

Dysregulation of Endogenous Opioid Emotion Regulation Circuitry in Major Depression in Women

Susan E. Kennedy, PhD; Robert A. Koeppe, PhD; Elizabeth A. Young, MD; Jon-Kar Zubieta, MD, PhD

Context: There is extensive evidence implicating dysfunctions in stress responses and adaptation to stress in the pathophysiological mechanism of major depressive disorder (MDD) in humans. Endogenous opioid neurotransmission activating μ -opioid receptors is involved in stress and emotion regulatory processes and has been further implicated in MDD.

Objective: To examine the involvement of μ -opioid neurotransmission in the regulation of affective states in volunteers with MDD and its relationship with clinical response to antidepressant treatment.

Design: Measures of μ -opioid receptor availability in vivo (binding potential [BP]) were obtained with positron emission tomography and the μ -opioid receptor selective radiotracer carbon 11-labeled carfentanil during a neutral state. Changes in BP during a sustained sadness challenge were obtained by comparing it with the neutral state, reflecting changes in endogenous opioid neurotransmission during the experience of that emotion.

Setting: Clinics and neuroimaging facilities at a university medical center.

Participants: Fourteen healthy female volunteers and 14 individually matched patient volunteers diagnosed with MDD were recruited via advertisement and through outpatient clinics.

Interventions: Sustained neutral and sadness states, randomized and counterbalanced in order, elicited by the cued recall of an autobiographical event associated with that emotion. Following imaging procedures, patients underwent a 10-week course of treatment with 20 to 40 mg of fluoxetine hydrochloride.

Main Outcome Measures: Changes in μ -opioid receptor BP during neutral and sustained sadness states,

negative and positive affect ratings, plasma cortisol and corticotropin levels, and clinical response to antidepressant administration.

Results: The sustained sadness condition was associated with a statistically significant decrease in μ -opioid receptor BP in the left inferior temporal cortex of patients with MDD and correlated with negative affect ratings experienced during the condition. Conversely, a significant increase in μ -opioid receptor BP was observed in healthy control subjects in the rostral region of the anterior cingulate. In this region, a significant decrease in μ -opioid receptor BP during sadness was observed in patients with MDD who did not respond to antidepressant treatment. Comparisons between patients with MDD and controls showed significantly lower neutral-state μ -opioid receptor BP in patients with MDD in the posterior thalamus, correlating with corticotropin and cortisol plasma levels. Larger reductions in μ -opioid system BP during sadness were obtained in patients with MDD in the anterior insular cortex, anterior and posterior thalamus, ventral basal ganglia, amygdala, and periamygdalar cortex. The same challenge elicited larger increases in the BP measure in the control group in the anterior cingulate, ventral basal ganglia, hypothalamus, amygdala, and periamygdalar cortex.

Conclusions: The results demonstrate differences between women with MDD and control women in μ -opioid receptor availability during a neutral state, as well as opposite responses of this neurotransmitter system during the experimental induction of a sustained sadness state. These data demonstrate that endogenous opioid neurotransmission on μ -opioid receptors, a system implicated in stress responses and emotional regulation, is altered in patients diagnosed with MDD.

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Author Affiliations:

Departments of Psychiatry (Drs Kennedy, Young, and Zubieta) and Radiology (Drs Koeppe and Zubieta) and the Molecular and Behavioral Neuroscience Institute (Drs Kennedy, Young, and Zubieta), University of Michigan, Ann Arbor.

THERE IS EXTENSIVE EVIDENCE implicating dysfunctions in stress responses and adaptation to stress in the pathophysiological mechanism of major depressive disorder (MDD) in humans. Systems acutely activated in response to stressors, such as those involving the central corticotropin-releasing hormone (CRH), appear to be ab-

normally activated in patients diagnosed with MDD.^{1,2} This chronic overactivation is felt to underlie the hypothalamic-pituitary-adrenal (HPA) axis neuroendocrine abnormalities observed in subgroups of patients with this illness,^{3,4} increases in proopiomelanocortin messenger RNA in the pituitary⁵ and in hypothalamic CRH messenger RNA,^{6,7} and decreases in CRH receptor concentrations in the frontal cor-

Table 1. Demographics and Psychophysiological Variables*

	MDD	Controls
Sample size	14	14
Age, y	35 ± 10	37 ± 9
Education, y	15 ± 2	16 ± 2
HAMD score	24 ± 3	0 ± 1
PANAS negative affect score		
Neutral state	6.3 ± 7.7	1.6 ± 2.4
Sadness state	11.8 ± 9.3	7.8 ± 4.5
PANAS positive affect score		
Neutral state	11.1 ± 7.3	14.6 ± 7.3
Sadness state	9.6 ± 7.2	10.9 ± 6.5
Plasma cortisol level, µg/dL		
Neutral state	7.5 ± 5.8	7.1 ± 2.8
Sadness state	6.4 ± 4.1	7.2 ± 2.9
Plasma corticotropin level, pg/mL		
Neutral state	4.2 ± 3.9	2.2 ± 0.98
Sadness state	3.7 ± 4.3	1.8 ± 0.52
No. of depressive episodes	7 ± 8	0
Duration of current episode, mo	17 ± 31	NA

Abbreviations: HAMD, Hamilton Depression Rating Scale; MDD, major depressive disorder; NA, not applicable; PANAS, Positive and Negative Affectivity Scale.

SI Conversion factors: To convert cortisol to nanomoles per liter, multiply by 27.59; corticotropin levels to picomoles per liter, multiply by 0.22.

*Data are expressed as mean ± 1 SD.

tex of those who commit suicide.⁸ Recent data have also linked the presence of HPA axis dysregulation with a poorer response to selective serotonin reuptake inhibitor (SSRI) antidepressants,⁹ a more protracted course of illness,^{1,10} and suicidal ideation and suicide attempts.^{11,12} By comparison, there is minimal information on other neurotransmitter systems involved in the stress response.

The present work focuses on the functional responses of μ -opioid receptor-mediated neurotransmission. μ -Opioid receptors are densely distributed in brain regions implicated in the response to stressors and emotionally salient stimuli. These include cortical areas, such as the rostral anterior cingulate and prefrontal cortex, thought to have a central role in the regulation and integration of emotionally significant stimuli.¹³⁻¹⁶ Reductions in the metabolic activity of the rostral anterior cingulate have been further associated with a poorer response to SSRI antidepressants.¹⁷ Subcortically, the μ -opioid system is known to have a prominent regulatory role in the striatopallidal pathway (nucleus accumbens, ventral pallidum) and associated circuits (eg, amygdala, thalamus, insular cortex) involved in the evaluation and response to salient stimuli, both rewarding and nonrewarding.^{15,18-24} In human subjects, in vivo μ -opioid receptor availability in the subamygdalar temporal cortex has been found to inversely correlate with the metabolic responses of this region to the presentation of a negative emotional challenge and with the ratings of negative affect experienced during that condition.²⁵

μ -Opioid receptor-mediated neurotransmission is typically activated in response to sustained or unpredictable stressful and noxious stimuli.²⁶⁻²⁸ In animal models, endogenous opioid peptides activating μ -opioid

receptors are centrally involved in the induction of stress-induced analgesia and the reduction of anxietylike responses to environmental adversity.²⁹⁻³² In health, these responses appear adaptive, reducing the physical and affective consequences of a challenge that threatens homeostasis.^{15,16,33} In MDD, an involvement of this neurotransmitter system is supported by findings of pronounced increases in the concentration, but not the affinity, of μ -opioid receptors in the prefrontal cortex, temporal cortex, and the basal ganglia of those with a history of depression who commit suicide.^{34,35}

Herein, we examined the status of the endogenous opioid system and μ -opioid receptors at baseline (a neutral state) and during the induction of a sustained sadness state in a sample of unmedicated patients diagnosed with moderate to severe MDD and a matched control group. In healthy women, this challenge has been associated with a deactivation of μ -opioid receptor-mediated neurotransmission, an effect correlated with the increases in negative affective state reported by the volunteers.¹⁶ It was hypothesized that patients diagnosed with MDD would demonstrate either a blunting of these responses or evidence of stress-induced endogenous opioid system overactivity, paralleling HPA axis alterations. Overactivity of μ -opioid neurotransmission would be observed with external imaging tools (positron emission tomography [PET] and a μ -opioid receptor selective radiotracer) as reductions in baseline μ -opioid receptor availability (or binding potential [BP]) and possibly further short-term reductions in receptor availability in response to the sustained sadness induction. Changes in BP are thought to reflect 1 or more processes associated with neurotransmitter activity (eg, competition of the radiotracer with the endogenous ligand, changes in receptor affinity after its interaction with the endogenous neurotransmitter, or receptor internalization and recycling)³⁶ and will be referred to herein as evidencing "activation" (reductions in BP) or "deactivation" (increases in BP) of μ -opioid receptor-mediated neurotransmission.

METHODS

SUBJECTS

Volunteers were 14 patients diagnosed with MDD and 14 healthy controls individually matched by age and educational level (**Table 1**). Subjects were right-handed women, 36 ± 9 years of age (mean ± SD), with a mean ± SD educational level of 16 ± 2 years. Volunteers had no personal history of acute or ongoing medical illness or substance abuse or dependence (including recent nicotine use [within 1 year] or history of nicotine dependence) and no family history of inheritable illnesses, except for MDD in the patient sample, ascertained by the Structured Clinical Interview for DSM-IV (nonpatient and patient versions³⁷). Patients were included who had Hamilton Depression Rating Scale scores (HAMD) (21-item)³⁸ greater than 20 (moderate to severe depression) but no psychotic symptoms or active suicidal ideation. None of the volunteers were taking psychotropic medications or hormonal treatments, including hormonal birth control, for at least 6 months; they were non-smokers and reported a history of regular menstrual cycles. Phase of the menstrual cycle was not controlled for because previous data demonstrated that μ -opioid receptor binding in vivo is not

influenced by the phase of the menstrual cycle³⁹ and because of logistical considerations (ie, to initiate antidepressant treatment in the MDD group as soon as possible following the PET scan). However, plasma levels of estradiol, which have been found to correlate with μ -opioid receptor BP in reproductive-aged women,^{39,40} were obtained at the time of scanning. All the procedures used were approved by the University of Michigan Investigational Review Board and Radiation Safety Committee. Written informed consent was obtained in all cases.

INDUCTION OF SUSTAINED SADNESS AND NEUTRAL STATES

Neutral and sadness states were initiated either at 5 or 45 minutes after radiotracer administration in a randomized, counter-balanced fashion. Volunteers were blind to the order of the experimental conditions until asked to self-induce neutral or sad emotional states. During the sadness condition, volunteers were instructed to focus on an event associated with a profound feeling of sadness that was selected and rehearsed prior to the actual imaging studies. These included the death of a friend or family member (4 patients, 11 controls); breakups with boyfriends or divorce (2 patients, 2 controls); arguments with boyfriends (1 patient, 1 control); illness of family members (4 patients); or other difficulties in life (3 patients). For the neutral state, they were asked to relax and passively pay attention to current sensory experiences but not to actively involve in other mental processes. Subjects were asked to report their experience every 10 minutes by rating from 1 to 5 the 5 items of the sadness subscale (sad, blue, downhearted, alone, lonely) of the Positive and Negative Affectivity Scale⁴¹ (PANAS) to ascertain their ability to maintain that emotional state. The complete PANAS was rated by the subjects at baseline, 45 minutes after tracer administration, and after completion of the study, the latter 2 retrospectively rating the preceding experimental period.

PET AND MAGNETIC RESONANCE IMAGING ACQUISITION

The PET scans were acquired with a Siemens ECAT Exact scanner (CTI, Knoxville, Tenn) in 3-dimensional mode with septa retracted (intrinsic full width at half maximum resolution, approximately 6 mm in plane and 5 mm in the z-axis), as previously described.¹⁶ Briefly, 370 to 555 MBq (10-15 mCi) of carbon 11 [¹¹C]-labeled carfentanil were administered to each subject at approximately 1:45 PM. Fifty percent of the [¹¹C]carfentanil dose was administered as a bolus and the remainder as a continuous infusion, using a computer-controlled pump to more rapidly achieve steady-state tracer levels. Twenty-two sets of scans were acquired over 100 minutes with an increasing duration (30 seconds up to 10 minutes). Times were decay-corrected and reconstructed using filtered back-projection with a Hanning 0.5 filter and included both measured attenuation and scatter corrections. The dynamic images were then coregistered using automated computer routines.⁴² Image data were transformed, on a pixel-by-pixel basis, into 3 sets of parametric maps: (1) a tracer transport measure (K_1 ratio), (2) 2 receptor-related measures, "neutral" and "sad" B_{max}/K_d (receptor concentration, B_{max} , divided by the affinity of the radioligand to the receptor, K_d), using a Logan graphical analysis,⁴³ and (3) the occipital cortex (an area devoid of μ -opioid receptors) as the reference region.

Anatomical magnetic resonance images (MRIs) were acquired prior to PET scanning on a 1.5-T scanner (Signa; General Electric, Milwaukee, Wis). Acquisition sequences were axial spoiled-gradient inverse-recovery prepared magnetic resonance (echo time=5.5 milliseconds; repetition time=14 milli-

seconds; image time=300 milliseconds; flip angle=20°; number of excitations=1; 124 contiguous images; 1.5-mm thick), followed by axial T2 and proton density images (repetition time=4000 milliseconds; echo time=20 and 100 milliseconds, respectively; number of excitations=1; 62 contiguous images; 3-mm thick). Magnetic resonance images were reviewed by a neuroradiologist to rule out gross structural brain abnormalities prior to PET scanning. K_1 and distribution volume ratio images for each experimental period and spoiled-gradient MRIs were coregistered to each other and to the International Consortium for Brain Mapping stereotactic atlas orientation.⁴⁴

HORMONE ASSAYS

Plasma levels of cortisol and corticotropin were obtained immediately prior to scanning, between 1:30 PM and 1:45 PM. Blood was collected on ice and centrifuged and separated within 30 minutes of drawing. All samples were stored at -80°C until assayed. Cortisol was assayed using Coat a Count kits (Diagnostic Products Corporation, Los Angeles, Calif) and corticotropin, using Allegro HS IRMA (Nichols Diagnostics, San Juan Capistrano, Calif). The intra-assay coefficient of variability (CV) for cortisol assay was 3.0% and the interassay CV, 5.2%. The intra-assay CV for corticotropin was 3% to 4% and the interassay CV, 7% to 8%.

ANTIDEPRESSANT TREATMENT

Patients diagnosed with MDD initiated treatment with fluoxetine hydrochloride (20 mg) after completion of the scanning protocol. Subjects were evaluated every 2 weeks thereafter for adverse effects (self-reported) and symptom severity (HAMD and Clinician Global Impression scale⁴⁵) until completion of the 10-week treatment protocol. At week 4, subjects who responded (more than a 50% reduction in HAMD scores) were maintained on the same dose of medication until completion of the protocol. Nonresponders at week 4 received 40 mg of fluoxetine hydrochloride until completion of the 10-week treatment protocol. Response at the end of the treatment period was defined as 50% or more reduction in HAMD scores from baseline levels. Seven subjects received 20 mg of fluoxetine hydrochloride throughout the study and 7 received 40 mg the last 6 weeks of the protocol.

IMAGE DATA ANALYSIS

Differences between conditions were mapped into stereotactic space using z maps of statistical significance with Statistical Parametric Mapping (SPM99) and Matlab software.⁴⁶ Only pixels with specific binding were included in the analyses (pixels with distribution volume ratio values >1.2 times the mean global image value as calculated with SPM99). To compensate for small residual anatomical variations across subjects and to improve signal-to-noise ratios, a 3-dimensional gaussian filter (full width at half maximum, 6 mm) was applied to each scan. For each subtraction analysis, 1-sample and 2-sample, 2-tailed *t* statistic values were calculated for each pixel using the smoothed pooled variance across pixels.⁴⁷ Significant differences and correlations were detected using a statistical threshold that controls a type I error rate at $P = .05$ for multiple comparisons, estimated using the Euler characteristic and the number of pixels in the gray matter and image smoothness⁴⁶; *z* scores were also deemed significant if they reached statistical thresholds after correction for the size of the cluster under consideration.⁴⁸ We also report regions with uncorrected *P* values $\leq .0001$ that did not reach statistical significance after full correction for multiple comparisons for the purposes of direct-

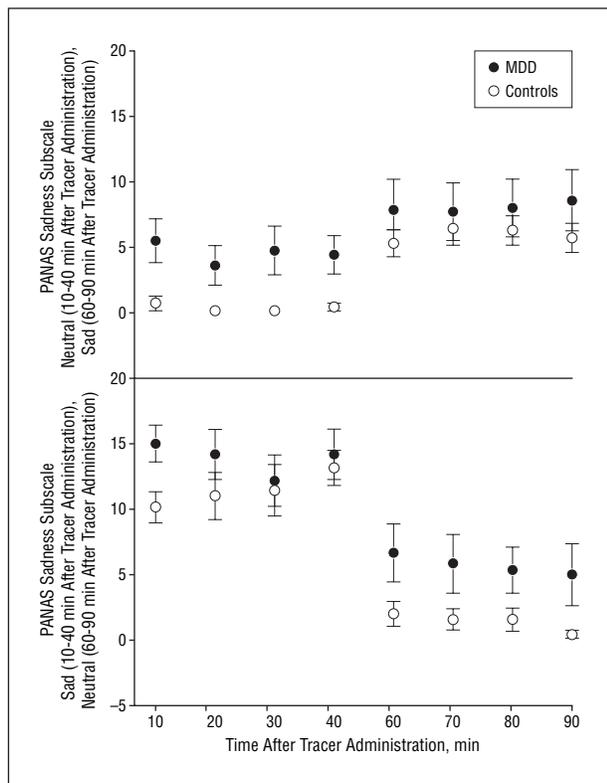


Figure 1. Time course of sadness ratings. Data show the mean \pm SEM for the Positive and Negative Affectivity Scale (PANAS) sadness subscale scores obtained every 10 minutes for the duration of the study. The order of conditions (sad, neutral) was randomized and counterbalanced between subjects. The top panel shows data from studies in which the sadness condition was performed second ($n=7$, patients with major depressive disorder [MDD]; $n=7$, controls), and the bottom panel shows data from studies in which the sadness condition was performed first ($n=7$, patients with MDD; $n=7$, controls), for a total of $n=14$ in each subject group.

ing future research. Only absolute data (B_{max}/K_d values, BP) were tested for statistical significance (that is, no further normalization of the data was applied to reduce interexperimental variability). Data for significant regions were then extracted from the images for the calculation of effect sizes, data plots, and regional correlation values (r) including voxels that reached a $P < .001$ for the significant clusters detected in the SPM99 analyses. Data are shown as mean \pm 1 SD.

RESULTS

Demographics and nonimaging variables are presented in Table 1. A trend toward higher plasma corticotropin levels was noted in the MDD group ($t_{21} = 1.8$; $P = .08$). The MDD group showed higher HAMD ($t_{26} = 29.2$; $P < .001$), neutral-state PANAS negative affect ($t_{24} = 2.1$; $P = .046$), and PANAS sadness subscale scores both during the neutral state ($t_{25} = 3.9$; $P = .001$) and during the sadness induction ($t_{25} = 2.2$; $P = .05$). No significant differences between groups were observed for plasma levels of estradiol (mean \pm SD, 74.6 ± 67.4 pg/mL [273.9 ± 247.4 pmol/L] in controls; 96.3 ± 57.4 pg/mL [353.5 ± 210.7 pmol/L] in MDD; $df = 23$; $P = .39$). The PANAS sadness subscale scores increased from 0.6 ± 1.1 (mean \pm SEM) during the neutral state to 7.9 ± 3.9 during the sadness state in controls and from 5.6 ± 4.5 (mean \pm SEM) to 11.8 ± 5.7 in the MDD

group (**Figure 1**). Ten subjects with MDD were classified as responders and 4, as nonresponders to fluoxetine treatment.

BASELINE MEASURES

Significantly lower neutral-state μ -opioid receptor BP was detected in the patients compared with the control group in the right posterior thalamus (x , y , and z coordinates in millimeters, -11 , -30 , and 5 ; $z = 7.52$; $P < .001$ after correction for multiple comparisons). Not reaching statistical significance, lower μ -opioid receptor BP was also noted in the left posterior thalamus (x , y , and z coordinates in millimeters, 16 , -31 , and 10 ; $z = 3.96$; $P > .05$). These corresponded to mean differences in μ -opioid receptor BP between groups of 14.7% and 15.5% in the right and left posterior thalamus, respectively (mean \pm SD BP, right, controls, 2.78 ± 0.48 , patients, 2.37 ± 0.27 ; left, controls, 2.61 ± 0.27 , patients, 2.28 ± 0.22) (**Figure 2**). Plasma levels of estradiol were further introduced as a covariate in the analyses, with no significant effects of estradiol or significant group \times estradiol interactions ($P > .05$).

No significant correlations were obtained between posterior thalamus μ -opioid receptor BP and measures of symptom severity in the MDD group (HAMD, Beck Depression Inventory⁴⁹ scores) ($P > .05$). However, significant and trend correlations were obtained between thalamic μ -opioid receptor BP and corticotropin and cortisol plasma levels acquired prior to scanning. Right thalamic BP was negatively correlated with plasma corticotropin levels ($r = -0.58$; $P < .05$) and at trend levels in the same direction with plasma cortisol levels ($r = -0.50$; $P = .08$). Left thalamic BP was also negatively correlated with corticotropin ($r = -0.61$; $P < .05$) and cortisol plasma levels ($r = -0.58$; $P < .05$) ($df = 13$ for all comparisons) (**Figure 2**).

μ -Opioid receptor BP in the right and left posterior thalamus was then examined as a function of response to 10 weeks of fluoxetine treatment in patients with MDD. The nonresponder group ($n = 4$) demonstrated lower BP values compared with responders ($n = 10$) in these regions (mean \pm SD, right thalamus, treatment responders, 2.48 ± 0.33 , nonresponders, 2.10 ± 0.24 ; $t = 2.11$; $P < .05$; left thalamus, responders, 2.37 ± 0.21 , nonresponders, 2.24 ± 0.18 ; $t = 1.95$; $P = .07$) (**Figure 2**).

RESPONSE TO SUSTAINED SADNESS INDUCTION

No evidence of μ -opioid system activation was observed in the healthy control group in response to the sustained sadness challenge (reductions in BP from the neutral to the sadness state) ($P > .05$ after correction for multiple comparisons). However, the MDD group demonstrated significant activation of μ -opioid neurotransmission in the left inferior temporal cortex (x , y , and z coordinates in millimeters, 27 , 2 , and -34 ; $z = 5.06$; $P < .01$ after correction for multiple comparisons; mean change, 11.8%). This activation was positively correlated with the PANAS negative affect scores attained during the sadness state ($df = 13$; $r = 0.67$; $P < .01$), but not with HAMD or Beck Depression Inventory scores or plasma levels of cortisol or corticotropin ($P > .05$). No

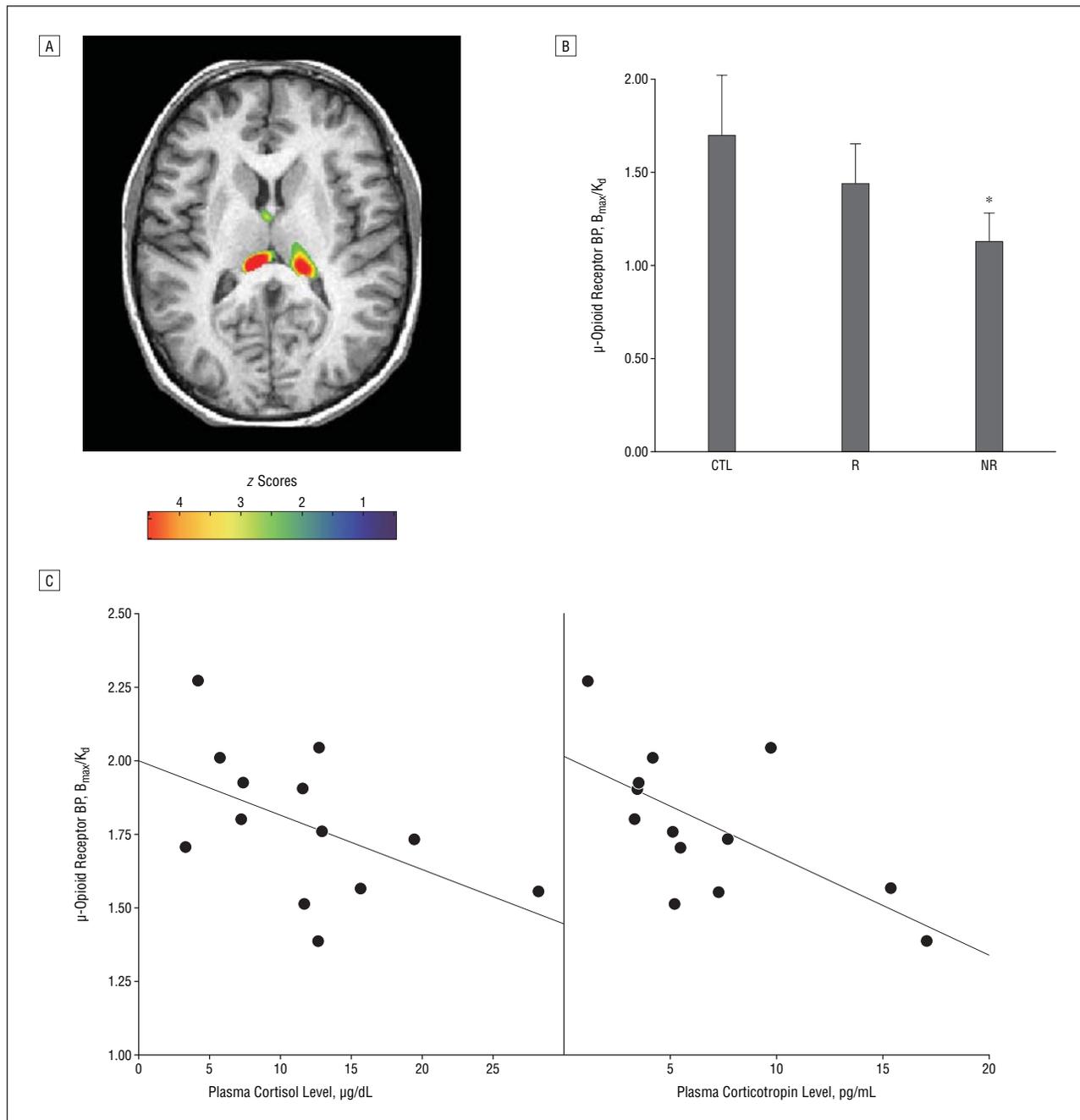


Figure 2. Lower regional μ -opioid binding potential (BP) during a neutral state in the posterior thalamus in subjects with major depressive disorder (MDD) compared with healthy controls. A, The z scores of statistical significance are represented by the pseudocolor scale under the image and are superimposed over an anatomically standardized magnetic resonance image in an axial view. Image data are displayed according to standard radiological convention so that the left side of the image corresponds to the right side of the brain. B, The mean neutral-state μ -opioid receptor BP in the right posterior thalamus. Error bars represent 1 SD from the mean. The nonresponder group demonstrated significantly lower BP values than responders in the right thalamus ($t=2.11$; $P<.05$). * $P<.05$ in comparison with healthy controls. C, Graphs of individual values for subjects with MDD for negative correlations between μ -opioid receptor BP during the neutral state in the left posterior thalamus and plasma cortisol ($r=-0.58$; $P<.05$; $df=13$) and corticotropin levels ($r=-0.61$; $P<.05$; $df=13$). Lines represent least squares linear regressions. B_{max} indicates receptor concentration; K_d , receptor affinity for radiotracer; CTL, controls ($n=14$); R, responders to 10 weeks of fluoxetine hydrochloride treatment ($n=10$); NR, nonresponders to 10 weeks of fluoxetine treatment ($<50\%$ decrease in Hamilton Depression Rating Scale score from week 1 to week 10) ($n=4$). To convert cortisol to nanomoles per liter, multiply by 27.59; corticotropin levels to picomoles per liter, multiply by 0.22.

significant difference in the magnitude of μ -opioid system activation in this region was detected between treatment responders and nonresponders, although nonresponders showed mean \pm SD changes in BP values that were slightly larger than the responders (responders, $9.6\% \pm 10.9\%$; nonresponders, $18.9\% \pm 9.2\%$; 2-tailed, unpaired $t=1.46$; $P>.05$). When plasma levels

of estradiol were introduced as a covariate, no significant effects of estradiol or significant group \times estradiol interactions were obtained ($P>.05$).

In the control group, the challenge was associated with a regional deactivation of μ -opioid neurotransmission (evidenced as increases in BP values from the neutral to the sadness state), as previously described.²⁴ This deactiva-

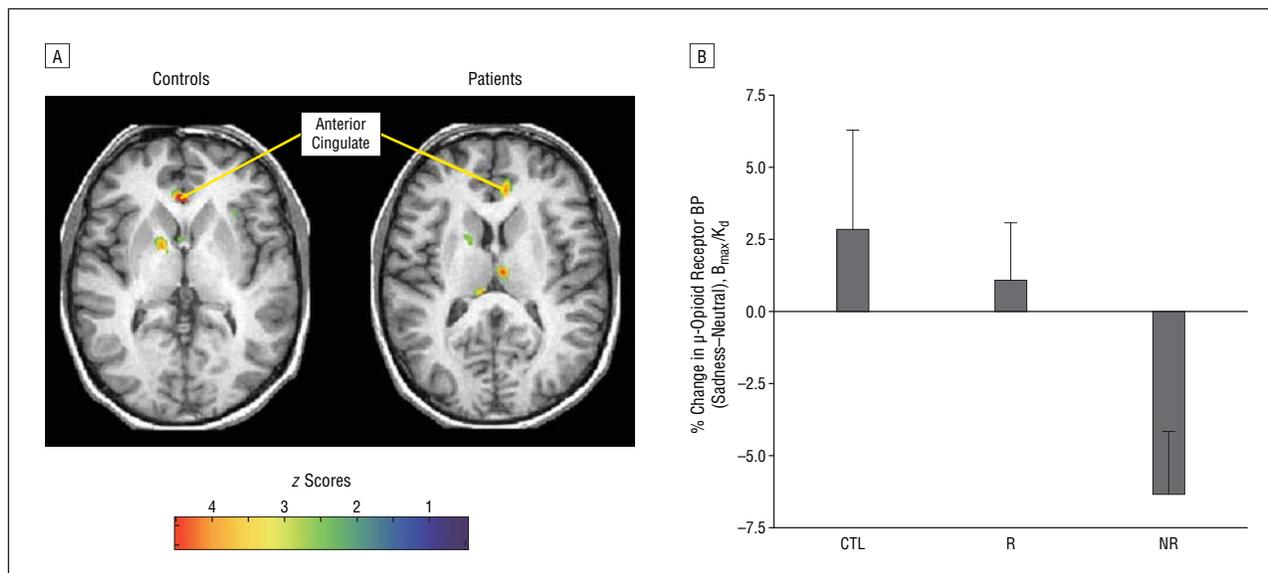


Figure 3. Responses of μ -opioid receptor-mediated neurotransmission in the anterior cingulate to a sustained sadness challenge as a function of treatment response. A, The z scores of statistical significance are represented by the pseudocolor scale on the lower part of the figure and are superimposed over an anatomically standardized magnetic resonance image in axial views. Image data are displayed according to standard radiological convention so that the left side of the image corresponds to the right side of the brain. B, The average percentage of change between binding potential (BP) values obtained in the neutral and sadness states within subjects. The error bars show the standard deviation from the mean percentage of change. B_{max} indicates receptor concentration; K_d , receptor affinity for radiotracer; CTL, controls (n=14); R, responders to 10 weeks of fluoxetine hydrochloride treatment (n=10); NR, nonresponders to 10 weeks of fluoxetine treatment (<50% decrease in Hamilton Depression Rating Scale score from week 1 to week 10) (n=4).

tion was localized in the rostral anterior cingulate (peak x, y, and z coordinates in millimeters, -3, 32, and 2; $z=5.47$; $P<.001$ after correction for multiple comparisons; mean change, 16.4%) (**Figure 3**). The magnitude of μ -opioid system deactivation was significantly correlated with the PANAS negative affect scores during the sadness state ($df=13$; $r=0.62$; $P=.02$). Plasma levels of estradiol, when introduced as a covariate, did not show effects or group \times estradiol interactions in this region ($P>.05$).

No significant deactivation in μ -opioid neurotransmission was observed in patients with MDD. However, the anterior cingulate region that registered significant μ -opioid system deactivation in controls showed subthreshold changes in the entire MDD group (x, y, and z coordinates in millimeters, -4, 32, and 2; $z=3.52$; $P>.05$ after correction for multiple comparisons) (Figure 3). Changes in μ -opioid receptor BP in this region in the patients with MDD were not correlated with the changes in affective ratings (PANAS scores) or symptom severity (HAMD and Beck Depression Inventory scores) ($P>.05$). However, significant differences in the direction of μ -opioid system activation were obtained between treatment responders and nonresponders. Treatment responders demonstrated mean deactivations in μ -opioid neurotransmission in this region, similar to the control group. Treatment nonresponders demonstrated the opposite response, mean activations of μ -opioid neurotransmission (reductions in BP in response to the sustained sadness state) (mean \pm SD change in BP, treatment responders, $-1.1\% \pm 6.0\%$, nonresponders, $6.3\% \pm 4.4\%$; $t_{12}=2.19$; $P<.05$) (Figure 3B). The level of μ -opioid system activation in this region was positively correlated with cortisol plasma levels acquired prior to scanning ($df=13$;

$r=0.61$; $P<.05$) but not with corticotropin levels ($df=13$; $r=0.20$; $P>.05$).

ACTIVATION AND DEACTIVATION OF μ -OPIOID RECEPTOR-MEDIATED NEUROTRANSMISSION DURING SUSTAINED SADNESS

We then tested whether activations (observed only in the MDD group) and deactivations (observed only in the healthy control group) reached statistically significant differences between patients and controls in voxel \times voxel, 2-sample t tests in SPM99. Results are summarized in **Table 2**. The comparison assessing μ -opioid system activation in patients with MDD, [(neutral_{MDD}-sad_{MDD})-(neutral_{CONTROL}-sad_{CONTROL})], yielded a number of regions with significantly greater μ -opioid system activation in the MDD group. These included the right anterior insular cortex, anterior and posterior thalamus, ventral basal ganglia (with separate peaks overlaying the nucleus accumbens and ventral pallidum), and bilaterally in the amygdala (extending to the inferior temporal cortex). Plasma level of estradiol was introduced as a covariate with no significant effects or group \times estradiol interactions obtained in these regions ($P>.05$). No significant effects were obtained for the comparison assessing significantly higher μ -opioid system activation in the healthy control group [(neutral_{CONTROL}-sad_{CONTROL})-(neutral_{MDD}-sad_{MDD})].

The subtraction [(sad_{CONTROL}-neutral_{CONTROL})-(sad_{MDD}-neutral_{MDD})], assessing whether μ -opioid system deactivation was more pronounced in the healthy control group, demonstrated significance in the inferior temporal cortex (Table 2). Not reaching statistical significance, results in the same direction were observed in the anterior cingulate, left anterior temporal cortex, right

Table 2. Changes in In Vivo Regional μ -Opioid Receptor Availability in MDD and Healthy Controls From Neutral to Sad States*

Region	Controls		MDD		Coordinates (x, y, z), mm†	Cluster Size‡	z Score	P Value
	Neutral	Sadness	Neutral	Sadness				
μ-Opioid System Activation, MDD > Controls								
Right anterior temporal cortex	1.28 ± 0.24	1.25 ± 0.23	1.27 ± 0.16	1.15 ± 0.19	-30, 4, -23	2698	5.38	.007
Left inferior temporal cortex	1.08 ± 0.25	1.08 ± 0.18	1.08 ± 0.21	1.03 ± 0.23	25, 4, -38	3799	5.59	.002
Right anterior insula	0.84 ± 0.21	0.86 ± 0.16	0.89 ± 0.25	0.87 ± 0.26	-38, -4, 17	392	5.07	.03
Right ventral basal ganglia	2.34 ± 0.45	2.36 ± 0.31	2.26 ± 0.44	2.18 ± 0.32	-15, 12, -2	739	5.04	.04
Right thalamus	1.77 ± 0.41	1.71 ± 0.33	1.80 ± 0.26	1.49 ± 0.26	-7, -5, 4	4062	5.62	.002
Right amygdala	1.74 ± 0.34	1.60 ± 0.23	1.80 ± 0.29	1.52 ± 0.36	-23, -2, -19	235	5.19	.02
μ-Opioid System Deactivation, Controls > MDD								
Anterior cingulate	1.22 ± 0.34	1.25 ± 0.29	1.12 ± 0.29	1.09 ± 0.21	-8, 36, 13	651	4.42	.38§
Left anterior temporal cortex	1.12 ± 0.36	1.15 ± 0.31	1.10 ± 0.30	1.05 ± 0.24	49, 7, -28	424	4.24	.63§
Left inferior temporal cortex	0.88 ± 0.29	0.93 ± 0.23	0.98 ± 0.31	0.86 ± 0.29	23, 5, -37	1073	5.59	.002
Left ventral basal ganglia	1.37 ± 0.43	1.38 ± 0.23	1.32 ± 0.23	1.28 ± 0.27	13, 0, -3	131	4.62	.19§
Right ventral basal ganglia	1.85 ± 0.45	1.77 ± 0.26	1.68 ± 0.38	1.65 ± 0.29	-16, 5, -1	570	4.32	.52§
Hypothalamus	1.37 ± 0.23	1.20 ± 0.28	1.23 ± 0.34	1.18 ± 0.32	1, -4, -10	495	4.93	.06§
Left amygdala	1.35 ± 0.42	1.31 ± 0.40	1.33 ± 0.30	1.26 ± 0.32	19, -1, -22	386	4.29	.55§

Abbreviations: B_{max} , receptor concentration; K_d , receptor affinity for radiotracer; MDD, major depressive disorder.

*Data are expressed as the mean ± 1 SD of binding potential values (B_{max}/K_d) in regions showing significant differences in μ -opioid system activation from neutral to sadness states between MDD and control groups. *P* values are corrected for multiple comparisons.

†Coordinates listed are the location of the peak in stereotaxic coordinates.

‡Cluster size is expressed in 1-mm³ voxels.

§*P* ≤ .0001 uncorrected for multiple comparisons.

and left ventral basal ganglia, hypothalamus, and left amygdala. No significant effects of plasma estradiol levels or interactions were obtained (*P* > .05). No significant effects were obtained for the comparison assessing higher μ -opioid system deactivation in the patient group [(sad_{MDD} - neutral_{MDD}) - (sad_{CONTROL} - neutral_{CONTROL})].

COMMENT

We describe the effects of an emotional challenge, the induction of a sustained sadness state, on the response of a stress-activated neurotransmitter system in women diagnosed with MDD and in matched healthy controls. In these studies, external measures of changes in endogenous opioid function (changes in the BP, or availability of μ -opioid receptors in vivo) were obtained with PET under neutral and sustained sadness conditions. Evidence of sustained sadness-induced activation (regional reductions in the BP measure) was obtained in the MDD group, while only deactivation (regional increases in BP) was observed in matched healthy controls. Neutral-state BP measures were additionally compared between groups, reflecting the in vivo availability of opioid receptors in the absence of an emotional challenge and demonstrating reductions in the BP measure in the thalamus of patients with MDD.

The self-induction of a sustained sadness state was associated with significant deactivation of μ -opioid receptor-mediated neurotransmission in the rostral anterior cingulate of healthy subjects, the magnitude of which correlated with the subjects' ratings of negative affect. Similar effects have been previously described using this and other challenges in women.^{16,33} These reductions in healthy controls are consistent with a dynamic role of the endogenous opioid system in regulating emotional

states. They further reflect the presence of a tonic activity of regional endogenous opioid neurotransmission under nonchallenged conditions (ie, during an emotionally neutral state), previously described in animal models⁵⁰⁻⁵³ and in humans.⁵⁴ Conversely, in the MDD sample, the sadness induction was associated with an activation of μ -opioid receptor-mediated neurotransmission, similar to that observed during experimental stress in other studies.^{15,55} This took place in the subamygdalar left inferior temporal cortex, a region previously involved in the μ -opioid receptor regulation of responses to affective stimuli.²⁵ A positive correlation was further obtained between the magnitude of μ -opioid system activation in this region and subjects' PANAS ratings of negative affect.

These data show that the engagement of stress-responsive neurotransmission (ie, the endogenous opioid and μ -opioid receptor system) differed between healthy subjects and patients diagnosed with MDD in the face of a negative affective challenge, both in the localization and direction of response. It further supports a role of the endogenous opioid system and μ -opioid receptors in interfacing stress responses and emotional regulation, as suggested by previous data in this area in rodents,^{29,56,57} nonhuman primates,^{30,38} and in humans.^{15,16,33,55}

In addition, it was observed that in the rostral anterior cingulate, a region implicated in mood regulation and sensory-emotional integration,⁵⁹⁻⁶² μ -opioid system responses differed between patients who subsequently responded to treatment and those who did not. Nonresponders to a 10-week course of an SSRI antidepressant demonstrated increases in μ -opioid system activation during the challenge, while responders displayed responses more closely related to those of controls (mean deacti-

vations of opioid neurotransmission). Interestingly, dysfunctions in the basal activity of this region have been previously associated with nonresponse to pharmacological treatment in MDD. Using PET to measure regional glucose metabolism, Mayberg et al⁶¹ reported lower baseline metabolism in this area in treatment nonresponders. Supporting the hypothesis that the activation of endogenous opioid neurotransmission in this region represents a central correlate of a dysfunction of stress responses, we observed a positive correlation between μ -opioid system responses in the anterior cingulate and cortisol plasma levels in patients with MDD.

The findings of lower in vivo availability of posterior thalamus μ -opioid receptors during the neutral state in patients with MDD compared with controls also supports the hypothesis of an overactivation (or alternatively, a down-regulation) of these receptors in MDD. This brain region forms part of circuits involved in the response to affective stimuli.⁶² Reductions in the availability of opioid receptors in this region have been described in clinical pain and during experimental stressful challenges.^{15,16,63,64} Correlations between μ -opioid receptor availability in the posterior thalamus and plasma levels of corticotropin and cortisol were additionally obtained. In addition, and similarly to the results obtained in the anterior cingulate cortex, the most pronounced reductions in μ -opioid receptor availability in the posterior thalamus were encountered in the nonresponders to the SSRI trial.

Finally, comparisons between the response of patients and controls to the sustained sadness induction challenge confirmed the presence of significant differences in the direction of μ -opioid receptor-mediated responses to the sustained sadness challenge. Significantly greater activation of μ -opioid neurotransmission was observed in the insular and inferior temporal cortices, anterior and posterior thalamus, ventral basal ganglia (including separate peaks overlaying the nucleus accumbens and ventral pallidum), and amygdala of patients with MDD compared with the control group. More deactivation of this neurotransmitter system was observed in the anterior cingulate cortex, amygdala, inferior temporal cortex, ventral basal ganglia, and hypothalamus of the healthy volunteers. These are regions and circuits involved in responses to affective challenges (recently reviewed by Phan et al⁶⁵) but also in the μ -opioid system regulation of stress,^{15,66,67} salient rewarding and nonrewarding stimuli,^{19,24,68} negative emotional states,^{16,25,29} emotional memory,²³ and the neuroendocrine stress response.⁶⁹ These results are consistent with an overlap between circuits and neurotransmitter systems (eg, the endogenous opioid) underlying the neurobiological substrates of emotion and stressors.^{13,22,70} They further provide an avenue of exploration to understand the substrates underlying the interaction of emotional dysregulation and the development of MDD with physical and emotional stressors.

The results reported herein obtained in female patients with MDD may or may not be generalizable to male patients with MDD. Sex differences have been observed in μ -opioid receptor availability during a baseline state⁷¹ and in μ -opioid receptor-mediated responses to a stressful challenge.^{33,40} In addition, while there does not ap-

pear to be a significant sex difference in the ability to self-induce sadness or happiness,^{44,72} healthy women display more neuronal activity in anterior limbic structures during transient sadness than healthy men.⁴⁴ Further studies are required to assess the role of endogenous opioid mechanisms in the regulation of negative affective states in male patients with MDD.

The endogenous opioid system and μ -opioid receptors appear to form part of a family of neurotransmitter systems, such as the CRH, noradrenergic, dopaminergic, and serotonergic, directly or indirectly altered in MDD and possibly other stress-associated pathological states. Further investigation of individual differences in the effects of antidepressant therapies on stress-induced μ -opioid system responses is warranted to further elucidate the pathophysiological mechanisms of this frequent and disabling illness.

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Correspondence: Jon-Kar Zubieta, MD, PhD, Department of Psychiatry, University of Michigan Medical School, Molecular and Behavioral Neuroscience Institute, 205 Zina Pitcher Pl, Ann Arbor, MI 48109-0720 (zubieta@umich.edu).

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Correction

Error in Figure Key. In the Original Article titled "Reduced Muscarinic Type 2 Receptor Binding in Subjects With Bipolar Disorder," published in the July issue of the ARCHIVES (2006; 63:741-747), there is an error in the key for **Figure 1 D**. The blue bars represent controls, the yellow bars represent subjects with major depressive disorder, and the green bars represent subjects with bipolar disorder. The figure and correct key are shown below.

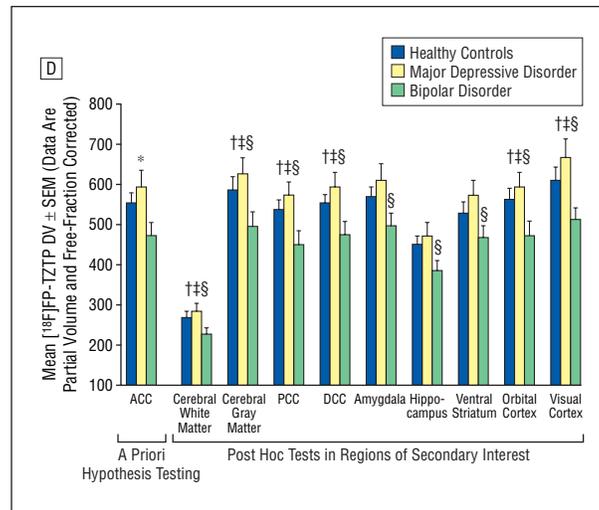


Figure 1. D, Regional [¹⁸F]FP-TZTP DV (fluorodopa F 18 [3-(3-[3-fluoropropyl]thio)-1,2,5-thiadiazol-4-yl]-1,2,5,6-tetrahydro-1-methylpyridine) in the primary and secondary structures of interest. Analysis of variance (ANOVA) significance at $P < .05$. ACC indicates anterior cingulate cortex; DCC, dorsal cingulate cortex; PCC, posterior cingulate cortex; *Bipolar disorder (BD) < healthy controls and BD < major depressive disorder (MDD) for t test $P < .05$; †ANOVA significance at $P_{\text{uncorrected}} < .05$; ‡BD < healthy controls $P_{\text{uncorrected}} < .05$; §BD < MDD $P_{\text{uncorrected}} < .05$.