



Supporting Online Material for

Prefrontal Regions Orchestrate Suppression of Emotional Memories via a Two-Phase Process

Brendan E. Depue,* Tim Curran, Marie T. Banich

*To whom correspondence should be addressed. E-mail: depue@colorado.edu

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Supporting Materials

S1: Methods

Participants

Eighteen English-speaking adults (N7 women) from 19-29 years of age participated in the study. Two participants (N1 female) were omitted from further analyses because of scanner malfunction or non-full head brain coverage, leaving a final N of 16.

Procedure

Anderson and Green's (2001) Think/No-Think paradigm was utilized using face-picture pairs (S1). Forty faces (female) previously normalized as having a neutral expression were used. Forty images were selected from the International Affective Picture Series (IAPS), negative in emotional content (S2). Pictures were selected at a median level of negative affect on a scale of 1-9 (mean = 4.1, SD = .55). Due to the IAPS having no relatedness scores, two independent raters selected pictures to have as minimal relatedness in content as possible, in order to eliminate potential grouping effects. The experiment was designed with E-Prime software, which was used to display the stimuli and record performance on a Dell laptop computer.

The experimental procedure was divided into three phases: training, experimental, and testing. In the training phase, participants learned to remember 40 face-picture pairs, which were displayed for 4 seconds. Participants first viewed each pair and, after 20 pairs, were shown a face and asked to select which of two pictures was originally paired with the face. Both pictures came from the training phase so that novelty of one choice could not be used as a potential cue for recognition. This procedure continued in sets of 20 until the participant could recognize the correct

picture previously paired with a face with 97.5% accuracy (39 items) over all 40 pairs (average training cycles: $M = 2.06$, $SD = .41$). In the experimental phase, participants saw the face for only 32 of the 40 pairs, half of these being relegated to the Think Condition, and half to the No-Think condition. In both conditions, a trial consisted of a face for 3.5 seconds, and then a 500 ms inter-trial interval. The color of a border around the faces indicated the condition: green for Think trials and red for No-Think trials.

Eighty fixation trials (4 sec) served as a low level baseline against which to compare experimental trials. These trials were pseudo-randomly interspersed throughout the course of the experimental phase. The pseudo-random trial design was “optimized” according to Wager and Nichols’ methodology for complex event related MR studies using more than one trial type (S3). This procedure ensures the maximal amount of “jitter” is instituted through experimental design rather than by using variable trial timing, which is difficult to institute in event-related design with a variety of trial types whose proportions cannot be predetermined, due to final accuracy. To ensure our deconvolution process did not result in activation bleeding from adjacent trials, we used FSL’s FLOBS (Analysis group, FMRIB, Oxford, UK, <http://www.fmrib.ox.ac.uk/fsl/>) to optimize our hemo-dynamic response function (HRF). FLOBS was used in incremental time steps (.5 secs) ranging from .5 – 2 seconds without any indication that our “original” HRF was insufficient.

Similar to Anderson and Green, in the Think condition, participants were told “Think of the picture previously associated with the face”, whereas in the No-Think condition they were told “Do not to let the previously associated picture come into consciousness” (S1). Within each condition (Think/No-Think), participants viewed the faces 12 times. The 8 faces not shown in the experimental phase served as a 0-

repetition behavioral baseline. During the experimental condition a video camera was used to view the participant's eye gaze to ensure that individuals did not simply shut their eyes or "look away" from the stimuli.

During the test phase, participants were shown each of the faces and told to write down a 3-5 word description of the picture associated with it. These descriptions were then scored as correct or incorrect by two independent judges (inter-rater reliability was .98). Because the IAPS pictures were carefully selected to minimize grouping effects, the rating of correct and incorrect was relatively simple to discern. If participants clearly remembered the picture with 3-5 words describing it, the picture was scored "remembered", whereas if the participants had no recollection or described the picture incorrectly, it was scored "forgotten". If there was not agreement between raters for an item, it was removed from the data set. These data provided the accuracy measures.

S2: Image acquisition and analysis

Image Acquisition

Functional MRI was performed on a 3-T GE scanner to acquire BOLD (blood oxygenation level-dependent) contrast using gradient echo T2*-weighted echoplanar imaging (EPI); (repetition time = 2000 ms; 256 mm field of vision, 64 x 64 matrix, 29 slices, 4-mm slice thickness, 0-mm slice gap; flip angle = 90°). Slices were oriented obliquely along the AC-PC line. The first four volumes from each run were discarded to allow for T1 equilibration effects. Additionally, two separate T1-weighted high-resolution structural scans were acquired in each subject for subsequent anatomic localization. Head movement was minimized using a custom-fitted head holder, consisting of polyurethane foam beads inflated to tightly mold around the head and neck.

Image Analysis

Data sets from 16 of our 18 subjects met our criteria for high quality and scan stability with minimum motion correction (< 2 mm displacement in any one direction) and were subsequently included in our fMRI analyses. Image processing and data analysis were performed using the FMRIB software library package FSL (Analysis group, FMRIB, Oxford, UK, <http://www.fmrib.ox.ac.uk/fsl/>). Standard pre-processing was applied; MCFLIRT – slice time correction/motion correction, BET – brain extraction, time-series prewhitening, registration and spatial normalization to the Montreal Neurological Institute (MNI) high-resolution 152-T1 template. Images were resampled into this space with 3-mm isotropic voxels and smoothed with a Gaussian kernel of 8-mm full-width at half-maximum to minimize noise and residual differences in gyral anatomy, resulting in an effective spatial resolution of 10.2 x 10.7 x 11.5 mm. Each normalized image was band-pass filtered (high-pass filter = 40 sec) to remove high frequency noise. FMRIB's improved linear model (FILM) was then applied from which statistical inferences were based on the theory of random Gaussian fields, and changes relative to the experimental conditions were modeled by convolution of single trial epochs with the canonical HRF to approximate the activation patterns (S4). Using multiple regression analysis, statistical maps representing the association between the observed time series (e.g., BOLD signal) and one or a linear combination of regressors for each subject were constructed. Group analysis was performed using the FMRIB software library package FSL's (Analysis group, FMRIB, Oxford, UK, <http://www.fmrib.ox.ac.uk/fsl/>) higher level FEAT analysis tool to yielded statistical parameter maps (SPMs) in which all subsequent analyses were performed. SPMs were thresholded on a voxel-wise basis at $Z_{2.81}$, $p.005$. To adjust for false positive errors on an area of activation basis, a cluster-wise

threshold was set at $p.05$ cluster size 120 as determined by Analysis of Functional Neuroimages' (AfNI) AlphaSim. This was determined by AlphaSim and the current literature regarding false positive activations in brain imaging data (S5, S6).

S3: Percent signal change confirmatory analysis

Percent signal change (ΔS) analyses were performed using FSL's (Analysis group, FMRIB, Oxford, UK, <http://www.fmrib.ox.ac.uk/fsl/>) Featquery signal change processing tool. Featquery was used to interrogate ΔS of a priori regions of interest (ROIs) previously defined by the literature reviewed. A priori ROIs included: medial frontal gyrus (mFG), middle frontal gyrus (MFG), superior frontal gyrus (SFG), inferior frontal gyrus (IFG), primary visual cortex (BA17), amygdala, and hippocampus. Areas outside of a priori regions were selected from SPMs (see section S2) at an increased threshold of $Z3.01$, $p.001$. These ROIs included thalamic nuclei and the fusiform gyrus. Next, associated ΔS was calculated using a 2mm^3 sphere (within data space) around the peak of activation within the ROIs based on our results of NT>T general SPMs. These peak based spheres were then interrogated within our modeled experimental paradigm to examine differences between NT and T conditions versus a fixation baseline. Parameter estimates were then converted to ΔS values before reporting. This is achieved by dividing the PE/COPE values by the mean image from filtered_func_data. These analyses yielded mean, maximum, minimum statistical values of ΔS across the time series within these ROIs for all subsequent analyses.

Supporting Material S4: Overall contrast and brain activation tables

This section shows the resultant brain imaging data from the overall analysis of NT>T trials regardless of recall accuracy. These analyses provide evidence that our constrained analyses (NTf>Tr) yielded similar results and did not select items that

may have been more visually stimulating or perceptually different on an individual basis. The overall similarity of results is shown in brain images selected with corresponding spatial relation to the results presented in the main body of the paper (Fig S1). SPMs were thresholded on a voxel-wise basis at $Z_{2.81}$, $p_{.005}$. To adjust for false positive errors on an area of activation basis, a cluster-wise threshold was set at $p_{.05}$ cluster size 120 as determined by AFNI's AlphaSim.

The following tables show the brain activations of both analyses (NT>T overall and NT forgotten>T remembered; Table S1; Table S2) as yielded by SPMs that were thresholded on a voxel-wise basis at $Z_{2.81}$, $p_{.005}$ and adjusting for false positive errors on an area of activation basis, cluster-wise threshold set at $p_{.05}$ cluster size 120 as determined by AFNI's AlphaSim.

The overall contrast, as compared to the constrained contrast (NTf>Tr), suggests that the brain regions involved in emotional memory suppression are remarkably similar whether or not trial inclusion is based on recall accuracy. Although, brain regions that reach significance in the two analyses overlap in BA area or gyral association, there are differences in specific anatomical location. Specific examples are apparent in prefrontal areas (SFG, MFG, mFG, and IFG), these areas are activated in both analyses yet specific anatomical proximity varies. We suggest that this is due to two primary reasons: (i) the increased variance associated with including approximately 40% more trials (NTr and Tf), and (ii) that the trials included (NTr and Tf) are not entirely related to successful suppression. These two factors likely include increased variance in the overall contrast that may shift anatomical localization of specific clusters/peaks of brain activation. That being noted, we feel that the specific localization of brain areas/clusters is most accurate in the condition in which

suppression is successful (NTf) and elaboration is successful (Tr), thus our analyses included in the main body of the text reflects that contrast (NTF>Tr).

Supporting Material S5: Correlational and time course analysis for brain regions

Correlational analyses were performed using standard Pearson correlation coefficient analysis and Pearson correlation significance on ΔS values provided by section S3 to examine the association between activity in different brain regions. Testing of Pearson correlation coefficient significance against other correlation coefficients was performed by Fisher's Z. Time course analyses were performed by linearly plotting signal change values for a given region across the four quartiles. Furthermore, analyses of ΔS values for NT trials were tested using a paired sample *t*-test against fixation baseline at each quartile for each ROI to assess significance.

Supporting Material S6: Hippocampal Activity Differentiates Behavioral Success

The present analyses were designed to corroborate the decrease in hippocampal activity as there has been debate in the recent literature about the degree to which the hippocampus may be activated during a fixation baseline (S7). These supporting analyses are not provided for other brain areas, because as far as we know, the decreases in activity below a fixation baseline that we observed in other brain areas are not common and hence can be more securely interpreted as suppression of activity.

Here we further provide evidence corroborating the idea that suppression of hippocampal activity is a critical mechanism in memory suppression. To establish this, we examined the percentage signal change of the hippocampal region indicated by the mask in Figure S2A. A three way ANOVA of condition (T, NT) x quartile (1,

2, 3, 4) x recall (forget, remember) (Fig S3) yielded a main effect of condition [$F(15)29.3, p.008$] and a main effect of recall [$F(15)6.9, p.009$], suggesting that hippocampal activity was less for NT than T trials as well as less for forgotten than remembered trials. The lower activity for NTf than Tf trials suggests that NT trials involve an active suppression mechanism. If no such mechanism were invoked, one would predict that hippocampal activity would be equivalent on these trial types as both have the same outcome: they are forgotten. A 2-way ANOVA restricted to NT trials with condition (NTf, NTr) x quartile (1, 2, 3, 4) yielded a trend for the main effect of condition [$F(15)2.64, p.06$], a main effect of quartile [$F(15)2.89, p.04$] and a trend for the interaction [$F(15)1.98, p.08$]. The main effect of condition replicates Anderson et al.'s result that NTf trials exhibit higher hippocampal activity than NTr trials. Our temporal analyses extends these findings by illustrating that this increased hippocampal activity occurs only during the first quartile while NTf trials exhibit less activity than NTr during the 3rd and 4th quartiles after repeated attempts at suppression.

Finally, we performed a 2x2 (NT vs T; forgotten vs remembered) ANOVA for hippocampal activity for just the final quartile. This analysis yielded two main effects [main effect of NT vs T; $F(63) 4.7, p.03$; main effect of forgotten vs remembered; $F(63) 20.12, p.00003$]. Paired t-tests indicated that ΔS in NTf trials (Fig S2B) was significantly *less* than NTr trials (Fig S2C); [$t(15)-2.08, p.02$]. This finding indicates that successful suppression of memory is associated with a significant modulation of hippocampal activity. We also observed a similar relationship for T trials in that hippocampal activity was significantly *greater* on Tr trials (Fig S2D) than Tf trials (Fig S2E); [$t(15)-3.78, p.002$]. These results suggest that the degree of hippocampal

activity indexes the strength of the memory representation that allows for or precludes subsequent recall.

Supporting Material S7: Correlational Analysis with Behavior

To further explore the nature of the suppressive mechanism that leads to decreased recall on NT trials, we created a behavioral suppression index for each participant. This index was the percentage recall on NT trials minus the percentage recall from baseline trials thus, the greater the value of this suppression index, the greater an individual's ability to suppress information on NT trials. We then performed a whole brain analysis (across the entire time course) to determine which brain region's activity correlated with the suppression index; SPMs were thresholded on a voxel-wise basis at $Z_{2.81}$, $p.005$. To adjust for false positive errors, a cluster-wise threshold was set at $p.05$ cluster size 120 as determined by AFNI's AlphaSim. The region that yielded a significant correlation was rMFG, such that increased activity in rMFG was associated with a larger suppression index (Fig S4). For the fourth quartile only, we found that decreased hippocampal activity predicted increased behavioral suppression (Fig S4). Furthermore, activity in rMFG correlated with decreased activity in the hippocampus (discussed in main paper). These findings suggest that the hippocampal deactivation observed on NTf trials results from cognitive control by prefrontal regions. We also include maximal correlations with behavioral suppression for each of the 3 brain regions within each phase (Fig S5), which illustrate that activity in rMFG and the hippocampus have the highest association with behavioral suppression.

S8: Correlation Matrix

This table presents the correlations coefficients of the association in activity across relevant brain regions (Table S3). Coefficients were determined quartile by

quartile and the highest observed correlation coefficient across all the quartiles is shown as the associations between rIFG and rMFG with posterior regions varied by quartile.

S9: Outlier Analysis

In order to address the potential that outlier might affect the correlations discussed in S8, we calculated, across participants, the range for ± 3 standard deviations away from the mean for ΔS across for each brain region (Table S4). Because no participant's ΔS fell above or below three standard deviations, no additional analyses were performed.

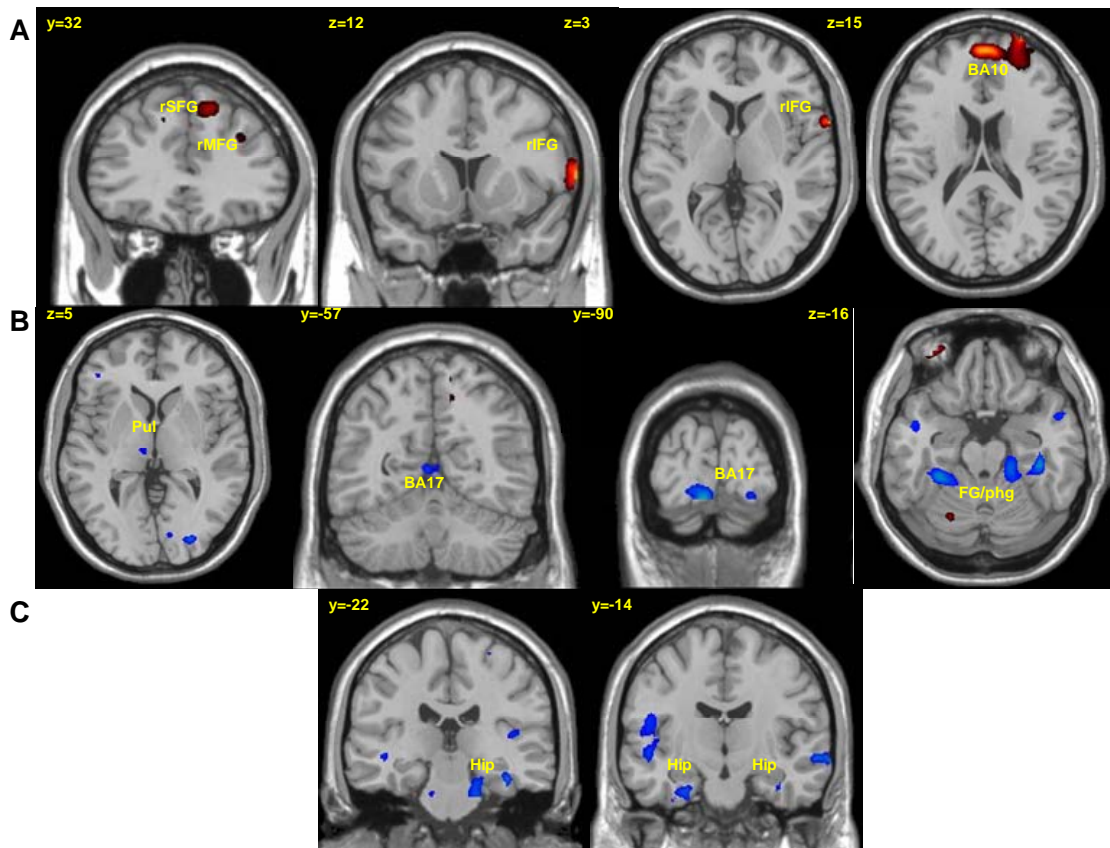


Fig. S1 Functional activation of brain areas involved in (A) cognitive control, (B) sensory representation of memory, and (C) memory processes. [rSFG=right Superior Frontal Gyrus, rMFG=right Middle Frontal Gyrus, rIFG=right Inferior Frontal Gyrus, phg=parahippocampal gyrus, Pul=pulvinar, FG=fusiform gyrus, Hip=hippocampus, Amy=amygdala]. Red indicates regions that exhibit greater activity for NT than T trials. Blue indicates regions that show significantly greater activity for T trials than NT trials. Conjunction analyses revealed that areas shown in Blue demonstrate increased activity for T trials above baseline *and also* decreased activity of NT trials below baseline.

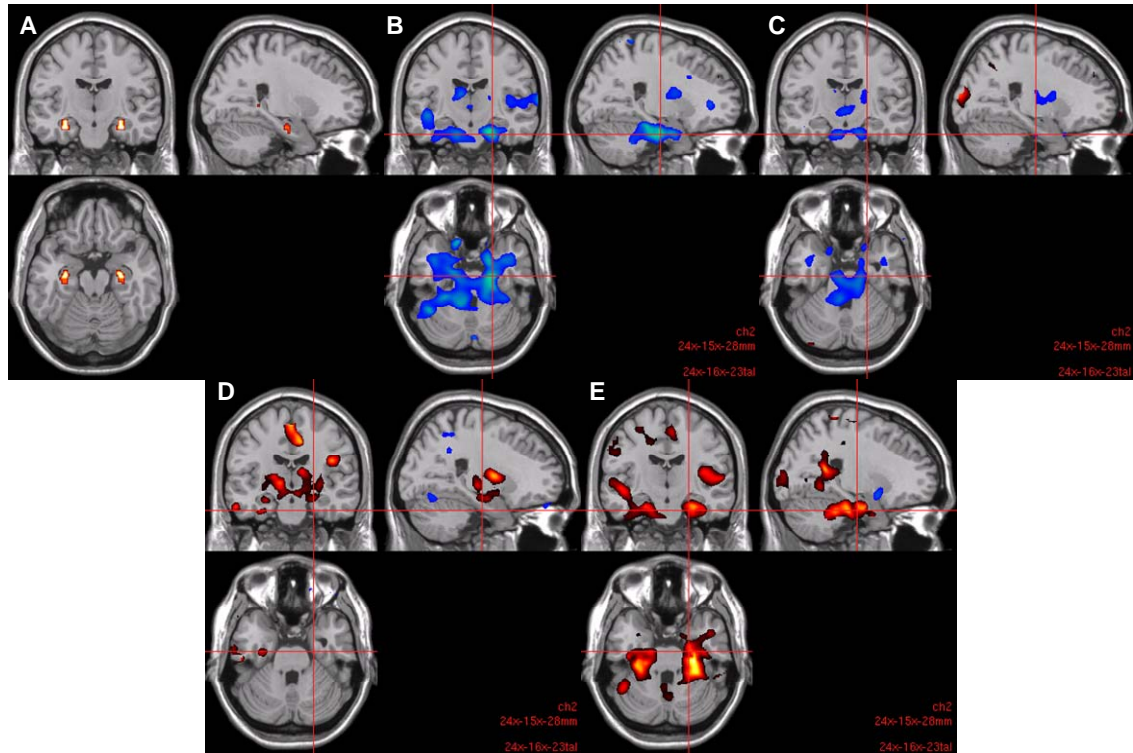


Fig S2. (A) The hippocampal mask used to extract percent signal change analysis. (B) Hippocampal activation for NT trials which were forgotten, (C) NT trials which were remembered, (D) T trials which were forgotten, (E) T trials which were remembered. For A, B, C, and D red indicates regions that exhibit greater activity than fixation baseline trials, whereas blue indicates regions that exhibit decreased activity from baseline trials. SPMs were thresholded at $Z_{2.52}$, $p.01$ to show the extent of hippocampal activation in all trial types.

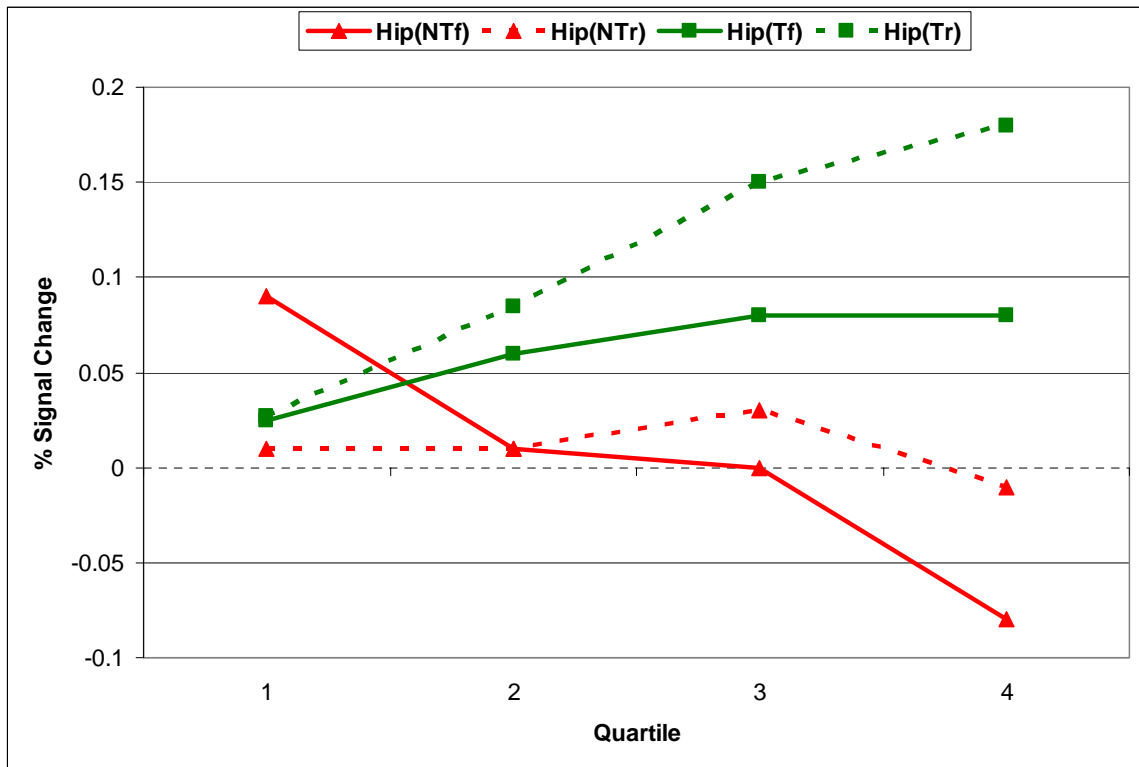


Fig S3. Percent signal change analysis in the hippocampus over all quartiles for NT trials that were forgotten (NTf - red solid line), NT trials that were remembered (NTr – red dashed line), T trials that were forgotten (Tf – green dashed line), and T trials that were remembered (Tr – green solid line).

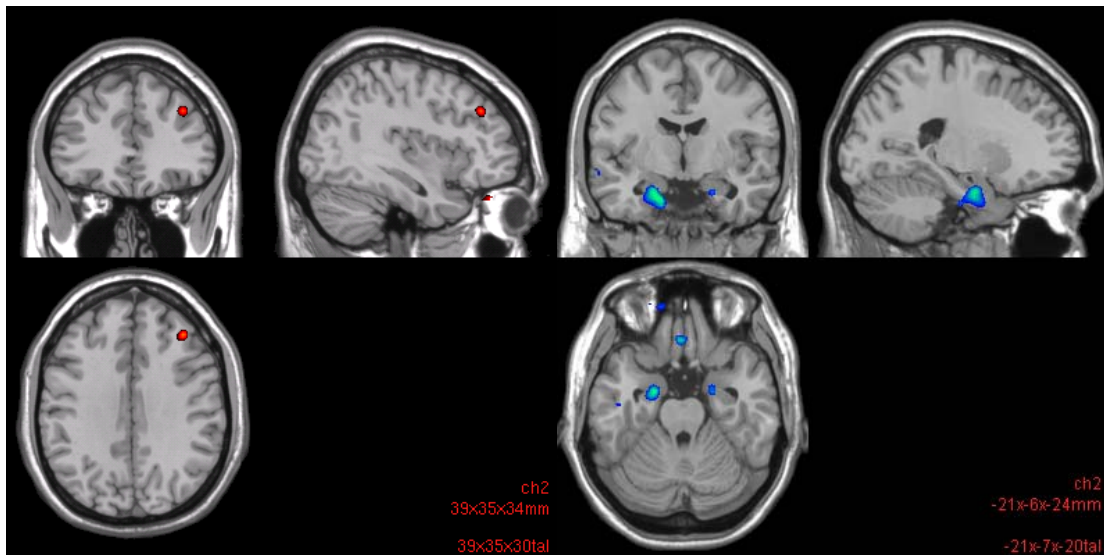


Fig S4. Brain activity correlated with behavioral suppression during the course of the experiment [(all quartiles) rMFG], and during the fourth quartile (hippocampus). Correlations are based on relative association of rMFG (increased activity with greater suppression) and hippocampus (decreased activity with greater suppression).

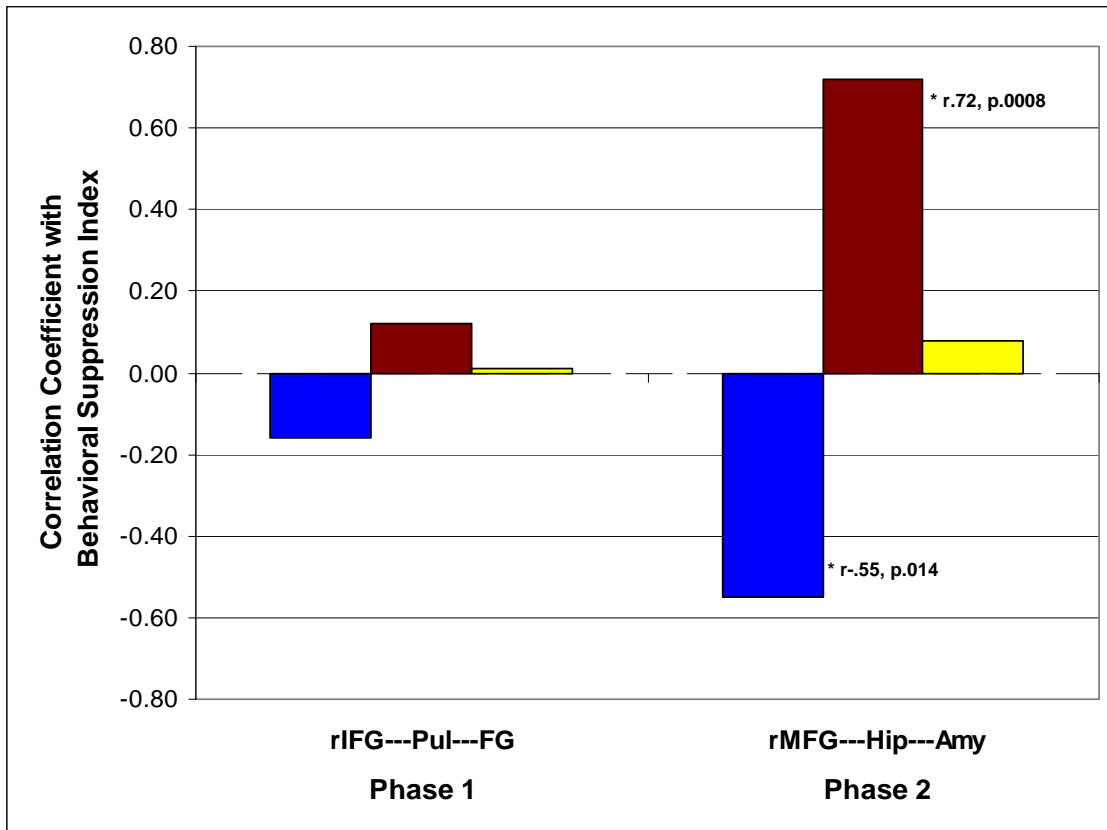


Fig S5 Highest correlations of brain activity (for an individual quartile) with behavioral performance during NT trials, by phase. Negative correlations indicate greater suppression of NT trials with increasing activity for control regions (rIFG, rMFG) whereas positive correlations indicate greater suppression of NT trials with decreasing activity for posterior regions (Pul, FG, Hip, Amy).

Putative Category	NT>T (Overall contrast: All trials)	BA	Z-score	x	y	z	Cluster size (# of Voxels)
<i>Control Regions</i>	Medial frontal gyrus	10	3.42	18	66	28	196
	Middle frontal gyrus	9/46	2.93	34	38	28	143
	Inferior frontal gyrus	47	-3.35	42	36	-8	120
	Superior frontal gyrus	8	3.54	20	32	52	249
<i>Visual Regions</i>	Cuneus	17/31	-3.24	-2	-66	10	318
	Fusiform gyrus	19	-3.65	40	-50	-10	185
	Occipital gyrus	19	-4.74	-34	-66	24	266
	Thalamus		-3.81	28	-28	8	256

<i>Memory/ Emotion Regions</i>	Hippocampus		-3.54	22	-16	-14	136
	Hippocampus/ Parahippocam pal gyrus		-4.09	-28	-34	-6	215
	Parahippocam pal gyrus	35	-3.67	16	-10	-26	214
<i>Other</i>	Lateral inferior parietal	40	3.44	64	-50	26	512
	Lateral inferior parietal	40	3.79	-56	-52	32	515
	Superior temporal gyrus	22	-3.90	-62	-24	4	233
	Superior temporal gyrus	21	-3.50	68	0	-6	149
	Cerebellum		-3.53	6	-52	-18	399
	Cerebellum		-4.22	-36	-50	-26	455

Table S1. Brain activations for the overall contrast of NT>T (all trials).

Putative Category	NT>T (NT forgotten>T remember)	BA	Z-score	x	y	z	Cluster size (# of Voxels)
<i>Control Regions</i>	Medial frontal gyrus	10	4.17	24	50	22	974
	Middle frontal gyrus	9/46	3.18	42	18	40	187
	Inferior frontal gyrus	47	3.15	58	18	4	192
	Superior frontal gyrus	9	3.58	14	26	52	153
<i>Visual Regions</i>	Cuneus	18	-3.73	-32	-68	28	273
	Cuneus	18	-4.05	34	-62	22	391
	Fusiform/ Lingual gyrus	17	-3.28	24	-70	-8	156
	Thalamus		-3.8	-8	-20	14	231
<i>Memory/ Emotion Regions</i>	Hippocampus/ Parahippocam pal gyrus/ Amygdala		-3.34	20	-14	-26	246
	Hippocampus/ Parahippocam pal gyrus		-3.19	-22	-25	-10	185
<i>Other</i>	Lateral inferior parietal	40	4.37	66	-46	28	859
	Lateral inferior parietal	40	3.59	-56	-54	32	253
	Fornix/ Corpus Collosum		-3.24	-10	-32	14	314
	Claustrom		-3.46	-32	2	16	173
	Cerebellum		-3.41	-2	-34	22	201

Table S2. Brain activations for the contrast of NT>T (only correct trials thus, trials that were suppressed in the NT condition, as compared to, trials that were remembered in the T condition).

	rBA10	rIFG	Pul	FG	rMFG	Hip	Amy
rBA10		.54	-.16	-.16	.75	-.09	-.22
rIFG			-.60	-.64	.07	-.52	-.51
Pul				.80	-.43	.59	.77
FG					-.44	.77	.88
rMFG						-.77	-.82
Hip							.84

Table S3. Maximal correlation coefficients between activity in different brain regions. The highest correlation observed across the four quartiles was used in correlation with rIFG and rMFG (blue = rIFG and associated posterior/sub-cortical brain areas, maroon = rMFG and associated posterior/sub-cortical brain areas and yellow = BA10 and rIFG, rMFG). All other values show associated correlations within the two-phase model.

<i>Outlier Analysis</i>	rBA10	rIFG	Pul	FG	rMFG	Hip	Amy
Mean	.13	.12	-.08	-.07	.06	-.09	-.12
Stand Dev	.19	.24	.15	.25	.14	.25	.23
Stand Dev ±3	-.44_.70	-.60_.84	-.53_.33	-.82_.68	-.26_.38	-.84_.66	-.81_.57
Minimum	-.33	-.23	-.51	-.58	-.17	-.76	-.54
Maximum	.43	.67	.12	.34	.33	.20	.24
Count	16	16	16	16	16	16	16

Table S4. Outlier analysis with calculated ±3 standard deviation range.

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