**Supplementary Methods**

The study flow chart is shown in figure S1.

*Image Acquisition*

All scanning used a 3 Tesla Trio TIM MRI scanner (Siemens, Berlin, Germany) using a 12-channel custom head coil located at the MR Research Center at the University of Pittsburgh. An axial, whole brain magnetization-prepared rapid gradient-echo (MPRAGE) sequence was collected pre-treatment with echo time (TE)=3430msec, repetition time (TR)=2300msec, flip angle (FA)=90°, inversion time (TI)=900msec, 1mm isotropic resolution, matrix=256×224, and 176 slices with GeneRalized Autocalibrating Partial Parallel Acquisition (GRAPPA) factor of 2. An axial, whole brain fluid attenuated inversion recovery (FLAIR) sequence was collected with TE=90msec, TR=9000msec, FA=150°, TI=2500msec, 1x1x3mm resolution, matrix=256x212, and 48 slices.

Axial, whole brain T2\*-weighted blood-oxygen level dependent (BOLD) sequences were acquired by using a gradient-echo echo planar imaging (EPI) sequence with TE=3400msec, TR=2000msec, FA=90o, 2x2x4mm resolution, matrix=128x128, and 28 slices. The emotion reactivity task was 117 volumes (4 minutes) and the reward task was 172 volumes (6 minutes).

*Image Preprocessing*

Image preprocessing was conducted by Statistical Parametric Mapping software (SPM12; <http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>). Interpolation was conducted using 4th degree B-spline interpolation, while normalized mutual information similarity metric (for coregistration between images of different types) and mutual information similarity metric (for motion correction) were utilized unless otherwise stated. The FLAIR was coregistered to the MPRAGE (affine transformation), and then the structural images were segmented into 6 tissue classes (probability maps are output per tissue class) using a multi-spectral approach. The number of Gaussian distributions to model white matter was set to two, as white matter hyperintensities (WMH) were present. This segmentation outputs a deformation field that could be used to coregister images to a standard anatomic space (Montreal Neurological Institute or MNI space). By thresholding the probabilities for gray matter, white matter, and cerebrospinal fluid at 0.1, we generated a mask that included any brain tissue. This was then filled using a standard hole-filling algorithm (imfill from MatLab) and then an image-closing algorithm was performed (imclose from MatLab). The structural images were then skull stripped. These were then normalized to MNI space and averaged across all subjects to create an average structural image to overlay all functional imaging results.

For the WMH calculation, we used an automated method using a fuzzy connected algorithm that required the MPRAGE and T2-weighted FLAIR images (1). We reported the normalized total WMH volume adjusting for total brain volume (WMH divided by total volume).

Functional images were motion-corrected (mean images as reference and rigid transformation). We coregistered the mean image to the skull-stripped MPRAGE and normalized them to MNI space (2mm isotropic resolution). A Gaussian kernel with full-width at half-maximum of 8mm was used for smoothing.

**Supplementary Results**

Groups did not differ on age, sex, race, education, MADRS at baseline, venlafaxine dosage, or dysphoria subscale (table S1). Further, groups did not differ on dosage, MADRS/dysphoria at the end of the trial, percent change in MADRS/dysphoria, change in euphoria, and side effects experienced (table S2).

*Emotional reactivity task*

There were no associations at baseline between MADRS and activation. Regardless of treatment groups, higher baseline right precuneus and left middle occipital gyrus (MOG) activation was associated with improvement of the total MADRS (supplement table 3 and Figure S2a), while lower baseline thalamus activity was associated with improvement on dysphoria (supplement table 3 and Figure S2b). Lower baseline left anterior insula and bilateral MFG activation were associated with improvement of dysphoria only in the BPN but not in the PBO group (supplement table 3 and figure S3).

*Gambling task*

Baseline activation (reward>neutral, loss>neutral, and reward>loss) was not associated with either baseline MADRS or dysphoria or their improvement and this was not dependent on treatment group. There was no association between increase in euphoria and acute increase in brain activation during this task. A decrease in activation in the left inferior temporal gyrus during the reward minus neutral contrast in the gambling task was associated with improvement in depression severity in both groups (supplemental table 3 and figure S4), while no association was found for the loss minus neutral contrast.

**Supplementary Discussion**

To understand baseline associations, we performed several exploratory analyses to investigate baseline activation and its association with baseline severity and total improvement (and whether they were dependent on group). In the emotion reactivity task, we found that independent of group, higher baseline activity in left MOG and right precuneus was associated with overall MADRS improvement, and lower thalamus activity was associated with dysphoria improvement. MOG and precuneus are activated during emotional reactivity task (2). Specifically, precuneus is responsible for an array of integrated functions, including visuo-spatial processing, episodic memory retrieval, and self-conscious or self-processing tasks (3). Higher precuneus activity during this task may reflect better emotional processing at baseline, which facilitates symptom improvement from either BPO or PBO augmentation. Thalamus has been found to show hyper-responsiveness to sad faces (4, 5) and to have higher resting functional connectivity with the default mode network (6) in major depressive disorder. Thus, lower thalamus activity in emotional reactivity task may suggest less severe form of depression at baseline, which is more amenable to further treatment. However, the result of baseline activity predicting treatment response should be interpreted with caution. In most research using baseline neuroimaging parameters to predict treatment response, patients are either medication-free or in acute depressed state without treatment. In our study design, our baseline refers to unremitted patients after a venlafaxine trial, meaning that their brain activation has been modulated by full-dose venlafaxine for 12 weeks. Therefore, our findings of the association between baseline brain activation and treatment improvement should not be interpreted as general bio-markers for treatment response in LLD. We conducted baseline association analyses was to validate the findings in the main results. In conclusion, the finding of decreased activation in MFG, left aINS, rACC, and dorsal ACC (dACC) predicting dysphoria symptom improvement only in BPN group complements our main result from early brain activation changes. This implies that low baseline and early increased activation in the similar brain regions (left aINS and MFG) are both associated with the treatment improvement in low dose buprenorphine augmentation therapy.

Reference

1. Wu M, Rosano C, Butters M, Whyte E, Nable M, Crooks R, Meltzer CC, Reynolds CF, Aizenstein HJ. A fully automated method for quantifying and localizing white matter hyperintensities on MR images. Psychiatry Research: Neuroimaging. 2006;148:133-142.

2. Fusar-Poli P, Placentino A, Carletti F, Landi P, Allen P, Surguladze S, Benedetti F, Abbamonte M, Gasparotti R, Barale F, Perez J, McGuire P, Politi P. Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. Journal of Psychiatry & Neuroscience : JPN. 2009;34:418-432.

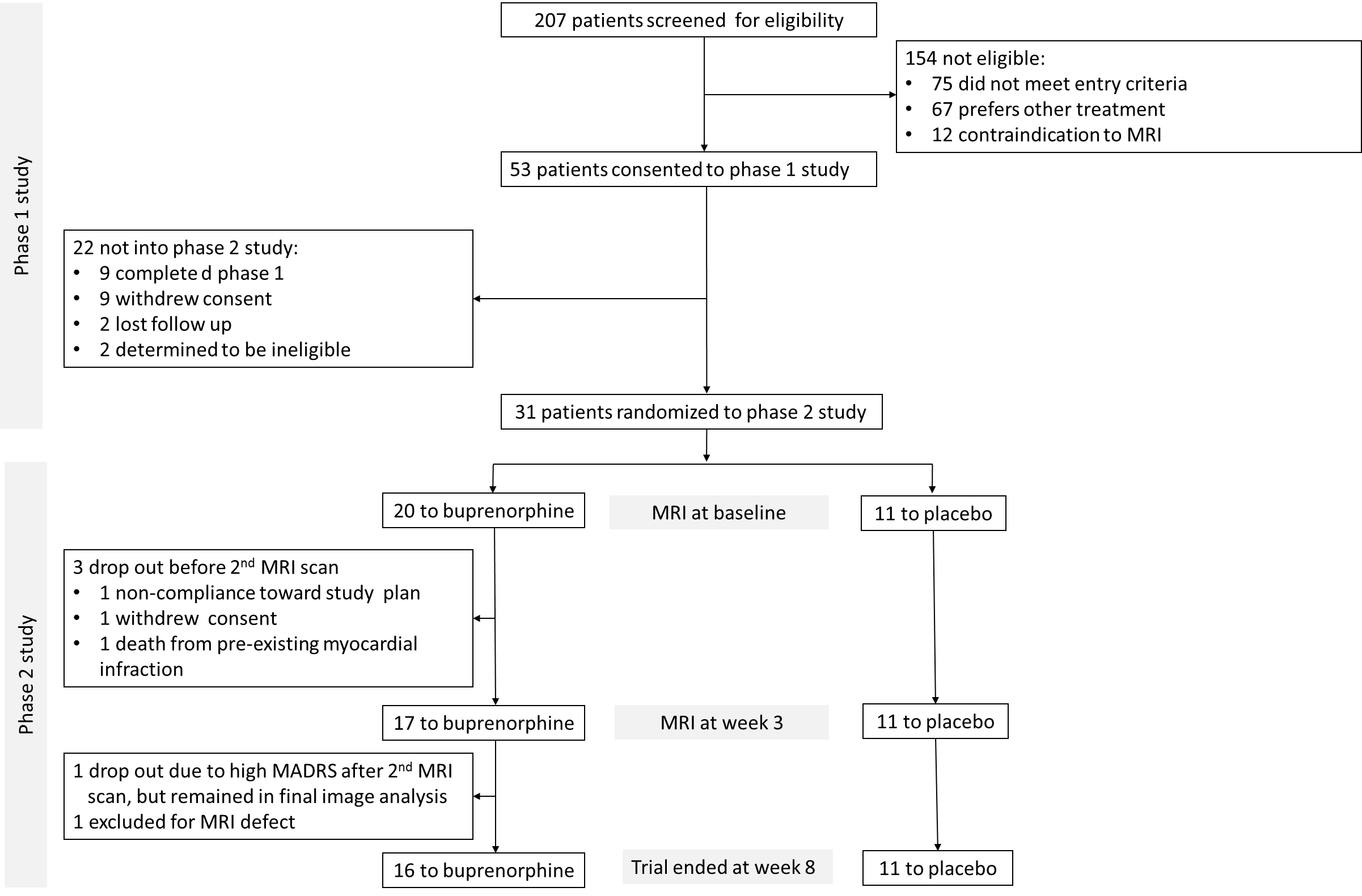
3. Cavanna AE, Trimble MR. The precuneus: a review of its functional anatomy and behavioural correlates. Brain. 2006;129:564-583.

4. Stuhrmann A, Suslow T, Dannlowski U. Facial emotion processing in major depression: a systematic review of neuroimaging findings. Biology of mood & anxiety disorders. 2011;1:10.

5. Fu CH, Williams SC, Cleare AJ, Brammer MJ, Walsh ND, Kim J, Andrew CM, Pich EM, Williams PM, Reed LJ. Attenuation of the neural response to sad faces in major depressionby antidepressant treatment: a prospective, event-related functional magnetic resonance imagingstudy. Archives of general psychiatry. 2004;61:877-889.

6. Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H, Reiss AL, Schatzberg AF. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. Biological psychiatry. 2007;62:429-437.

Figure S1. Flow chart of study



|  |  |  |  |
| --- | --- | --- | --- |
| Table S1. Baseline Clinical and Demographic Characteristics | | | |
|  | Buprenorphine  (N=16) | Placebo  (N=11) | Statistics |
| Age, years | 65±10 | 66±8 | *t* = -0.26, *p* = 0.79 |
| Sex (M/F) | 10/6 | 8/3 | **χ2** = 0.31, *p* = 0.58 |
| Ethnicity |  |  |  |
| White, n (%) | 14 (87.5%) | 11 (100%) | **χ2** = 1.49, *p* = 0.22 |
| African American, n (%) | 2 (12.5%) | 0 |  |
| Education, years | 15±3 | 16±3 | *t* = -0.97, *p* = 0.34 |
| MADRS pre-treatment | 21±6 | 27±5 | *t = 0.95, p =* 0.35 |
| MADRS post-treatment with venlafaxine | 24±6 | 27±5 | *t* = -1.08, *p* = 0.29 |
| Venlafaxine dosage at baseline (mg/day) | 267±63 | 286±45 | *U* = 74.50, *p* = -0.64 |
| Dysphoria subscale score at baseline | 12±3 | 10±3 | *t* = 1.59, *p* = 0.12 |
| MMSE, Mini Mental State Examination; MADRS, Montgomery–Åsberg Depression Rating Scale. | | | |

|  |  |  |  |
| --- | --- | --- | --- |
| Table S2. Clinical Characteristics and Change Scores During Augmentation Pharmacotherapy with Buprenorphine or Placebo | | | |
|  | Buprenorphine  (N=16) | Placebo  (N=11) | Group Differences |
| Buprenorphine/Placebo dosage at completion | 0.5±0.2 | 0.6±0.2 | *t* = -0.57, *p* = 0.57 |
| MADRS score at completion | 18±9 | 15±9 | *t* = 0.89, *p* = 0.38 |
| MADRS change during the trial (%) | 13±48 | 23±43 | *U* = 83.5, *p* = -0.20 |
| Dysphoria subscale at completion | 10±5 | 8±5 | *t* = 0.78, *p* = 0.44 |
| Dysphoria change during the trial (%) | 18±41 | 16±43 | *U* = 81.5, *p* = 0.30 |
| Euphoria VAS increase | 0.3±0.6 | 0±0 | *U*=71, *p*= 0.79 |
| ASEC increase | -1±5 | -3±4 | *U*= 73, *p*= 0.47 |
| ASEC, antidepressant side effect checklist; VAS, visual analogue scale | | | |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table S3. Association between baseline brain activity in fMRI task and symptom improvement | | | | | | | |
| Region | BA | MNI coordinates | | | Cluster Size\* | Peak t | |
|  |  | x | y | z |  | |
| **Emotional reactivity task** | | | | | |
| **Baseline brain activity and change in MADRS** | | | | | |
| Left middle occipital gyrus | 18,31 | -34 | -68 | 22 | 360 | 5.28 | |
| Right precuneus | 7,31 | 18 | -56 | 34 | 544 | 4.96 | |
| **Baseline brain activity and change in dysphoria** | | | | |
| Thalamus |  | 0 | -8 | 12 | 263 | 4.57 | |
| **Baseline brain activity and change in dysphoria in**  **buprenorphine group** | | | | |
| Left aINS, MFG, rostral ACC | 10 | -36 | 44 | 6 | 2280 | -5.62 | |
| Left dorsal ACC | 32 | 22 | 50 | 6 | 214 | -5.19 | |
| **Gambling task** |  |  |  |  |  |  | |
| **Decreased brain activity and change in MADRS** | | | | | | | |
| Left ITG |  |  |  |  |  |  | |
| aINS, anterior insula; ACC, anterior cingulate gyrus; MFG, middle frontal gyrus; ITG, inferior temporal gyrus; BA, Brodmann area; \* SnPM correction with p-uncorrected cluster forming threshold at 0.001 and FWE at 0.05. | | | | | | |

Figure S2. Baseline activation in emotional reactivity task was associated with end-of-trial symptom improvement across all subjects. (Top) Association of increased baseline right precuneus activity and MADRS improvement (Bottom) Association of decrease baseline thalamus activity and dysphoria improvement.



Figure S3. Association between baseline left anterior insula and bilateral middle frontal gyrus activation and dysphoria improvement, which was dependent on group.



Figure S4. Change (decrease) in left inferior temporal activity in gambling task (reward>neutral) was associated with MADRS improvement across all individuals. No significant association was found in the gambling task contrast of loss minus neutral (loss>neutral).

