Research Paper

Real-time functional magnetic resonance imaging neurofeedback training of amygdala upregulation increases affective flexibility in depression

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Background: Decreased affective flexibility is associated with depression symptoms, and it has been suggested that common interventions may target this mechanism. To explore this hypothesis, we evaluated whether real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf) training to increase the amygdala responses during positive memory recall resulted in both symptom improvements, as has been observed previously, and flexibility to decrease amygdala reactivity in response to a cognitive task among patients with major depressive disorder (MDD). **Methods:** In a double-blind, placebo-controlled, randomized clinical trial, adults with MDD received 2 sessions of rtfMRI-nf training to increase their amygdala (experimental group) or parietal (control group) responses during positive autobiographical memory recall. We evaluated signal changes in the amygdala during both the positive memory neurofeedback and a subsequent counting condition. **Results:** We included 38 adults with MDD, including 16 in the experimental group and 22 in the control group. In the experimental group, amygdala activity increased ($t > 2.01$, df < 27, $p < 0.05$, $d > 0.5$) and depressive symptoms decreased (–8.57, 95% confidence interval [CI] –15.12 to –2.59; $t_{13} = -3.06$, $p = 0.009$, $d = 1$). Amygdala activity during the count condition decreased after rtfMRI-nf (–0.16, 95% CI –0.23 to –0.09; $t_{396} = 4.73$, $p < 0.001$, $d = 0.48$) and was correlated with decreased depression scores ($r = 0.46$, $p = 0.01$). We replicated previous results and extended them to show decreased amygdala reactivity to a cognitive task during which no neurofeedback was provided. **Limitations:** The count condition was reported by participants as negative, but emotionality or accuracy during this condition was not assessed. **Conclusion:** These results suggest that nominally targeting unidimensional change in neural mechanisms could have implications for bidirectional control, increasing the likely reach and explanatory framework for how common depression interventions work. **Trial registration:** ClinicalTrials.gov NCT02709161.

Introduction

Affective flexibility, or the ability to transition into and out of affective states, is decreased in depression, prospectively predicts future depressive symptomatology and has been suggested to be implicitly targeted by common interventions for depression, which do not nominally have flexibility as their goal.[1–3] If flexibility, rather than unidimensional change, was the actual target of interventions, it could radically change how we think about how these interventions work. Common explanations purported to differentiate interventions — like challenging negative thinking specifically, decreasing sadness, increasing control over negative emotion, increasing happiness or increasing motivation — could suggest the need for different types of interventions to match different symptom clusters. A flexibility explanation would instead suggest a common mechanism by which many depression interventions operate, decreasing the potential utility of affective valence–related personalization in intervention and increasing potential mechanistic explanatory power.

To understand whether this flexibility explanation is likely, we examined whether a highly regimented, programmatic intervention, constructed to target a singular neural mechanism in a unidirectional manner, actually targets affective flexibility. Specifically, we considered whether real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf) to increase amygdala reactivity to positive stimuli also affects the amygdala's response to stimuli that are not positive, as well as a broader array of depressive symptoms that are unlikely to be related to positive affective reactivity alone. The amygdala is considered critical for affective experiences,[4,5] and its response to positive and negative stimuli has been observed to change during recovery from major depressive disorder (MDD),[6–9] but the interventions for which it has been examined do not purport to affect a single unidirectional

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mechanism. For example, amygdala activity is generally increased in response to negative information and decreased in response to positive information in depression;[10] after successful treatment with selective serotonin reuptake inhibitors (SSRIs), amygdala hyperactivation to negative stimuli decreases while amygdala activation to positive self-referential words increases, but SSRIs affect a wide variety of neural mechanisms and it is unclear whether changes in flexibility could result from mechanistic effects in a single direction.[11,12]

The effects of working to increase amygdala reactivity on other types of stimuli would not only improve our causal understanding of the nature of the intervention but would be practically important, as we have suggested that increased emotional reactivity is undesirable at times and can cause some of the problems seen in depression. Indeed, depression is characterized by sustained amygdala reactivity to a cognitive task following an emotional stimulus.[13,14] In support of flexibility as a mechanism of intervention, we have found that cognitive training geared toward increasing recruitment of prefrontal control decreases amygdala reactivity to emotional words.[15]

It has been shown previously that patients with depression have an attenuated amygdala hemodynamic response during positive memory recall, in comparison with healthy controls.[16] Following up on that result, we have shown that, using rtfMRI-nf, patients with MDD are able to increase their amygdala response while recalling positive autobiographical memories,[17,18] and the degree of amygdala regulation success is associated with clinical improvements.[17] Within a larger clinical trial structured to replicate this general result, we evaluated the explicit goal of examining whether amygdala activity to a subsequent nonemotional task ("count backward from 300 by a number"), during which no feedback was provided, is also affected. This count condition was originally included to allow the amygdala signal to return to baseline between happy and rest blocks. Although patient reports suggested that participants found the count condition negative and stressful at the study's beginning, by the end, participants found it to be calming and some reported using counting backward to manage their depression at follow-up. We hypothesized that amygdala activity during the count condition would decrease across sessions in the experimental group, relative to the control group. We also hypothesized that change in amygdala reactivity to happy and count conditions would account for the same variance in change in depressive severity, supporting a flexibility, rather than valence-specific explanation for recovery.

Methods

Participants

We recruited participants from the community via advertisements and through the University of Pittsburgh's Pitt+Me's registry of research participants. Screening evaluations were performed at the University of Pittsburgh and included the depression module of the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*[19] and the Mini International Neuropsychiatric Interview.[20] Exclusion criteria included general exclusions for research involving magnetic resonance imaging, psychosis, bipolar disorder, major medical or neurologic disorders, exposure to any medication (other than antidepressants) that were likely to influence cerebral function or blood flow within 3 weeks, drug or alcohol abuse within the previous year or lifetime alcohol or drug dependence (excepting nicotine). Unsuccessful current antidepressant medications were not an exclusion, but participants had to be stable on their medication (defined as maintaining the same dose for at least 3 weeks or, for fluoxetine, 6 weeks). All participants were naive to rtfMRI-nf.

Procedure

Participants completed 1 screening visit and 2 rtfMRI-nf training sessions, as well as an assessment of symptoms via Beck Depression Inventory (BDI-II)[21] before starting protocolized cognitive behavioural therapy (not reported here). The rtfMRI-nf sessions occurred about 1 week apart from each other. All study visits took place at the University of Pittsburgh. We conducted a follow-up visit 1 week after the final neurofeedback session, during which participants completed the BDI and then underwent their first session of cognitive behavioural therapy. Participants completed the BDI at each study visit, which served as the primary outcome measure.

Under double-blind conditions (participants and experimenters), participants were randomly assigned to receive rtfMRI-nf from 1 of 2 regions of interest defined as 7-mm spheres in Talairach space, namely the left amygdala for the experimental group (coordinates –21, –5, –16) or the left horizontal segment of the intraparietal sulcus for the control group (coordinates –42, –48, 48), a region putatively not involved in emotion regulation.[22] One team member (T.H.) wrote a script that randomized each participant to 1 of the 2 neurofeedback targets according to the number that was given to them when they were enrolled in the study. One team member (M.S.) was responsible for enrolling participants and another (S.L.) was in charge of running the scans; both were blinded to participants' group assignment. Group membership was only disclosed later to 2 members (K.Y. and L.C.) for the purpose of running group analyses. This sample size allowed detection of effect sizes of 0.95 and greater for between-group differences and of 0.75 and greater within the experimental group at a power of 0.8. Our aim was to be able replicate the effect size of our previous study (d = 1.03) for changes in BDI scores.[17]

rtfMRI-nf paradigm

We instructed participants to retrieve positive memories while attempting to increase their hemodynamic activity in the assigned region. Each neurofeedback run consisted of sequential 40-second blocks of rest (static thermometer, neurofeedback not provided), instructions to upregulate the assigned region while retrieving happy memories (happy condition; moving thermometer, neurofeedback provided) and counting backward from 300 by a given 1-digit integer of

3 or higher (count condition; static thermometer, neurofeedback not provided). Each rtfMRI-nf session consisted of 6 fMRI runs, each lasting 8 minutes and 40 seconds, as follows: a baseline run, a practice run, 3 training runs and a transfer run; no neurofeedback was provided during the first and last runs. Details on the paradigm, the different runs and imaging parameters can be found in Appendix 1, Box 1, available at www.jpn.ca/lookup/doi/10.1503/jpn.220208/tab-related-content.

Imaging was conducted using a Siemens 3T Prisma scanner and Turbo BrainVoyager neurofeedback software (Brain Voyager). We computed the neurofeedback signal for each happy condition as the fMRI percent signal change relative to the average fMRI signal for the preceding rest block, updated every 2 seconds and displayed as a thermometer. To reduce fluctuations from noise in the fMRI signal, the bar height was computed at every time point as a moving average of the current and 2 preceding values. These percent signal change values were averaged over each run and used as a performance measure. We defined neurofeedback success as the mean percent signal change in the region of interest from the initial baseline run to the final transfer run. Higher scores indicated more activity after training relative to baseline.

Differences from previous publications

Despite the similarity of the training paradigm with our previous publications,[17,23] there are several differences worth noting. Previous studies were conducted using a GE Discovery MR750 3T scanner, whereas we used a Siemens Prisma 3T scanner for this study. Our previous publications used custom rtfMRI-nf software for stimulus presentation and AFNI's (Analysis of Functional NeuroImages) real-time features for neurofeedback implementation.[25] In the current study, we used commercially available software, Turbo BrainVoyager (version 3.2) for neurofeedback implementation, and E-prime-2 for stimulus presentation. Our primary outcome measure was the BDI, while in our previous study we also used the Montgomery–Åsberg Depression Rating Scale and the Hamilton Depression Rating Scale. We made this change so that self-report of symptoms could be collected at multiple time points without the need to return to the laboratory. Of note, the BDI was collected in our previous randomized controlled trial (RCT) and showed similar results to those obtained with the other tools.[17] Finally, our previous studies were all conducted in Tulsa, Oklahoma. The participants from the current study came from the Pittsburgh metropolitan area.

Statistical analysis

We used R (version 3.5.0) and SPSS (version 27) for statistical analyses. Auto-correlation structures in models were decided after model comparisons (Table S4). To evaluate change in BDI we used a multivariable linear mixed-effects model with fixed factors of visit (fMRI visits 1 and 2, and 1-week follow-up) and group (experimental, control); we included participants as a random effect. We evaluated the neurofeedback training effect via a similar linear mixed-effects model with the fixed factors of run (baseline, practice, training runs, transfer), visit (fMRI visits 1 and 2), and group (experimental, control), with participants included as a random effect. We analyzed the neurofeedback training effect on regional percent signal change during happy versus rest and count versus rest. We performed associated t tests to characterize differences underlying main effects and interactions. We calculated the association between residualized neurofeedback success (difference in the percent signal changes between visit-1 baseline and visit-2 transfer) and residualized BDI scores at follow-up to account for baseline. We also analyzed difference scores to be consistent with the literature.[27] The threshold for statistical significance was set at $p = 0.05$ in 2-tailed tests.

Ethics approval

Participants gave written informed consent to participate in the study after receiving a complete description of it and received financial compensation for the fMRI study visits. The research protocol was approved by the University of Pittsburgh Institutional Review Board and registered on ClinicalTrials.gov (NCT02709161; see Appendix 1, Table S1). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Results

The final sample included 38 right-handed adults (aged 18–55 yr) with MDD, including 16 in the experimental group and 22 in the control group (Appendix 1, Figure S1). During the screening visit (Table 1 and Appendix 1, Table S2), the groups did not differ significantly in any characteristic (age, sex, education, depression severity, anhedonia). The experimental group included 11 females and the control group included 18 females. Average depression scores were in the moderate-to-severe range. At follow-up, 4 BDI scores were missing in the control group and 2 were missing in the experimental group. When missing, we used the data that we had for linear mixed models over time but excluded these participants for

Table 1: Beck Depression Inventory scores for each group and visit among patients with major depressive disorder

	Mean ± SD		
Group	rtfMRI-nf session 1	rtfMRI-nf session 2	Follow-up
Experimental, $n = 16$	30.00 ± 10.08	26.19 ± 10.11*	20.57 ± 8.68*
Control, $n = 22$	28.09 ± 7.19	27.41 ± 9.07	26.39 ± 10.09

rtfMRI-nf = real-time functional magnetic resonance imaging neurofeedback; SD = standard deviation.
*Significant difference from baseline, $p < 0.05$.

tests investigating overall clinical improvements. Appendix 1, Table S3 provides a comparison of the characteristics of this sample with the previously published RCT.[17]

Linear mixed modelling showed a significant main effect of visit ($F_{2,66.44} = 7.16$, $p = 0.004$), with a decrease in BDI scores over time between the first neurofeedback visit and follow-up (–4.38, 95% confidence interval [CI] –7.91 to –0.84; $t_{31} = -2.52$, $p = 0.02$). In the experimental group, scores at the second rtfMRI-nf visit (–3.81, 95% CI –5.28 to –2.34; $t_{15} = -5.52$, $p < 0.001$, $d = 0.38$) and at follow-up (–8.57, 95% CI –15.12 to –2.59; $t_{13} = -3.06$, $p = 0.009$, $d = 1$) decreased from the first rtfMRI-nf session. In the control group, BDI scores did not change significantly ($t_{21} < -0.55$, $p > 0.58$). The interaction of group × visit was not statistically significant ($p = 0.09$) and the effect size for the interaction was small enough that we did not deem it sufficiently clinically significant to investigate ($f = 0.21$).

None of the participants met BDI criteria for treatment response (i.e., at least 50% decrease in BDI scores) at the time of follow-up. However, 3 participants in the experimental group met the criteria for remission (BDI score < 13), compared with none in the control group ($n = 38$, $\chi^2_1 = 2.10$, $p = 0.15$), and 8 participants in the experimental group achieved a minimal clinically significant difference in BDI scores (defined as a 20% decrease)[28] compared with 4 participants in the control group ($n = 38$, $\chi^2_1 = 2.74$, $p = 0.1$).

Neurofeedback performance

In the contrast of happy versus rest conditions, there was a significant main effect of group ($F_{1,36.06} = 16.17$, $p < 0.001$), and a group × run interaction ($F_{5,279.71} = 2.33$, $p = 0.04$) on signal change in the amygdala (Figure 1A). The experimental group had significantly elevated amygdala activity, compared with the control group, overall (0.21, 95% CI 0.16 to 0.27, $t_{395} = 7.77$, $p < 0.001$, $d = 0.79$) and in every run averaged on both visits except baseline ($t > 2.89$, df < 66, $p < 0.005$, $d > 0.72$). In the experimental group only, all runs averaged on both visits showed increased amygdala activity relative to baseline ($t > 2.01$, df < 27, $p < 0.05$, $d > 0.5$); in the control group, no run was significantly different from the baseline across both visits in the amygdala ($t < -0.81$, df > 34, $p > 0.42$, $d < 0.16$).

This same model showed a significant main effect of group ($F_{1,33.4} = 4.66$, $p = 0.038$) with intraparietal activity as the dependent variable (Figure 1C). The average percent signal change in the parietal region was higher overall in the control group than in the experimental group (0.14%, 95% CI 0.07% to 0.21%; $t_{395} = 3.82$, $p < 0.001$, $d = 0.39$). The group × visit interaction was not statistically significant ($p = 0.06$), and the effect size for the interaction was small enough that we did not deem it sufficiently clinically significant to investigate ($f = 0.2$).

In the contrast of count versus rest, there was a significant main effect of group ($F_{1,39.9} = 8.64$, $p = 0.005$) and run ($F_{5,228.5} = 2.64$, $p = 0.02$) on percent signal change in the amygdala (Figure 1B). The experimental group exhibited significantly lower amygdala activity than the control group (–0.16, 95% CI –0.23 to –0.09; $t_{396} = 4.73$, $p < 0.001$, $d = 0.48$).

This same model, with intraparietal activity as the dependent variable (Figure 1D), showed a significant effect of run ($F_{5,286.18} = 2.51$, $p = 0.03$), reflecting a significant increase in reactivity in the parietal region between transfer and baseline (0.17, 95% CI 0.06 to 0.27; $t_{67} = 3.23$, $p = 0.002$, $d = 0.28$). This increase was evident in both groups.

Association between regional change during happy and count contrasts and clinical change

As shown in Table 2, using linear regression to get residualized scores and subtraction to compute differences, we evaluated the association between change in BDI scores and amygdala signal change during the happy versus rest and count versus rest conditions. Lower residual depressive symptomatology (i.e., more improvement) was associated with increased amygdala reactivity during the happy neurofeedback condition (Appendix 1, Figure S2A). The correlation with intraparietal success was in the opposite direction (Appendix 1, Figure S2D). In the count condition, decreased signal change in the amygdala from baseline to transfer was associated with lower residual depressive symptomatology or clinical improvement (Appendix 1, Figure S2B), with nearly the same strength of association as during the happy neurofeedback condition. Again, the correlation with the percent signal change in the intraparietal region was in the opposite direction (Appendix 1, Figure S2E). Finally, the percent signal change in the amygdala between the happy neurofeedback and count condition was significantly and negatively correlated (Appendix 1, Figure S2C); percent signal change in the intraparietal region was not correlated during the happy and count conditions (Appendix 1, Figure S2F).

To determine whether amygdala activity in the count condition was a mediator of the association between amygdala activity in the happy condition or neurofeedback success and residual BDI changes, we performed a mediation analysis using bootstrapping (obtained via the R mediate function from the psych package), which showed that when neurofeedback success and amygdala activity during the count condition were included in the same model, the effect of neurofeedback success was no longer significant, suggesting that residual amygdala activity during happy and count conditions explain the same part of variance of residual BDI changes (Figure 2).

Discussion

Amygdala neurofeedback resulted in increased affective flexibility in both increasing amygdala reactivity during positive autobiographical happy memory recall, as we have previously observed,[17] and in decreasing amygdala reactivity during a nominally nonemotional task. Upregulating amygdala reactivity and its nonmanipulated downregulation during subsequent cognitive processing explained similar variance in change in depressive symptomatology. These results have implications for the causal role of the amygdala in affective flexibility, for neurofeedback as a clinical intervention and for other clinical interventions.

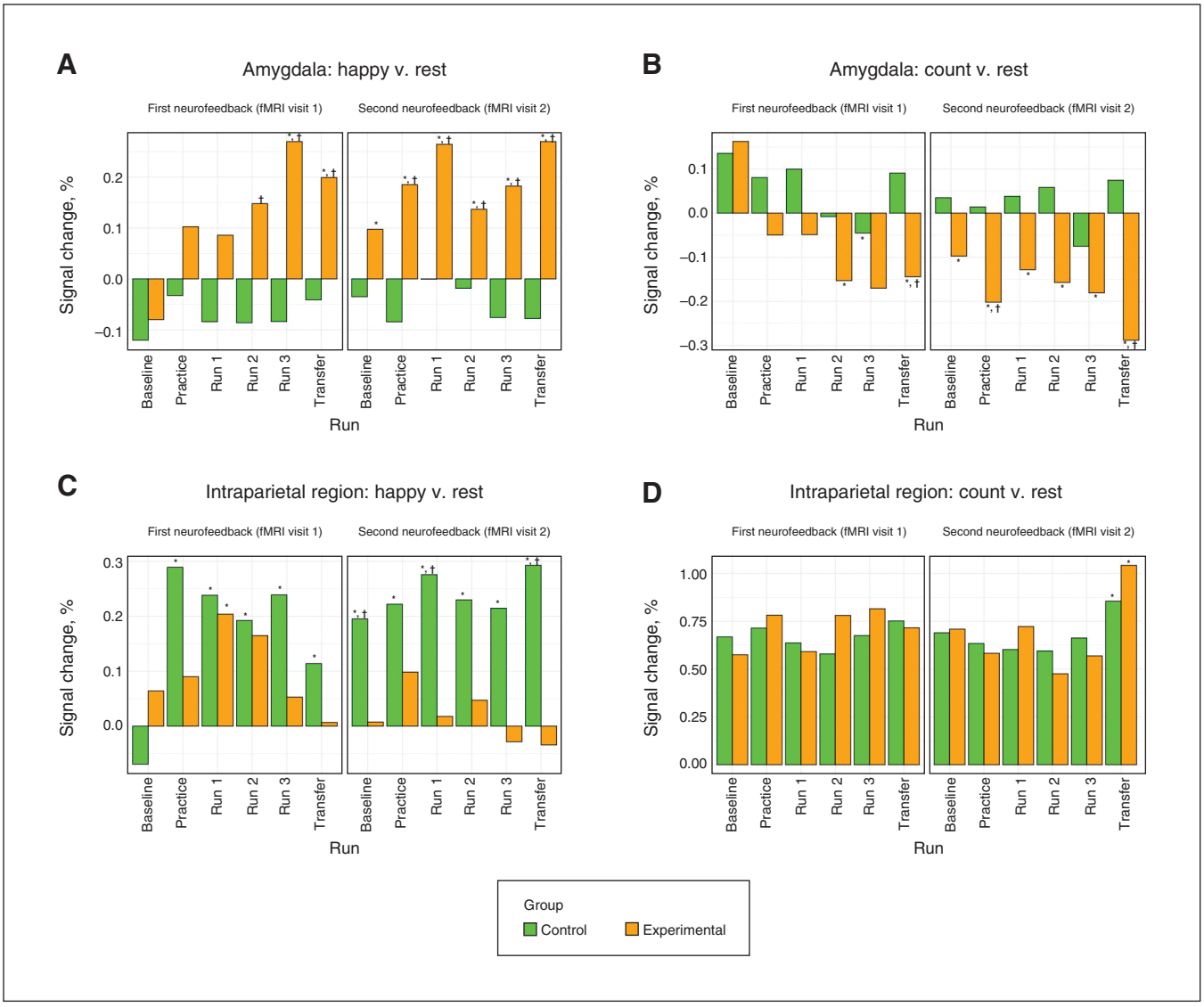

Figure 1: Regional percent signal change for each run of each neurofeedback visit by group for (A) the left amygdala, happy versus rest condition, (B) left amygdala, count versus rest condition, (C) the left horizontal segment of the intraparietal sulcus, happy versus rest condition and (D) the left horizontal segment of the intraparietal sulcus, count versus rest condition. *Significant difference ($p < 0.05$) from the initial baseline run (pre-neurofeedback), computed with paired *t* tests. †Significant difference ($p < 0.05$) in the corresponding run between groups, computed with paired *t* tests. fMRI = functional magnetic resonance imaging.

Table 2: Association between percent signal changes in the amygdala and intraparietal regions in happy and count contrasts and clinical change, computed with residualized scores and differences

	Residualized scores		Differences	
Correlation	*r*	*p* value	*r*	*p* value
BDI scores and happy v. rest signal in the amygdala	–0.35	0.08	–0.47	0.01
BDI scores and happy v. rest signal in the control region	0.47	0.01	0.37	0.06
BDI scores and count v. rest signal in the amygdala	0.35	0.08	0.46	0.01
BDI scores and count v. rest signal in the control region	–0.29	0.13	–0.38	0.05
Count v. rest and happy v. rest signal in the amygdala	–0.66	< 0.001	–0.65	< 0.001
Count v. rest and happy v. rest signal in the control region	–0.002	0.99	–0.06	0.72

BDI = Beck Depression Inventory.

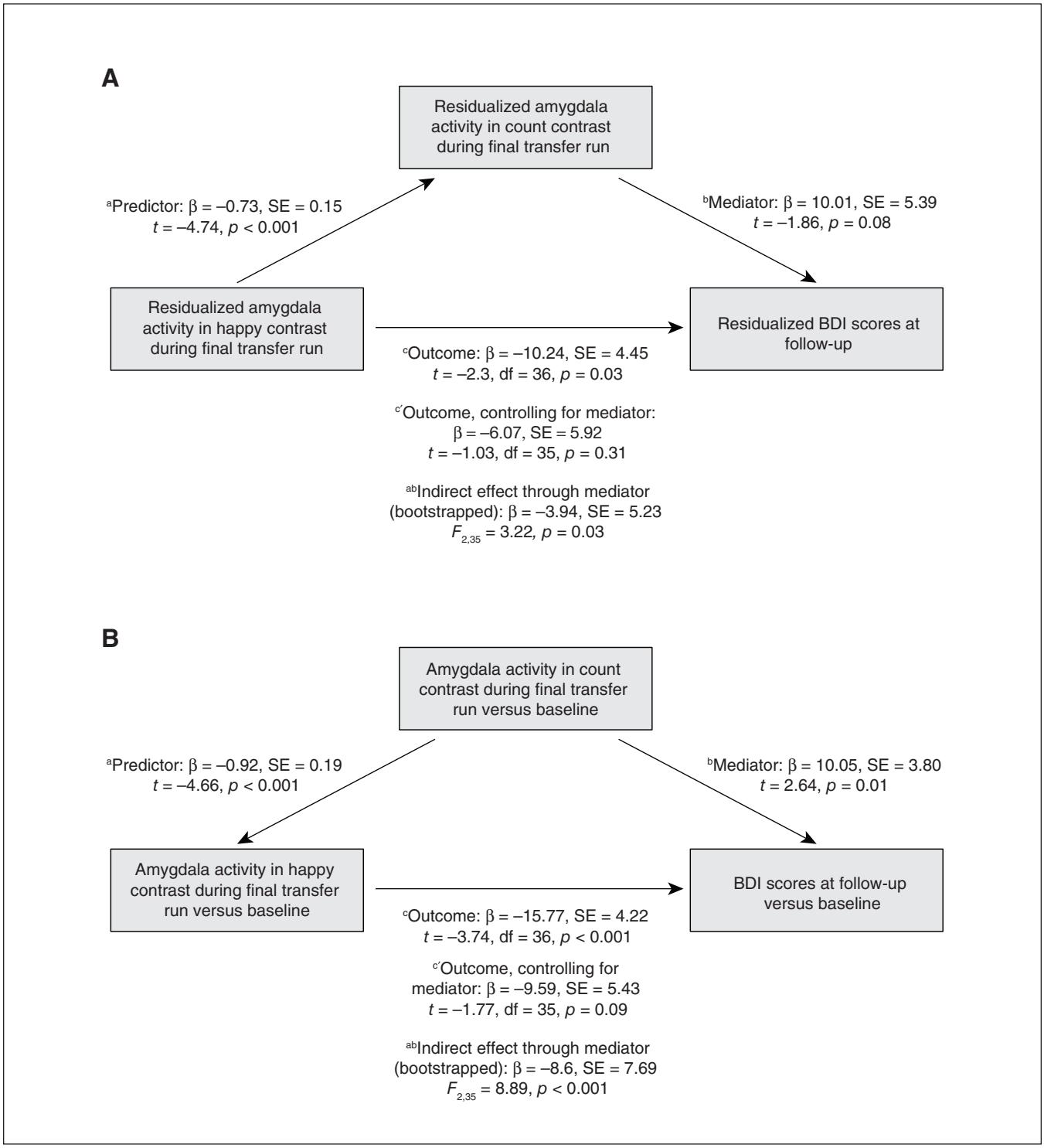

Figure 2: Mediation model between neurofeedback success (percent signal change in the amygdala in the happy condition), percent signal change in amygdala in the count condition and Beck Depression Inventory (BDI) scores at follow-up on (A) residualized scores and (B) differences between transfer or follow-up versus baseline. [a]The predictor is amygdala activity during the final transfer run from baseline in the happy condition or neurofeedback success. [b]The mediator is amygdala activity during the final transfer run from baseline in the count condition. [c]The outcome is change in depressive symptoms (c denotes the relationship between predictor and outcome, and c′ denotes the same relationship after removing the effect of the mediator). [ab]The indirect effect of predictor on outcome through mediator obtained via bootstrapping. Regression analyses using change in BDI score as the dependent variable and amygdala activity changes in either the happy or count condition as the predictor show adjusted R^2 of the same value (0.09), suggesting that both predictors explain the same variance of the dependent variable.

As hypothesized, amygdala activity decreased to below baseline levels during the count condition in the experimental group, in this sample as in the one analyzed in our original RCT (Appendix 1, Box 3).[17] Given that these results were found in the context of a cognitive condition that some participants reported as stressful or negative, with no feedback provided, suggests that the ability of rtfMRI-nf to increase amygdala reactivity to positive information affects flexibility more generally.

We considered 2 other possible interpretations. The count condition could have involved downregulating the amygdala even without neurofeedback. However, the fact that this effect (lower amygdala activity over time in the count condition) was not observed in the control group suggests otherwise. Amygdala training can generalize to other tasks, as has previously been shown in our original RCT.[17] Alternately, decreased amygdala reactivity in the count period among patients in the experimental group could be entirely due to the blood oxygenation level–dependent undershoot generated by the increase in amygdala activity during the happy condition. However, the data in the experimental group were better accounted for by using 2 regressors for the happy and count conditions, rather than just a happy condition regressor with an undershoot alone (Figure S3). This result suggests that the undershoot explanation is not as strong as a 2-process model for the current data.

Thus, the current results regarding the count condition lend further support to the idea that participants are learning adaptive control of their amygdala response, which may generalize to other situations or tasks. Our results specifically suggest that by changing how a brain mechanism functions in response to emotion (i.e., increasing responses to positive stimuli), interventions can also change how it responds during cognitive processing (i.e., decreasing amygdala activity during a counting task). This explanation adds evidence to the contention that common, nominally unidirectional interventions may actually function by increasing flexibility,[3] and may speak to the utility of either personalizing interventions to nominal symptom clusters or developing novel interventions directed at increasing flexibility.

The current work has independent clinical importance as well, as we replicated our previous result that amygdala activity during positive autobiographical memory recall can be increased via neurofeedback training and that this phenomenon is associated with improvements in depressive symptoms, with comparable effect sizes from the first scan to our follow-up assessment ($d = 1$ here and $d = 1.03$ in our previous RCT), despite different sample severities, medication status and anhedonia (see Appendix 1, Table S3 for a comparison of this sample with an unmedicated sample and analyses showing no interaction of the medication status with the effects related to this intervention).[17]

Our results also have implications for neurofeedback trials. They suggest that as the count condition is an active regulatory process, it is not an appropriate baseline from which to calculate neurofeedback values; using the rest condition, as we have done here, is appropriate, rather than the count condition as has been done previously.[29,30] Of additional methodological note, given that the intraparietal neurofeedback condition was associated with worsening depression and that there was a positive correlation between intraparietal neurofeedback success and BDI score change, a sham control condition or comparison to a currently available treatment may be a more optimal control for future studies.

Limitations

We provided only 2 sessions of rtfMRI-nf and the analysis of the amygdala signal in the count versus rest condition showed only a main effect of group. We selected this dosing based on the positive effects of 2 sessions in our previous publications, as well as financial and participant burden considerations. However, given that the correlation between the change in BDI scores and the change in amygdala signal found in the regression could argue for higher dosing, studies varying the number of neurofeedback sessions could help to optimize learning effects and determine whether more sessions would show a group × visit × run interaction and whether a greater number of sessions would induce a more pronounced reduction in symptoms. Anecdotally, during the first session, some participants reported the count condition as being negative. Including assessment of the emotionality of the count condition in future studies could help disambiguate cognitive from emotional effects on the count task. We did not measure accuracy during the count task and therefore cannot know whether this change yielded behavioural improvement during cognitive processing. Finally, results suggest that the benefits associated with this intervention are transferable to other tasks. Nevertheless, for this interpretation to be validated, future studies could also include follow-ups to assess quality of life to determine the extent to which these benefits are transferable to patients' daily lives. It may also be informative to investigate which symptoms or behaviours are affected by the neurofeedback intervention, as well as to include a measure of emotional flexibility to verify that this variable is indeed affected.

Conclusion

We provide evidence that training in upregulation of amygdala reactivity is associated with bidirectional amygdala flexibility, which, in turn, is associated with MDD symptom reduction. The study thus replicates our previous result that fMRI neurofeedback training in upregulation of amygdala activity during positive autobiographical memory recall is associated with clinically important improvements in depressive symptoms, and extends its interpretation to suggest that future research in this paradigm explicitly evaluate flexibility as a mechanism.

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Competing interests: Greg Siegle reports royalties from a patent licensed to Apollo Neuroscience for a device to decrease stress during cognitive tasks and patents for reactive vibroacoustic stimulation (WO2017173436A1 PCT/US2017/025702 16/596351). He is senior advisor to the Neurocognitive Therapies and Translational Research special interest group of the Association for Behavioral and Cognitive Therapy.

Contributors: Greg Siegle, Theodore Huppert and Kymberly Young contributed to the conception and design of the work. Sair Lazzaro, Marlene Strege, Gia Canovali and Scott Barb contributed to data acquisition. Laurie Compère, Greg Siegle, Scott Barb and Kymberly Young contributed to data analysis. Laurie Compère, Greg Siegle, Sair Lazzaro and Kymberly Young contributed to interpretation of data. Laurie Compère drafted the manuscript. All of the authors drafted the manuscript, revised it critically for important intellectual content, gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Data sharing: All of the individual participant data collected during the trial after deidentification are available through the National Institute of Mental Health Data Archive (https://nda.nih.gov).

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