Relationship Between Amygdala Responses to Masked Faces and Mood State and Treatment in Major Depressive Disorder

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Context: Major depressive disorder (MDD) is associated with behavioral and neurophysiological evidence of mood-congruent processing biases toward explicitly presented, emotionally valenced stimuli. However, few studies have investigated such biases toward implicitly presented stimuli.

Objective: To investigate differential amygdala responses to sad, happy, and neutral faces presented below the level of explicit conscious awareness using a backward masking task in unmedicated participants with MDD and healthy controls (HCs).

Design: Initial cross-sectional design followed by a longitudinal treatment trial using functional magnetic resonance imaging.

Setting: Psychiatric outpatient clinic at the National Institute of Mental Health.

Participants: We studied 22 unmedicated, currently depressed people with MDD (dMDD), 16 unmedicated individuals with MDD in full remission (rMDD), and 25 HCs.

Intervention: Ten dMDD participants underwent 8 weeks of antidepressant treatment with the selective serotonin reuptake inhibitor sertraline hydrochloride.

Main Outcome Measures: Amygdala region-of-interest and whole-brain analyses evaluated the hemodynamic response during exposure to masked sad vs masked happy faces, to masked sad vs neutral faces, and to masked happy vs neutral faces.

Results: The dMDD participants showed greater amygdala responses than HCs to masked sad faces, whereas HCs showed greater amygdala responses to masked happy faces. The bias toward sad faces also was evident in rMDD participants relative to HCs and did not differ between dMDD and rMDD participants. This processing bias reversed toward the normative pattern in dMDD participants after sertraline treatment.

Conclusions: Emotional-processing biases occur in amygdala responses to sad faces presented below the level of conscious awareness in dMDD or rMDD individuals and to happy faces in HCs. By influencing the salience of social stimuli, mood-congruent processing biases in the amygdala may contribute to dysfunction in conscious perceptions and social interactions in MDD. Our data suggest, however, that the negative bias resolves and a positive bias develops in patients with MDD during selective serotonin reuptake inhibitor treatment.

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A MOOD-CONGRUENT PROcessing bias toward negatively valenced emotional stimuli is a consistent pathophysiological feature of major depressive disorder (MDD). This bias is evident in behavioral measures that evaluate memory and attention[1-4] as well as in neurophysiological indexes.[5-10] For example, neurophysiological responses to explicitly presented sad faces are exaggerated in the amygdala in depressed patients compared with healthy controls (HCs),[11] and this abnormality normalizes after antidepressant drug treatment.[12]

The amygdala plays a pivotal role in evaluating the emotional salience of sensory stimuli through participation in 2 distinct types of distributed networks: one involving cortical regions that allow conscious or explicit stimulus perception and the other involving subcortical structures that allow rapid nonconscious assessment of stimulus features.[13,14] Notably, healthy individuals show greater amygdala responses to happy vs sad faces when stimuli are presented below the level of conscious awareness. This finding suggests the existence of a normal positive-processing bias that is supported by subcortical networks that mediate rapid automatic emotional evaluations.[15] Thus, the negative-processing bias that characterizes MDD might also be mediated by this rapid nonconscious processing network involving the amygdala.

Our study used functional magnetic resonance imaging (fMRI) to test the hypothesis that the amygdala response to emo-
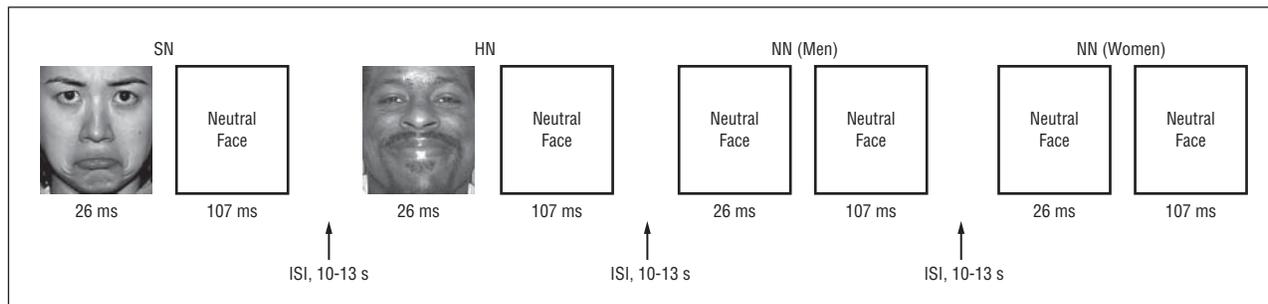
Figure 1. Design of the backward masking task. Two faces were shown as part of each trial presentation. Examples of the masked face event types are shown with placeholders to indicate the presentation of a neutral face. Closed-mouth images were used to reduce nonspecific differences in the stimulus features across stimulus types. HN indicates masked happy face followed by an unmasked neutral face; ISI, interstimulus interval; NN, masked neutral face followed by an unmasked neutral face; and SN, masked sad face followed by an unmasked neutral face.

tional stimuli presented below the level of conscious awareness would show a negative-processing bias in unmedicated participants with MDD. First, this bias was evaluated between currently depressed people with MDD (dMDD) and HCs. Second, the dependence of this emotional-processing bias on current mood state was assessed by comparing amygdala responses between dMDD participants, HCs, and individuals with MDD in full remission (rMDD). Finally, the sensitivity of the processing bias to antidepressant treatment was evaluated by comparing the amygdala responses in dMDD participants before and after 8 weeks of sertraline hydrochloride treatment.

METHODS

PARTICIPANTS

Twenty-two dMDD participants,[16] 16 rMDD participants,[16] and 25 HCs completed the fMRI protocol. Volunteers between the ages of 18 and 50 years were recruited through the clinical services of the National Institute of Mental Health or newspaper advertisements in the Washington, DC, metropolitan area. Participants underwent a screening evaluation before enrollment that involved a medical and psychiatric history, laboratory testing, drug screening, physical examination, and neuromorphological MRI. Only right-handed individuals were selected, as assessed with the Edinburgh Handedness Inventory.[17] The psychiatric diagnosis was established using the Structured Clinical Interview for *DSM-IV*[18] and a semistructured interview with a psychiatrist. The Family Interview for Genetic Studies[19] was used to screen for family history of psychiatric disorders.

Participants were excluded if they had (1) serious suicidal ideation or behavior, (2) major medical or neurological disorders, (3) exposure to drugs likely to influence cerebral blood flow or neurological function within the past 3 weeks (8 weeks for fluoxetine hydrochloride), (4) a history of drug or alcohol abuse within the past year or a lifetime history of drug or alcohol dependence,[16] (5) current pregnancy or breastfeeding, or (6) general MRI exclusion criteria. Additional exclusions applied to the HCs included a history of any major psychiatric disorder or having a first-degree relative with a mood or an anxiety disorder. Additional exclusions applied to the rMDD participants included having experienced a depressive episode or having received psychotropic medications within 3 months before MRI. After receiving a complete explanation of the study procedures, all participants provided written informed consent as approved by the National Institute of Mental Health Institutional Review Board. Individuals received financial compensation for their participation.

Intelligence testing and mood ratings were performed using the Wechsler Abbreviated Scale of Intelligence,[20] Hamilton Depression Rating Scale (HAM-D),[21] Automatic Thoughts Questionnaire (ATQ),[22] Inventory of Depressive Symptomatology: Self-Rating (IDS-SR),[23] State-Trait Anxiety Inventory–State (STAI-S),[24] State-Trait Anxiety Inventory–Trait (STAI-T),[24] and Thought Control Questionnaire (TCQ).[25]

ANTIDEPRESSANT DRUG TREATMENT

Ten of the dMDD participants underwent a second MRI after 8 weeks of treatment with the selective serotonin reuptake inhibitor sertraline. To control for test-retest and other nonspecific order effects, 10 HCs underwent additional MRI after the same interval. After the baseline MRI study in the pretreatment condition, the dMDD participants received sertraline hydrochloride (50 mg/d for 3 days and then titrated to 100 mg/d as tolerated). After 3 weeks of follow-up, the dose was increased or decreased as clinically indicated. All participants received a stable sertraline dose for at least 4 weeks before the posttreatment MRI study. At the posttreatment study, the mean (SD) sertraline dose was 105 (50) mg/d (range, 50-200 mg/d). Additional information regarding patient selection for the treatment study (eMethods and eFigure; http://www.archgenpsychiatry) and a comparison of the participants who received treatment vs those who did not appears in the "eResults" section of the supplemental text.

fMRI DATA ACQUISITION

Images were obtained using a General Electric 3.0-T scanner (GE Signa, Milwaukee, Wisconsin) with an 8-channel phased-array head coil using an echoplanar imaging pulse sequence (39 continuous slices; echo time=20 milliseconds; repetition time=2000 milliseconds; flip angle=90°; matrix=64×64; field of view=22 cm; voxel dimensions=3.4×3.4×3.0 mm³). A total of 290 fMRIs were acquired in each of four 10-minute runs during the backward masking task. The first 4 images of each run were discarded to allow for steady-state tissue magnetization. To provide an anatomical framework for analysis of the fMRIs, high-resolution anatomical images were also obtained using a fast spoiled gradient echo sequence (128 axial slices; echo time=2.7 milliseconds; repetition time=780 milliseconds; flip angle=12°; matrix=224×224; field of view=22 cm; 1.2-mm-thick, in-plane resolution=0.98 mm²).

fMRI BACKWARD MASKING TASK

Participants underwent fMRI while performing a novel version of a backward masking task using a slow–event-related design (**Figure 1**). Before each run, participants were shown 2

neutral target faces. They were instructed to remember the faces and to respond as quickly as possible to indicate whether a target face appeared during the current trial. They used a button box with their right hand and pressed the *1* button if the face shown was one of the 2 target faces or the *2* button if the target face was not shown. Target faces displayed neutral, sad, or happy expressions, and participants judged whether a target face was present based on the identity of the person pictured, irrespective of emotional expression. Individuals demonstrated their understanding of the task by performing an abbreviated version using flash cards before undergoing fMRI. Each task trial displayed faces in pairs, including a 26-millisecond masked face immediately followed by a 107-millisecond masking face to inhibit explicit perception of the first face.[26,27] Each face stimulus was presented in the masked position and followed by a neutral stimulus for the following pairings: sad-neutral (SN), happy-neutral (HN), or neutral-neutral (NN). In addition, a neutral face stimulus was presented in the masked position and followed by an emotional face stimulus for the following pairings: neutral-sad (NS) and neutral-happy (NH). A sad or happy face stimulus in the masked position was never also presented in the unmasked position. The SN, HN, NS, and NH stimulus types were presented 8 times and the NN type 16 times within each run in a pseudorandomized mixed-trial design. Each run used different target faces and emotional face stimuli from distinct actors. The data from the 4 runs were combined so that each stimulus type was presented a total of 32 times for stimulus pairs that included an emotional face and 64 times for pairs including only neutral faces. Within a single trial, the identity of the masked face was never the same as the identity of the masking face, but the 2 face stimuli always depicted the same sex. The sex for all stimulus pairings was balanced across runs. A 10- to 13-second interstimulus interval was selected to allow the hemodynamic response to return to baseline before the next stimulus presentation.

Stimuli were presented using E-Prime software (Psychological Software Tools, Pittsburgh, Pennsylvania) on a Monarch Hornet PC computer (Monarch Computer Systems, Tucker, Georgia) with a cathode ray tube monitor at 75 Hz and a cloned projection display to participants in the scanner gantry. Accuracy of the presentation times was verified using a photodiode and oscilloscope. Face stimuli were obtained from the NimStim Set of Facial Expressions.[28] For the third experiment, the dMDD participants and HCs performed the same task before and after treatment using unique happy and sad face stimuli for the separate sessions.

ASSESSMENT OF BEHAVIORAL PERFORMANCE DURING fMRI

Behavioral data were analyzed using SPSS version 14.0 statistical software (SPSS Inc, Chicago, Illinois). Accuracy of responses and reaction times for identifying whether each face stimulus was a target was recorded using E-Prime software. The efficacy of the backward masking was assessed by categorizing each participant's response to a stimulus event according to target detection accuracy. Individuals were debriefed after the fMRI study and asked about their experience performing the task.

fMRI PROCESSING AND STATISTICAL ANALYSES

Functional imaging analyses were performed using the general linear model within SPM5 statistical software (Wellcome Trust Centre for Neuroimaging, London, England; http://www.fil.ion.ucl.ac.uk/spm). Whole-brain fMRI volumes were realigned to the first volume, coregistered to each participant's anatomical study, and normalized to fit the Montreal Neuro-

logical Institute's standard brain template. The data were smoothed with a gaussian kernel (8 mm; full width at half maximum) that was high-pass filtered with a cutoff period of 128 seconds to correct low-frequency artifacts and corrected for serial correlations by choosing an autoregressive model of the order 1. The nonspecific effects of global fluctuations in blood oxygen level–dependent (BOLD) signals were removed using global normalization. Realignment parameters were modeled in the analysis as regressors to control for motion artifacts. We excluded runs from the analyses in which the participant showed movement of more than one-half-voxel (1.5-mm) translation or 1.25° rotation. Imaging data from 3 or 4 runs were included for all individuals.

Because the masked and unmasked stimuli for each stimulus pair were presented too closely in time to model the hemodynamic response to each component separately, the fMRI data were modeled as event-related correlates of the combined stimulus pairs, and the hemodynamic responses to the different pair combinations were compared.[29] This method allowed us to evaluate the effect of varying the emotional expression of the masked face while the expression of the unmasked face remained constant (ie, SN-HN, SN-NN, and HN-NN).

Single participant SPM images of the voxel *t* values were generated by computing the difference maps between emotional conditions (eg, masked SN vs masked HN, or SN-HN). At the group level, these difference maps were compared within an amygdala region of interest to evaluate significant interactions and main effects through random-effects analysis of the β-weight values obtained from the single-participant analyses. The voxelwise statistical analysis within the amygdala was constrained using the "small-volume correction" option within SPM5 to reduce the likelihood of type I error. Results included differences in amygdala activation that remained significant after applying false–discovery rate error correction for multiple comparisons or consisted of clusters of 10 or more contiguous voxels at a threshold of $P < .05$ (uncorrected) within the amygdala region of interest. To assess group differences in other regions, an exploratory whole-brain analysis was performed post hoc. The significance threshold was set at a cluster of 10 or more contiguous voxels for which the voxel was at $P < .001$. Coordinates were transformed from Montreal Neurological Institute coordinates to the stereotaxic array of Talairach and Tournoux.[30] Anatomical localization was performed using stereotaxic atlases.[30,31]

Data analyses were divided into 3 experiments and presented in the order conducted. First, we tested the hypothesis that the amygdala response to emotional stimuli presented below the level of conscious awareness would show a negative-processing bias in MDD by comparing the difference in BOLD response between dMDD participants and HCs. We anticipated that the difference between groups would show the greatest effect size in the direct comparison of masked sad vs masked happy faces. Second, we characterized the influence of mood and clinical state by performing the same contrasts in a separate cohort of participants with MDD who underwent fMRI while unmedicated and in remission. In the third experiment, we evaluated the effects of treatment on emotional-processing biases in a longitudinal assessment of dMDD participants who underwent fMRI before and during antidepressant pharmacotherapy.

In experiment 1, the fMRI data acquired from the dMDD participants (n=22) and HCs (n=25) were compared using 2-sample *t* tests to evaluate the difference between groups for masked sad vs masked happy faces (SN-HN). To assess the specificity of the responses to each type of masked stimulus (happy or sad), post hoc *t* tests also evaluated differences in the amygdala response to masked sad vs masked neutral faces (SN-NN) and to masked happy vs masked neutral faces (HN-NN). Finally, to assess the specificity of the results for stimuli pre-

Table 1. Participant Demographic Characteristics and Clinical Symptom Rating Scores

Group	No. of Participants	Women, %	Mean (SD)									
			Age, y	WASI	HAM-D	ATQ	IDS-SR	STAI-S	STAI-T	TCQ-D	TCQ-W	TCQ-P
HC	25	60	28.8 (6.7)	121.9 (9.3)	0.0 (0.2)	2.5 (3.0)	3.2 (3.3)	26.9 (7.1)	27.0 (6.4)	15.4 (3.1)	7.6 (1.5)	6.9 (1.1)
rMDD	16	69	30.8 (9.8)	124.9 (12.6)	1.0 (1.4)[a]	7.8 (7.3)[a]	5.8 (3.7)[a]	28.1 (6.8)	35.9 (9.1)[a]	14.6 (3.6)	8.6 (2.6)	7.1 (1.3)
dMDD	22	55	31.1 (7.8)	120.4 (13.7)	24.0 (6.3)[b,c]	64.7 (23.9)[b,c]	32.4 (7.2)[b,c]	50.9 (8.3)[b,c]	61.1 (6.2)[b,c]	12.2 (1.9)[b,c]	11.0 (2.6)[b,c]	10.1 (2.9)[b,c]

Abbreviations: ATQ, Automatic Thoughts Questionnaire; dMDD, currently depressed people with major depressive disorder; HAM-D, Hamilton Depression Rating Scale; HC, healthy controls; IDS-SR, Inventory of Depressive Symptomatology: Self-Rating; rMDD, individuals with major depressive disorder in full remission; STAI-S, State-Trait Anxiety Inventory–State; STAI-T, State-Trait Anxiety Inventory–Trait; TCQ-D, Thought Control Questionnaire–Distraction subscale; TCQ-P, Thought Control Questionnaire–Punishment subscale; TCQ-W, Thought Control Questionnaire–Worry subscale; WASI, Wechsler Abbreviated Scale of Intelligence.
[a] rMDD scored higher than HC on the HAM-D (*P*=.003), ATQ (*P*=.003), IDS-SR (*P*=.02), and STAI-T (*P*=.001).
[b] dMDD scored higher than HC on the HAM-D, ATQ, IDS-SR, STAI-S, STAI-T, TCQ-D, TCQ-W, and TCQ-P (all *P*<.001).
[c] dMDD scored higher than rMDD on the HAM-D (*P*<.001), ATQ (*P*<.001), IDS-SR (*P*<.001), STAI-S (*P*<.001), STAI-T (*P*<.001), TCQ-D (*P*=.11), TCQ-W (*P*=.009), and TCQ-P (*P*=.001).

Table 2. Participant Demographic Characteristics and Mood Assessment Rating Scores in Pretreatment vs Posttreatment Conditions for dMDD Groups

Group	No. of Participants	Women, %	Mean (SD)									
			Age, y	WASI	HAM-D	ATQ	IDS-SR	STAI-S	STAI-T	TCQ-D	TCQ-W	TCQ-P
dMDD-pre	10	60	33.2 (5.0)	122.3 (13.1)	24.8 (5.8)	72.8 (21.0)	31.7 (5.4)	50.6 (8.9)	62.3 (3.3)	12.0 (1.9)	11.9 (1.0)	10.4 (3.0)
dMDD-post	10	60	33.2 (5.0)	122.3 (13.1)	6.4 (6.0)[a]	18.1 (13.1)[a]	13.6 (9.2)[a]	35.7 (10.7)[a]	43.1 (9.4)[a]	14.7 (2.7)[b]	10.7 (2.1)[a]	9.0 (2.1)[a]

Abbreviations: dMDD-post, currently depressed people with major depressive disorder, posttreatment; dMDD-pre, currently depressed people with major depressive disorder, pretreatment. See Table 1 for additional abbreviations.
[a] Scores were lower in the dMDD-post vs dMDD-pre condition on the HAM-D, ATQ, IDS-SR, and STAI-T (all *P*<.001), STAI-S (*P*=.003), TCQ-P (*P*=.02), and TCQ-W (*P*=.01).
[b] Scores were higher in the dMDD-post vs dMDD-pre condition on the TCQ-D (*P*=.04).

sented below the level of conscious awareness, we compared the BOLD response to unmasked sad vs unmasked happy faces (NS-NH).

In experiment 2, the fMRI data obtained from the individuals in experiment 1 were compared with those obtained from the 16 unmedicated rMDD participants to evaluate the mood state dependence of the emotional-processing biases found in experiment 1. The rMDD participants performed the same backward masking task. A 2-way repeated-measures analysis of variance (ANOVA) was used to analyze hemodynamic differences across conditions (SN, HN, and NN) and groups (dMDD, rMDD, and HCs).

When emotion × group interactions were significant, post hoc analyses were performed using SPSS version 14.0. The β-weight values were extracted at the peak voxel within a cluster for each participant and compared across groups using independent *t* tests to characterize specific interaction effects.

In experiment 3, 10 dMDD participants underwent subsequent fMRI after sertraline treatment. To control for test-retest and other nonspecific order effects, 10 HCs underwent additional fMRI after the same interval. Time × group ANOVAs were performed with the data from the contrasts found in experiment 1 to provide the most specific information regarding whether the response to sad faces (ie, SN-NN) or happy faces (ie, HN-NN) accounted for abnormalities identified in the SN vs HN contrast. Paired *t* tests were used to characterize the significance of differences within the dMDD participant group before vs during treatment and within the HC group across the same interval.

RESULTS

Demographic and clinical characteristics of the study participants appear in **Table 1** and **Table 2** and eTable 1 and eTable 2. Groups were similar regarding sex composition, mean age, and mean intelligence quotients. Of the samples from individuals with MDD, 13 dMDD and 3 rMDD participants had not taken psychotropic drugs. For participants who previously had received antidepressant medications, the mean (SD) drug-free period was 21 (23) months and 50 (54) months for the dMDD and rMDD groups, respectively. The mean (SD) age at onset was 16.7 (6.0) years and 18.3 (4.3) years for the dMDD and rMDD groups, respectively.

CLINICAL RATINGS

The 1-way ANOVAs revealed a significant effect of group on mean scores for HAM-D ($F_{2,60}=274$; $P<.001$), ATQ ($F_{2,60}=120$; $P<.001$), IDS-SR ($F_{2,60}=221$; $P<.001$), STAI-S ($F_{2,59}=69.0$; $P<.001$), and STAI-T ($F_{2,54}=123$; $P<.001$) and 3 subscales of the TCQ: Distraction ($F_{2,58}=61.3$; $P=.001$), Worry ($F_{2,58}=65.6$; $P<.001$), and Punishment ($F_{2,58}=65.1$; $P<.001$) (Table 1).

For the dMDD participants who underwent treatment, ratings of illness severity significantly decreased (Table 2). Nine of these 10 individuals with MDD were considered treatment responders (ie, ≥50% improvement on HAM-D scores), and 7 of the 10 were considered to have gone into remission during treatment (ie, HAM-D scores in the nondepressed range [≤7]).[32] Nevertheless, independent *t* tests showed that scores for dMDD participants posttreatment remained higher than those of the unmedicated rMDD participants on the

Table 3. Differential Accuracy Measures for Detecting Target Faces When Stimuli Were Presented in the Masked vs the Masking Face Position[a]

	Target Face Position, %		
	First	Second	Neither
Correct detection	24.4[b]	76.6	. . .
Correct rejection	. . .	. . .	74.6
Incorrect detection (false-alarm rate)	. . .	. . .	25.4
Incorrect rejection	75.6	23.4	. . .

[a] Masked refers to the first position; masking, second position. Categorization of the participant's response to each stimulus event was divided into 4 categories: (1) correct detection: the participant correctly identified the face as a target stimulus when shown in the first or second face position; (2) correct rejection: the participant correctly rejected the presence of a target stimulus when a target face was not present in either position; (3) incorrect detection (false-alarm rate): the participant indicated that a target face was present when no target face was shown in either face position; (4) incorrect rejection: the participant failed to identify the presence of a target face when it was shown in either the first or second face position. Ellipses indicate the category of the participant's response was not an option for that target face position (eg, correct detection was not a possible category if the target face was not presented in either position [neither]).
[b] A direct comparison showed no significant difference in the correct detection rate for a target face in the first position vs the incorrect detection rate for a target face in neither position. These data demonstrate the efficacy of the backward masking paradigm.

HAM-D ($t_{24}=3.55$; $P=.002$), ATQ ($t_{24}=2.59$; $P=.02$), IDS-SR ($t_{24}=3.06$; $P=.005$), STAI-S ($t_{24}=2.24$; $P=.04$), STAI-T ($t_7=2.10$; $P=.047$), TCQ-Worry ($t_{24}=2.21$; $P=.04$), and TCQ-Punishment ($t_9=2.92$; $P=.007$) scales.

fMRI BEHAVIORAL PERFORMANCE

During debriefing, no participants reported an awareness of seeing 2 face presentations. Participants performed at chance level in the identification of target faces when presented in the masked position (**Table 3**). A paired *t* test comparing the correct detection rate with the incorrect detection rate (false-alarm rate) showed that individuals did not differ in their response to a target face presented in the first position vs when no target face was presented ($t=0.15$; $P=.88$). An ANOVA also revealed no difference between groups ($F_{2,59}=2.6$; $P=.09$). These data imply that the experimental method succeeded at masking the masked face stimuli so that they were not consciously perceived.

An ANOVA (5 stimulus types × 3 groups) revealed a group difference in detection of the target face in the NN stimulus pair ($F_{2,59}=4.39$; $P=.02$). Both HCs and rMDD participants were more likely to detect the neutral target face in the masked position than dMDD participants. No other significant between-group difference was found.

An ANOVA also revealed a significant difference between groups in reaction time to target masked sad faces ($F=10.4$; $P<.001$). Post hoc tests showed that the rMDD participants were faster than dMDD participants ($P=.02$) and HCs ($P<.001$), whereas dMDD participants responded faster to masked sad faces than HCs ($P=.02$). In HCs, reaction time was faster to target masked happy faces than to target masked sad faces ($t=3.6$; $P<.001$).

fMRI RESULTS

In experiment 1, the dMDD participants and HCs differed in the left and right amygdala response to SN-HN ($t_{45}=3.00$; $P=.002$, and $t_{45}=2.80$; $P=.004$, respectively; **Figure 2**A and B). These results remained significant after false-discovery rate corrections for multiple comparisons ($P=.02$, bilaterally). Post hoc *t* tests showed that the magnitude of the difference between SN-HN in the left amygdala ($P=.002$) and right amygdala ($P=.003$) was greater in dMDD participants than in HCs. Similarly, the difference in the left ($P=.02$) and right amygdala ($P=.02$) between SN-NN was greater in dMDD participants than in HCs (Figure 2C and E). In contrast, the difference in the left amygdala between HN-NN was greater in HCs than in dMDD participants ($P=.007$; Figure 2D).

Post hoc assessments addressed the relationships between depression severity and behavioral performance in the amygdala response to masked sad or happy faces. In dMDD participants, the HAM-D scores correlated inversely with the amygdala response to masked happy faces ($r=-0.45$; $P=.04$) so that the amygdala response to HN-NN decreased as depression severity increased (**Figure 3**). We found no significant relationship between depression severity and the amygdala response to SN-NN. Nevertheless, in dMDD participants, the reaction time to masked sad faces was inversely correlated with the right amygdala response to SN-HN ($r=-0.53$; $P=.01$) (eFigure). Additional correlational analyses of the relationship between reaction time and amygdala response are reported in the "eResults" section of the supplemental text.

In the exploratory whole-brain analyses performed post hoc, the hemodynamic response to SN-HN was greater in the left hippocampus in dMDD participants than in HCs ($t_{45}=3.91$; $P<.001$) and greater in the left thalamus in HCs than in dMDD participants ($t_{45}=3.52$; $P<.001$) (eTable 3). In the post hoc assessment of hemodynamic responses to unmasked sad vs unmasked happy faces (NS-NH), no significant difference was found between groups in the amygdala region of interest. In the whole-brain analysis of the same contrast, however, the BOLD response to NS-NH was greater in the left temporopolar cortex in dMDD participants than in HCs ($t_{45}=4.40$; $P<.001$) and greater in HCs than in dMDD participants in the superior frontal gyrus ($t_{45}=4.92$; $P<.001$), right and left precentral gyrus ($t_{45}=4.37$; $P<.001$ and $t_{45}=3.96$; $P<.001$), postcentral gyrus ($t_{45}=4.24$; $P<.001$), middle temporal gyrus ($t_{45}=4.17$; $P<.001$), and parietal operculum ($t_{45}=3.82$; $P<.001$) (eTable 4).

In experiment 2, the 2-way repeated-measures ANOVA comparing hemodynamic differences across conditions (SN, HN, and NN) and groups (dMDD, rMDD, and HCs) showed a condition × group interaction ($F_{3,92}=4.19$; $P=.007$; Figure 2F and G). Post hoc *t* tests indicated that the magnitude of the difference in the amygdala hemodynamic response to SN vs HN was greater in the dMDD and rMDD groups than in HCs ($P=.002$ and $P=.04$, respectively; Figure 2H).

In additional post hoc *t* tests, the dMDD group showed a greater amygdala response to SN than NN ($P=.02$), whereas HCs showed no such effect ($P=.51$); the difference between groups was significant ($P=.01$; Figure 2I). In contrast, the HCs showed higher amygdala activity in
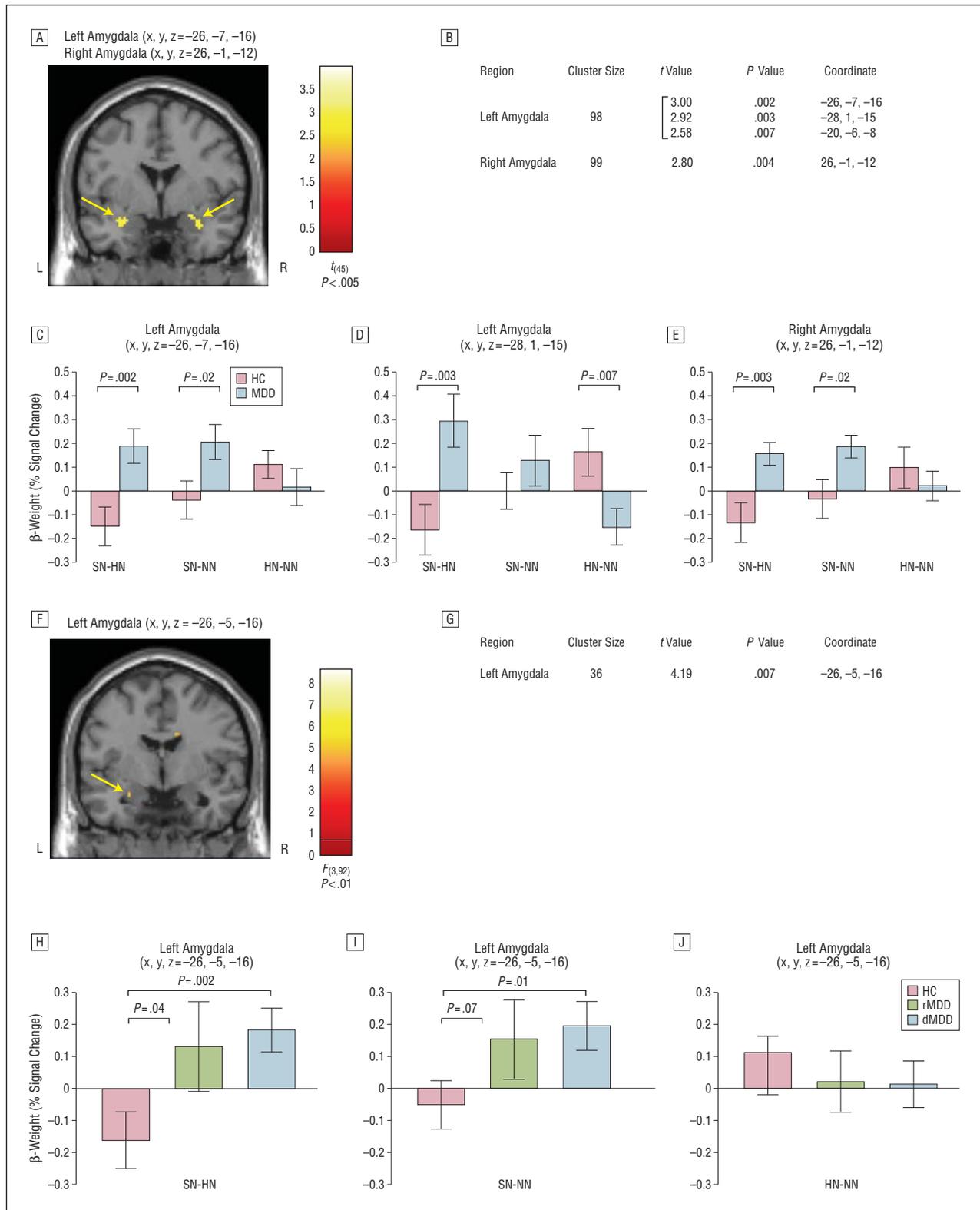
Figure 2. Neuroimaging results for experiments 1 and 2. A, Voxels in the bilateral amygdala indicate differences in the hemodynamic response to masked sad vs masked happy faces (SN-HN) between currently depressed people with major depressive disorder (dMDD) and healthy controls (HCs), shown on a coronal slice located 1 mm posterior to the anterior commissure. B, Coordinates of peak voxel t value signifying the difference in the amygdala response to SN-HN for dMDD participants vs HCs that correspond to the stereotaxic array of Talairach and Tournoux as the distance in millimeters from the origin (anterior commissure), with positive x value indicating right, positive y value indicating anterior, and positive z value indicating dorsal. Cluster size indicates contiguous voxels (P<.05). Contrast β-weights are shown for specified contrasts in dMDD vs HCs for loci identified in the left (C and D) and right amygdala (E). F and G, The location in the left amygdala shows a diagnosis × task interaction. Contrast β-weights are shown for specified contrasts (H, I, and J) in HCs, individuals with major depressive disorder in full remission (rMDD), and dMDD participants from the locus identified in the analysis of variance. HN-NN indicates masked happy vs masked neutral faces; SN-NN, masked sad vs masked neutral faces.
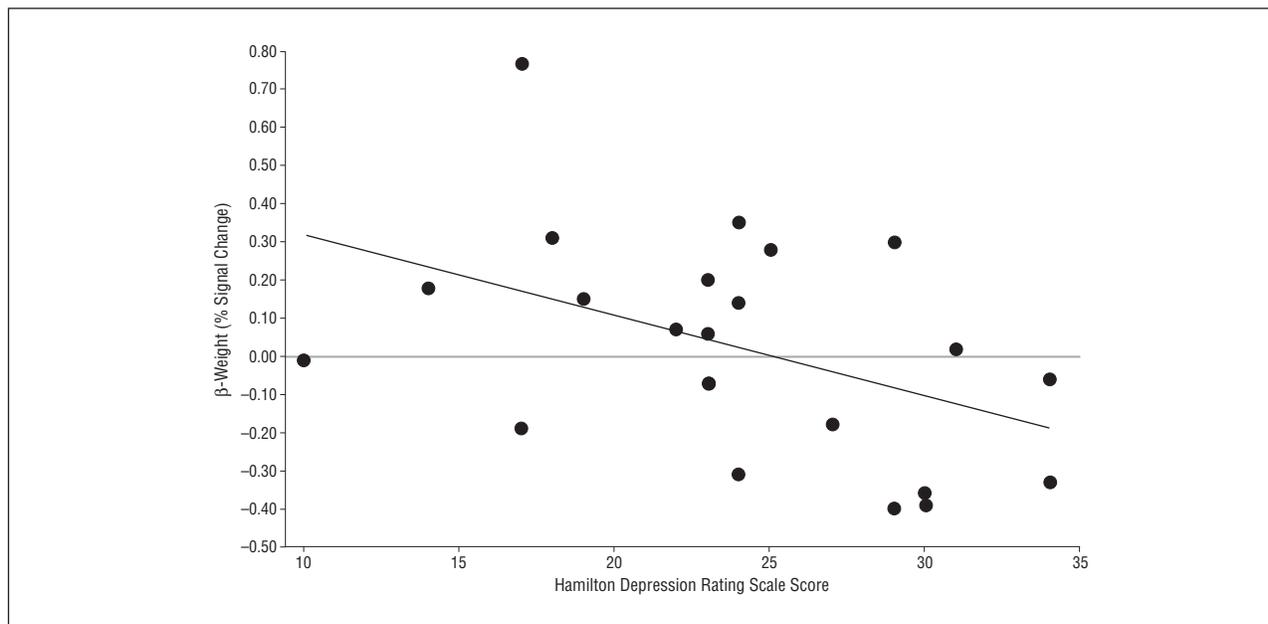
Figure 3. Relationship between depression severity and amygdala response to masked happy vs masked neutral faces (HN-NN). Correlation is shown between depression severity (Hamilton Depression Rating Scale score) in currently depressed participants (n=22) and the β-weight value at the peak voxel response to HN-NN in the right amygdala (26, −1, −12) (r=−0.45; P=.04).

response to HN than NN (P=.04), whereas the dMDD and rMDD participants showed no such difference (P=.87 and P=.83, respectively); the difference across groups was not significant (Figure 2J). The dMDD and rMDD groups did not differ significantly in their amygdala response to any task condition.

In experiment 3, after treatment, the dMDD participants showed a reduced response to SN-NN in the right amygdala (t_9=3.26; P=.005; **Figure 4**A and B) and elevated activity in response to HN-NN in the left amygdala (t_9=2.59; P=.01; Figure 4A and C).

A time × group ANOVA for responses to SN-NN revealed a significant interaction in the right amygdala (t_{18}=2.21; P=.02; Figure 4D and E). Individual comparisons performed post hoc showed a reduction in the amygdala response to SN-NN in the dMDD group in the pretreatment vs posttreatment conditions (P=.04) with no significant change across time in HCs (P=.17). These post hoc comparisons excluded a single HC whose contrast β-weight value exceeded 3 SDs beyond the mean.

<div style="text-align:center">**COMMENT**</div>

These data demonstrate that negative emotional-processing biases occur automatically, below the level of conscious awareness, in unmedicated, currently depressed people with MDD. Both dMDD and rMDD participants showed greater amygdala activity than HCs when processing masked sad vs masked happy faces. The dMDD participants also responded faster than HCs to masked sad faces, despite being unaware of the masked face (Table 3). In contrast, HCs showed greater responses in the amygdala and faster behavioral responses to masked happy faces than to masked sad or neutral faces, consistent with other evidence that healthy individuals show a processing bias toward positively valenced stimuli.[15,33,34]

This nonconscious processing of emotional stimuli is consistent with evidence that the amygdala contains cells that are tuned selectively to specific stimulus characteristics, facilitating early detection of biologically salient information.[35] The coordinates for the emotional-processing biases found in this article (Figure 1) appear to implicate specifically the lateral nucleus of the amygdala,[31] which receives monosynaptic projections from the sensory cortices that allow conscious or explicit stimulus perception and from the subcortical structures that support rapid nonconscious assessment of stimulus features.[13,14] The rapid response system facilitates detection of and behavioral adaptation to stimuli that are novel, threatening, rewarding, or socially significant.[36-42] The exploratory whole-brain analysis (eTable 3) implicated the hippocampus and thalamus in the extended anatomical network that, together with the amygdala, responds to nonconscious stimuli. Projections from the hippocampus to the amygdala provide input during emotional processing about the environmental context,[43] whereas the thalamus plays a role in gating the transmission of sensory information to other brain regions based on the anticipated salience of this information within the behavioral context.[44] Nonconscious, emotional, mood-congruent processing biases in the amygdala in people with MDD may negatively influence their conscious perceptions of experiential stimuli and affect social interactions.

The comparison of hemodynamic responses to unmasked sad vs unmasked happy faces showed the specificity of our amygdala results to masked stimuli, as we found no difference across groups in the amygdala response to explicitly presented emotional faces. This suggests that using backward masking confers an advan-
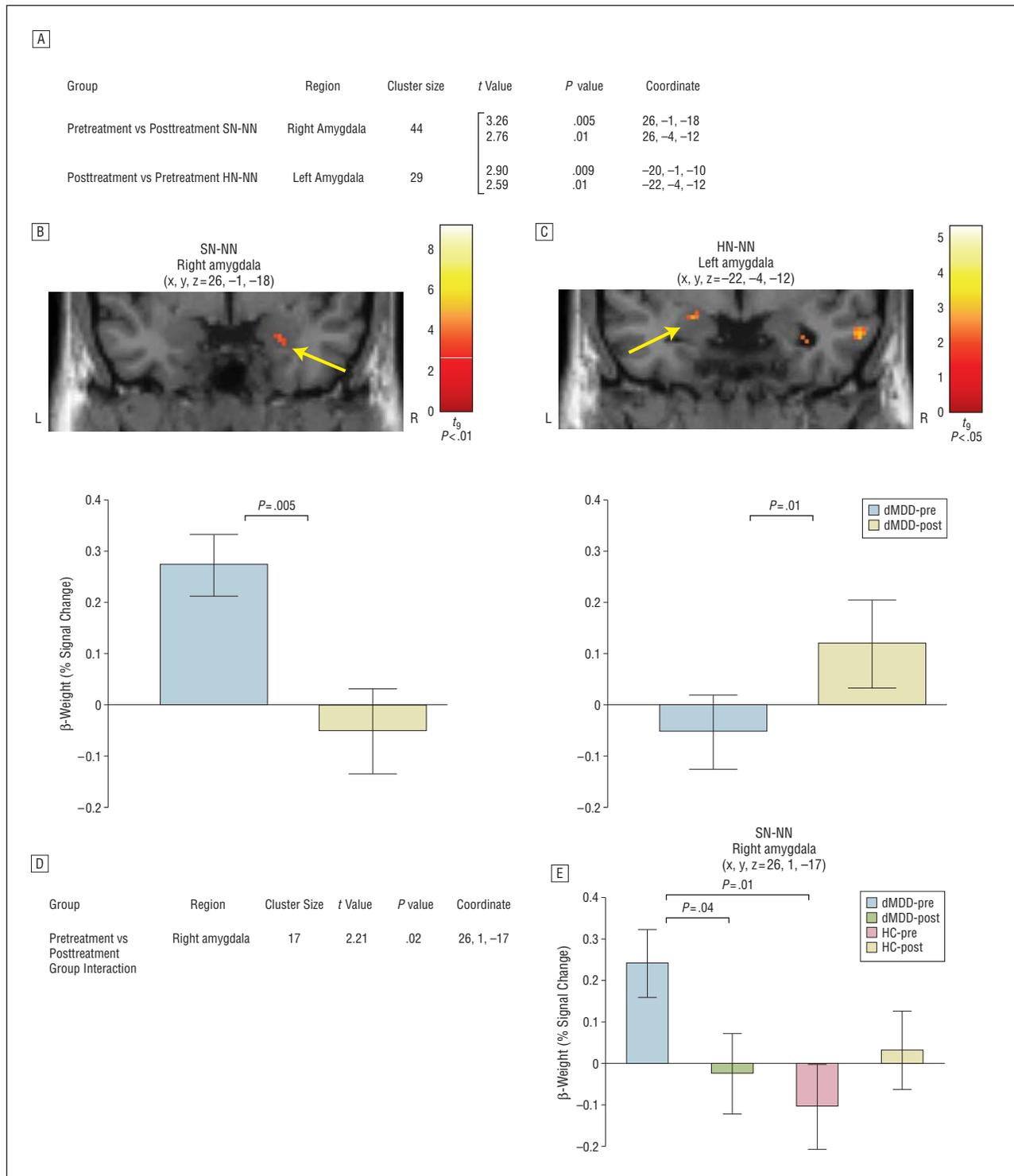
Figure 4. Neuroimaging results for experiment 3. A through C, Areas in the amygdala where the hemodynamic response to masked sad vs masked neutral (SN-NN) and masked happy vs masked neutral (HN-NN) faces differed in patients with major depressive disorder (MDD) after sertraline hydrochloride treatment vs the pretreatment baseline. β-weights are shown for specified contrasts for identified loci (A). D and E, Location in the right amygdala shows a time × group interaction. β-weights are shown for the specified contrasts as obtained during the 2 functional magnetic resonance imaging time points for the healthy controls (HCs) and MDD groups. dMDD indicates currently depressed people with MDD; dMDD-pre, dMDD before treatment.

tage in identifying emotional-processing biases involving the amygdala in MDD.

The emotional-processing abnormalities found in unmedicated dMDD participants extended to unmedicated rMDD participants, suggesting that MDD is associated with a traitlike bias toward processing negative stimuli independently of current mood state. Nevertheless, although rMDD participants met the criteria for full remission, they showed an elevation of trait anxiety ratings and negative thought patterns (Table 1). These symp-

tom clusters appear endophenotypic in individuals who develop MDD[45] and conceivably relate to the persistent emotional-processing bias observed in our study. This finding is suggestive of an "illness-congruent" processing bias in people in remission that may be a biomarker for the vulnerability to depressive relapse and recurrence within MDD.

This pattern of amygdala activity reversed during treatment, however, as the response bias toward masked sad faces disappeared (Figure 2B) and a bias toward masked happy faces developed in individuals with MDD who were receiving treatment (Figure 2C). Previous studies reported that the amygdala response to unmasked sad faces[12] or masked fearful faces[46,47] attenuated during treatment, whereas our study was the first, to our knowledge, to identify a reciprocal increase in amygdala activity in response to masked happy faces with a concomitant decrease in amygdala activity in response to masked sad faces associated with treatment. These findings thus provide the first evidence of a nonconscious negative-processing bias toward sad faces in unmedicated people with MDD that resolves, while a positive-processing bias emerges, during treatment.

Our results appear compatible with the hypothesis[48] that antidepressant drugs exert their primary therapeutic mechanism by normalizing the negative bias in information processing. This hypothesis was based partly on evidence that in healthy individuals the short-term administration of citalopram enhanced the amygdala response to happy faces,[49] and in depressed individuals the single-dose administration of reboxetine enhanced the behavioral responses to positively valenced stimuli.[50] Longitudinal studies are needed to assess whether the ability of antidepressant drugs to reduce relapse vulnerability and improve clinical outcomes relates directly to the attenuation of the automatic amygdala response to negative stimuli.

Notably, Suslow et al[5] reported that patients with MDD who were receiving antidepressant medication but were persistently depressed showed hemodynamic responses in the right amygdala that were exaggerated to masked sad faces and blunted to masked happy faces. Although that study did not include an unmedicated sample for comparison, when their data are considered with ours, the combined results suggest that the decrement in right amygdala responses to masked sad faces that we found (Figure 4) during pharmacotherapy may depend on treatment effectiveness. Nine of the 10 participants we studied posttreatment showed good clinical responses, so we could not compare neurophysiological effects between responders and nonresponders.

The importance of laterality effects also is raised by these data, although neither study addressed laterality effects specifically. Suslow et al[5] observed evidence of emotional-processing biases in the right but not in the left amygdala in medicated, currently depressed patients with MDD. Our study, to our knowledge the first to examine the responses to masked happy or sad stimuli in the amygdala in unmedicated dMDD participants and the first to examine this phenomenon in unmedicated rMDD participants, additionally found that these emotional-processing biases exist in both groups in the left amygdala. Moreover, in our longitudinal study the normal positive-processing bias that emerged posttreatment was significant only in the left amygdala, whereas the attenuation of the negative-processing bias was significant in the right (Figure 4).

Some researchers have suggested that emotional-processing biases are limited to late or controlled information processing in MDD.[51-53] In contrast, our data suggest that these biases are also evident at an automatic or early processing level. Studies suggesting that processing biases in depression are limited to late or controlled processing have used behavioral assessments of attention and memory for anxiety-related or socially threatening stimuli, or sad words. Depressed patients exhibit a specific bias toward sad stimuli but have not consistently shown processing biases toward socially or physically threatening stimuli.[54] In this study,[53] the bias was for sad words shown for 500 to 1000 milliseconds. However, an implicit emotional-processing bias was not found toward briefly presented sad words shown for 14 milliseconds.[53] Verbal stimuli may require longer processing times to detect their emotional salience. Given the biological salience of faces, face stimuli can be processed rapidly, within the time frame needed for backward masking techniques.[14] Also, the effects of antidepressant medication were not controlled for in these studies. Antidepressant treatment has been shown to decrease negative emotional information processing in depressed patients, so it is plausible that previous studies were unable to detect a processing bias earlier during information processing owing to confounding medication effects.

Several limitations of our study merit comment. First, we did not address the generalizability of these findings to other mood disorders or to other antidepressant drug classes. Second, the rMDD and dMDD samples were not the same participants studied in distinct illness phases, and the rMDD sample had fewer participants with comorbid anxiety disorders than the dMDD sample. Finally, the longitudinal component of this study did not include a placebo arm, so causal evidence for a pharmacotherapeutic effect could not be established by the results.

In conclusion, our findings provide behavioral and neurophysiological support for an emotional-processing bias in depression toward negatively valenced stimuli presented below the level of conscious awareness that persists independently of the current mood state. Developmental studies are needed to explore whether this processing bias constitutes a potential endophenotype in MDD and to characterize its relationship to the emergence of depressive episodes.

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