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eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.
**eAppendix 1. Methods**

**1. Subjects**

In addition to completing physical and neurological examinations, study participants were screened using the Mini International Neuropsychiatric Interview. Inclusion criteria included the diagnosis of MDD, 17-item Hamilton Depression Rating Scale (HDRS) scores >12 and excluded suicidal ideation, comorbid conditions (medical, neurological or psychiatric, substance abuse or dependence), the use of psychotropic agents and pregnancy. Because MDD and anxiety disorders share risk factors, we permitted current anxiety disorder diagnoses of generalized anxiety, panic, agoraphobia, social phobia, and specific phobia. We excluded left-handed individuals and patients who have used any centrally acting medications, nicotine, or recreational drugs within the past 2 months. None of the patients had taken antidepressant medications for at least 6 months prior to enrollment in the study. Written informed consent was obtained in all cases. All of the procedures used were approved by the University of Michigan Investigational Review Board for Human Subject Use and the Radioactive Drug Research Committee. Data was collected and stored using Research Electronic Data Capture (REDCap).

**Authorized deception procedure:** During the consent process, subjects were not told of the purpose of the study (the study of placebo mechanisms), nor the manipulations in expectations that took place in the study by the labeling of placebos as active or inactive. To resolve this ethical dilemma, we followed the recommendations of Miller et al. and Martin and Katz by incorporating the following information into the consent form: “You should be aware that the investigators have intentionally withheld certain aspects of the study. This is necessary to obtain valid results. However, an independent research committee has determined that this consent form describes the major risks or benefits of the study. The investigators will explain the withheld...
aspects of the study to you at the end of your participation”. Upon completion of the study, or if the subjects wished to discontinue the study at any point, the purpose of the study and the use of placebos were explained to the subjects.

2. Neuroimaging Methods

Immediately after each 1-week of placebo treatment participants were positioned in the PET scanner gantry (Siemens HR+, Knoxville, Tennessee) and 2 i.v. (antecubital) lines were placed. A light forehead restraint was used to eliminate intrascan head movement. Two 90 min PET scanning sessions, with and without i.v. placebo administration, were completed. Images were acquired in 3-dimensional mode (reconstructed full-width/half-maximum resolution, approximately 5.5 mm in plane and 5.0 mm axially), with the septa retracted and scatter correction. $[^{11}\text{C}]$carfentanil was synthesized at high specific activity by the reaction of $[^{11}\text{C}]$methyl iodide and a normethyl precursor as previously described 6. $15 \pm 1 \text{ mCi} (555 \pm 55 \text{ MBq})$ were administered in each scan, with cold masses of $< 0.03 \text{ µg/kg}$ for carfentanil. These doses ensured that the compounds were administered in tracer quantities, that is, subpharmacological doses occupying less than 0.5% of the available receptors and were not associated with side-effects. Fifty percent of the radiotracer doses were administered as an initial tracer bolus and the remaining 50% by continuous infusion for the remainder of the study to more rapidly achieve steady-state levels. For each study, 21 sets of dynamic scans were acquired with an increasing duration (four 30-second frames, three 1-minute frames, two 2.5-minute frames, eight 5-minute frames, and four 10-minute frames).

Images were reconstructed using iterative algorithms (brain mode; Fourier rebinning algorithm with ordered-subsets expectation maximization, 4 iterations, and 16 subsets; no
smoothing) into a 128x128-pixel matrix in a 28.8-cm-diameter field of view. Attenuation correction was performed through a 6-minute transmission scan (Ge$^{68}$ source) obtained before the PET study and with iterative reconstruction of the blank/transmission data, followed by segmentation of the attenuation image. Small head motions during PET were corrected by an automated computer algorithm for each subject before analysis, and the images were coregistered with the same software$^7$. Time points were then decay corrected during reconstruction of the PET data. Image data were then transformed on a voxel-by-voxel basis into 2 sets of parametric maps, a tracer transport measure (K$_1$ ratio) and a receptor-related measure (non-displaceable binding potential, BP$_{ND}$, or receptor availability in vivo$^8$). To avoid the need for arterial blood sampling, these measures were calculated using a modified Logan graphical analysis$^9$, using the occipital cortex (an area devoid of μ-opioid receptors) as reference region. Using the bolus-continuous infusion protocol described above, the slope of the Logan plot becomes linear 5~7 min post-tracer administration and is proportional to the receptor concentration divided by its affinity for the radiotracer [BP$_{ND}$ + 1, or (f$_2$Bmax/K$_d$) +1]$^{10}$. Bmax is the receptor concentration and K$_d$, the receptor-ligand dissociation constant. The term f$_2$ refers to the concentration of free radiotracer in the extracellular fluid and is considered to represent a constant and very small value. Reductions in the in vivo availability of receptors, the BP$_{ND}$ measure, after an acute challenge (i.e., placebo administration) are thought to reflect processes, such as competition between radiotracer and endogenous ligand, associated with neurotransmitter release$^{11}$.

Anatomical MRI studies were acquired on a 3-T scanner (General Electric, Milwaukee, Wisconsin). Acquisition sequences were axial spoiled gradient recall inverse recovery prepared magnetic resonance [echo time, 3.4 milliseconds; repetition time, 10.5 milliseconds; inversion
time, 200 milliseconds; flip angle, 25°; number of excitations, 1; using 124 contiguous images, 1.5-mm thickness]. The \( K_1 \) and BP\( \text{ND} \) images for each experimental period and the anatomical MRI were coregistered to each other and to the Montreal Neurological Institute (MNI) stereotactictic atlas orientation 12.

2.1 Data Analysis. Differences between conditions were mapped into stereotactic space with \( t \) maps of statistical significance using SPM8 (Wellcome Department of Cognitive Neurology, University College, London, England) and Matlab (MathWorks, Natick, Massachusetts) software, with a general linear model and correction for multiple comparisons. No global normalization was applied to the data, and therefore the calculations presented herein are based on absolute BP\( \text{ND} \) (\( f_2 \) Bmax/ \( K_d \)) estimates. Only regions with specific \( \mu \)-opioid receptor binding were included in the analyses (voxels with BP\( \text{ND} >0.1 \)) 13. To compensate for small residual anatomic variations across subjects and to improve signal-to-noise ratios, a 3-dimensional gaussian filter (full-width/ half-maximum resolution, 6 mm) was applied to each scan. Subtraction analyses were performed on \( \mu \)-opioid receptor images to assess main effects. For each subtraction analysis, 1-sample, 2-tailed \( t \) values were calculated for each voxel by using the pooled variance across voxels 14. Regression analyses between the subtracted images and the placebo response variables were performed on a voxel-by-voxel basis, including sex, order effects and QIDS-16SR screening scores as nuisance covariates. \textit{A priori} hypothesized regions implicated in stress and mood regulation 15-20, namely sgACC, NAc and AMY, were deemed significant at \( p<0.001 \), and an extent threshold (\( K \)) > 10 voxels. Regions of interest (ROI) were created using the WFU PickAtlas SPM toolbox 21. Bilateral amygdala ROIs were generated using the Automated Anatomical Labeling atlas, and the Brodmann area 25 was used as the sgACC ROI (both ROI dilated once). Bilateral NAc were defined as 6 mm radius spheres

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centered at ±8, 8, -8 (MNI coordinates) (eFigure 1). For other regions, significant differences were detected using a p<0.05 FWE corrected\(^{22}\). Significant regional data were extracted using MarsBaR\(^{23}\), for quantification of regional changes in BP\(_{ND}\), graphing and determination of correlation coefficients (Pearson/Spearman correlations at p<0.05). Data are expressed as the mean ± 1 S.D., unless otherwise indicated. Measures of MOR availability \textit{in vivo} (binding potential, BP\(_{ND}\))\(^{8}\) at baseline, and the reductions in BP\(_{ND}\) during i.v. placebo administration (activation of endogenous opioid neurotransmission) were then related to clinical placebo and antidepressant responses. All statistical analyses were controlled by sex, order effects and QIDS-16SR pre-randomization scores.
**eAppendix 2. Results**

*Patient Characteristics*

Hamilton Depression Rating Scale (HDRS-17) scores were (mean ± S.D.) 21 ± 4.5 and QIDS-16SR, 16 ± 4.7 immediately prior to the 2-week experimental placebo phase. All participants, except for one, had at least partial follow-up data and 71% (25/35) completed the 10-week antidepressant trial. Patients who dropped off from the study were not significantly different from completers in their severity scores (QIDS-16SR: Dropped off (n=10): 15.6 ± 5.2; Completers (n=25): 15.7 ± 4.6; t=-0.6, p=0.9) or placebo responsiveness (ΔQIDS-16SR: Dropped off (n=10): -0.1 ± 3.2; Completers (n=25): 2.3 ± 5.3; t=-1.3, p=0.2; ΔPIBS: Dropped off (n=10): 52.2 ± 25.1; Completers (n=25): 39.4 ± 26.7; t=1.2, p=0.2). In almost all cases dropping off took place in the initial 4 weeks of the antidepressant administration. Reasons included: fear or reluctance to take medication, long distance to appointments and/or small compensation during the trial period.

*Sex effects on baseline µ-opioid BP_{ND} and placebo-induced changes in µ-opioid BP_{ND}*

Because a significant effect of sex was found on behavioral placebo responses we further investigated the potential effect of sex on baseline µ-opioid BP_{ND} and placebo-induced endogenous opioid release. Women showed significantly greater baseline µ-opioid BP_{ND} in the NAc/ventral pallidum region (right: 10, 8, 2; 264 mm^3; z = 3.49; left: -14, 2, -6; 1140 mm^3; z = 4.10), compared to men. Women also showed significantly greater placebo-induced endogenous opioid release in the right NAc (6, 8, -6; 528 mm^3; z = 5.26) and AMY (24, 0, -26; 672 mm^3; z = 4.93) (eFig. 3).
Effect of history of psychotropic medication on behavioral placebo effects, baseline $\mu$-opioid

$BP_{ND}$ and placebo-induced changes in $\mu$-opioid $BP_{ND}$

Of the 35 patients studied, 12 of them were experiencing their first depression episode (FDE) of less than two years of duration and were psychotropic-medication naïve. The remaining 23 patients had at least one previous episode of depression [recurrent depression (RD)] or persistent symptoms of depression for more than two years [persistent depressive disorder (PDD)]. All the patients in the RD/PDD group had taken at least one psychotropic medication in the past, except for one subject (who was excluded from the analyses). There were no significant differences between the two groups with respect to age, depression severity at baseline or placebo responsiveness.

Compared with MDD patients in the RD/PDD group, psychotropic naïve patients experiencing their first depression episode showed greater opioid $BP_{ND}$ in the dorsal putamen (-22, 6, 0; 8920 mm$^3$; 4.77), in addition to many cortical regions that did not meet statistical thresholds after correction for multiple comparisons (not shown). Patients in the RD/PDD group (and a history of psychotropic medication use) showed greater i.v. placebo-induced opioid release in the AMY (-24, 0, -18; 416 mm$^3$; 4.28) and NAc (-4, 12, 0; 152 mm$^3$; 3.6), compared to first episode (psychotropic naïve) patients. However, with the current samples we were unable to differentiate whether these effects are caused by the individual previous psychotropic medication history or disease chronicity, questions that would need to be tested in future studies.
**eTable.** Mixed Effects Model of QIDS-16SR Scores Over the 10-Week Open-Label Antidepressant Trial (n = 35)

<table>
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<th>Fixed Effects of Sustained Placebo Model</th>
<th>Estimate</th>
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<tr>
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<td>Placebo Responsiveness by Week Interaction</td>
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<td>QIDS-16SR Scores at Screening</td>
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<td>-1.91, 0.80</td>
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<tr>
<td>Week</td>
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<td>0.08</td>
<td>-0.71, -0.41</td>
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<td>Placebo Responsiveness by Week Interaction</td>
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<td>0.07</td>
<td>-0.19, 0.09</td>
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<tr>
<td>QIDS-16SR Scores at Screening</td>
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<td>0.14</td>
<td>0.15, 0.70</td>
</tr>
</tbody>
</table>

*p-value < 0.05 by z-test

Note: significant placebo response by week interaction effects indicate that decreases in depressive symptoms over time were larger among those with greater placebo responsiveness.
eFigure 1. Regions of Interest: Amygdala (Green); Nucleus Accumbens (Red), and Subgenual Anterior Cingulate Cortex (Blue)
**eFigure 2.** Estimated Mean (± SEM) Depression Level (QIDS-16SR Score) by Placebo Group (Responders Versus Nonresponders) Over 10 Weeks of Antidepressant Treatment (n = 35)

*Note: Means reported for screening are observed rather than predicted because this variable was a covariate in the model reported in eTable 1, rather than a repeated measure of the outcome. The means at screening have been weighted for the amount of data contributed over follow-up by participants.*
**eFigure 3.** Voxel-by-Voxel Effects of Sex on Baseline $\mu$-Opioid $\text{BP}_{\text{ND}}$ (Left) and Placebo-Induced Decrease in $\text{BP}_{\text{ND}}$ (Right)

Compared to men, women showed significantly greater $\mu$-opioid $\text{BP}_{\text{ND}}$ in the bilateral ventral striatum (left) and greater placebo-induced endogenous opioid release in the right NAc and AMY bilaterally (right).
eReferences


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