Study of Naltrexone-Induced Blockade of Antidepressant Effects

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Placebo Effects in Major Depression: A pharmaco-fMRI Intervention

INTRODUCTION:

This NARSAD Young Investigator Award application proposes to define the neural and molecular bases of placebo effects in Major Depression. This proposal would be a perfect extension to my on-going study aimed at developing and piloting a functional magnetic resonance imaging (fMRI) task to investigate the neural signature of placebo effects in Major Depression, which will be used in this application. This study would also be an ideal add-on to a current clinical trial at Dr. Zubieta´s laboratory investigating the role of opioid and cannabinoid interactions in placebo effects in depression. These results will provide robust preliminary data, to inform the design of a randomized controlled trial (RCT) that would use imaging-based biomarkers of placebo effects, to be submitted as an R01 application to the National Institute of Mental Health.

BACKGROUND & STUDY GOAL:

The placebo effect – a positive psychophysiological response attributed to inert treatments – is an important yet seemingly misunderstood phenomenon in conventional medical science. While placebo effects are as old as healing itself, it is still unknown why certain diseases, such as depression, show remarkably and increasingly high placebo rates (Walsh et al., 2002), hindering the development of novel therapeutics and predictors of treatment response. In RCTs of antidepressants for adults, response rate to placebo ranges from 25-60%, compared to 50% response rate to antidepressant medication (Quitkin, 1999). The failure to differentiate between placebo and antidepressant responses has caused large pharmaceutical companies to reduce or discontinue research focused on treatments for depression and other mental illnesses (Cressey, 2011). Furthermore, only one landmark published study has investigated the neural correlates of placebo effects in depression (Mayberg et al., 2002). Therefore, there is a critical need to define biomarkers of placebo effects in depression and translate this knowledge into novel marker-informed clinical trial designs.

Preliminary studies: Neuroimaging offers a precise and objective way to characterize the neural and molecular basis of the placebo response in humans. We have already established the utility of positron emission tomography (PET) with the μ-opioid selective ligand [^{11}C] carfentanil to investigate the neurobiology of placebo effects using a pain model [for a review, (Pecina and Zubieta, 2014)]. Additionally, we have used the same methodology to define the neural bases of placebo effects in patients with Major Depressive Disorder (MDD) (PI: Zubieta). This study demonstrated that improvement of depressive symptoms in response to placebo administration was positively correlated with placebo-induced opioid release in the medial thalamus, anterior cingulate cortex (ACC), nucleus accumbens, and amygdala. These data show that placebo administration in patients with MDD is associated with increased opioid neurotransmission in regions implicated in emotion regulation. More recently, I have developed and piloted an fMRI experiment to characterize the cognitive mechanisms involved in placebo effects in patients with MDD. This fMRI task is specifically designed to record and modulate mood improvement by providing simulated neurofeedback in the context of an intravenous placebo administration with expectations of fast-acting antidepressant effects. In a pilot study using this task, patients with MDD who reported acute mood improvement in response to simulated positive neurofeedback showed increased blood oxygen level dependent (BOLD) responses in the ACC (Fig. 1), and in particular the rostral ACC (rACC), a reliable marker of treatment response in depression (Pizzagalli, 2011), and placebo analgesic effects (Petrovic et al., 2002). In summary, these preliminary studies demonstrate: 1) the contribution of the opioid system to the formation of placebo effects in MDD; and 2) increased rACC BOLD responses in patients who reported acute mood improvement induced by simulated positive neurofeedback with expectations of fast-acting antidepressant effects. Still, the opioid modulation of placebo-induced acute mood improvement and rACC BOLD responses in patients with MDD has not been investigated, which justifies the research proposed in this application.

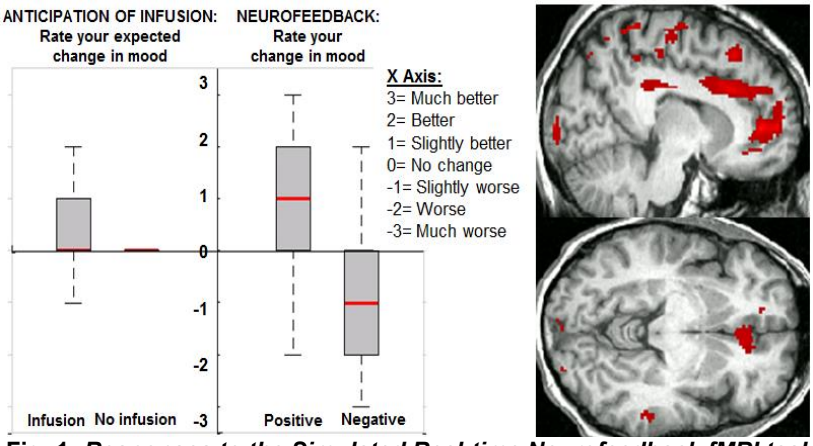


Fig. 1: ***Responses to the Simulated Real-time Neurofeedback fMRI task:*** **Simulated positive neurofeedback during this task resulted in 1) Acute mood improvement (t=-2.8, p=0.03) (left), and 2) Increased BOLD response in the rostral ACC (and also the dorsal and subgenual ACC) in placebo responders (n=3) compared to non-responders (n=3) (right).**

Based on this preliminary evidence I hypothesize that placebo effects in patients with Major Depression take placebo during opioid modulation of rACC activity, and therefore can be partially or totally blocked using the selective μ-opioid antagonist naloxone. To test our central hypothesis, we will pursue the following aims:

AIM 1: Evaluate the effect of naloxone on acute mood improvement and rACC BOLD activity induced by simulated positive neurofeedback with expectations of fast-acting antidepressant effects. We hypothesize that naloxone-induced blockade of μ-opioid receptors will reverse the acute mood improvement and increased rACC BOLD activity induced by positive neurofeedback.

AIM 2: Determine the extent to which individual differences in the rACC BOLD activity induced by simulated positive neurofeedback with expectations of fast-acting antidepressant effects predict acute mood improvement. We hypothesize that increased rACC BOLD activity induced by positive neurofeedback will be associated with greater acute mood improvement. If AIM 1 and 2 are significant, we will pursue AIM 3: Define the role of the rACC BOLD activity induced by positive neurofeedback as a mediator of the effect of group (naloxone versus placebo) in acute mood improvement.

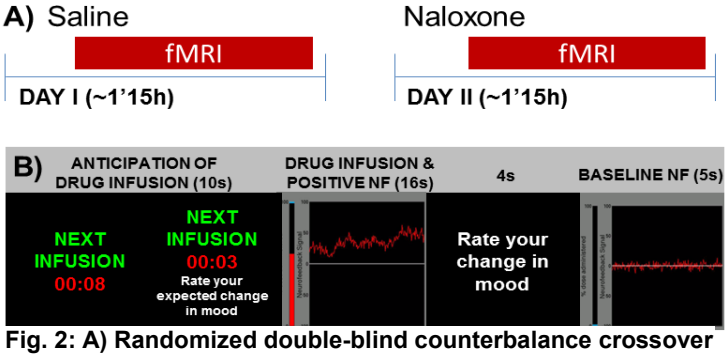


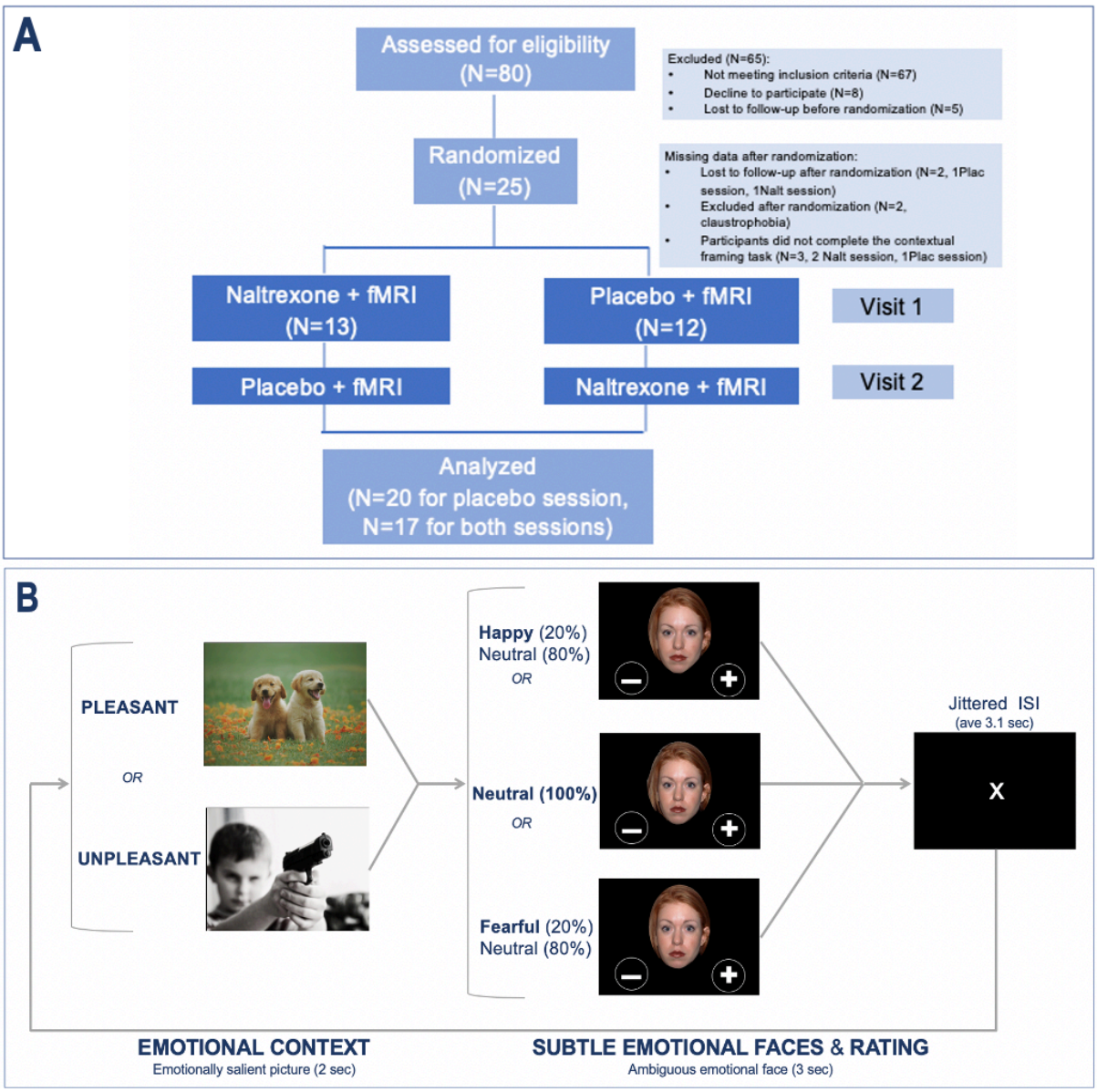
Fig. 2: A) Randomized double-blind counterbalance crossover design of naloxone versus saline. B) Simulated Real-Time Neurofeedback fRMI Task.

Research design: We will recruit 20 un-medicated patients with Major Depression aged 18–55 years with a current moderate-to-severe first depressive episode (Hamilton Depression Rating Scale scores >14). We will use authorized deception (Miller et al., 2005) to inform patients that the purpose of the study is to investigate the effects of a fast-acting intravenous antidepressant treatment on real-time brain signal as well as to assess the test-retest reliability of this procedure (this is to justify a second experiment). After assessing eligibility, patients will receive continuous infusion of the "fast-acting intravenous antidepressant", which will be either naloxone (NARCAN ® injection, naloxone hcl injection. Endo Pharmaceuticals, Inc.) or saline (0.9% Sodium Chloride Injection). As previously described (Sprenger et al., 2011), naloxone will be administered in bolus of 0.15 mg naloxone per kg bodyweight or the same amount of saline, fifteen minutes before the experiment (Fig. 2). Because of the relatively short half-life of naloxone, an additional constant intravenous infusion (dose of 0.2 mg/kg/h or saline for the duration of the fMRI experiment) will be administered. To control for the effects of naloxone on blood pressure, the participants' blood pressure will determined directly before and 5 minutes after drug application and at the end of the experiment.

Inside of the scanner, participants will undergo the "Simulated Real-Time Neurofeedback" fMRI task (Fig.2), which consist of periods of anticipation of "drug" or "drug-free" infusion (10s), followed by periods of simulated positive or negative neurofeedback (16s). Before the experiment, patients will be trained to expect positive neurofeedback and acute mood improvement in response to each "drug" infusion and negative neurofeedback and acute mood-worsening in response to each "drug-free" infusion trial. The experiment will consists of six 7-minute runs of 12 trials counterbalanced within and between runs. After the experiment, we will assess the task's credibility and patients who did not believe the experiment will be excluded from the study. All newly collected fMRI data will be analyzed using standard procedures (Ashburner, 2007). At the group-level, a random effects analysis will determine the main effects of the valance (positive versus negative neurofeedback) resulting in statistical parametric maps (t or F statistics), which will be thresholded with height and extent values generated by Monte Carlo simulations with 3dClustSim to protect against overall type I error at $p < 0.05$. To control for potential confounders, sex and depression severity will be entered as covariates in statistical models. The whole-brain analyses will be complemented by hypothesis-driven analyses in the rACC. Average BOLD signal within the rACC will be extracted and regressed against variables of interest.

Expected Outcomes and Impact: First, we expect to demonstrate that naloxone will reverse the acute mood improvement and increased rACC BOLD activity induced by positive neurofeedback with expectations of mood improvement (AIM 1). Second, we expect to demonstrate that increased rACC BOLD activity induced by positive neurofeedback will be associated with greater acute mood improvement (AIM 2). Third, we expect to demonstrate that rACC BOLD activity induced by positive neurofeedback mediates the effect of group (naloxone versus saline) on acute mood improvement (AIM 3).The results from this study are expected to have an important impact on our ability to understand the neurobiology of placebo effects in Major Depression, to detect novel target for drug development and to develop imaging-based biomarkers of placebo effects.

Figure 1. Study design and contextual framing fMRI task.



Participants underwent a randomized, double-blind, placebo-controlled, cross-over study of one dose of oral 50mg naltrexone hydrochloride (ReVia® [package insert]. Toronto, ON: Teva Canada Limited; 2015) (onset of action: ≥15 minutes; peak effect: ~1 hour; duration: ~24 hours) or matching placebo (Figure 1A). The two sessions occurred within 7-10 days from one another. Naltrexone or placebo pills were ingested 60 minutes before the fMRI scanning session. Participants were informed that the purpose of the study was to investigate the effects of opioid blockade on emotional processing. They were also informed about naltrexone, including its pharmacological properties, its general clinical use, and its possible side effects. However, they were not informed about the potential effects of context.

Of the 25 enrolled participants who received the naltrexone/placebo pill, two dropped out prior to scanning – one who was claustrophobic and another who had a positive pregnancy test. We excluded three additional participants who did not complete their placebo session. The final sample size for the contextual framing task was 20 for the placebo session, and 17 for the naltrexone session (thee participants did not complete the fMRI session naltrexone session, but their results from their placebo session were still included in the analysis). The final sample size for the antidepressant placebo fMRI task was 20 for both sessions.

<u>Contextual Framing fMRI Task</u>

The contextual framing task (Figure 1B) was iteratively refined as a simpler version of a similar task utilized by Mobbs and colleagues [39]. In this mixed block/event-related design, participants saw an emotionally evocative/salient contextual image for 2 seconds (Context phase) followed by the rating of the valence of an ambiguous emotional expression for 3 seconds (Emotion & Rating). The jittered inter-trial interval was ~ 3 seconds (2-6s range, S.D.=1.1)].

For the contextual images, we used a total of 72 emotionally evocative color photographs (36 pleasant and unpleasant images) from the International Affective Picture System (IAPS) database [44], which contains normalized scores of valences (pleasant = 9, unpleasant =1) and arousal (high = 9, low = 1) for all the images. Based on these scores, we selected 72 contextual images with high or low valence scores (pleasant > 7.3 and unpleasant < 3.4) and moderate arousal score (less than 6) to avoid excessively salient stimuli. This normalized image set provided the image-context for influencing the rating of ambiguous faces.

We then generated three types of emotional faces with ambiguous expressions from the NIM-STIM database (http://www.macbrain.org/resources.htm). First, we selected images of 48 individuals' faces that had a neutral facial expression. Then, the neutral face image was morphed with the same individuals' face image with a happy or fearful expression to generate ambiguous faces using a face-morphing software (FantaMorph 2.5). To maintain the ambiguity of facial expression, we merged morphed only 20% of happy or fearful face to with the neutral face.

Prior to the fMRI scan, participants received the following instructions: "You have two choices for rating each face: + (positive), indicating that the person seems happy, pleased, or delighted; or - (negative), indicating that the person seems sad, afraid, angry, or disgusted. You will see faces only for a brief moment, so please respond as fast as possible. The pictures of faces will alternate with other kinds of pictures." To avoid participants' guessing the premise of the task, we added the following instruction: "Pay attention to these non-face pictures because you'll be asked about them afterwards. But remember that your main task is to rate the facial expressions." Participants reported their responses by clicking either the left or right index finger one of the buttons of the 5-button response box in each hand. They were instructed to rate the facial expression by pressing a right index finger to select positive ("+") or a left index figure to select negative ("-") during the first run and the opposite during the second run.

Participants completed 144 trials (72 trials with each unique context picture, repeated twice) for each visit (Placebo and Naltrexone session). The task lasted approximately 20 minutes. Three types of ambiguous faces appeared equal times, and the sex of the face was randomized. The trial orders were optimized by using the easy-optimize-x tool that was implemented in MATLAB [45].

Antidepressant Placebo fMRI Task (See figure 2 in study protocol).
Participants were informed that the purpose of the study was to investigate the neural effects of a "fast-acting antidepressant" compared to a "conventional antidepressant". In addition, participants were told that the experimental procedures consisted in the administration of multiple antidepressant infusions during an fMRI scanning session where we recorded their brain activity, which we called neurofeedback.

Before entering into the scanner, a certified nurse placed an fMRI intravenous compatible line in the participant's arm for the "antidepressant" infusions. In addition, patients watched a fragment of the task to be displayed inside of the scanner and were informed that the positive neurofeedback signal (higher tracing) reflected the effectiveness of the drug infusion and might result in mood improvement during the experimental session, whereas the baseline neurofeedback signal was unlikely to cause mood improvement. Once in the scanner, an MRI compatible pump, controlled from outside the scanning room by pushing the "go" trigger, delivered the saline to the participant during the scanning session. The infusion was manually started at a given flow rate and volume, at the beginning of each run.

Then, participants completed the Antidepressant Placebo fMRI Task, which features two putative components of the placebo effect: the expectancy and reinforcement condition, each followed by an expectancy and mood rating cue, respectively. The expectancy condition involves two "antidepressant" infusion cues – described as a "fast-acting" and a "conventional antidepressant"– and two no-infusion cues – described as periods of equipment calibration. During the "antidepressant" infusion cue (4s), a bar is filled at four 1s-periods

representing 0%, 33%, 66% and 100% of the dose administered. During the calibration no-infusion cue (4s) the bar remains empty. For the reinforcement condition (10s) we used sham neurofeedback as a secondary reinforcer of the “antidepressant” effects. In the high-reinforcement condition sham neurofeedback is positive 88% of the trials (vs. 12% baseline). In the low-reinforcement condition, sham neurofeedback is positive 25% of the trials (vs. 75% baseline). Participants rated their expected and actual change in mood (YES/NO) in response to each infusion/neurofeedback signal respectively by using a keypad and their index fingers.

The combination of the expectancy and reinforcement manipulation results in 4 different conditions: 1) Infusion cue + High reinforcement, 2) Infusion cue + Low reinforcement, 3) Calibration cue + High Reinforcement and 4) Calibration cue + Low Reinforcement. Each condition is color coded as red, blue, yellow and green respectively (Fig.1). Random jitters were included between trials and between the expectancy and the reinforcement manipulation. The jitter duration was randomly selected from an exponential distribution bounded between 0.33 and 2 s. One jitter length was sampled from the uniform distribution with bounds 4-6 s to increase randomness.

MRI Data Acquisition

Structural and functional MR images were collected at the University of Pittsburgh Magnetic Resonance Imaging Center on a 32-channel parallel receive-transmit head coil on a 3T Siemens PRISMA scanner (Munich, Germany). All scanning was conducted in the afternoon ~ 1h after receiving either placebo or naltrexone. A sagittal, whole brain 3D magnetization prepared rapid gradient echo (MPRAGE) with repetition time (TR)=2400ms, echo time (TE)=2.22ms, flip angle (FA)=8deg, inversion time (TI)=1000ms, field of view (FOV)=300x320, 208 slices, 0.8mm isotropic (0.4mm space between slices), with Generalized Autocalibrating Partial Parallel Acquisition (GRAPPA) factor of 2, and lasted ~6min 38 sec. An axial, whole brain (including cerebellum and brainstem) echo planar (EPI) T2*-weighted functional images were collected to measure the blood oxygen-level dependent (BOLD) response with TR=1000ms, TE=30ms, FA=45º, FOV=95x95, 60 slices, 2.3mm isotropic (no spaces), multiband factor of 5, and 1200 volumes (two 10-minute runs). Participants were scanned for 90 minutes and this task started at approximately minute 65.

MRI Preprocessing

Acquired images were preprocessed using functions in the following software packages: NiPy [46], AFNI [47], BrainWavelet Toolbox [48] and the fMRI software library (FSL, [49]). We have previously detailed this preprocessing pipeline elsewhere [50]. Specific methods and programs used were outlined below.

Anatomical images first underwent gradient unwarping, and then were registered to the MNI152 template using both affine and nonlinear transformations methods implemented in FLIRT (FSL) and FNIRT (FSL), respectively. A mask of the brain was also created by removing the non-brain voxels from the anatomical images using BET (FSL) for later use in functional image co-registration.

The functional images underwent slice timing and motion correction simultaneously using NiPy’s four-dimensional registration algorithm SpaceTimeRealign. Running both simultaneously ensured that motion artifacts would not be reintroduced into the data in later processing. Non-brain voxels in the images were removed by masking low intensity voxels, calculated from the field map, and using brain extraction function BET (FSL). After intensity-normalizing every voxel to have a mean of 1000, wavelet despiking was performed using the BrainWavelet Toolbox with the spike threshold set to 10. The resulting image was aligned and warped to their anatomical images, resampled to 3mm isotropic voxels, and warped into MNI152 standard space. A 7mm full-width at half maximum kernel was used to smooth the images spatially and a high-pass filter was applied to remove signal slower than 0.008 Hz. Lastly, the images were intensity normalized by rescaling the intensity by 100 divided by voxel mean.

MRI Analysis

MRI First Level Analysis of the Contextual Framing Task. We constructed regressors for emotion and context type, and their interaction terms. The emotion regressor was coded as one 1, for happy faces, 0 for neutral faces, and -1 for fearful faces. Similarly, the context regressor was coded as 1 for pleasant and -1 for unpleasant context. The interaction term included two regressors, one for the effect of context on happy faces (1 for pleasant context/happy faces, -1 for unpleasant context/happy faces, 0 for the remaining conditions), and

another one for the effect of context on fearful faces (1 for pleasant context/fearful faces, -1 for unpleasant context/fearful faces, 0 for the remaining condition). We also included a trial regressor of no interest.

We aligned the boxcar regressors with trial onset (length = trial duration) and convolved then with the hemodynamic response function. We then used the resulting time-series to regress against BOLD signal for each run and participant, and constructed contrasts for each regressor (i.e., emotion, context type, emotion x context type interaction).

MRI First Level Analysis of the Antidepressant Placebo fMRI Task: We constructed four event regressors: infusion event, expectancy ratings event, neurofeedback event and mood ratings event. We constructed two additional regressors for the expectancy manipulation, coded as 1 or -1 (“antidepressant” infusion cue or calibration no-infusion cue, respectively) and the reinforcement manipulation, coded as 1 or - 1 (positive or baseline sham neurofeedback, respectively). The expectancy manipulation regressor was aligned to the infusion event and the expectancy ratings event, whereas the reinforcement manipulation event was aligned to the neurofeedback event and the mood ratings event. For all models we convolved boxcar regressors with the HRF and the used general linear models using FSL FEAT for each run and participant.

Group Level Statistical Analysis. We conducted group level voxel-wise analyses using randomize in FSL. We used threshold-free cluster enhancement to determine significant clusters ($1-p > 0.05$). For each contrast we conducted one-sample t-tests during the placebo session only. We then conducted paired samples t-test for each contrast (Placebo > Naltrexone and Naltrexone > Placebo).