Modulatory effects of dynamic fMRI-based neurofeedback on emotion regulation networks during adolescence

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Abstract

This study used real-time fMRI-based neurofeedback (NF) to modulate functional connectivity patterns in the emotional regulation networks in a sample of adolescent girls. Adolescence is a developmental period which brings along changes at multiple levels, such as hormonal changes, improvements in socio-emotional processing, as well as ongoing brain maturation and functioning. It has been suggested that these changes increase the risk for the individual. For example, early, difficulties with emotion regulation have been linked to a range of mental health problems, such as anxiety. Here we successfully trained participants to modulate the functional coupling of the prefrontal cortex and the amygdala towards a more negative connectivity pattern, which resembles the connectivity pattern found in the mature brain. These brain-based changes were related to changes at the behavioural level. We also found that the modulation largely depends on the specific neurofeedback implementation, which provides important insights for future NF training approaches.

Introduction

This study used real-time functional magnetic neuroimaging-based neurofeedback (fMRI-NF) to modulate functional connectivity patterns in the emotional regulation networks in a sample of adolescent girls. Adolescence is marked by a multitude of changes on both the neural level (e.g. hormonal changes and brain maturation) and the behavioural level (e.g. improvements in cognitive abilities and socio-emotional behaviour) (Blakemore, 2008; Burnett, Sebastian, Cohen Kadosh, & Blakemore, 2011; Cohen Kadosh, Linden, & Lau, 2013; Linscott & van Os, 2013), and it has been suggested that these complex, adolescence-associated transformational processes may increase the risk for developing mental health problems (Haller, Cohen Kadosh, & Lau, 2013; Haller, Cohen Kadosh, Scerif, & Lau, 2015; Keshavan, Giedd, Lau, Lewis, & Paus, 2014; Paus, Keshavan, & Giedd, 2008), with the most common being anxiety. Adolescents with anxiety experience difficulties in emotion regulation, leading to problems with friendships, poor school performance, and long-term mental health difficulties (Trentacosta & Fine, 2010). On the latter issue, paediatric anxiety predicts lifelong persistent mental health problems, which are estimated to cost the UK taxpayer £8.6 billion annually (Beddington, Cooper, & Field, 2008; *The economic case for improving efficiency and quality in mental health - No health without mental health: A cross-Government mental health outcomes strategy for people of all ages*, 2011).

To regulate emotions effectively it is necessary to recognise emotional content and cues in social interactions, and then select and implement appropriate responses (Ahmed, Bittencourt-Hewitt, & Sebastian, 2015). Research has shown that these processes develop continuously throughout the first two decades of life (Burnett et al., 2011; Cohen Kadosh, Johnson, Dick, Cohen Kadosh, & Blakemore, 2013; Dumontheil, Hassan, Gilbert, & Blakemore, 2010). This is significant, as the prolonged developmental change suggests that there could be a window of opportunity for intervention, which may be particularly effective due to age-specific high social saliency and plasticity. Accordingly, there is a strong rational to gain a better understanding of the underlying mechanisms for regulating emotions during this unique developmental period to devise efficient and targeted strategies for early support and intervention. One such intervention could be NF, a neuroimaging technique which makes use of the latest progress in real-time image analysis to train participants in the self-regulation of neural networks.

In the human brain, the regulation of emotions relies on a network of brain regions, comprising the prefrontal cortex (PFC) and the amygdala, as well as additional regions in the orbitofrontal, frontal and cingulate cortex (Kohn et al., 2014; Ochsner & Gross, 2005). Whereby a particular focus has been placed on the PFC-amygdala relationship, a relationship that manifests in both structural and functional connections (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Davidson, Putnam, & Larson, 2000; H. Kim, Somerville, Johnstone, Alexander, & Whalen, 2003; M. J. Kim & Whalen, 2009; Kohn et al., 2014; Quirk & Beer, 2006). With regards to the regulatory aspect of the PFC-amygdala connection, the functional coupling between the two regions has been theorized to reflect top-down PFC regulation of amygdala reactivity (Hare et al., 2008; Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003; H. Kim et al., 2003; Pezawas et al., 2005), with increased PFC responses leading to decreases in amygdala activation (M. J. Kim et al., 2011). Interestingly, it has been shown that this specific functional connectivity (fc) pattern only emerges during adolescence (Dougherty, Blankenship, Spechler, Padmala, & Pessoa, 2015; Gee et al., 2013; Wu et al., 2016), with research showing that children at the beginning of adolescence (up until around 10 years of age) exhibit predominantly positive, not negative, connectivity between these regions during emotion processing tasks, along with improving emotion regulation abilities ((Dumontheil, Gilbert, Frith, & Burgess, 2010; Dumontheil, Hillebrandt, Apperly, & Blakemore, 2012; Silvers et al., 2012), see also (Ahmed et al., 2015) for an overview of developmental changes in the adolescent emotion regulation network).

For many psychiatric and neurological diseases neurofeedback (NF), the reflection of one's own brain activity, constitutes a promising intervention to alleviate symptoms by (re-) shaping maladaptive neural activity. In the case of emotion regulation NF can aid the modulation of networks underlying this cognitive process, e.g. PFC-amygdala fc. Currently, only fMRI allows to target subcortical structures precisely and account of inter-individual or age-related differences. Previous investigations suggest that fMRI-based fc-NF represents a promising approach to train individuals in the self-modulation of brain regions or networks (Johnston, Boehm, Healy, Goebel, & Linden, 2010; Koush et al., 2013, 2017; Scharnowski & Weiskopf, 2015; Weiskopf et al., 2004; Zilverstand, Sorger, Zimmermann, Kaas, & Goebel, 2014), particularly in the emotion processing domain ([adults: (Johnston et al., 2010; Koush et al., 2017; Paret et al., 2014; Zotev, Phillips, Young, Drevets, &

Bodurka, 2013); [children: (Cohen Kadosh et al., 2016)). Building upon these we used fMRI-based NF of PFC-amygdala fc to modulate neural and behavioural measures relevant for emotion regulation in a sample of adolescent girls.

Results

Comparison of different fc-NF implementations with regard to their effectiveness in modulating PFC-amygdala fc

First, we compared three different fc-NF implementations (NF reinforcing negative fc; weighted NF reinforcing negative fc; NF reinforcing positive fc; **Supplementary Methods;** N = 5 per implementation) regarding their effectiveness in modulating PFC-amygdala fc (i.e. partial correlation between PFC and amygdala against CST). Specifically, three aspects capturing different quantitative and qualitative characteristics were investigated: I) the strength of the correlation, II) the frequency of negative correlations and III) the direction of activity change within negative correlations.

As regards the strength of the correlation, i.e. strength of PFC-amygdala fc, **Fig. 1A** displays the average correlations for fc-NF and no-NF at each volume within a mini-block. Correlations are averaged across mini-blocks within one condition (fc-NF, no-NF), NF runs (1, 2, 3, 4) and individuals. For the fc-NF implementation reinforcing negative fc we did not find a significant difference between fc-NF and no-NF at any volume ($p > 0.1$). However, for the weighted fc-NF implementation reinforcing negative fc, fc-NF leads to more negative, i.e. less positive, correlations than no-NF (volumes 16, 17: $p < 0.05$; volumes 9, 13, 14, 18: $p < 0.1$). To the contrary, the fc-NF implementation reinforcing positive fc fc-NF leads to more positive, i.e. less negative, correlations than no-NF (volumes 9, 10, 11, 12, 14: $p < 0.05$; volumes 6, 13: $p < 0.1$). To further explore these findings, we investigated the relationship between the average correlation at each volume and the number of fc-NF volumes within the correlation window (**see Fig. 2B** for methodological aspects and **Fig. 1B** for analyses results). For the fc-NF implementation reinforcing negative fc we observed a positive relationship between the number of fc-NF volumes in the correlation window and the average correlation ($r_{(40)} = 0.37$, $p = 0.02$). The opposite is true for the weighted fc-NF implementation reinforcing negative fc ($r_{(40)} = -0.72$, $p < 0.001$), which means that the more fc-NF volumes are included in the correlation window, the less positive the resulting correlation (**Fig. 1B**). For comparison, no significant relationship was observed for the fc-NF implementation reinforcing positive fc ($r_{(40)} = 0.22$, $p = 0.16$). The relationship between average correlation and the number of fc-NF volumes in the correlation window was significantly different between the weighted fc-NF implementations reinforcing negative fc, and the other

two implementations (both p's < 0.001), whereby the other two implementations did not differ notably from each other regarding this aspect (p = 0.48).

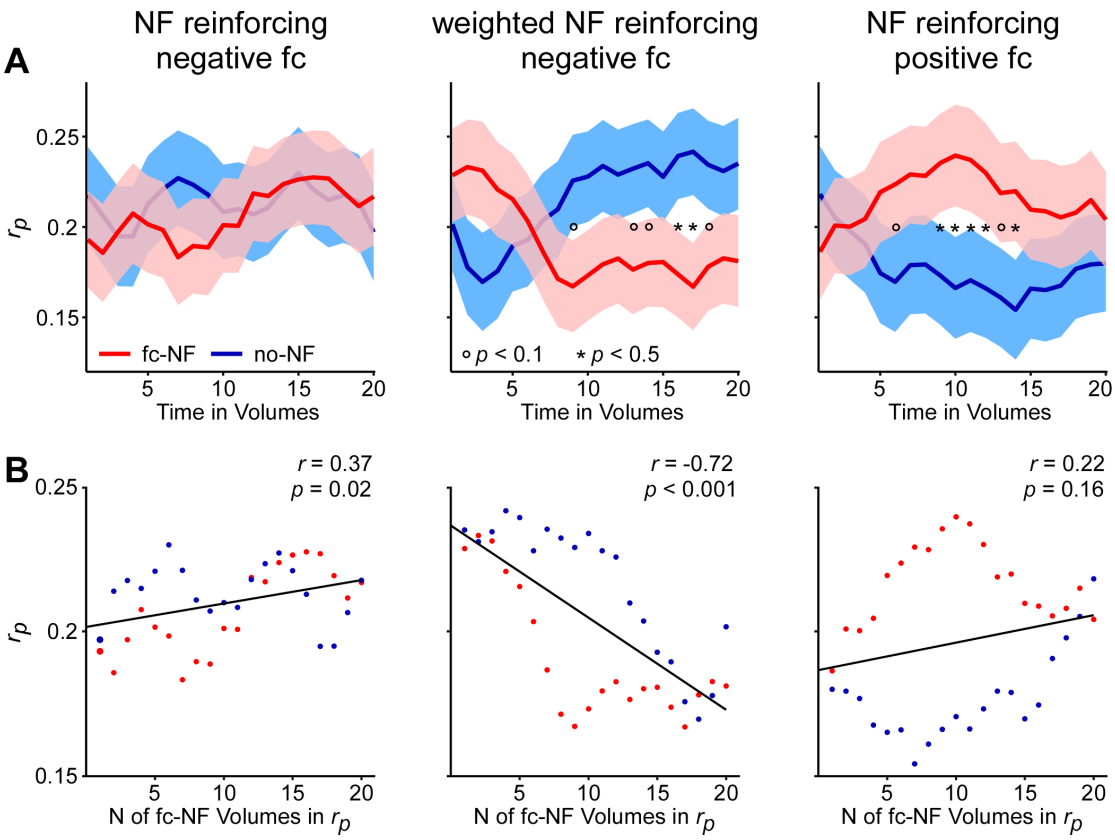

Fig. 1 Difference in average partial correlations between fc-NF and no-NF for each fc-NF implementation (N = 5 per implementation). **(A)** Means and standard error for the correlations for fc-NF (red) and no-NF (blue) at each volume within a mini-block. Correlations are averaged across mini-blocks within one condition (fc-NF, no-NF), NF runs (1, 2, 3, 4) and individuals. **(B)** Relationship between average correlation at each volume and the number of fc-NF volumes within the correlation window. Relationship is described using Pearson correlations r.

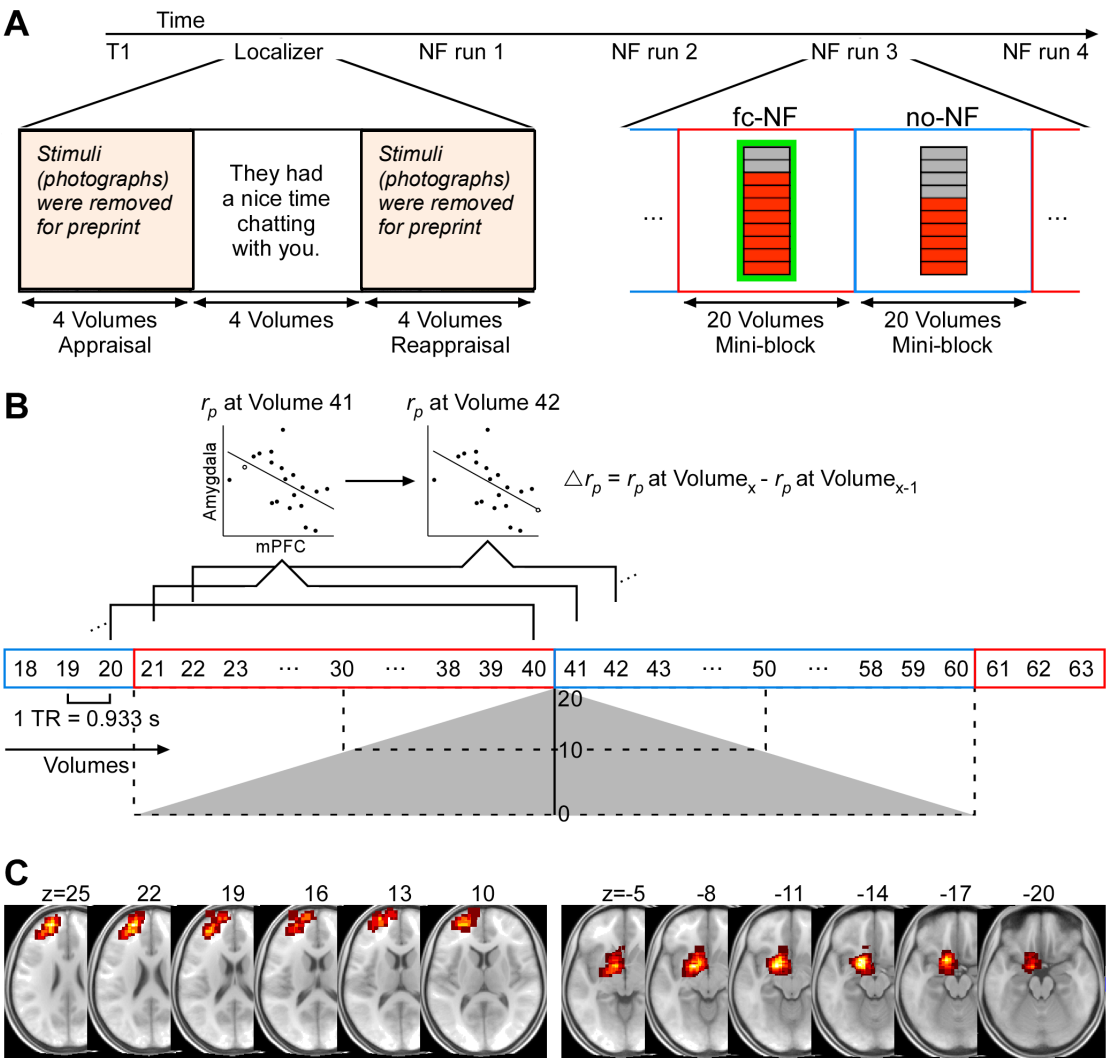

Fig. 2 Schematic representation of the experimental procedure. **(A)** The MRI session consisted of one structural scan, a functional localizer and four identical runs of the NF task. To activate the key regions involved in emotion regulation a social scene task was performed during the localizer. The task lasted 9 min. and comprised thirty trials. Each trial included emotional appraisal (4 volumes), a positively valenced statement (4 volumes), reappraisal (4 volumes), rating of change in thoughts and feeling from appraisal to reappraisal (5 volumes) and a fixation cross (1 volume). Based on the individual's localizer activity three regions of interests (ROIs; left dorsolateral prefrontal cortex (PFC), left amygdala, left corticospinal tract (CST)) were defined. Each of the four NF runs started with a rest period (20 volumes), followed by seven fc-NF and seven no-NF mini-blocks (each 20 volumes), presented in alternating order. During the no-NF mini-blocks a non-changing thermometer was shown, whereas during the fc-NF mini-blocks the same thermometer was framed green and the 'temperature', reflecting PFC-amygdala fc, was updated with every TR. **(B)** Detailed view of the procedure during the NF task. Each fc-NF and no-NF mini-block comprised 20 volumes. Fc-NF was based on partial correlations *rp* between the PFC and amygdala activity, against CST activity, whereby correlations were obtained from a moving window comprising 20 subsequent volumes. Thus, the ratio of fc-NF to no-NF volumes in the correlation window was updated with every incoming TR, e.g. for fc-NF mini-blocks, the ratio of fc-NF:no-NF volumes in the correlation window is 1:19 at the first volume of the mini-block, 2:18 at the second volume of the mini-block and 20:0 at the last volume of the mini-block, and vice

versa for no-NF blocks. The grey triangle illustrates the number of fc-NF volumes within a correlation, the remaining volumes are thus no-NF volumes. **(C)** Probabilistic maps of subject-specific ROIs superimposed on the group average structural images in MNI space. ROIs were selected based on the individuals' localizer activity, whereby the search for the local maxims originated from the key regions involved in emotion regulation, the left PFC (left side of the Fig.) and the left amygdala (right side of the Fig.). The centre of the probabilistic map of the PFC ROIs (MNI coordinates x=-20, y=50, z=24) is classified as Left Middle Frontal Gyrus and the centre of the probabilistic map of the amygdala ROIs (x=-17, y=-1, z=-14) is classified as Left Amygdala using the SPM anatomy toolbox (Eickhoff et al., 2005).

The observed differences between fc-NF and no-NF on the strength of PFC-amygdala fc can be attributed either to a strengthening of the existing fc pattern (e.g., negative correlations become more negative), or to a change in the original fc pattern (e.g., positive correlations become negative). To address this issue, we analysed the frequency of negative correlations. Because if fc-NF, compared to no-NF, modulates the frequency of the negative correlations in a similar way than the strength of the correlation this speaks for a change of the original fc pattern, whereas the absence of such modulation rather suggests a strengthening of the original existing fc pattern. **Fig. 3A** displays the average of the frequency of negative correlations for fc-NF and no-NF at each volume within a mini-block. Frequency of negative correlations is averaged across mini-blocks within one condition (fc-NF, no-NF), NF runs (1, 2, 3, 4) and individuals. Compared to no-NF, fc-NF leads to significantly more negative correlations, i.e. less positive correlations, for both, the fc-NF implementation reinforcing negative fc (volume 6: $p < 0.05$ and volume 7: $p < 0.1$) and the weighted fc-NF implementation reinforcing negative fc (volumes 11, 17: $p < 0.05$ and volumes 10, 13, 14, 16: $p < 0.1$). To the contrary, for the fc-NF implementation reinforcing positive fc significantly less negative correlations, i.e. more positive correlations, were observed during fc-NF compared to no-NF (volume 11: $p < 0.05$; volumes 9, 10: $p < 0.1$). Furthermore, we observed a negative relationship between the number of fc-NF volumes in the correlation window and the average of the frequency of negative correlations for both, the fc-NF implementation reinforcing negative fc ($r_{(40)} = -0.31$, $p = 0.05$) and the fc-NF implementation reinforcing positive fc ($r_{(40)} = -0.32$, $p = 0.05$; **Fig. 3B**). To the contrary, a positive relationship was found for the weighted fc-NF implementation that reinforced negative fc ($r_{(40)} = 0.47$, $p < 0.01$), i.e. more negative correlations with more fc-NF volumes within the correlation window. The relationship between average of the frequency of negative correlations and the number of fc-NF volumes in the correlation window was significantly different between the weighted fc-NF

implementations reinforcing negative fc and the other two implementations (both p's < 0.001), whereby the other two implementations did not differ notably from each other regarding this aspect (p = 0.96).

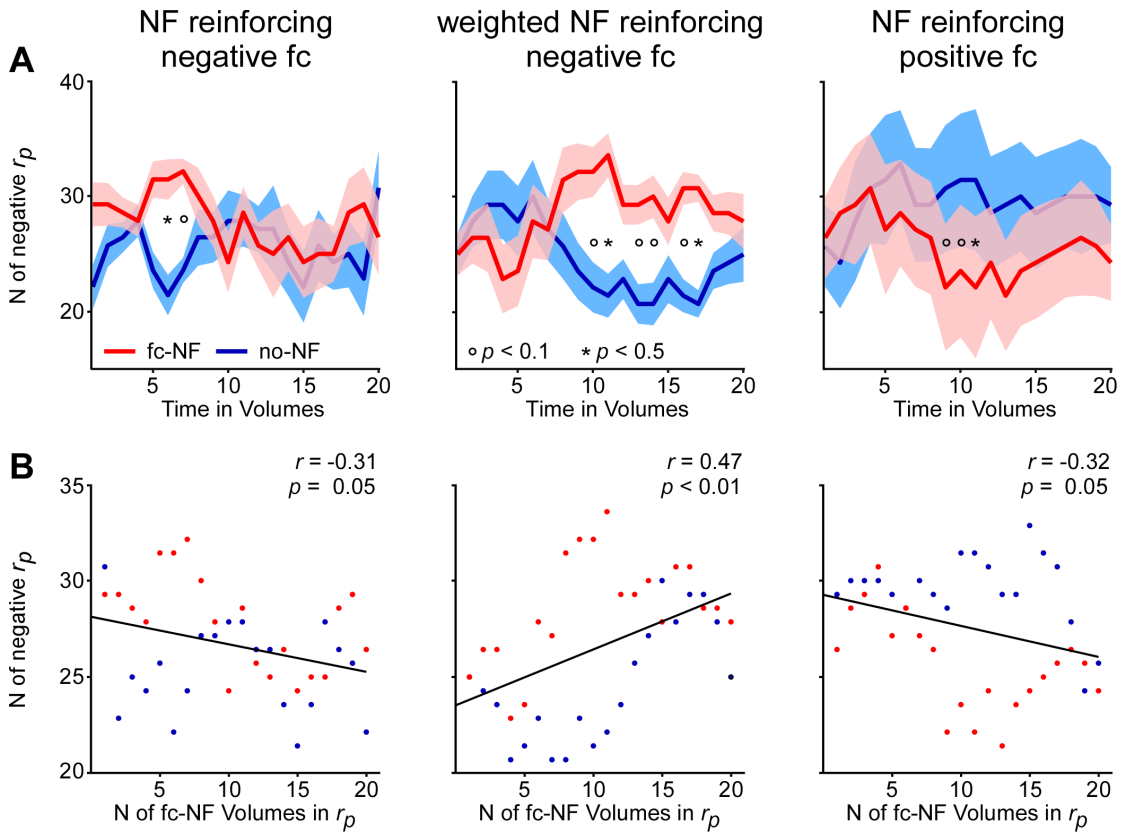

Fig. 3 Difference in average of the frequency of negative correlations between fc-NF and no-NF for each fc-NF implementation (N = 5 per implementation). **(A)** Means and standard error for the frequency of negative correlations for the fc-NF (red) and no-NF (blue) condition at each volume within a mini-block. Correlations are averaged across mini-blocks within one condition (fc-NF, no-NF), NF runs (1, 2, 3, 4) and individuals. **(B)** Relationship between the average of the frequency of negative partial correlations at each volume and the number of fc-NF volumes within the correlation window. Relationship is described using Pearson correlations r.

The preceding analyses indicate that fc-NF modulates the strength of the correlation by modulating the frequency of negative correlations. To further investigate the underlying neural mechanisms of these changes, we explored the direction of activity change within negative correlations. To this end, negative correlations were classified as one of four possible scenarios (scenario 1 – PFC up-regulation & amygdala down-regulation, scenario 2 – PFC down-regulation & amygdala up-regulation, scenario 3 – PFC & amygdala up-regulation and scenario 4 – PFC & amygdala down-regulation, **Supplementary Fig. 1**) and the relationship between the distribution of the scenarios and the number of fc-NF volumes within the correlation

window compared between the three fc-NF implementations. **Fig. 4A** illustrates the distribution of the scenarios at each volume for fc-NF and no-NF mini-blocks. For the fc-NF implementation reinforcing negative fc, the number of fc-NF volumes in the correlation window was positively related to scenario 1 occurrences, i.e. the task-relevant, mature scenario of PFC up-regulation & amygdala down-regulation, (r = 0.39, p = 0.01) and negatively related to scenario 2 occurrences, i.e. the less mature scenario of PFC down-regulation & amygdala up-regulation, (r = -0.50, p < 0.01; **Fig. 4B**). The same pattern, but enhanced, was observed for the weighted fc-NF implementation reinforcing negative fc (scenario 1: r = 0.70, p < 0.001; scenario 2: r = -0.64, p < 0.001). However, the reversed pattern was found for the fc-NF implementation reinforcing positive fc (scenario 1: r = -0.44, p < 0.01; scenario 2: r = 0.55, p < 0.001). Comparisons across the three fc-NF implementations revealed that the fc-NF implementation reinforcing positive fc differs significantly from the other two fc-NF implementations (scenario 1: both p's < 0.001; scenario 2: both p's < 0.001), whereby the other two implementations did not differ from one another regarding this aspect (scenario 1: p = 0.05; scenario 2: p = 0.37).

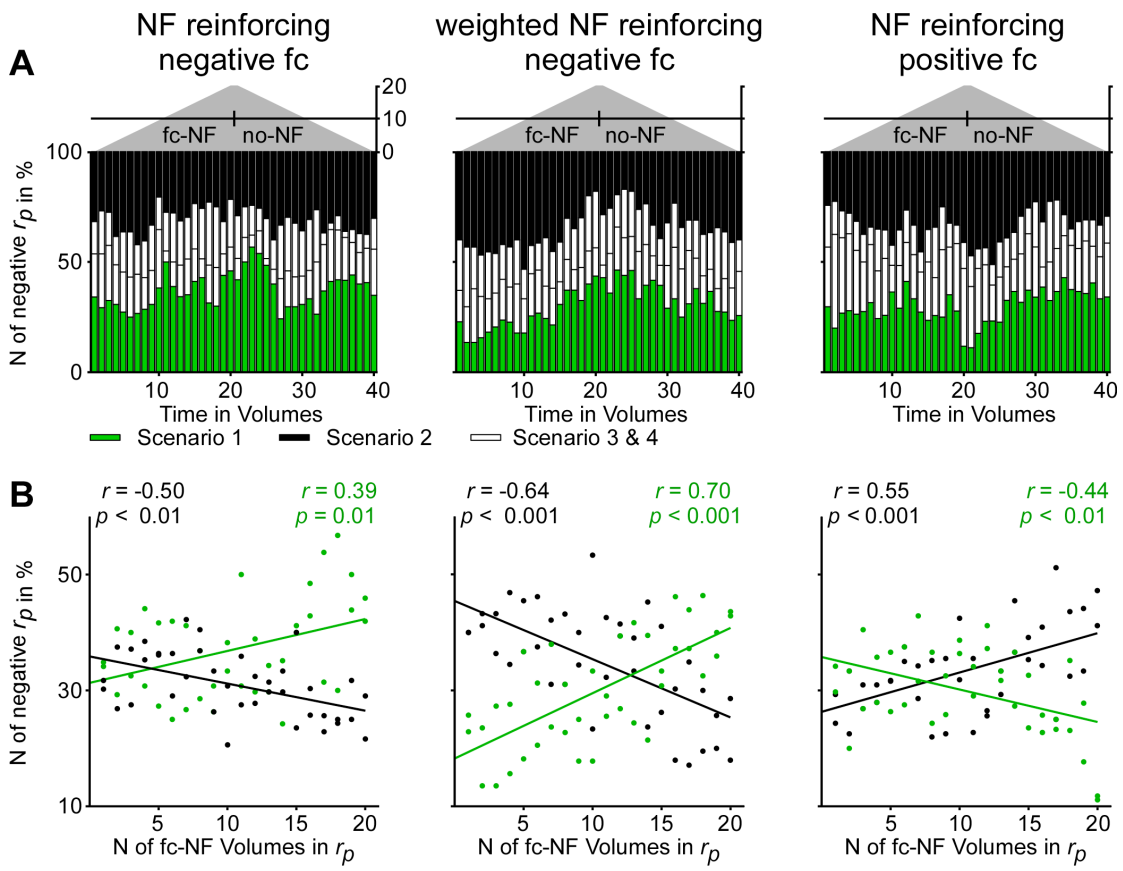

Fig. 4 Direction of activity change within negative correlations for each fc-NF implementation (N = 5 per implementation). **(A)** Percentage of negative correlations

between PFC and amygdala for scenario 1 (PFC up-regulation & amygdala down-regulation; green), scenario 2 (PFC down-regulation & amygdala up-regulation; black) and scenario 3 and 4 (white). Percentages are averaged across mini-blocks within one condition (fc-NF, no-NF), NF runs (1, 2, 3, 4) and individuals. Fc-NF and no-NF conditions are visualised subsequently and the number of fc-NF volumes within each correlation window highlighted on top in grey. **(B)** Relationships between the number of fc-NF volumes within the correlation window and the percentage of negative correlations belonging to scenario 1 (PFC up-regulation & amygdala down-regulation; green) or scenario 2 (PFC down-regulation & amygdala up-regulation; black). Relationships are described using Pearson correlations *r*.

Taken together, the comparison of the three different fc-NF implementations demonstrated, that although both, the fc-NF implementation reinforcing negative fc and the weighted fc-NF implementation reinforcing negative fc, modulate PFC-amygdala fc similarly at a qualitative level, only the latter one leads to a detectable change in the frequency of negative correlations, which is why only for this fc-NF implementation a significant modulation of PFC-amygdala fc in the desired direction could be observed. These findings demonstrate the superiority of the weighted fc-NF implementation reinforcing negative fc regarding the effectiveness of modulating PFC-amygdala fc in the desired, i.e. more negative, direction.

fc-NF induced changes on behavioural and neural level

Aiming at further exploring the effectiveness of fc-NF we probed changes on neural level, behavioural level and their interactions induced by one session of fc-NF in twenty-seven adolescent girls.

Most importantly, the effects of the weighted fc-NF implementation on neural level (i.e., strength of the correlation, frequency of negative correlations and direction of activity change within negative correlations) could be replicated (**Supplementary Fig. 2**). To evaluate changes on neural level beyond fc-NF effects we further investigated practice-related changes on neural level. Here, practice-related change was quantified as slope of the linear regression for NF run 1 to NF run 4 (for the average fc within each NF run, **Supplementary Fig. 3A**). While thirteen individuals showed a negative trend (change in the desired direction, average slope = -.023, *SD* = .019), fourteen individuals a positive trend (change in the undesired direction, average slope = .031, *SD* = .025), which is why at the group average level no significant change in fc could emerge (average slope = .0052, *SD* = .035, bootstrapping 1000, $p > .01$, **Supplementary Fig. 4**).

At the group level, pre-post comparisons of cognitive and psychological measures revealed no substantial changes on the behavioural level (**Supplementary Table 2**, **Supplementary Fig. 4**).

With regards to interactions on the neural and behavioural level, we first investigated whether changes on neural level and behavioural level were related. As defined above, change on the neural level was quantified as the slope of the linear regression for NF run 1 to NF run 4 (for the average fc within each run, **Supplementary Fig. 3A**). Change on behavioural level was defined as the difference between behavioural measures obtained before and after the MRI session. We found that change in fc was negatively related with change in thought control ability (TCAQ; **Fig. 5D**; $r_{(23)} = -.37$, $p = .09$; after the removal of two outliers identified based on bootstrapping the Mahalanobis distance ($r_{(21)} = -.48$, $p = .03$). Hence, the larger the change towards negative fc, the better the thought control ability after the MRI session, when compared to the baseline measure.

Moreover, we were interested whether, within this scope, relevant neural measures (i.e. initial fc, fc-NF-related change, and change in fc) could be predicted by behavioural measures obtained before the MRI session. Correlation analysis revealed that initial fc (i.e. average of the first two mini-blocks, **Supplementary Fig. 3B**) is positively related with trait anxiety (STAIT T; **Fig. 5A**; $r_{(27)} = .36$, $p = .07$; after the removal of two outliers identified based on bootstrapping the Mahalanobis distance: $r_{(25)} = .59$, $p = .002$) and negatively related with thought control ability (TCA-Q: **Fig. 5B**; $r_{(23)} = -.36$, $p = .09$; after the removal of two outliers identified based on bootstrapping the Mahalanobis distance: $r_{(21)} = -.66$, $p = .001$). In other words, initial fc is more negative in individuals with lower trait anxiety and individuals with better ability to control thoughts. Moreover, we found that the fc-NF-related change, i.e. difference between no-NF and fc-NF (**Supplementary Fig. 3C**), is negatively related to state anxiety obtained before the MRI session (STAI S; **Fig. 5C**; $r_{(24)} = -.41$, $p = .05$; after the removal of one outlier identified based on bootstrapping the Mahalanobis distance: $r_{(23)} = -.55$, $p = .007$). This suggests that individuals with lower state anxiety benefit more from the fc-NF than individuals with high state anxiety. None of the behavioural measures obtained before the MRI session could

predict the change in fc (correlation analysis, all *p's* > .1), indicating a more complex relationship.

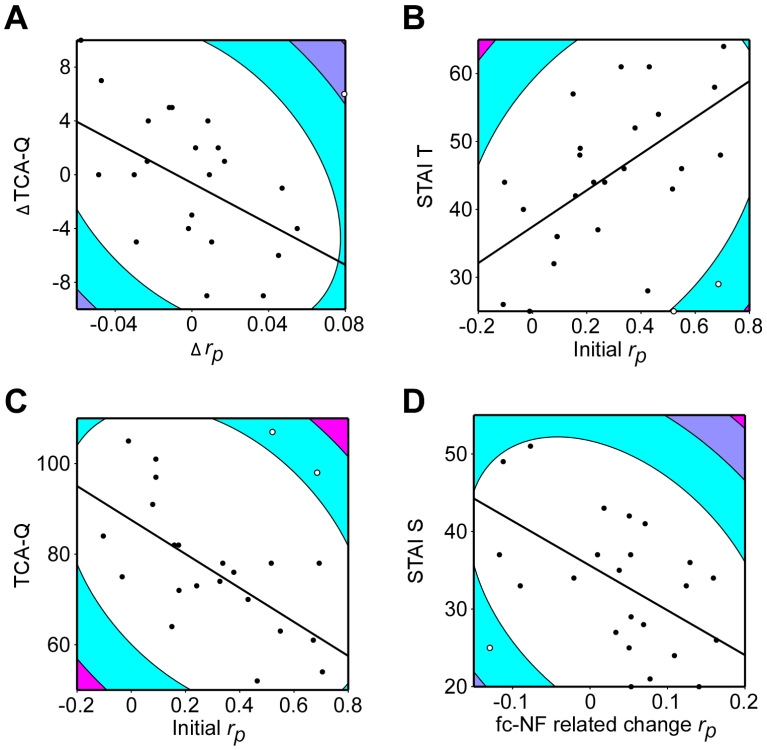

Fig. 5 Associations between behavioural and neural level. (**A**) Association between change in fc and change in thought control ability (TCA-Q; N = 23. Association between the initial fc and trait anxiety (STAI T; N = 27; **B**) as well as thought control ability (TCA-Q; N = 23; **C**) obtained before the MRI session. (**D**) Association between fc-NF-related change and state anxiety (STAI S; N = 24) obtained before the MRI session.

Discussion

We examined the effectiveness of fMRI-based NF of PFC-amygdala fc in modulating key neural and behavioural signatures for emotion regulation in a preclinical sample of adolescent girls. Firstly, our results highlight that NF implementations need to be designed carefully to be effective. Furthermore, we showed that a well-designed fc-NF implementation aids PFC-amygdala fc modulation in the developing brain. Critically, we also highlighted the interrelatedness of changes at the neural and behavioural level.

Differential effects of fc-NF implementations

We compared three fc-NF implementations and found that they modulated PFC-amygdala fc differentially. As regards the PFC-amygdala fc strength, for the weighted fc-NF implementation reinforcing negative fc PFC-amygdala fc strength was more negative, i.e. task-relevant and more mature direction, when fc-NF was present compared to its absence, while for the fc-NF implementation reinforcing positive fc the opposite pattern emerged. In view of the frequency and quality of negative correlations, we found, that for both fc-NF implementations reinforcing negative fc, the number of fc-NF volumes in the correlation window was positively related to the frequency of negative correlations and the percentage of desired negative correlations (PFC up-regulation & amygdala down-regulation), and negative related to the percentage of undesired negative correlations (PFC down-regulation & amygdala up-regulation). The reversed pattern was found for the fc-NF implementation, which reinforced positive fc. These results therefore demonstrate that only fc-NF implementations reinforcing negative PFC-amygdala fc support the task-relevant, more mature negative fc pattern and hamper the less useful, immature positive fc pattern. However, the absence of a fc-NF-related difference in PFC-amygdala fc strength for the fc-NF implementation reinforcing negative fc suggests that the NF implementation was not effective. It has been recommended, that effective NF implementations should be 1. non-evaluative and supportive, 2. meaningful, and 3. specific (Hattie & Timperley, 2007; Lotte, Larrue, & Mühl, 2013). Considering that younger children and anxious individuals of all ages exhibit generally lower levels of negative PFC-amygdala fc, it is plausible to suggest that a fc-NF implementation ranging from zero, i.e. no correlation, to minus one, i.e. maximum negative correlation, was too unspecific. In such a case, the naturally more rarely occurring negative correlations do not receive enough reinforcement. On

a more general note, this suggests that individually tailored NF implementations should enhance the modulation of task-specific activity patterns even more.

Concurrent behavioural and neural changes

While demonstrating NF-related effects on neural level is highly relevant, one of the key aims of NF is to attain transfer effects. To examine this aspect, we investigated whether fc-NF training with the most effective fc-NF implementation led to changes on neural and behavioural level. Here we did not find a significant change in any obtained measures on group level. This could be due to several reasons, such as the large inter-individual differences in neural and behavioural measures, or the relatively short training time (4 runs). We note though that, based on previous work, longer training does not seem to necessarily result in significant behavioural changes (Koush et al., 2017). This indicates that rather than increasing NF training time, it may be more beneficial to link the NF training more closely to cognitive-behavioural training approaches, such as cognitive behavioural therapy.

Despite the absence of significant changes at the group level, we found several cross-subject neural-behaviour correlations. Specifically, we found that trait anxiety levels were positively correlated with initial fc, with lower trait anxiety levels predicting more negative fc. This result is in line with the finding that lower levels of PFC-amygdala fc can predict anxiety levels in anxious participants (M. J. Kim et al., 2011). We further extended this finding here by showing that baseline state anxiety levels were negatively correlated with NF-related change, i.e. as lower the baseline state anxiety as higher the NF related change in fc. This finding suggests that one approach to increase NF success could be to ensure that participants, especially those with high levels of state anxiety, are relaxed and comfortable during the intervention. Another approach, recently developed in the framework of electroencephalography-based NF, is to only initiate the next trial if the participant is in a “good” state (i.e. relaxed, low state anxiety), which could be assessed either behaviourally or by means of an identified a biomarker (Meinel, Castaño-Candamil, Reis, & Tangermann, 2016). Our results further indicate that fc-NF affects more general cognitive and thought control abilities rather than specific affective responses that were targeted within the reappraisal network. That is, we found that baseline thought control abilities were negatively related to the initial fc, i.e. the higher the thought control ability levels, the more negative the initial fc. In addition, we found that change in fc over the NF runs was negatively associated to the

change in thought control abilities, i.e. change towards a more negative fc is related to increase in thought control abilities. Together this suggests that the fc-NF training implemented did not only have an effect on anxiety levels, but was also related to and reinforced an individual's ability to control their thoughts. We note though that, due to the correlational nature of our results, it is currently not possible to draw a causal interpretation in terms of whether initial levels of PFC-amygdala fc enabled better thought control, or vice versa. Further studies are now needed to pinpoint the specific characteristics of the relationship between neural and behavioural level, as this might guide the focus of future research. For example, if it were to be found that only participants with high levels of thought control abilities benefit from the NF training, then future studies could focus on thought control exercises a-priori the NF training in order to maximise the effect.

Form a translational point of view, our results highlight the possibility of using fc-NF to shape connectivity patterns in the developing brain while they emerge, with the possibility of diverting maladaptive patterns before they become hardwired (Cohen Kadosh, Linden, et al., 2013). This is non-trivial, given that as many as one in four children have increased levels of worry and fear as they enter adolescence and that paediatric anxiety is often referred to as a gateway disorder, which predicts lifelong mental health problems (Keshavan et al., 2014). The current study showed that while connectivity patterns in our sample of participants were predominantly positive, participants were nevertheless able to decrease fc when fc-NF was provided compared to no-NF. This is crucial as it has been shown that anxious individuals, much like younger children exhibit lower levels of negative PFC-amygdala fc or even positive PFC-amygdala fc (M. J. Kim et al., 2011). Thus, on a broader scale, our results provide first evidence for a possible clinical application that aims to shape fc patterns non-invasively in the developing brain while they emerge. We note however that the interventional potential of this fc-NF implementation depends among other on whether fc-NF will affect the PFC-amygdala relationship similarly in other samples (i.e. different age groups, gender).

We were able to show that fc-NF can be used to modulate PFC-amygdala fc in the developing brain, whereby the nature of the modulation largely depends on the fc-NF implementation. Moreover, we found that NF training using a task-relevant and specific fc-NF implementation shapes the PFC-amygdala functional circuit towards

a more negative fc pattern and that neural and behavioural level are related. Together, our results tentatively suggest that the more controlled, calmer and non-anxious the individual, the more beneficial the fc-NF training. Whether these insights could offer possible points of intervention to increase the effectiveness of NF for all individuals remains to be further explored.

Materials and Methods

Participants

Forty female adolescent participants (mean age = 15.7 years; SD = 1.3 years) were recruited from local schools in the Oxfordshire/Gloucestershire area. All participants had normal or corrected-to-normal vision and reported no history of neurological and psychiatric disorders (determined via self-report). Informed written consent was obtained from the primary caregiver and informed written assent was obtained from the adolescent. Participants received an Amazon voucher (£20) for their participation. The study was approved by the Central Oxfordshire Ethics Committee (MSD-IDREC-C2-2015-023), and in accordance with the Declaration of Helsinki. This work is registered as preclinical trial ClinicalTrials.gov Identifier: NCT02463136.

fMRI data acquisition

FMRI data acquisition was performed on a 3 T Siemens MAGNETOM Prisma MRI scanner (Siemens AG, Erlangen, Germany) using a standard 32-channel head matrix coil. First, a high-resolution structural scan was acquired, which was followed by functional imaging during the localizer task and the NF task (**Supplementary Methods**).

Localizer task

A social scenes task (Haller, Raeder, Scerif, Cohen Kadosh, & Lau, 2016) served as a localizer, as it was expected that this task activates the key regions involved in emotion regulation (**Supplementary Methods**).

Neurofeedback task

The NF task consisted of four identical runs lasting 4.8 min each. Stimulus presentation was controlled with BrainStim 1.1.0.1 (open source stimulation software, Maastricht University, http://svengijsen.github.io/BrainStim/). Each run began with a fixation cross that was displayed for 20 volumes (18.66 s), followed by seven fc-NF mini-blocks (20 volumes each) and seven no-NF mini-blocks (20 volumes each), which were presented in alternating order, with the start condition being randomized and counterbalanced across individuals. During both fc-NF and no-NF mini-blocks, a ten-segment thermometer was presented at the centre of the screen on a dark grey background. During the no-NF mini-blocks, the ‘temperature’ of the thermometer was frozen at segment six (i.e. 60%) throughout the block,

whereas during the fc-NF mini-blocks, the thermometer featured a green frame and the 'temperature' was updated with every TR (**see Fig. 2A**). The change in thermometer temperature was a direct reflection of PFC-amygdala fc (see section *Online MRI data analysis*). We first evaluated three different fc-NF implementations with regard to their effectiveness of modulating PFC-amygdala fc in a subsample of the participants (N = 15, N = 5 per implementation, **Supplementary Methods**), before investigating the effects of one fc-NF implementation on neural and behavioural level in more detail (N = 27). All participants were asked to up regulate the thermometer by revisiting positive thoughts and emotions (**Supplementary Methods**).

Self-report questionnaires

Immediately prior to the MRI session, participants completed several self-report questionnaires, which will be referred to as 'behavioural measures'. Specifically, we assessed psychological variables using the Emotion Regulation Questionnaire (CERQ), the Mood and Feelings Questionnaire (MFQ), Social Anxiety Scale for Adolescents (SAS-S) and the State-Trait Anxiety Inventory (STAI-T, STAI-S), as well as cognitive variables via the Thought Control Questionnaire (TCQ) and Cognitive Thought Control Ability Questionnaire (TCAQ). These represent established self-report measures, which are frequently used in psychological testing settings and were used here to provide baseline measures of state-dependent effects prior to the MRI session. The questionnaires CERQ, MFQ, SATI S, TCQ, TCQ-A were then repeated after the MRI session in order to assess changes on these measures. Participants also completed a Demographic and Health Questionnaire and the Wechsler Abbreviated Scale of Intelligence.

Online MRI data analysis

To enable real-time fMRI-based neurofeedback, MR images were passed from the MRI console computer to the real-time computer via a direct TCP/IP network link using the Server Message Block (SMB) network layer. Following completion of the structural scan, anatomical images were processed using BrainVoyager QX 2.8.2 (Brain Innovation, Maastricht, The Netherlands). Each 3D volume was corrected for B0 in-homogeneities (4-cycle bias field estimation), followed by stripping the brain from the skull and separating bone and cerebro-spinal fluid (2-cycle iteration).

Functional images obtained during the localizer and the NF runs were processed in real-time using Turbo-BrainVoyager 3.2 (Brain Innovation, Maastricht, The Netherlands). To correct head motion each volume was realigned to the first volume of the localizer. Each realigned volume was smoothed with a three-dimensional Gaussian kernel of 8 mm full-width-half-maximum. After completion of the localizer, three regions-of-interest (ROIs) were selected based on the GLM t-statistics as well as information from the processed structural scan (left PFC, left amygdala, left CST).[1] For the left PFC and the left amygdala the centre of the ROIs (12 mm^3, 6 x 6 x 6 voxel cube) defined as the local maximum of individual's localizer activity of three contrasts (appraisal > fixation, reappraisal > fixation, reappraisal > appraisal) at the threshold t = 3 overlaid on the individuals structural images. Probability maps of the selected ROIs across subjects are depicted in **Fig. 2C**.

During the NF task, PFC-amygdala fc was calculated in real-time. PFC-amygdala fc was defined as the partial correlation (r_p) between PFC and amygdala activity, against CST activity. Correlations were based on a moving window comprising 20 subsequent volumes (**Supplementary Discussion**), which were updated with every incoming volume. By design (length of fc-NF and no-NF mini-blocks as well as correlation window were set to 20 volumes) the ratio of fc-NF to no-NF volumes in the correlation window changed with every incoming TR, e.g. for fc-NF mini-blocks, the ratio of fc-NF:no-NF volumes in the correlation window is 1:19 at the first volume of the mini-block, 2:18 at the second volume of the mini-block and 20:0 at the last volume of the mini-block, and vice versa for no-NF blocks. Calculations were performed using a custom-made plugin for Turbo-BrainVoyager, which also provided a direct TCP/IP based link between the real-time analysis software and the stimulus application BrainStim. PFC-amygdala fc was displayed via thermometer during the fc-NF blocks.

Offline MRI data analysis

In addition to the real-time analysis we analysed the MRI data offline. While online and offline analysis of the MRI data follow a similar pre-processing pipeline, offline algorithms tend to be more robust. Offline analysis of the MRI data was performed using SPM12 (FIL, Wellcome Trust Centre for Neuroimage, UCL, London, UK). Here head motion was corrected by realigning the functional time series of the localizer or

[1] We note that the current study focused on left-lateralized ROIs as those were reliably activated in all subjects in the localiser task, whereby a lateralization in favour of the left hemisphere is in line with the literature (Ochsner & Gross, 2005)(*25*).

the NF runs to its first volume. Due to motion artefacts (motion exceeded 3 mm on any axis) three individuals were excluded from all analysis. Each individual's structural image was registered to their mean functional image and segmented, in order to normalize structural and functional images to the Montreal Neurological Institute (MNI) template. Finally, normalized functional images were smoothed with a three-dimensional Gaussian kernel of 8 mm full-width-half-maximum. To increase the consistency between online and offline analysis we used the same ROIs. Therefore, the ROIs defined in Turbo-BrainVoyager during the MRI session were transformed from DICOM to NIFTI format, from radiological to neurological convention and from voxel to mm space. Subsequently, ROIs were normalized to the MNI template by applying the same transformation matrix used for the subject-specific normalization of structural and functional images. Time series were extracted from the three ROIs using MarsBaR 0.44 (Brett, Anton, Valabreque, & Poline, 2002) and PFC-amygdala fc calculated using the same procedure as during real-time fc-NF, i.e. partial correlation between PFC and amygdala activity, against CST activity.

Acknowledgements

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Disclosures

The authors declare no financial or non-financial competing interests or potential conflicts of interests.

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Supplementary Materials for: Modulatory effects of dynamic fMRI-based neurofeedback on emotion regulation networks during adolescence

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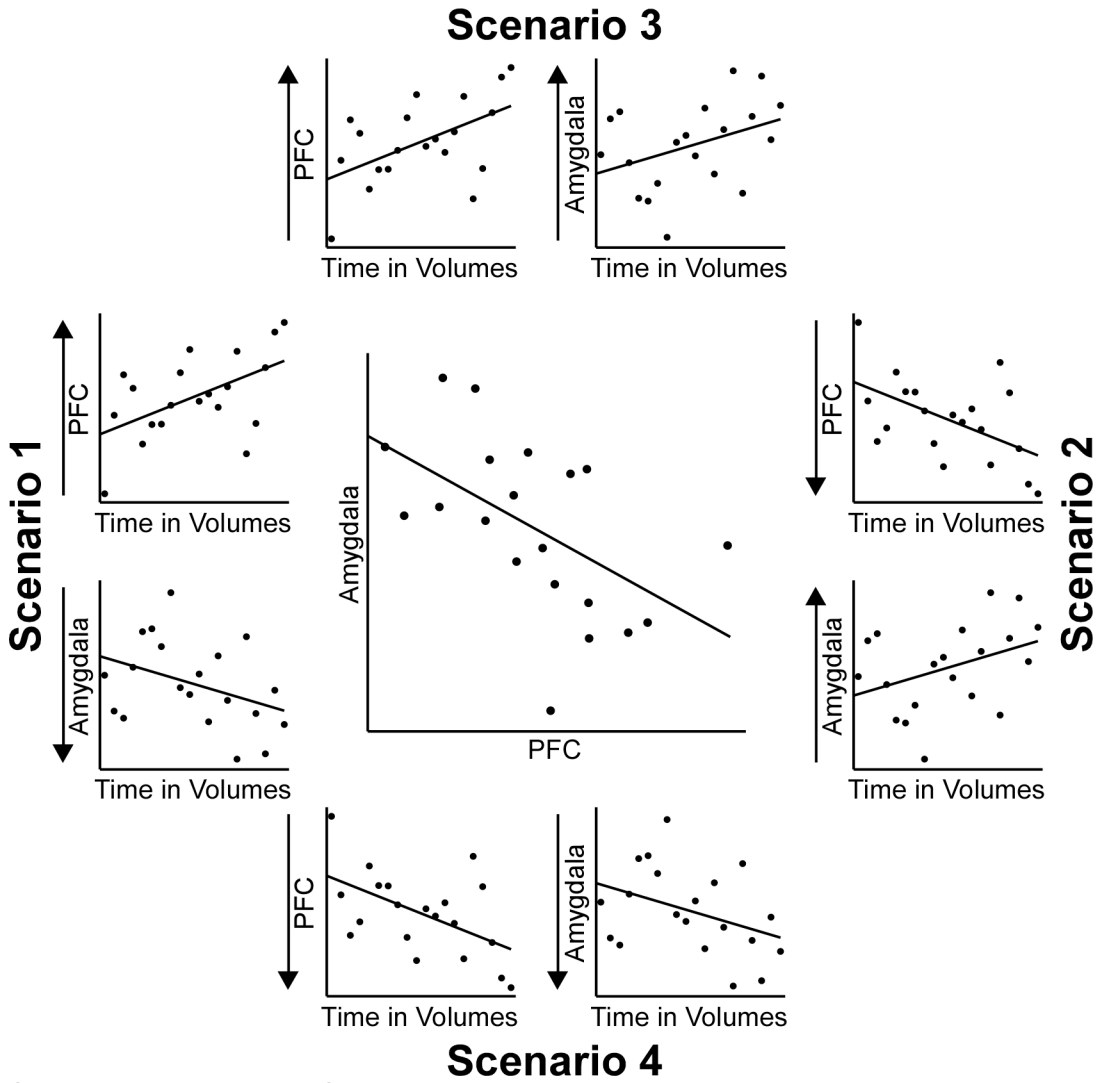
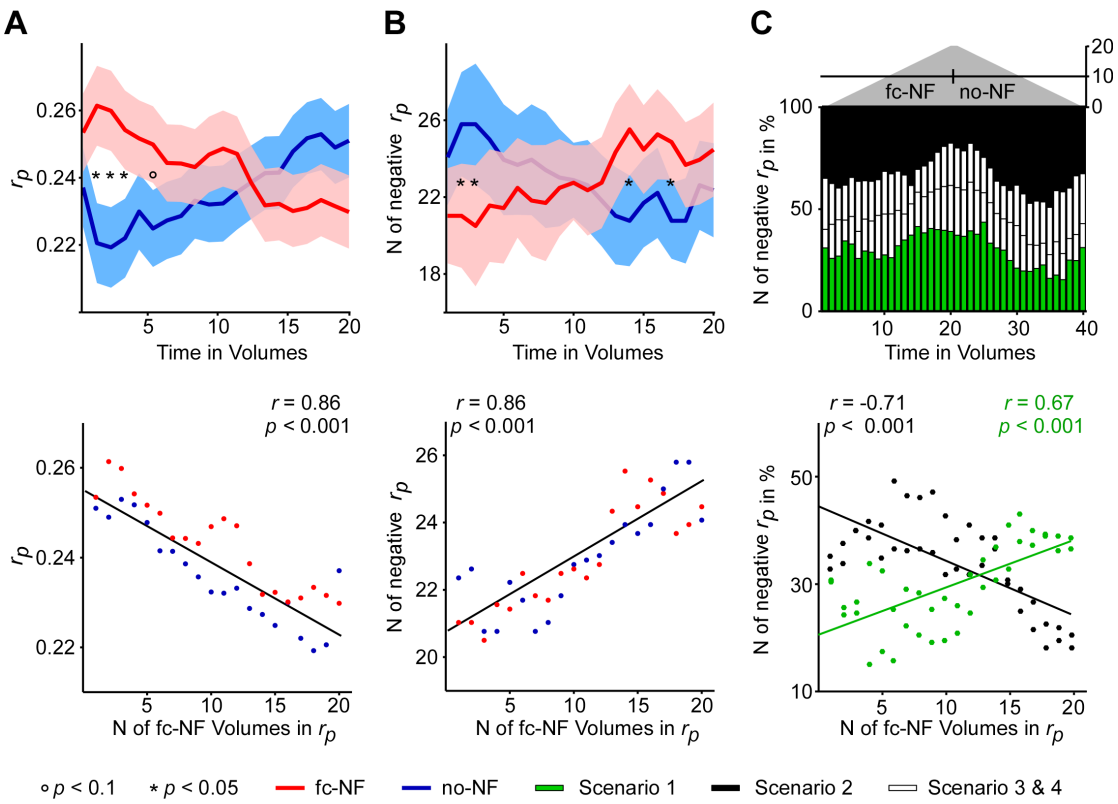
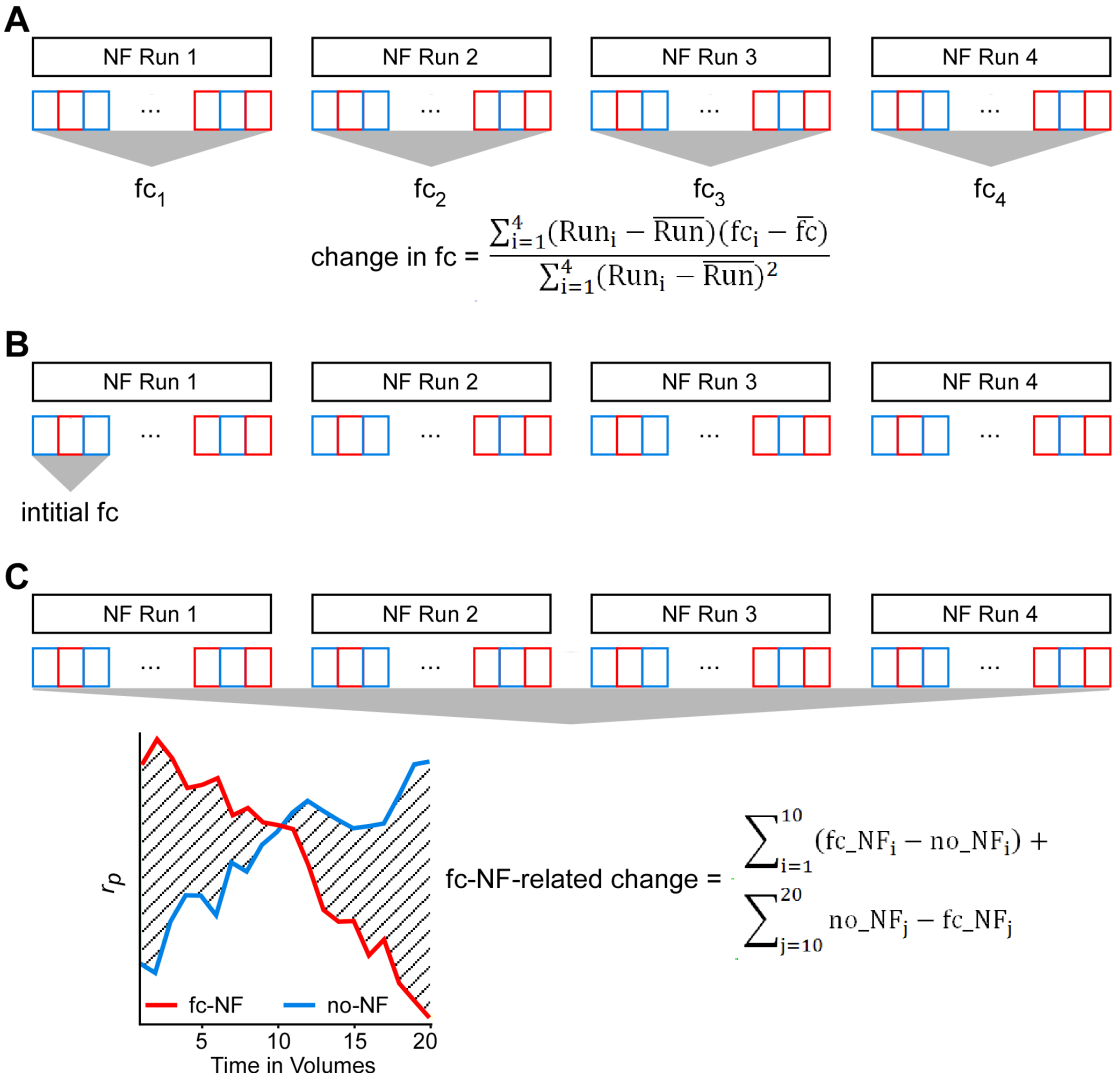
Supplementary Figures



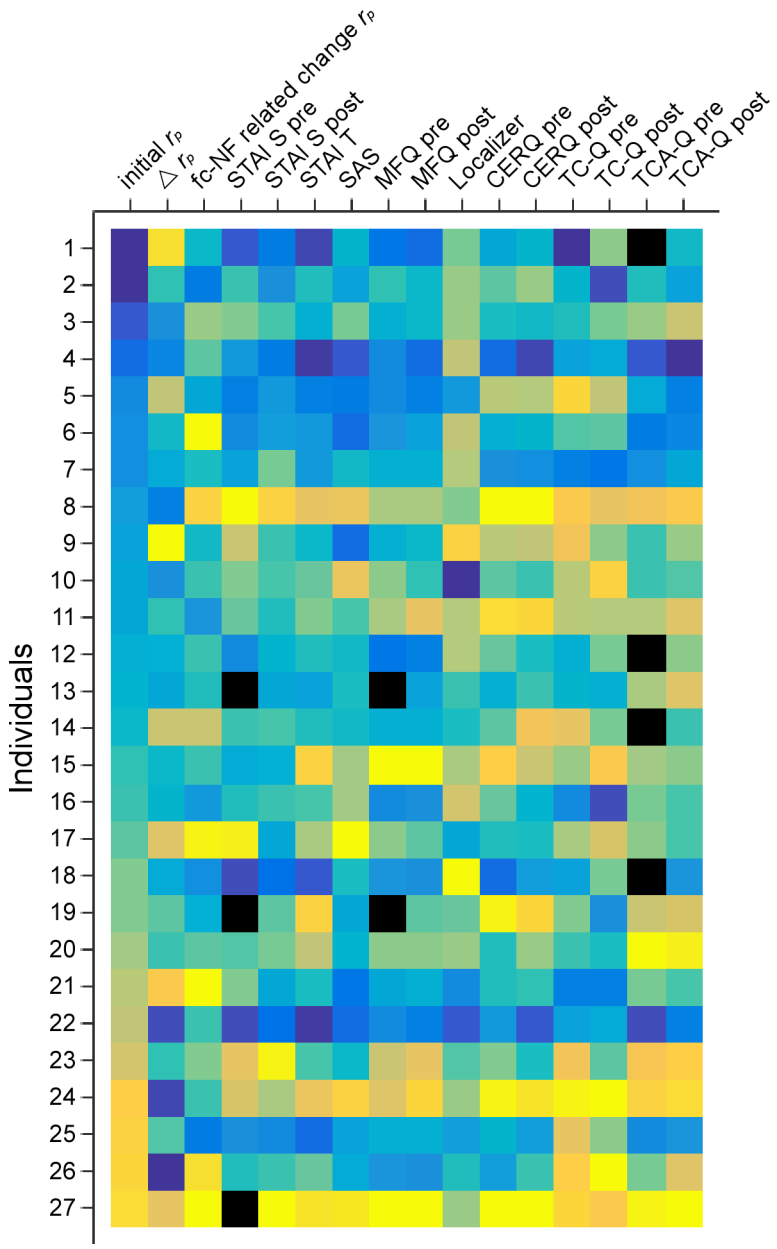
Supplementary Fig. 1 Schematic representation of the procedure used to quantify the direction of activity change within a negative correlation. **Centre**: exemplary negative partial correlation between PFC and amygdala activity accounting for the activity in the CST is illustrated. The direction of activity change within this correlation was determined for the PFC and the amygdala separately. In detail, both, the PFC and the amygdala, were correlated separately with time while accounting for the activity in the CST. Theoretically four different scenarios are possible: scenario 1 – PFC up-regulation & amygdala down-regulation (left), scenario 2 – PFC down-regulation & amygdala up-regulation (right), scenario 3 – PFC & amygdala up-regulation (top) and scenario 4 – PFC & amygdala down-regulation (bottom).



Supplementary Fig. 2 Replication of fc-NF-related effects on neural level for the fc-NF implementation reinforcing negative fc weighted (N = 27). (**A**) Difference in average correlations between fc-NF and no-NF (top). Means and standard error of the correlations for the fc-NF (red) and no-NF (blue) at each volume within a mini-block. Correlations are averaged across mini-blocks within one condition (fc-NF, no-NF), NF runs (1, 2, 3, 4) and individuals. Relationship between average correlations at each volume and the number of fc-NF volumes within the correlation window (bottom). Relationship is described using Pearson correlations *r*. (**B**) As (A), but for the frequency of negative partial correlations. (**C**) Percentage of negative correlations between PFC and amygdala for scenario 1 (PFC up-regulation & amygdala down-regulation; green), scenario 2 (PFC down-regulation & amygdala up-regulation; black) or scenario 3 and 4 (white; top). Percentages are averaged across mini-blocks within one condition (fc-NF, no-NF), NF runs (1, 2, 3, 4) and individuals. Fc-NF and no-NF conditions are visualised subsequently and the number of fc-NF volumes within each correlation window highlighted on top in grey. Relationship between the number of fc-NF volumes within the correlation window and the percentage of negative correlations belonging to scenario 1 (PFC up-regulation & amygdala down-regulation; green) and scenario 2 (PFC down-regulation & amygdala up-regulation; black; bottom). Relationships are described using Pearson correlations *r*.



Supplementary Fig. 3 Illustration of quantification of neural measures. (**A**) Visualization of change in fc, i.e. slope of the linear regression for NF run 1 to NF run 4 (for the average fc within each NF run. (**B**) Illustration of initial fc, i.e. average fc of the first two mini-blocks. (**C**) Visualization of the fc-NF-related change (hatched) on exemplary data from one subject. Due to the dynamic character of the fc-NF, the fc-NF-related change is composed of two parts. For fc-NF mini-bocks (red) the ratio of fc-NF:no-NF volumes in the correlation window is 1:19 at the first volume of the mini-block, 2:18 at the second volume of the mini-block and 20:0 at the last volume of the mini-block. Consequently, in fc-NF mini-blocks volumes 1 to 10 are dominated by no-NF volumes, and volumes 11 to 20 are dominated by fc-NF volumes. Exactly the opposite is the case for no-NF mini-blocks.



Supplementary Fig. 4 Maturity comparison by obtained measure at the neural and behavioural level. Each measure (columns) is z-transformed and scaled to their maximum absolute value (blue higher maturity level is represented in blue, less maturity level is represented in yellow, missing data are visualized in black). Individuals (rows) are sorted by their initial fc (r_p).

Supplementary Tables

Supplementary Table 1 Summarized overview of the main research findings

	NF reinforcing negative fc	**weighted NF reinforcing negative fc**	**NF reinforcing positive fc**
Is there a difference in the **strength** of the PFC-amygdala correlations between fc-NF and no-NF and, if so, in which direction? (see Fig. 2A)	No	Yes (desired)	Yes (undesired)
Has the number of fc-NF volumes in the correlation window an influence on the **strength** of the PFC-amygdala correlations and, if so, in which direction? (see Fig. 2B)	Yes (undesired)	Yes (desired)	No
Is there a difference in the **number of negative** PFC-amygdala correlations between fc-NF and no-NF and, if so, in which direction? (Fig. 3A)	Yes (desired)	Yes (desired)	Yes (undesired)
Has the number of fc-NF volumes in the correlation window an influence on the **number of negative** PFC-amygdala correlations and, if so, in which direction? (see Fig. 3B)	Yes (undesired)	Yes (desired)	Yes (undesired)
Has the number of fc-NF volumes in the correlation window an influence on the **number of negative more mature** PFC-amygdala correlations and, if so, in which direction? (see Fig. 4A)	Yes (desired)	Yes (desired)	Yes (undesired)
Has the number of fc-NF volumes in the correlation window an influence on the **number of negative less mature** PFC-amygdala correlations and, if so, in which direction? (see Fig. 4B)	Yes (desired)	Yes (desired)	Yes (undesired)

Supplementary Table 2 Cognitive and psychological measures

	Pre	*Post*	*Difference*
CERQ	*N = 27; M = 60.52; SD = 11.09*	*N = 27; M = 60.93; SD =*	$t_{(26)} = -.442$; $p = .662$
Localizer	*N = 27; M = 2.51; SD = 0.37*		
MFQ	*N = 25; M = 5.44; SD = 4.81*	*N = 27; M = 5.56; SD =*	$t_{(24)} = -.778$; $p = .444$
SAS-S	*N = 27; M= 49.15; SD = 15.01*		
STAI S	*N = 24; M = 32.79; SD = 8.61*	*N = 27; M = 31.78; SD =*	$t_{(23)} = 1.784$; $p = .088$
STAI T	*N = 27; M = 43.52; SD = 11.49*		
TC-Q	*N = 27; M = 61.04; SD = 8.67*	*N = 27; M = 60.70; SD =*	$t_{(26)} = .251$; $p = .804$
TCA-Q	*N = 23; M = 78.91; SD = 15.37*	*N = 27; M = 79.85; SD =*	$t_{(22)} = .041$; $p = .968$

Supplementary Methods

fMRI data acquisition

Before the experimental tasks a high-resolution structural scan was obtained from each subject using a T1-weighted magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequence (TR = 1900 ms, TE = 3.97 ms, FoV = 192 x 192 mm^2, filp angle = 8°, slice thickness = 1 mm, sagittal). Functional measures comprised the localizer and four NF runs. The localizer comprised 570 and each fc-NF run 310 2D multiband gradient echo planer imaging volumes (Todd et al., 2016) (2.0 x 2.0 x 2.0 mm voxels, 0.57 mm gap, TR = 933 ms, TE = 33.40 ms, FoV = 192 x 192 mm^2, flip angle = 64°, 72 slices, Multi-band factor = 6, fat saturation, transverse slices with phase encoding in the A >> P direction). At the beginning of the localizer and each fc-NF run ten additional volumes were acquired, but not analysed, in order to avoid saturation effects.

Localizer task

The localizer task lasted 9 min. and comprised thirty trials. Each trial started with a social scene presented for four volumes (3.73 s). Scenes depicted negative, rejecting social situations viewed from the perspective of a (female) protagonist depicted from the back. The participants were instructed to interpret the scene freely (appraisal). This appraisal event was followed by a positively valenced interpretative statement (4 volumes, 3.73 s), after which the same scene was shown again for four volumes (3.73 s). For the duration of the second scene presentation, participants were instructed to reappraise the social scene in accordance with the interpretative statement (reappraisal). Finally, participants were asked to rate how much they were able to change (reappraise) their thoughts and feelings from the first to the second presentation of the scene. Participants indicated their perceived success on a Likert scale ranging from no change (1) to much change (4) via button press on each trial. A fixation cross was presented for one volume between two trials. Stimulus presentation was controlled via BrainStim 1.1.0.1 (open source stimulation software, Maastricht University, http://svengijsen.github.io/BrainStim/).

Different NF display implementations

The representation of the functional connectivity varied for the three groups. Specifically, whereas the bottom of the scale was always zero, the top of the scale was set to -1 (maximal negative correlation) for group N, to -0.3 (chosen based on

the cumulative distribution of partial correlations r_p between the PFC, amygdala and corticospinal tract from pilot data) for group N+ and to +1 (maximal positive correlation) for group P. Hence, assuming the thermometer shows two out of ten segments as highlighted, a change towards three out of ten indicates a change towards a more negative correlation by 0.1 for **group N**, a more negative correlation by 0.03 for **group N+**, and a change towards a more positive correlation by 0.1 for **group P**, a change towards, and vice versa for a thermometer change towards one out of ten.

Neurofeedback task – Instructions

All participants were blind to their NF display parameters and were instructed as follows: *"You will see a thermometer with a green rim on the screen. The red bars show how much the regions that are important for emotions are active. Your job is to get these regions as active as possible! So try to get this thermometer up as much as possible. Similar to the task before, try to control your thoughts towards a positive feeling. When the thermometer does not have a green rim, the thermometer is not working. However, even if the thermometer is not working, your task will be the same and we are still measuring how much your brain is active. The two different thermometers will alternate."* Debriefing revealed that, in order to change their thoughts and emotions towards a more happy mood, participants thought about certain people, things that happened in the past and things that they would like to happen in the future.

Supplementary Discussion

Length of the correlation window

The sliding correlation time-window design (20 volumes per mini-block) in the current study allowed us to differentially assess the effect of fc-NF vs no-NF in greater detail. We found that the PFC-amygdala fc changed as a function of fc-NF volumes within a correlation window. That is, as more fc-NF volumes were included in the correlation window, the less positive the resulting partial correlation. We chose this time-window based on a previous study by Zilverstand and colleagues (Zilverstand, Sorger, Zimmermann, Kaas, & Goebel, 2014), who found that the functional connectivity measures in a well-controlled, simple motor task were weaker and less reliable for a shorter (12 s) window but not qualitatively different form a longer (26 s) window. Given the higher reliability of the longer correlation windows, we matched the size of our time-windows with the length of the mini-blocks. This allowed us to directly assess the NF-related changes as a function of the ratio of fc-NF:no-NF volumes in the correlation window by considering gapless each data point. Our results demonstrate that the strength of the fc-NF-related modulation in fc correlates with the number of fc-NF volumes in the correlation window. This finding is not only of theoretical interest, but also has important practical implications. Specifically, it suggests that with regard to clinical applications the relation between task window and correlation window should be adapted, so that the length of the task window exceeds the correlation window, in order to further enhance the here observed fc-NF-related modulations.

References

Todd, N., Moeller, S., Auerbach, E. J., Yacoub, E., Flandin, G., & Weiskopf, N. (2016). Evaluation of 2D multiband EPI imaging for high-resolution, whole-brain, task-based fMRI studies at 3T: Sensitivity and slice leakage artifacts. *NeuroImage*, *124*, 32–42. http://doi.org/10.1016/j.neuroimage.2015.08.056

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