by rt-fMRI. Therefore, during amyg-fMRI NF (Pre- and Post-Training), feedback for both groups was driven by amygdala activity. The only difference between the groups was during the Training session, in which the EFP-Test group received online-feedback driven by the amyg-EFP and the EFP-Sham group received feedback unrelated to their own brain activity. Before and after all NF sessions, participants performed a backward masking task while being scanned by fMRI. Four subjects (2 EFP-Test; 2 EFP-Sham) were unable to meet experiment schedule and were excluded from analysis. The final analysis included 14 subjects.

Backward Masking

Participants were instructed to identify backward-masked photographs of either a person or an object presented for either 33 or 83ms. Admon et al. (17) found that high amygdala reactivity to short presentation (33ms) among a-priori healthy individuals, predicted more stress symptoms following trauma. One EFP-Sham subject didn't participate and was excluded from analysis.

Experiment 3

Forty participants were randomly assigned either to the EFP-Test (*n=16, aged: 22-33*), EFP-Sham *(n=12; aged 23-32)* or no-treatment (*n=12; aged 23-34)* group. Amygdala related behavioral modifications were tested based on a well-established association between performance in an emotional conflict Stroop task and down-regulation of amygdala activity (18). All groups performed two sessions of this task with a one-hour break between sessions. During the break the EFP-Test underwent amyg-EFP NF, the EFP-Sham underwent sham-NF and the no-treatment group had no particular task. NF in experiment 3 was performed using a visual scenario with the fMRI-NF design of experiment 2 (Figure S3). Participants in the EFP-Test and EFP-Sham had no knowledge of their assignment. During the emotional conflict 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. *'Emotional adaptation'* is measured by the difference in response times between the low-conflict (two consecutive incongruent stimuli [ii]) and high-conflict (incongruent stimulus following congruent stimulus [ci]) conditions. A low score [ii-ci] indicates better implicit emotion regulation (18).

Results

Experiment 1: The amyg-EFP neural validity.

The simultaneous EEG/fMRI recordings indicated that the amyg-EFP reliably predicted amygdala fMRI-BOLD activity. A whole-brain random effects general liner model (RFX-GLM) analysis using the amyg-EFP signal for all participants (i.e. EFP-Test and EFP-Sham) as a regressor revealed that the amyg-EFP signal correlated with the BOLD activity of the right amygdala (Peak Talairach coordinates:

x=23, y=-2; z=-17, FDR-corrected p<0.05, n=23) (Figure 1D). Remarkably, the amyg-EFP signal in this study correlated with amygdala-BOLD activity in the region of interest (ROI) used to develop the model (Peak-coordinates: x=20, y=-5; z=-17). Importantly, the analysis revealed no differences between the EFP-Test and EFP-Sham groups in the BOLD correlates of the amyg-EFP. As anticipated, the amyg-EFP also correlated with activity in additional brain regions, including premotor, somatosensory and associative-visual cortexes (Table S1). An additional whole brain analysis using conventional EEG markers of general arousal (Alpha & Theta) as regressors, verified that the amyg-EFP is a unique marker providing valuable information of limbic sub-cortical activity beyond what could be obtained using conventional EEG (Figure S4).

Analysis of amyg-EFP signal modulations during NF relative to Rest indicated that, as hypothesized, the EFP-Test group responded differently than the EFP-Sham group (Figure S5). A two-way repeated measures ANOVA revealed a group (EFP-Test/EFP-Sham) by condition (Rest/NF) interaction *(F(1,22)=4.34, p<0.05)*. Planned-comparisons (19) further showed that while the EFP-Test group down-regulated the amyg-EFP signal during NF relative to Rest (*F(1,22)=5.8, p<0.03; Rest[mean±sd]=0.04±0.05 ;NF=-0.34±0.71*), the EFP-Sham group did not (*F(1,22)=0.59, p>0.45; Rest=0.01±0.09; NF=0.16±0.37*) (See figure S6 for changes in all EEG frequencies). Also consistent with our assumptions, changes in amyg-EFP correlated with changes in amygdala-BOLD activity during NF relative to Rest (NF-Rest) (*r=0.47, p<0.03*); with participants who significantly reduced amyg-EFP amplitude during NF relative to Rest, also exhibiting a simultaneous reduction in amygdala-BOLD activity (*T(9)=-2.24, p<0.05; mean(NF-Rest)=-0.06±0.08)*. Importantly, this reduction was not observed in other, non-targeted brain areas (See supplemental material).

Experiment 2: Neural effects of amyg-EFP NF

Similar to the first experiment, only the EFP-Test group learned to down-regulate the amyg-EFP during NF at the Training session (Figure S7). To test whether this learned amyg-EFP regulation led to improved down-regulation of amygdala-BOLD activity, we performed an ROI analysis on the

amygdala functional cluster used for the initial amyg-EFP model development. We compared the amygdala-BOLD activity observed during fMRI-NF before amyg-EFP NF (Pre-Training), to that observed during fMRI-NF Post-Training. As expected, following amyg-EFP NF only the EFP-Test group exhibited improved down-regulation of amygdala fMRI-BOLD activity, relative to Pre-Training. A triple Group (EFP-Test/EFP-Sham) by Time-Point (Pre-Training/Post-Training) by Condition (Rest/NF) interaction *(F(1,12)=18.66, p<0.001)* Indicated that as expected, prior to amyg-EFP NF neither the EFP-Test nor the EFP-Sham groups were able to reduce amygdala activity during amyg-fMRI NF (All p>0.2). In contrast, Figure 2A shows a Group*Condition interaction (*F(1,12)=62.29, p<0.001*), indicating that after amyg-EFP NF only the EFP-Test group successfully reduced amygdala-BOLD activity during NF relative to Rest (*F(1,12)=15.67, p<0.002; Rest=-0.027±0.27, NF=-0.18±0.36*). Surprisingly, Post-Training amygdala activity of the EFP-Sham group increased during fMRI-NF relative to Rest (*p(Bonferroni)<0.001 Rest=-0.09±0.32, NF=0.19±0.33*). To further explore other brain-regions that might have also been modulated along the amygdala, whole-brain RFX-GLM analysis was conducted on the Group*Condition interaction effect at the Post-Training session. This analysis revealed that several temporal and pre-frontal regions reacted similarly to the amygdala (Figure 2B; Table S2). Follow-up analysis testing the training effect (NF<Rest) within each group further revealed areas such as the dACC and pre-SMA that might have been involved in amygdala down-regulation (Tables S3 & S4).

The neural effect of the NF training was further assessed by conducting an ROI analysis on the amygdala weighted beta during viewing of backward-masked stimuli, before and after all NF sessions. Figure 2C shows that as hypothesized, following amyg-EFP NF only the EFP-Test group showed reduced reactivity of the amygdala relative to before training (EFP-Test: *F(1,11)=4.90, p<0.05; Pre-Training 1=0.03±0.12; Post-Training=-0.11±0.07;* EFP-Sham: *F(1,11)=0.17, p>0.68; Pre-Training=0.03±0.10; Post-Training=0.01±0.15)*. Pre-Training, no differences were found between the

groups (*F(1,11)=0.01, p>0.92).* However, the ANOVA did not reveal a significant Group*Time-Point interaction *(F(1,11)=1.44, p>0.25*), preventing a definite dissociation between the groups.

Experiment 3: Behavioral effect of amyg-EFP NF.

Similarly to the first two experiments (Figures S5 & S7), a Group*Condition interaction (F(1,26)=4.78, p<0.04) revealed that only the EFP-Test group learned to volitionally down-regulate the amyg-EFP (EFP-Test: *F(1,26)=11.28, p<0.003; Rest=-0.33±1.29, NF=-0.62±1.23;* EFP-Sham: *F(1,26)=0.01, p>0.98; Rest=-0.62±0.68, NF=-0.62±0.60).* As hypothesized, following amyg-EFP NF the EFP-Test group showed improved implicit emotion regulation while the EFP-Sham and the no-treatment groups did not. A Group*Time-Point interaction *(F(2,37)=4.45, p<0.02)* (figure 2D) indicated that following NF, only the EFP-Test group showed a lower emotional adaptation score relative to before NF (EFP-Test*: F(1,37)=6.18, p<0.02; Before=3.72±30.66; After=-20.43±23.*26; EFP-Sham: F(1,37)=1.31, p>0.25; *Before=-4.54±25.06; After=8.34±36.*57; no-treatment: (*F(1,33)=1.49, p>0.23; Before=-9.79±34.78; After=3.88±35.17*). Pre-Training, no differences were found between the groups (All p>0.28).

Discussion

The current work demonstrated a novel, mobile and low-cost method for local neuro-modulation of deeply located limbic activity. Simultaneous EEG\fMRI indicated that the amyg-EFP model reliably predicts fMRI-BOLD activity in the amygdala ROI, for which it was originally developed (Figure 1D). Prospective fMRI scanning convincingly showed that learned amyg-EFP down-regulation is causally related to improved down-regulation of amygdala-BOLD activity via fMRI-NF and to reduced amygdala reactivity to visual stimuli (Figure 2). Behavioral evidence demonstrated the positive effect of amyg-EFP NF on implicit emotion regulation, pointing to the therapeutic potential of this new approach (Figure 2D).

Pushing the spatial limits of EEG

Source estimation of EEG is considered an ill-posed problem (20). This problem becomes even more detrimental when aiming to locate sources in deep sub-cortical regions. The EFP model introduced a novel data-driven approach to enable the prediction of fMRI-BOLD activity using only EEG (13). However, when forsaking a-priori hypotheses, this data-driven method also suffers from a higher risk of false discovery. By conducting simultaneous EEG/fMRI on a new sample, the current study validated that the amyg-EFP can indeed predict amygdala-BOLD activity (Figure 1D). The fact that this prediction is included in the ROI used to develop the model is both reassuring and remarkable. Our results do not imply however, that amygdala-BOLD activity is the sole origin of the amyg-EFP signal and we do not claim to directly measure amygdala neuronal activity from scalp electrodes. Rather, the additional BOLD correlates of the amyg-EFP revealed by the whole-brain analysis (Figure 1D; Table S1) suggest that the amyg-EFP reflects the activity of a network of regions, including areas of the limbic and salience systems in which the amygdala is considered a major hub (21). This claim is further supported by the results of the fMRI-NF showing that as expected, additional brain regions were involved in successful amygdala down-regulation (Figure 2B; Table S2). These regions, including the posterior-insula and subgenual-ACC, were previously associated with salience processing (22) and affect regulation (23), respectively. This result once again coincides with the notion that amygdala down-regulation requires the recruitment of a widely distributed neural system that possibly involves multiple levels of regulation (24). Further research could optimize the specificity of the amyg-EFP by recording EEG simultaneously with amygdala targeted fMRI-NF. Integrating intracranial electrical measurements could further enhance the model specificity and overcome the current models base towards low frequencies (Figure 1B) resulting from the fact that slow frequencies are more prominent when recording scalp-EEG. Such research could also enable the development of additional EFP models denoting other limbic regions and provide a library of localized activation reflecting multiple processes for mobile and highly accessible brain-based diagnosis and treatment.

Therapeutic implications of the EFP approach

The results of the current study showing that participants are able to learn down-regulation of the amygdala in a single session are consistent with previous fMRI-NF studies (25,26). However, the neural effect of amygdala targeted NF was thus far mainly estimated on the basis of observed neural changes during an actual fMRI-NF session or alternatively, using subsequent resting-state fMRI (25-27,7-8). In the current study we have gone further by training participants with amyg-EFP NF guided by auditory feedback outside the MRI scanner and subsequently tested their performance in a different context using fMRI-NF with visual feedback (Figure S2). Our findings indicate that training in one modality and context can be transferred to a completely different context and even a different modality, as long as the same localized brain activity is targeted (Figures S7 & 2A). In addition, beyond previous studies we tested whether this learned amygdala regulation could facilitate adaptive response in an entirely different fMRI task. Our results indicate an acquired plasticity of the amygdala by showing that learned regulation via NF was later transferred to a greater reduction in amygdala reactivity in response to backward masked stimuli (Figure 2C). Lastly, while the behavioral effects of amygdala targeted NF were previously examined using explicit measurements such as self-report questionnaires, our work exhibits implicit behavioral modifications in a task known to involve amygdala activity (18). Relative to the control groups, following amyg-EFP NF participants exhibited a greater improvement of implicit regulation, as indicated by the change in performance on the emotional conflict task (Figure 2D). Failure to implicitly regulate emotions was previously associated with stress related psychopathologies (28). Taken together, these results demonstrate the potential of amyg-EFP NF to affect both neural processes and behavioral manifestations of emotion regulation in healthy individuals. Such acquired regulation may facilitate adaptive mechanism of coping with stress, hence reducing vulnerability to its consequences. However, these assumptions must be further investigated using multiple NF sessions, testing its effect before and after real-life trauma.

One might argue that the relatively weak statistical effect found for the reduced amygdala reactivity in the backward masking (Figure 2C) limits the conclusions that may be drawn. Readers should bear in

mind however, that this effect was tested on a healthy population of normal baseline performance. Testing these effects on a clinical population with abnormal amygdala activity at baseline should presumably result in stronger effects. Moreover, testing the effects of explicit regulation training such as NF on implicit regulation skills is also assumed to produce weaker effects. As such, the finding of both implicit neural and behavioral transfer effects after a relatively short training period is quite impressive.

The introduction of an EEG based method for amygdala activity modulation holds great clinical potential. Showing that the learned skill of amygdala down-regulation could facilitate adaptive neural plasticity and manifest as improved emotion regulation, further demonstrates the huge clinical implications of this novel method. The future development of additional EFP models denoting different brain regions could provide a library of localized activity for mobile and low cost brain based diagnosis and treatment. Implementing the amyg-EFP model in NF, the current study clearly demonstrates the prospects of such approaches. EFP-NF holds the potential to reach *'anyone anywhere',* in the form of home stationed bed-side treatment for recent trauma patients or stress resilience training for trauma prone individuals.

Acknowledgments

Funding:

This project was supported by the following grants:

US Department of Defense - grant agreement no. W81XWH-11-2-0008.

The European Union's Seventh Framework Programmed for research, technological development and demonstration under grant agreement no. 602186.

Mafaat, IDF, FP7-EU, I-Core cognitive-studies. Grant agreement no. 693210.

Author contributions:

T.H and J.N.K conceived and designed the study. J.N.K, A.C and A.O.B collected the data. G.J, S.K, L.I and J.N.K programmed the real-time imaging data analysis methods. L.I design and programmed the real-time fMRI paradigm. Y.M.H and N.I constructed the common model amyg-EFP. J.N.K, G.G, I.K, A.C and A.G analyzed the data. A.E designed the emotional conflict task. J.N.K and T.H wrote the paper.

Financial disclosure:

The authors report no biomedical financial interests or potential conflicts of interest.

References

1. Johnston T, van Reekum CM, Urry HL, Kalin NH, Davidson RJ (2007): Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. *J Neurosci* 27(33): 8877-8884.

2. Ochsner KN, Bunge SA, Gross JJ, Gabrieli JD (2002): Rethinking feelings: An fMRI study of the cognitive regulation of emotion. *J. Cognitive Neurosci* 14(8): 1215-1229.

3. Admon R, Milad MR, Hendler T (2013): A causal model of post-traumatic stress disorder: disentangling predisposed from acquired neural abnormalities. *Trends Cogn Sci*. 17(7): 337-347.

4. Weiskopf N (2012): Real-time fMRI and its application to neurofeedback. *Neuroimage*, *62*(2): 682-692.

5. Gruzelier JH (2013): EEG-neurofeedback for optimising performance. I: A review of cognitive and affective outcome in healthy participants. *Neurosci. Biobehav.* R.44: 124-141.

6. Zotev V, Krueger F, Phillips R, Alvarez RP, Simmons WK, Bellgowan P, *et al.* (2011): Self-regulation of amygdala activation using real-time fMRI neurofeedback. *PloS one*, 6(9): e24522.

7. Scheinost D, Stoica T, Saksa J, Papademetris X, Constable RT, Pittenger C, *et al.* (2013): Orbitofrontal cortex neurofeedback produces lasting changes in contamination anxiety and resting-state connectivity. *Translational psychiatry*, 3(4): e250.

8. Young KD, Zotev V, Phillips R, Misaki M, Yuan H, Drevets WC, *et al.* (2014): Real-time FMRI neurofeedback training of amygdala activity in patients with major depressive disorder. PloS One, 9: e88785.

9. Birbaumer N, Ruiz S, Sitaram R (2013): Learned regulation of brain metabolism. *Trends Cogn. Sci.* 17(6): 295-302.

10. Peniston EG, Kulkosky PJ (1991): Alpha-theta brainwave neurofeedback for Vietnam veterans with combat related post-traumatic stress disorder. *Medical Psychotherapy*. 4(1): 47-60.

11. Quaedflieg CWEM, Smulders FTY, Meyer T, Peeters F, Merckelbach H, Smeets T (2015): The validity of individual frontal alpha asymmetry EEG neurofeedback. *Social cognitive and affective neuroscience*, nsv090.

12. Kinreich S, Podlipsky I, Intrator N, Hendler T (2012): Categorized EEG neurofeedback performance unveils simultaneous fMRI deep brain activation. In: Langs *et al*. editors. *Machine Learning and Interpretation in Neuroimaging*. Berlin Heidelberg: Springer, pp 108-115.

13. Meir-Hasson Y, Kinreich S, Podlipsky I, Hendler T, Intrator N (2014): An EEG Finger-Print of fMRI deep regional activation. *NeuroImage*.102: 128-141.

14. Kinreich S, Podlipsky I, Jamshy S, Intrator N, Hendler T (2014): Neural dynamics necessary and sufficient for transition into pre-sleep induced by EEG NeuroFeedback. *NeuroImage*, 97: 19-28.

15. Shibata K, Watanabe T, Sasaki Y, Kawato M (2011): Perceptual learning incepted by decoded fMRI neurofeedback without stimulus presentation. *Science*. 334(6061): 1413-1415.

16. Sulzer J, Haller S, Scharnowski F, Weiskopf N, Birbaumer N, Blefari M (2013): Real-time fMRI neurofeedback: progress and challenges. *Neuroimage*, 76: 386-399.

17. Admon R, Lubin G, Stern O, Rosenberg K, Sela L, Ben-Ami H, Hendler T (2009): Human vulnerability to stress depends on amygdala's predisposition and hippocampal plasticity. *Proceedings of the National Academy of Sciences*, 106(33): 14120-14125.

18. Etkin A, Egner T, Peraza DM, Kandel ER, Hirsch J (2006): Resolving emotional conflict: a role for the rostral anterior cingulate cortex in modulating activity in the amygdala. *Neuron*, 51(6): 871-882.

19. Rosenthal R, Rosnow RL (1985): *Contrast analysis: Focused comparisons in the analysis of variance*. CUP Archive.

20. Grech R, Cassar T, Muscat J, Camilleri KP, Fabri SG, Zervakis M, *et al.* (2008): Review on solving the inverse problem in EEG source analysis. *Journal of neuroengineering and rehabilitation*, 5(1): 25.

21. Pessoa L, Adolphs R (2010): Emotion processing and the amygdala: from a'low road'to'many roads' of evaluating biological significance. *Nature Reviews Neuroscience*, *11*(11): 773-783.

22. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, *et al.* (2007): Dissociable intrinsic connectivity networks for salience processing and executive control. *The Journal of neuroscience*, 27(9): 2349-2356.

23. Goldin PR, McRae K, Ramel W, Gross JJ (2008): The neural bases of emotion regulation: reappraisal and suppression of negative emotion. *Biol. Psychiatry*. 63(6): 577-586.

24. Ochsner KN, Gross JJ (2008): Cognitive emotion regulation insights from social cognitive and affective neuroscience. *Curr. Dir. Psychol.* Sci. 17(2): 153-158.

25. Paret C, Kluetsch R, Ruf M, Demirakca T, Hoesterey S, Ende G, Schmahl C (2014): Down-regulation of amygdala activation with real-time fMRI neurofeedback in a healthy female sample. *Frontiers in behavioral neuroscience*: *8.*

26. Brühl AB, Scherpiet S, Sulzer J, Stämpfli P, Seifritz E, Herwig U (2014): Real-time neurofeedback using functional MRI could improve down-regulation of amygdala activity during emotional stimulation: a proof-of-concept study. *Brain topography*, *27*(1), 138-148

27. Zotev V, Phillips R, Young KD, Drevets WC, Bodurka J (2013): Prefrontal control of the amygdala during real-time fMRI neurofeedback training of emotion regulation. PLoS One 8: e79184.

28. Etkin A, Schatzberg AF (2011): Common abnormalities and disorder-specific compensation during implicit regulation of emotional processing in generalized anxiety and major depressive disorders. *Am. J.* Psychiat. 168(9): 968-978.

Figure legends

Figure 1: The amyg-EFP prediction model. The recorded EEG data (A) is multiplied by the common model coefficient matrix (B) to produce the predictor of the right amygdala BOLD activity (C). **(A)** The EEG data used for the model is a Time/Frequency matrix recorded from electrode Pz including all frequency bands in a time window of 12 seconds. **(B)** The common model coefficients matrix. {CH]x[FQ]x[DELAY]x[TIME]. fMRI-BOLD activity at time *T* can be predicted by the EEG using the frequency intensity *FQ* of electrode *CH* in delay *D* from *T*. In our case, *CH* includes a selected single electrode (Pz). **(C)** The predicted right amygdala BOLD activity time curse. Further details regarding the construction process of the amyg-EFP is available in the supplementary material. **(D) Experiment-1 whole brain amyg-EFP correlates**. Correlation maps obtained from whole-brain RFX-GLM analysis using as a predictor the amyg-EFP during NF are shown in axial sagittal and coronal views (left to right). The area which was used originally (on a different group) to develop the amyg-EFP model is marked by a green square. See table S1 for whole brain peak correlates in more regions.

Figure 2: amyg-EFP NF Outcome Measures. **(A) Experiment 2, Post-Training rt-fMRI results.** The group*condition interaction *(F(1,12)=64.7,p<0.001)* shows that as expected, following amyg-EFP NF training, only the EFP-Test group (red bars) was able to down-regulate amygdala activity during NF (solid fill) relative to Rest (dashed fill). The EFP-Sham group in contrast, showed increased amygdala activity during NF relative to Rest. Prior to amyg-EFP-NF training, neither the EFP-test nor the EFP-sham groups were successful in down regulating amygdala BOLD activity during NF relative to Rest (All p>0.2). **(B)** Coronal, axial and sagittal views of BOLD activity interaction map (Group[Test/Sham]*Condition[NF/Rest]) obtained by whole brain RFX-GLM analysis (n=14) conducted on the post amyg-EFP NF trainig rt-fMRI. The green square (**1**) indicates the amygdala ROI for which the amyg-EFP model was originally developed. Note that the same ROI was targeted in the fMRI-NF. The whole brain activation maps show that frontal and temporal areas were involved in the

down regulation of amygdala activity (**2**- Subgenual ACC, **3**- Posterior insula). **(C) Experiment 2, Backward masking:** Changes in mean amygdala peak activation for EFP-Test and EFP-Sham groups obtained in response to stimuli presented for 33ms during the backward masking task prior to NF training (dashed fill) and following NF training (solid fill). Average beta (peak activation) were extracted from the ROI that was targeted in the fMRI NF training. Only the EFP Test group showed reduced amygdala reactivity after the NF training. The EFP-Sham group showed no significant changes between time points. **(D) Experiment 3 reaction times (ms) in the emotional conflict task with relation to NF training.** A low score indicates higher `*emotional adaptation*` (Y-axis) and thus better emotion regulation. A significant interaction *(F(2,37)=4.45, p<0.02)* was found between group (EFP-Test/EFP-Sham/no-treatment) and time (Before_NF/After_NF) showing that after NF (solid fill) only the EFP-Test group (red) exhibited improved emotion regulation relative to before NF training (dashed fill).*p<.05; **p<.01;***p<.001