Real-Time Functional Magnetic Resonance Imaging Amygdala Neurofeedback Changes Positive Information Processing in Major Depressive Disorder

Supplement

Supplementary Methods

Real-Time fMRI Neurofeedback Paradigm

The rtfMRI-nf amygdala emotional training protocol has been previously implemented (1, 2). The amygdala and intraparietal regions were defined as spheres of 7mm radius in the stereotaxic array of Talairach and Tournoux (3). Participants were informed that they would be assigned to receive neurofeedback from one of two brain regions; one region involved in emotional processing or another region independent of emotional processing which may be difficult to regulate. They were informed to maintain the strategy of positive memory recall even if they felt it was ineffective at raising their brain activity, though they could change the positive memories utilized or the aspects of the memories focused on. Upon completion of Visit 4, participants were informed as to which condition they were assigned, and participants in the intraparietal rtfMRI-nf condition were offered the opportunity to return to the lab to repeat the rtfMRI-nf experiment with the amygdala as the target region-of-interest.

The selection of a control task for rtfMRI-nf experiments is challenging, and no consensus has yet been reached as to the optimal approach. Studies utilizing out of scanner control conditions (4), control conditions in which the neurofeedback bar remains static (5), or no control condition (examining only within-subject changes; (6, 7)) run the substantial risk of false positives as control participants know they are not receiving feedback, and experimenter blinding is impossible. Therefore improvements evident in the active relative to the control group may be due to experimenter bias or the appeal of a novel, technology-based intervention and not to gaining control over the target region. Sham control conditions in which the neurofeedback signal is either artificially created or derived from other participants' data (8) run the risk of participants detecting the non-contingency between their efforts and the resulting neurofeedback signal thereby discouraging performance. Control conditions using neurofeedback from a different region are best suited to determine a) specificity of the

procedure; whether feedback from the target region is necessary for enhanced control of that region and b) whether changes in mood ratings are due to feedback from the target region or due to a placebo effect. Therefore, for our rtfMRI-nf protocol, we employed a control condition in which subjects received rtfMRI-nf from the horizontal segment of the intraparietal sulcus while recalling positive autobiographical memories, a region implicated in number and not in emotional processing and which is independent of amygdala activity (9-12).

For each of the Rest, Happy, and Count blocks within a run, cues were presented on the screen using both text and color icons to indicate each condition. During the Happy Memory Condition, the cue “Happy” and two color bars (red, blue) were displayed on the screen. The red bar represented the actual neurofeedback signal, which was updated continuously by changing the height of the bar either upwards or downward based on the corresponding level of BOLD activity. This neurofeedback signal was also indicated by a number shown above the red bar representing the percent signal change within the target region. During this condition, participants were instructed to retrieve and contemplate positive autobiographical memories while also attempting to increase the level of the red bar to the fixed target level displayed by the blue bar. Because the Happy Memories condition required memory recall and rumination on those memories could potentially not be stopped quickly (13), two control conditions were implemented to distract participants’ attention from contemplating positive memories and to dampen the activation of the emotion regulation network. During the Count condition, the participants were shown the cue “Count” with the specific instruction to count backwards from 300 by subtracting a specified integer (9, 3, 4, 6, 7, and 9 for Baseline, Practice, Run 1, Run 2, Run 3, and the Transfer run, respectively). During the Rest condition, participants were presented with the cue “Rest” and were asked to relax and breathe regularly while looking at the display screen. No bars were displayed during the Count and Rest conditions.

The rtfMRI-nf procedure consisted of eight fMRI runs each lasting 8 minutes and 40 seconds; a resting run, a baseline run in which no neurofeedback information was provided, a

practice run, three training runs, a final transfer run in which no neurofeedback information was provided, and a final Rest run. During the Rest runs, a resting-state paradigm was employed and participants were instructed to clear their minds and not think of anything in particular while fixating on the display screen. All subsequent runs consisted of alternating blocks of Rest (5 blocks lasting 40 seconds each), Count (4 blocks lasting 40 seconds each), and Happy (4 blocks lasting 40 seconds each). The Baseline run served as a measure of amygdala activity during positive memory recall prior to rtfMRI-nf training. Participants were instructed simply to recall positive memories when the cue “Happy” appeared. No bars were presented. During the Practice run, participants were given an opportunity to become comfortable with the neurofeedback procedure. For the first three Happy Memory blocks participants were instructed to recall and contemplate positive memories prepared with help from the experimenter prior to entering the fMRI environment, and then, for the last Happy condition block, to use the one memory that elevated their mood to the greatest extent. Thus, the Practice run allowed participants to accommodate to the neurofeedback task and evaluate the emotional impact of the prepared happy memories within the experimental setting. During the subsequent three Training runs participants were encouraged to use various memories and to switch memories in order to help them raise the red bar. Because our preliminary experiments indicated that the activation level of the left amygdala could be as high as a 2% BOLD signal change, the target level of the blue bar was set to 0.5%, 1.0%, 1.5% and 2.0% for PR, R1, R2, and R3, respectively. During the Transfer Run, participants were instructed to perform the same task as during neurofeedback training, but rtfMRI-nf information was not provided. The transfer run was performed to assess the transfer of the learned control and to check whether the training effect generalized to situations where no neurofeedback was available. The procedure on Visit 3 was identical to that on Visit 2.

Data Acquisition and Online Analysis

A standard 8-channel receive-only head coil array was used for fMRI data collection. A single-shot gradient-recalled EPI sequence with Sensitivity Encoding (SENSE) was employed for fMRI. The following EPI imaging parameters were used: field-of-view/slice=240/2.9mm, axial slices per volume=34, acquisition matrix=96x96, repetition/echo time=2000/30 ms, SENSE acceleration factor R=2 in the phase encoding (anterior-posterior) direction, flip angle=90°, sampling bandwidth=250 kHz, number of volumes=263. Three EPI volumes (6 sec) were added at the beginning of each fMRI run to allow the fMRI signal to reach steady state, and were excluded from data analysis. The EPI images were reconstructed into a 128x128 matrix, in which the resulting fMRI voxel volume was 1.875x1.875x2.9mm^3. Additionally, simultaneous pulseoximetry and respiration waveforms were recorded (with 50 Hz sampling) for each fMRI run. A T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence with SENSE was used to provide an anatomical reference for the fMRI analysis. It had the following parameters: field-of-view=240mm, axial slices per slab=128, slice thickness=1.2 mm, image matrix=256x256, repetition/echo time=5/1.9ms, acceleration factor R=2, flip angle=10°, delay/inversion time=1400/725 ms, sampling bandwidth=31.2 kHz.

The image data analyses were performed using Analysis of Functional NeuroImages (AFNI, http://afni.nimh.nih.gov/). The neurofeedback was implemented using the custom real-time fMRI system utilizing the real-time features of AFNI (14) and a custom developed graphic user interface (GUI) software. The regions-of-interest, defined as described above, were transformed to the EPI image space using each subject's high-resolution MPRAGE structural data. The resulting regions-of-interest in the EPI space contained approximately 140 voxels each. We performed a visual inspection of the regions-of-interest (both the intraparietal and amygdala regions in all participants to maintain the blind) prior to the start of neurofeedback.

Supplementary Results

Neurofeedback Performance

Paired-sample t-tests were performed to examine whether activity significantly increased from the pre-neurofeedback baseline run in each group and run. In the control group, there was no run in which *amygdala* activity significantly differed from baseline ($ts(14)<1.14$, $ps>0.27$ $ds<0.21$). In the experimental group amygdala activity during the Visit 2 practice run was not significantly elevated above baseline ($ts(17)=1.74$, $p=0.10$, $d=0.28$) while all subsequent runs (including Visit 3 baseline and both transfer runs) were ($ts(17)>2.67$, $ps<0.02$, $ds>0.61$). In the *intraparietal* region, the control group significantly increased intraparietal activity from baseline during run 3 and the final transfer run at Visit 3 ($ts(14)>2.42$, $ps<0.02$, $ds>0.69$). In the experimental group, there was no run in which intraparietal activity differed from baseline ($ts(17)<1.67$, $ps>0.12$, $ds<0.36$). An independent-samples t-test comparing neurofeedback success in the experimental group (for amygdala activity) versus the control group (for intraparietal activity) was not significant ($t(31)=1.49$, $p=0.15$, $d=0.21$), indicating that by study end the control group was as effective at regulating hemodynamic activity in the intraparietal region as the experimental group was at regulating activity in the amygdala.

Despite equivalent neurofeedback success with the assigned region, only in the experimental group did depressive symptoms significantly diminish (See Table S1). Furthermore, residual MADRS scores at the final visit were significantly correlated with residual amygdala activity during the final transfer run ($\beta=-15.5$, $t=3.09$, $p=0.004$; adjusted $R^2=0.21$). While the association with intraparietal success was in the same direction, this association was not significant ($\beta=-2.46$, $t=0.81$, $p=0.43$; adjusted $R^2=0.09$) and was significantly different from the model examining the association between residual *amygdala* activity and residual MADRS scores ($z=2.66$, $p=0.004$). Collectively, these results support the hypothesis that enhanced

control of amygdala activity led to the clinical effects, and not simply gaining control over hemodynamic activity more generally.

Psychobiological Correlates with Neurofeedback Success

Examining correlates of neurofeedback success could help guide future efforts towards understanding who this treatment might be effective for. Therefore, we examined correlations between neurofeedback success in the experimental group and performance on the ETB, baseline amygdala activity during the BMT, and demographic factors. No significant correlations were found between baseline performance on any ETB measure (largest $r=0.16$, $p=0.37$) or baseline amygdala activity (largest $r=0.26$, $p=0.13$). With respect to demographic factors, we obtained evidence compatible with our previous findings (2) of a negative correlation between neurofeedback success and both the “Difficulty Describing Feelings” subscale of the TAS ($r=0.33$, $p=0.059$) and the length of the current depressive episode ($r=-0.35$, $p=0.042$).

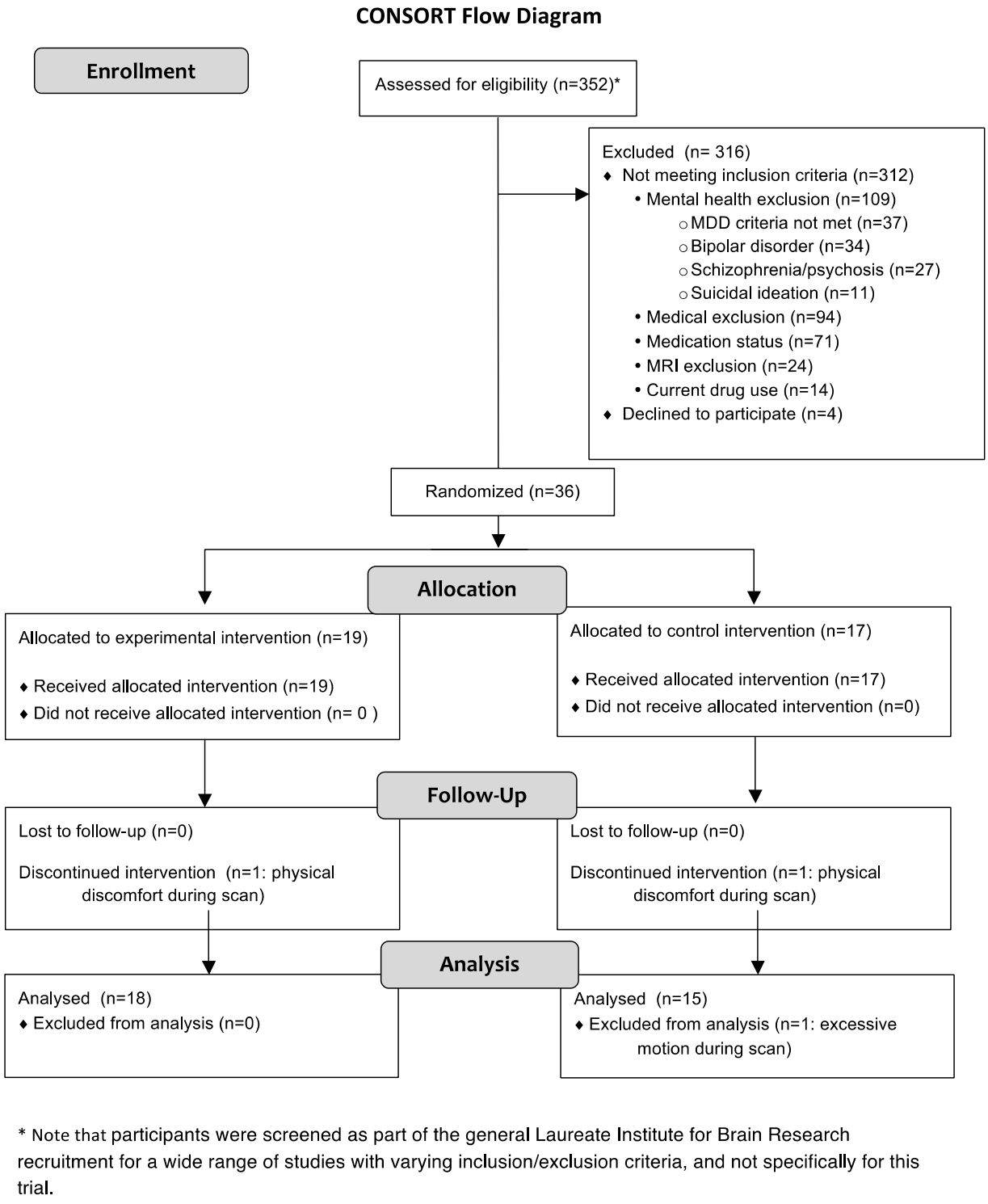

Figure S1: CONSORT Flow Diagram. Flow diagram of the progress through the phases of the parallel randomized clinical trial of two groups, including enrollment, intervention, allocation, follow-up, and data analysis.

Table S1: Clinical and Demographic Characteristics for Each Group

Sample Characteristics		
	Experimental Group	**Control Group**
n [n female]	18 [12]	16 [12]
Age	32 (12)	31 (9)
MADRS		
Baseline	24 (10)	24 (7)
Follow-up	12 (9)	22 (8)*
Change Effect Size (d)	1.89	0.30
HDRS-21		
Baseline	19 (8)	19 (5)
Follow-up	10 (7)	17 (5)*
Change Effect Size (d)	1.84	0.42
BDI-II		
Baseline	27 (11)	27 (13)
Follow-up	16 (10)	24 (12)*
Change Effect Size (d)	1.87	0.24
MDE Length in Months	30 (56)	34 (49)
Number of Episodes		
1	5 (28%)	2 (13%)
2	2 (11%)	2 (13%)
3 or more	11 (61%)	12 (67%)
Previous Number of Antidepressants		
None	7 (39%)	4 (25%)
1-2	6 (33%)	7 (44%)
3 or more	5 (28%)	5 (31%)
Time since Last Antidepressant (months)	33 (34)	31 (35)
Co-Morbid Diagnosis		
None	6 (33%)	7 (44%)
PTSD	6 (33%)	3 (19%)
GAD	5 (28%)	7 (44%)
Social Phobia	3 (17%)	5 (31%)

Numbers in parentheses indicate one standard deviation of the mean, except where a percent sign is present indicating the percent of participants that fall into that category.

* indicates a significant difference from the experimental group at $p<0.05$.

Abbreviations: BDI-II = Beck Depression Inventory; GAD = generalized anxiety disorder; HDRS-21 = 21 item Hamilton Depression Ratings Scale; MADRS = Montgomery-Asberg Depression Rating Scale; MDE = major depressive episode; PTSD = posttraumatic stress disorder

Table S2: Comparison of Happy and Surprise Faces during the FERT

	Accuracy				Reaction Time			
	Surprise	*Happy*	*t-value*	*p value**	*Surprise*	*Happy*	*t-value*	*p value**
Baseline								
active group	70.8 (6.53)	72.5 (4.46)	0.91	0.37	1668 (223)	1658 (332)	0.11	0.92
control group	74.8 (5.87)	77.9 (6.00)	1.48	0.15	1639 (170)	1690 (233)	0.71	0.49
combined	72.8 (6.61)	75.2 (5.95)	1.57	0.12	1654 (208)	1674 (313)	0.31	0.76
Follow-up								
active group	73.8 (5.64)	76.1 (6.09)	1.18	0.25	1412 (277)	1430 (368)	0.17	0.87
control group	74.9 (6.64)	78.4 (5.55)	1.62	0.12	1614 (199)	1665 (265)	0.62	0.54
combined	74.4 (5.89)	77.3 (6.86)	1.70	0.10	1513 (241)	1548 (319)	0.51	0.61
Change from Baseline								
active group	3.00 (6.19)	3.60 (5.97)	0.30	0.77	-256 (192)	-228 (254)	0.37	0.71
control group	0.10 (6.94)	0.5 (7.95)	0.15	0.88	-25 (151)	-25 (211)	0.00	1.00
combined	1.6 (6.44)	2.1 (6.89)	0.31	0.76	-141 (193)	-126 (262)	0.27	0.79

* Note that in no case was the difference between happy and sad faces significant, providing further justification for combining these data into a single positive faces variable

Table S3: Regions Where Hemodynamic Activity Differed between Groups in the Change from Baseline to Follow-up in Response to Implicitly Presented Sad (SN-NN) and Happy (HN-NN) Face Presentation

Area	x, y, z[a]	Cluster Size[b]	F-value	Percent Signal Change	
				Experimental Group	*Control Group*
HN-NN faces					
R middle frontal G / BA 9	34, 32, 25	62	16.2	0.05 (0.01)	0.01 (0.01)
L ACC	-18 32, 2	52	13.9	0.05 (0.01)	-0.07 (0.02)
R PCC	4, -34, 23	57	17.1	-0.06 (0.01)	0.01 (0.02)
L anterior insula	-31, 10, 18	45	11.8	0.05 (0.01)	-0.03 (0.01)
R anterior insula	39, 11, 9	132	12.3	0.10 (0.02)	-0.01 (0.01)
L superior temporal G	-55, -36, 11	61	12.5	0.05 (0.01)	-0.04 (0.02)
SN-NN faces					
R PCC	22, -46, 27	36	11.4	-0.04 (0.01)	0.01 (0.01)
R caudate	29, -43, 13	129	15.7	0.04 (0.01)	0.01 (0.01)
L globus pallidus	-24, -17, 2	38	6.03	0.05 (0.01)	-0.01 (0.01)
R thalamus	15, -29, 14	59	13.1	0.05 (0.01)	0.01 (0.01)
L precuneus	-18, -41, 46	56	11.5	-0.05 (0.01)	-0.02 (0.01)

[a]Coordinates correspond to the stereotaxic array by Talairach and Tournoux (3).

[b]Cluster size refers to the number of contiguous voxels for which the voxel t statistic corresponds to $p_{corrected}<0.05$.

Abbreviations: ACC = anterior cingulate cortex; BA = Brodmann area; G = gyrus; L= left; PCC = posterior cingulate cortex; R= right

Supplementary References

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