

## RESEARCH ARTICLE

# Pretreatment Differences in BOLD Response to Emotional Faces Correlate with Antidepressant Response to Scopolamine

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## Abstract

**Background:** Faster acting antidepressants and biomarkers that predict treatment response are needed to facilitate the development of more effective treatments for patients with major depressive disorders. Here, we evaluate implicitly and explicitly processed emotional faces using neuroimaging to identify potential biomarkers of treatment response to the antimuscarinic, scopolamine.

**Methods:** Healthy participants (n=15) and unmedicated-depressed major depressive disorder patients (n=16) participated in a double-blind, placebo-controlled crossover infusion study using scopolamine (4 µg/kg). Before and following scopolamine, blood oxygen-level dependent signal was measured using functional MRI during a selective attention task. Two stimuli comprised of superimposed pictures of faces and houses were presented. Participants attended to one stimulus component and performed a matching task. Face emotion was modulated (happy/sad) creating implicit (attend-houses) and explicit (attend-faces) emotion processing conditions. The pretreatment difference in blood oxygen-level dependent response to happy and sad faces under implicit and explicit conditions (emotion processing biases) within a-priori regions of interest was correlated with subsequent treatment response in major depressive disorder.

**Results:** Correlations were observed exclusively during implicit emotion processing in the regions of interest, which included the subgenual anterior cingulate ( $P < .02$ ) and middle occipital cortices ( $P < .02$ ).

**Conclusions:** The magnitude and direction of differential blood oxygen-level-dependent response to implicitly processed emotional faces prior to treatment reflect the potential to respond to scopolamine. These findings replicate earlier results, highlighting the potential for pretreatment neural activity in the middle occipital cortices and subgenual anterior cingulate to inform us about the potential to respond clinically to scopolamine.

**Keywords:** Biomarker, antimuscarinic, mood disorders, anticholinergic

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## Introduction

Currently utilized antidepressant agents produce varied clinical responses in patients with major depressive disorder (MDD), and often multiple trials are necessary before an effective therapy is identified (Insel and Wang, 2009; Simon and Perlis, 2010). As a result, patients often go many months without experiencing relief of their symptoms. There is a crucial need both to identify novel agents that provide a more rapid clinical onset of antidepressant effects and to develop methods that can predict the potential for patients to respond to specific antidepressant agents. The identification of biomarkers associated with treatment response in a rapid antidepressant paradigm carries potential both to identify critical neurobiology associated with treatment outcome and guide the development of novel antidepressant agents (Zarate et al., 2013). An important aspect of biomarker development includes the replication of findings in independent cohorts as well as evaluations of the specificity or generalizability of a result.

While pretreatment activity and posttreatment change (Pizzagalli, 2011) in neural activity observed in the subgenual anterior cingulate cortex (sgACC) and other areas of the rostral anterior cingulate cortex has been linked to antidepressant treatment response in MDD (Drevets et al., 1997; Mayberg et al., 1997, 1999, 2005; Covington et al., 2010; Pizzagalli, 2011), neural activity in response to sad faces early in treatment (2 weeks) in sgACC predicted subsequent clinical response (Keedwell et al., 2009, 2010). Importantly, in these same studies, activity in areas of the occipital cortex at the same 2-week period, together with sgACC, also correlated with subsequent antidepressant response. These findings highlight the potential for neural activity within the sgACC and visual cortices to reflect subsequent clinical outcome, and if assessed prior to treatment, activity in these brain regions may function as biomarkers of subsequent antidepressant response.

The cholinergic neurotransmitter system is implicated in mood disorders (Janowsky et al., 1972a, 1972b; Sitaram et al., 1982; Dube et al., 1985; Janowsky et al., 1994), and the muscarinic-cholinergic antagonist scopolamine when administered intravenously produces rapid antidepressant effects (within 3 days) in patients with MDD (Furey and Drevets, 2006; Drevets and Furey, 2010). Importantly, this agent offers clinical efficacy in a fraction of the time required for conventional antidepressant options. This rapid response also offers a unique opportunity to quickly evaluate potential biomarkers of treatment outcome (Drevets et al., 2012). Previously, we demonstrated that pretreatment levels of neural activity in middle occipital cortex (MOC), when performing a face emotion working memory task, predicted treatment outcome to scopolamine in MDD (Furey et al., 2013). Importantly, this effect was specific to an emotional processing condition, as no predictive value was observed when the same faces were included in a face-identity working memory task. The change in neural response in the MOC to these same task conditions measured acutely post-scopolamine administration also correlated with treatment outcome, and the direction of correlation reversed relative to the pretreatment correlation. Thus, brain regions reflecting the potential to respond to treatment also were modulated by acute scopolamine in a manner that correlated with treatment response.

The most consistently reported cognitive feature of mood disorders is a negative emotion-processing bias where patients demonstrate a bias towards processing negative emotional stimuli over positive stimuli (Bradley et al., 1995, 1996; Mogg et al., 1995; Murphy et al., 1999; Erickson et al., 2005; Harmer,

2008; Koster et al., 2010). This bias is evident in behavioral studies (Bradley et al., 1995, 1996; Murphy et al., 1999; Erickson et al., 2005) as well as functional neuroimaging studies (Sheline et al., 2001; Fu et al., 2004; Gotlib et al., 2004; Victor et al., 2010, 2012), where patients demonstrate differential neural responses to sad and happy faces under implicit (Sheline et al., 2001; Victor et al., 2010) and explicit (Surguladze et al., 2005; Keedwell et al., 2010; Siegle et al., 2012) emotion-processing conditions. While these studies often focus on amygdala (Sheline et al., 2001; Victor et al., 2010), differential emotional processing in mood disorders also has been reported in prefrontal cortical regions (Davidson et al., 2003; Fu et al., 2004; Victor et al., 2012), including sgACC (Keedwell et al., 2009, 2010; Laxton et al., 2013), and visual processing areas (Surguladze et al., 2005; Keedwell et al., 2009, 2010).

In addition to being affected in mood disorders, the cholinergic system also is implicated in stimulus-processing mechanisms (Furey, 2011). The cholinergic neurotransmitter system influences neural activity extensively via widespread projections throughout the cerebral cortex (reviewed in Sarter et al., 2005), including visual processing areas and the ACC. Moreover, the cholinergic transmission to these regions is influenced by structures involved in evaluating the salience of emotional stimuli, such as the amygdala (reviewed in Price, 2010). In healthy individuals, cholinergic activity differentially modulates neural responses in visual cortices based on emotional content (Bentley et al., 2003), and thus the cholinergic dysfunction characterized in mood disorders may contribute to the emotional processing biases observed in this population.

The purpose of the current study was to assess neural estimates of an emotional processing bias as a potential biomarker of rapid treatment response to scopolamine within brain regions previously found to effectively predict treatment response in patients with MDD. One goal was to assess the generalizability vs selectivity of emotion-processing tasks to elicit neural activity that reflects the potential for antidepressant response to scopolamine. We expected that, in an independent patient sample, the magnitude of the emotional processing bias prior to treatment in sgACC and in MOC would predict subsequent treatment outcome to scopolamine.

## Methods

### Participants

Fifteen healthy volunteers (female=9, age [mean±SD] =32.5±6.9 years) and 16 currently depressed and unmedicated patients with MDD (female=13, age [mean±SD] = 32.4±8.9 years), without psychotic features, were enrolled in this study; diagnosis was confirmed by the Structured Clinical Interview for Axis I DSM-IV Disorders (First et al., 1997) and an unstructured interview conducted by a psychiatrist. All participants were evaluated at the National Institute of Mental Health outpatient clinic. Inclusion criteria for the depressed sample were the diagnosis of MDD, a Montgomery-Asberg Depression Rating Scale (MADRS) score of ≥20, and a current major depressive episode of at least 4 weeks duration. Exclusion criteria included other Axis I disorders (excepting anxiety disorders for MDD patients and a history of substance abuse within the past year for all participants), exposure to psychotropic or other medications likely to affect central nervous system or cholinergic function within 3 weeks (8 weeks for fluoxetine), and suicidal ideation suggestive of high suicide risk (for additional exclusion

criteria, see Furey and Drevets, 2006; Drevets and Furey, 2010). Healthy volunteers also were excluded for the presence of any psychiatric disorder or a family history of mood disorder in a first-degree relative. The study was approved by the Combined Neuroscience Institutional Review Board of the National Institutes of Health. All subjects provided written informed consent. Clinical findings associated with these patients have been published previously (Furey and Drevets, 2006; Drevets and Furey, 2010).

## Study Design

Using a double-blind, placebo-controlled, crossover design, participants received a 15-minute intravenous infusion of either a placebo saline solution or 4.0 µg/kg of scopolamine in each of 7 sessions. A single-blind, lead-in session was used in which all subjects received placebo infusion. Participants subsequently were randomized by the NIH outpatient pharmacy into either a P/S or S/P sequence, whereby P constituted a series of 3 placebo infusions and S comprised a series of 3 scopolamine infusions (Figure 1; for additional details, see Furey and Drevets, 2006; Drevets and Furey, 2010). Infusion sessions were scheduled 3 to 5 days apart. Nonpregnancy was established prior to each session for female participants.

Immediately prior to each infusion, psychiatric assessments were conducted that included the MADRS (Khan et al., 2002). Study end interviews were obtained 3 to 5 days after the final infusion to provide the final clinical assessment and establish treatment response. Treatment response magnitude was calculated as a percent change at study end relative to the pretreatment MADRS score, obtained prior to the infusion or scanning in session 1. Patients also were characterized as demonstrating: (1) full response ( $\geq 50\%$  reduction in MADRS score from baseline); or (2) nonresponse ( $< 50\%$  reduction) (Nierenberg and DeGecco, 2001).

## Functional Imaging

Pretreatment functional MRI scans were obtained following the single-blind placebo infusion in session 1 and during sessions 2 and 5 to ensure that imaging data were acquired following scopolamine infusions (Figure 1). Thus, all participants were scanned on 3 occasions including a pretreatment placebo condition, and the post-scopolamine scan always occurred following the first drug administration. This procedure ensured that data were acquired following drug administration without compromising the double-blind nature of the study. After the infusion

and before scanning, a 45-minute waiting period followed to allow the peak cognitive effects to develop and the peak side effects (ie, drowsiness) to diminish (Safer and Allen, 1971). A 3-tesla, General Electric scanner (GE Signa, Milwaukee, WI) was used to obtain an echo-planar imaging sequence for blood oxygen-level-dependent (BOLD) data acquisition (TE=24; TR=2500; sagittal slices=35; voxel dimensions=3.75×3.75×3.5 mm; 200 time-points per run) and a spoiled gradient echo sequence for the anatomical data (matrix=256×256, number of sagittal slices = 128 to 140 (to obtain full brain coverage). Four images were discarded from the beginning of each echo-planar imaging acquisition to allow for steady-state tissue magnetization.

## Selective Attention Task

During scanning, participants performed the selective attention task (Figure 2). For each trial, participants were shown 2 stimuli comprised of superimposed images of faces and houses presented side-by-side. Participants were instructed to attend to either the face or the house component of the stimuli and to perform a matching task based on the attended stimulus component. For example, when participants attend to faces, the task is to determine if the faces in the 2 stimuli are of the same person or different people. Trials were separated by a 1500-ms inter-stimulus interval. Every 4 to 7 trials, the cue changed (2500ms), which instructed participants to shift attention to the other stimulus component. Under both attention conditions, the emotion in the face stimuli was modulated (happy or sad), creating conditions of explicit (attend to faces) and implicit (attend to house) emotion processing (Figure 2A). For each trial, both faces expressed the same emotion. The attentional target (faces or houses) was randomized at the beginning of each run. (B) Task runs alternated with control runs where stimuli were presented in the same spatial and temporal manner, but here the unattended stimulus component was phase scrambled. Thus, when matching based on faces, the house component of each stimulus is phase scrambled to provide visual noise with the same contrast and spatial frequency information as in the intact stimulus condition. Participants were instructed to perform a matching task based on the intact stimulus component. Every 4 to 7 trials, the participants would be cued, and then the intact component would shift from faces to houses or vice versa. When viewing faces, emotional expressions (happy and sad) were used but did not influence response requirements. For both tasks, accuracy and reaction time were obtained.

## Imaging Data Analyses

Echo-planar images were registered, smoothed, time-corrected, and normalized to the mean. AFNI (Cox, 1996) was used to conduct multiple regression analysis using 3dDeconvolve to estimate BOLD response when attending to faces (AF) and attending to houses (AH), when faces were happy (AFh, AHh), and when faces were sad (AFs, AHs). Results of statistical analyses were spatially normalized to the stereotaxic array of Talairach and Tournoux (Talairach and Tournoux, 1998). Regressors were designed so that only those trials that included a response were included in the multiple regression analysis.

## Emotional Processing Bias Analysis

The differences in BOLD signal between happy and sad emotions during explicit (AFh-AFs) and implicit (AHh-AHs) processing conditions were calculated, producing estimates of

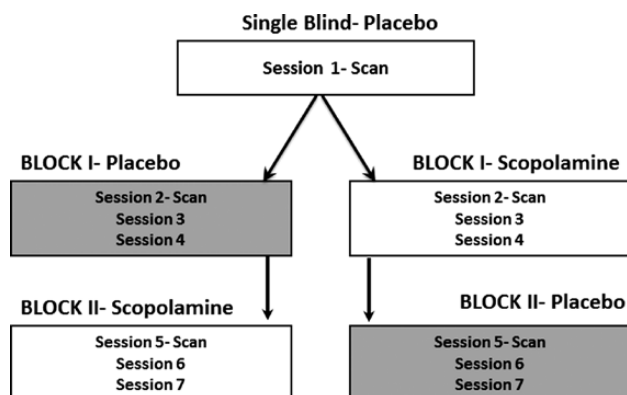
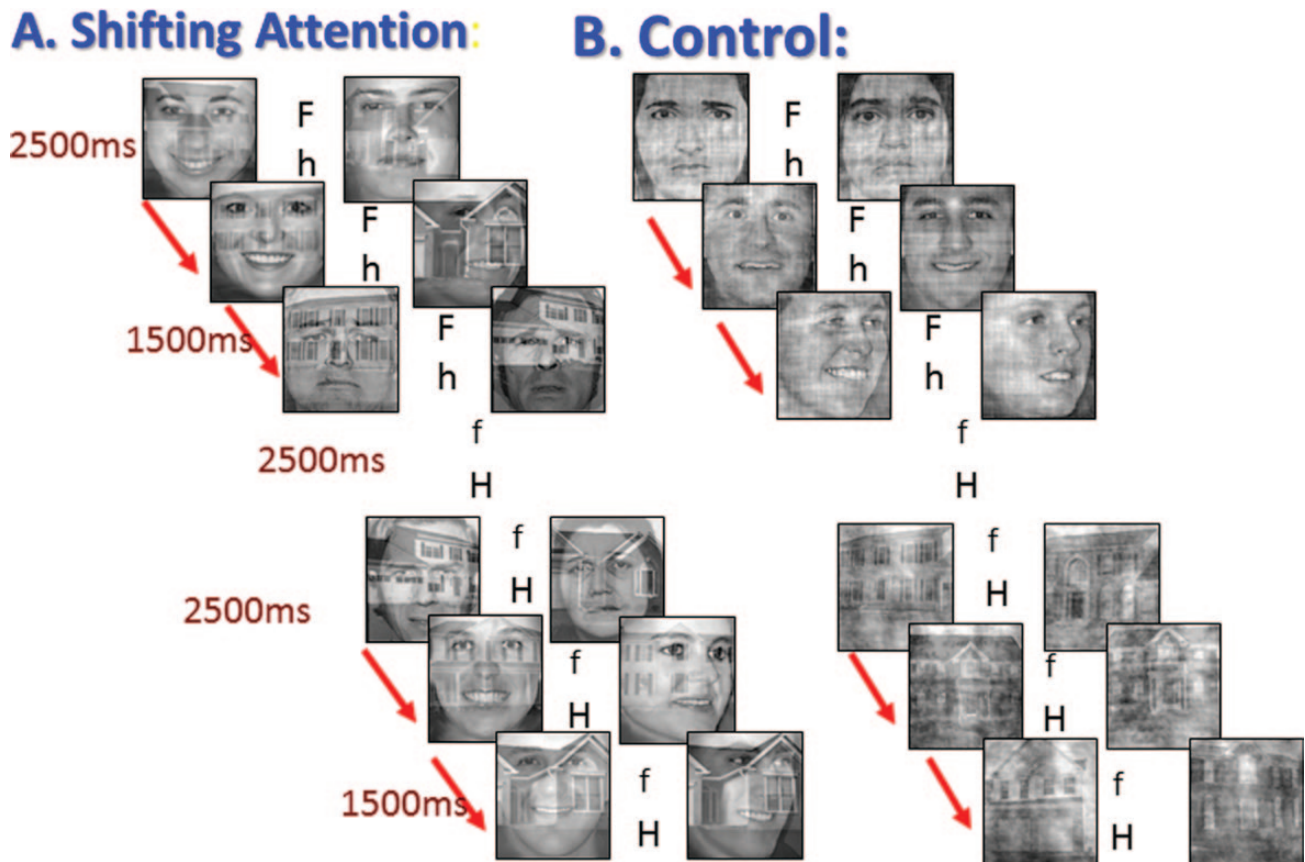


Figure 1. Experimental design.



**Figure 2.** Selective attention task with emotion modulation. (A) Under both attention conditions, the emotion in the face stimuli was modulated (happy or sad), creating conditions of explicit (Fh, attend to faces) and implicit (fH, attend to house) emotion processing. (B) Task runs alternated with control runs where stimuli were presented in the same spatial and temporal manner, but here the unattended stimulus component was phase scrambled. Approximately 80 face (most with multiple emotions and 2–3 orientations) and 80 house (each with -3 orientations) stimuli were used to create the stimulus set for each session. Multiple orientations were used so that on matching trials, participants could not perform a simple pattern match. The same stimulus set was used to create the attention and control tasks. All complex stimuli were unique, but individual face and house pictures were used multiple times to complete the stimulus set for a session. Unique sets of face and house stimuli were used for each of the 3 scanning sessions. Face stimulus sets included images from a laboratory library, the Ekman stimulus set (Young, 2002), Karolinska Directed Emotional Faces (Lundqvist et al., 1998), and the MacBrain Face Stimulus Set (NimStim). Development of the MacBrain Face Stimulus Set was overseen by Nim Tottenham and supported by the John D. and Catherine T. MacArthur Foundation Research Network on Early Experience and Brain Development. Please contact Nim Tottenham at tott0006@tc.umn.edu for more information concerning the stimulus set. All face emotion stimulus sets have been normed.

emotional processing biases under 2 processing conditions. For the primary analysis, these 2 conditions were correlated individually (ie, 2 separate correlations) with the subsequent magnitude of treatment response (defined above) using AFNI software (Cox, 1996) (3dRegAna). To determine the selectivity of observed effects to the selective attention conditions, the difference between BOLD response to happy and sad faces as measured during the control task (thus only for the control face condition) was calculated. This difference also was correlated with subsequent treatment response. For these analyses, the search volume was restricted to 3 ROIs (left and right MOC, sgACC) (Figure 3). Significance was defined voxel-wise as  $P < .01$ , and the extent criteria for significance was based on a small volume correction (SVC) that included the combined regions of interest, defined as  $\geq 12$  contiguous voxels (as determined by AFNI software, ClustSim,  $P < .025$  to adjust the significance level based on the 2 analyses).

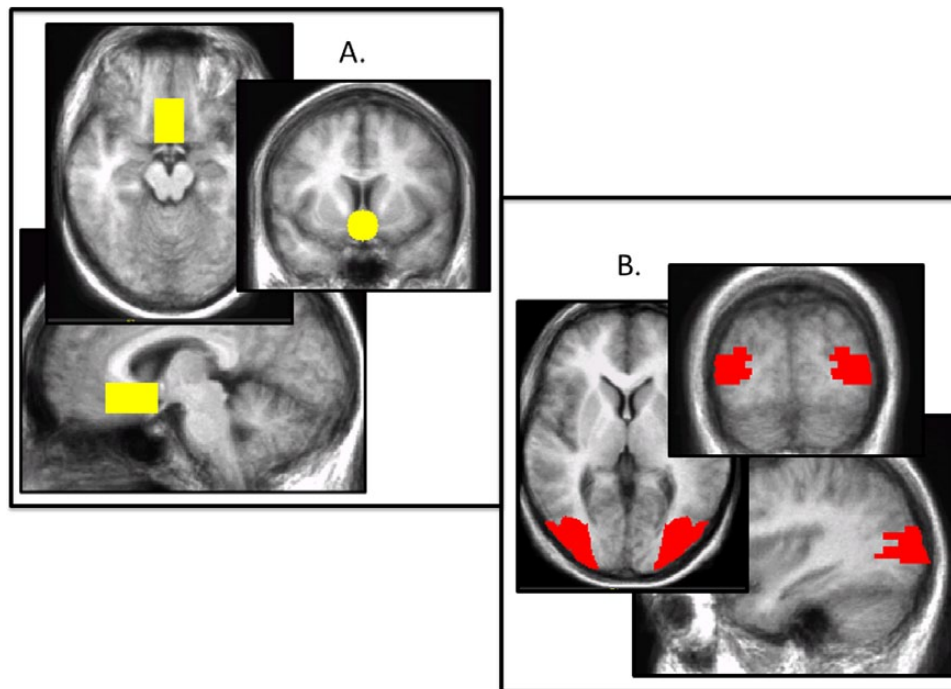
### Placebo Analysis

For conditions (implicit and/or explicit processing) that produced significant correlations in the primary analysis described above, the specificity of the effects to treatment response was

evaluated by correlating baseline BOLD measures with the magnitude of the subsequent placebo response. This analysis was designed to assess whether BOLD signal correlated selectively with treatment response, or if BOLD signal also was related to nonspecific change in depression severity (ie, placebo response). Given the carry-over antidepressant effects of scopolamine (Furey and Drevets, 2006; Drevets and Furey, 2010), this analysis was restricted to those participants who received placebo in the first treatment block. As nonsignificance of the placebo analysis strengthens the findings reported here (ie, to reduce the chance of a type 2 error), we relaxed the significance criterion to be less stringent. Significance was defined as voxel-wise  $P < .10$  for the placebo analysis only; the extent criterion for significance was determined by AFNI software, ClustSim ( $P < .10$ ) based on the same combined ROIs and was defined as  $\geq 40$  contiguous voxels.

### Healthy Volunteer Comparisons

For the conditions (implicit and/or explicit processing) that produced significant correlations in the primary analyses described above, the emotional processing bias also was calculated from BOLD data acquired from healthy volunteers. BOLD response in the depressed group was compared with healthy volunteers in



**Figure 3.** Regions of interest (ROI). The subgenual prefrontal cortex ROI (A) was defined to encompass anterior cingulate cortex areas that included the most rostral portion of BA 24, the infralimbic cortex (BA 25), and the putative prelimbic cortex (BA 32pl) areas implicated in neuroimaging and neuropathological studies of mood disorders (Drevets et al., 1997; Mayberg et al., 1999; Ongur et al., 2003; Mayberg et al., 2005; Price and Drevets, 2010). A cylindrical ROI was defined that encompassed this cortex within the stereotaxic atlas of Talairach and Tournoux (Talairach and Tournoux, 1998). Thus, the cylindrical volume was 3 cm in length, 2 cm in diameter, and situated with long axis at  $x = 0$  (midline) and  $z = -8$  mm between the coronal planes centered at 3 and 33 mm anterior to the anterior commissure. The middle occipital cortex (MOC) regions (B) were defined anatomically using the AFNI drawing plug-in.

the same regions in which correlations were observed; thus, the mask created from the pretreatment patient analysis (ie, voxels reflecting a correlation between BOLD and treatment outcome) was applied to select the voxels from the healthy volunteer group, and a mean across the region was calculated to reflect the average difference in BOLD response to happy and sad faces. A 1-sample  $t$  test was used to determine if a significant processing bias was evident in healthy volunteers (group mean compared with 0, representing no processing bias), and the patient and healthy groups were compared using a  $t$  test. For these analyses, significance was defined as  $P < .005$ .

### Whole Brain Analysis

A whole-brain analysis also was conducted to compare the healthy volunteer emotional processing bias with that observed in patients outside of the defined ROIs using a  $t$  test. Significance was defined as  $>25$  voxels ( $P < .05$ ) based on a voxel level significance of  $P < .005$ , with an extent criteria corrected for the sum of the cortical and subcortical volume.

### Performance Data Analysis

Reaction time (RT) was determined for each task condition. Behavioral processing biases were estimated by calculating the difference in RT under implicit (AHh-AHs) and explicit (AFh-AHs) emotion-processing conditions. To determine if a performance bias was evident at baseline, each RT bias estimate was analyzed using 1-sample  $t$  test (ie, significance would imply that performance was dependent on emotion). Significance was defined as  $P < .025$  to correct for the number of comparisons within each of the 2 performance measures. Baseline biases were compared between healthy volunteers and patients, and

changes following acute scopolamine were compared using  $t$  tests ( $P < .025$ ).

## Results

The mean ( $\pm$ SD) MADRS score pretreatment in patients with MDD was 30 ( $\pm 3.8$ ). Five MDD subjects (31%) had a comorbid anxiety disorder that arose secondary to the primary MDD diagnosis based upon ages at onset. Overall, the patients showed a significant reduction in depression severity as indicated by a decrease in MADRS score from baseline to study end ( $t = 4.7$ ,  $P < .001$ ). The percent reduction in MADRS scores ranged from -5% to 93%, with a mean decrease of 14.4 points. Image data from 2 MDD participants were excluded from analysis, one due to movement in the scanner that exceeded 1 voxel and one due to inadequate behavioral performance (chance level) indicating that they were not performing the task. Of the 14 patients (females = 11, mean age [ $\pm$ SD] = 32 [ $\pm 8.9$ ]) whose image data were included in the analysis, 7 showed a clinical response following scopolamine (ie,  $\geq 50\%$  reduction in MADRS score) and 7 were either partially or nonresponsive (reductions in MADRS of  $< 50\%$ ). For subjects receiving scopolamine first (S/P), there was no worsening in depression severity between the end of the scopolamine block and the end of the study ( $P > .50$ ). One subject discontinued the study after 5 infusions (the participant did receive the full series of scopolamine infusions and was included in these analyses as a nonresponder). The other participants completed all 7 infusions. Three additional participants failed to provide the final assessment (assessment 8, which occurs 3 to 5 days following the seventh infusion); they were included in the analysis using the last observation carried forward (2 of whom received scopolamine in the first series). The clinical findings for the patients

included in this analysis are among those reported previously (Furey and Drevets, 2006; Drevets and Furey, 2010).

### Emotional Processing Bias Analysis

Treatment response magnitude correlated significantly with the difference in BOLD response (ie, emotion processing bias) only under implicit processing (AH) (Table 1). In the sgACC, a positive correlation ( $P < .025$  SVC; Figure 4) was observed, so that those patients with relatively larger BOLD response to happy vs sad faces during implicit emotional processing at baseline subsequently showed larger clinical responses to scopolamine. Conversely, a negative correlation ( $P < .025$  SVC; Figure 5) was observed in the right MOC, so that those patients manifesting larger BOLD response to sad vs happy faces during implicit processing at baseline subsequently showed larger clinical responses. No correlation was observed within the ROIs between the magnitude of placebo response and the emotional processing bias during implicit emotional processing ( $n=6$ ), despite the loosening of significance criteria (ie, voxel-wise  $P < .10$ ; extent correction  $P < 0.10$ ). While the emotional processing bias during implicit emotion processing did not correlate significantly with placebo response, this finding was limited by the small sample size.

In contrast, during explicit emotional processing (AF) no significant correlation was observed within the predefined ROIs between treatment response magnitude and an emotional processing bias (happy vs sad). Moreover, during the control task no correlation was observed between treatment outcome and the emotional processing bias. Thus, the effect is specific to the implicit emotional processing condition.

### Healthy Volunteer Comparison

For the implicit processing condition, the baseline emotion processing bias (happy – sad) was compared between healthy volunteers and patients. Group means for the 2 ROIs showed no significant difference ( $P > .40$ ). To assess whether the healthy volunteers had a significant emotion processing bias, BOLD estimates were compared with zero in a 1-group *t* test; no significant effect was observed ( $P > .40$ ).

### Whole Brain Analysis

A group difference in the BOLD estimate of the emotional processing bias during the implicit processing condition was observed in the dorsal anterior cingulate cortex (voxel  $P$ -value  $< .005$ ; number of voxels = 16; centered at Talairach coordinates ( $x, y, z$ ) = -1, 21, 22; Broadman Area 24), but the region size did not meet the extent criteria for significance ( $>25$  voxels). While the

control group showed relatively larger BOLD response to happy faces and the MDD group manifested relatively larger response to sad faces at baseline in this region, this result did not meet whole brain correction.

### Performance Data

An emotional response bias was observed for reaction time during implicit emotional processing ( $t=2.6, P=.02$ ), where patients responded more quickly when unattended faces were sad than when they were happy. No response bias was observed for RT under explicit processing conditions ( $P=.48$ ) in patients, and no response bias was observed in healthy participants for implicit ( $P=.13$ ) or explicit ( $P=.13$ ) conditions. The implicit processing bias in patients was no longer evident acutely following scopolamine administration ( $P=.18$ ), although when compared directly to the bias measured at baseline, the comparison did not reach statistical significance ( $P=.19$ ). No bias estimate correlated with treatment response ( $P > .25$ ).

### Discussion

The results of this study demonstrate that the differential neural response that reflects an emotional processing bias, specifically during the implicit processing of emotion, may provide a biomarker of antidepressant response to the antimuscarinic agent, scopolamine. We observed that those patients who showed higher levels of neural activity at baseline to implicitly processed sad relative to happy faces in MOC subsequently manifested the larger clinical response to scopolamine. Moreover, greater neural activity in response to happy vs sad faces in the sgACC similarly predicted larger clinical response. These effects were specific to implicit emotion processing, when participants are attending away from the emotional faces, and thus reflect a processing bias associated with automatic emotional processing. These findings suggest that the magnitude and direction of an emotional processing bias may reflect the potential to respond clinically to scopolamine. Importantly, neural activity associated with the emotional processing bias during implicit processing at baseline did not predict placebo response, suggesting that these effects are specific to clinical improvement following scopolamine. As the patterns of correlation in MOC and sgACC mirror each other, these findings may indicate that a distributed brain network differentially responds to implicitly processed emotion in a manner that correlates with subsequent treatment outcome to scopolamine. This finding calls for more specific network analyses that address this possibility.

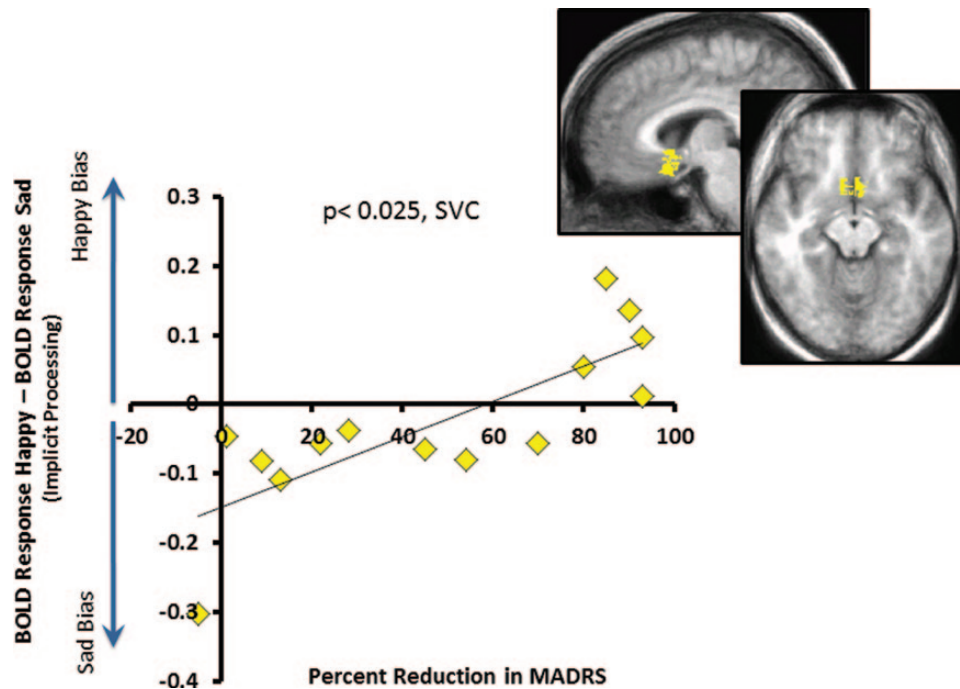
An emotion processing bias also was evident in the behavioral performance data, with patients responding more rapidly

**Table 1.** Center of Mass Locations in Middle Occipital Cortex and Anterior Cingulate Cortex That Show Correlation Effects between the Emotional Processing Bias (Happy – Sad) and Subsequent Antidepressant Response to Scopolamine in Patients with Major Depressive Disorder

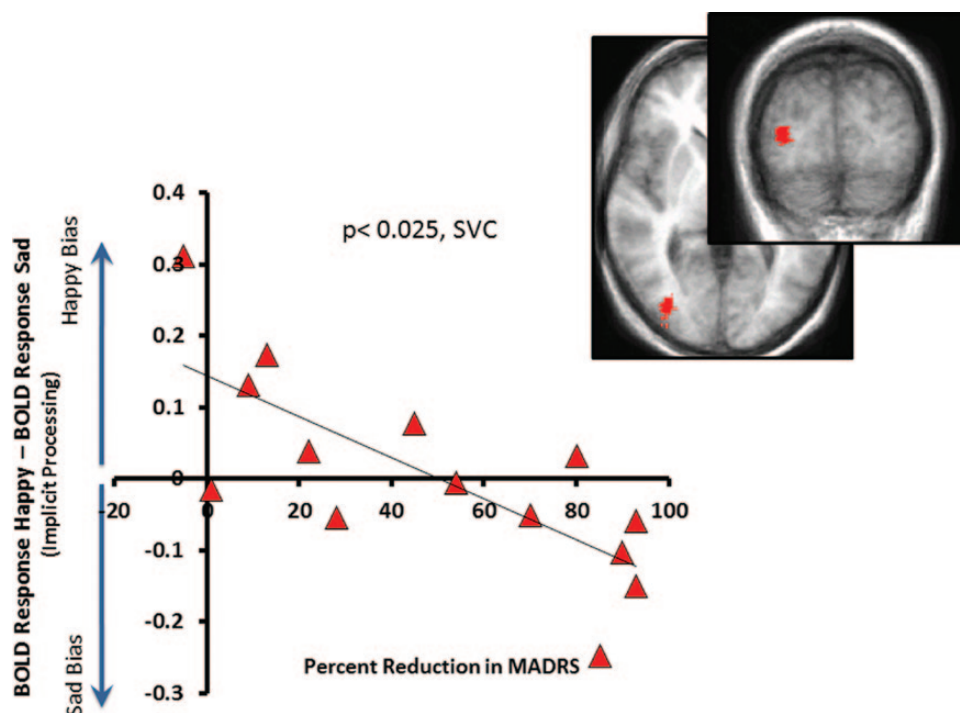
Task Condition	Left MOC		sgACC	
	TA Coordinate (voxel count)	t value	TA Coordinate (voxel count)	t value
Baseline				
Implicit emotion processing (attention to houses)	+32, -79, +6 (13)*	3.6	-1, +12.9, -.9 (21)*	3.0
Explicit emotion processing (attention to faces)	NA		NA	

MOC, middle occipital cortex; NA= no significant finding; sgACC, subgenual anterior cingulate cortex; TA, coordinates based on the Talairach and Tournoux Coplanar Stereotaxic Atlas of the Human Brain.<sup>37</sup>

\*Voxel level significance  $< 0.01$ ; small volume correction  $P < .025$  ( $>12$  voxels).



**Figure 4.** Voxels within the subgenual anterior cingulate cortex (sgACC) region of interest (ROI) that show a correlation between treatment response to scopolamine (change in Montgomery-Asberg Depression Rating Scale [MADRS]) and the difference in blood oxygen level dependent (BOLD) response between implicitly processed (attention to houses) happy and sad faces (ie, emotion processing bias) at baseline (voxel  $P < 0.01$ ; small volume corrected [SVC]  $P < .025$ ). The region within the ROI showing the correlation is superimposed on the TT brain template (Cox, 1996). The representative scatter plot (subject values represent the mean beta as averaged across significant voxels) and the best-fit line also are presented.



**Figure 5.** Voxels within the right middle occipital cortex (MOC) region of interest (ROI) that show a correlation between treatment response to scopolamine (change in Montgomery-Asberg Depression Rating Scale [MADRS]) and the difference in blood oxygen level dependent (BOLD) response between implicitly processed (attention to houses) happy and sad faces (ie, emotion processing bias) at baseline (voxel  $P < .01$ ; small volume corrected [SVC]  $P < .025$ ). The region within the ROI showing the correlation is superimposed on the TT brain template (Cox, 1996). The representative scatter plot (subject values represent the mean beta as averaged across significant voxels) and the best-fit line also are presented.

when stimuli contain sad faces than happy faces under implicit emotion processing conditions. The behavior effect was selective to the implicit processing condition, as no bias was evident

between emotional expressions under explicit processing conditions or during the control task. Healthy participants showed no behavioral emotion processing bias.

Previously, we showed in an independent cohort of patients with MDD that baseline neural activity selectively during an emotion working memory task (and not when viewing the same faces but performing an identify working memory task) in a similar, spatially overlapping area in MOC also predicted treatment outcome to scopolamine (Furey et al., 2013). Thus, 2 distinct tasks similarly predicted treatment outcome, and the predictive task conditions were selective to specific emotion processing requirements. Together, these findings suggest that neural activity per se is not sufficient to predict treatment outcome, but rather the task-specific processing demands associated with the neural response are critical. While each independent study indicated relevant selectivity of task conditions, the processing demands defined by the tasks are quite different. Additional work will be necessary to better define the specificity of processing demands that will uncover the relationship between neural response and treatment outcome. Our current data also provide a replication of the earlier finding, together pointing to MOC as a brain region that may prove to be a critical biomarker of treatment response to scopolamine, and the extant literature suggests that this may extend to other forms of antidepressant treatment (Keedwell et al., 2009, 2010). Thus, the results indicate that pretreatment neural activity in MOC associated with processing emotional faces under various task conditions may offer a biomarker of treatment response to scopolamine.

That these effects are specific to implicit processing, when attending away from the emotional faces, speaks to the relevance of automatic or bottom-up emotion processing to these findings. Evidence suggests that stimulus-driven, implicit processing of face emotion leads to differential levels of activity in the sgACC in MDD (Laxton et al., 2013) and in visual cortical regions in healthy (Bentley et al., 2003), supporting the implication of these regions in our findings.

Others also have identified biological measures that correlate with antidepressant treatment response. Several meta-analyses have reported that clinical status observed at 2 weeks into conventional antidepressant treatment is predictive of subsequent clinical response (Szegedi et al., 2009; Gorwood et al., 2013), allowing for clinical management decisions to be made earlier in the course of treatment. Clinical symptomatology and functional neuroimaging measures have been evaluated as markers of antidepressant response to conventional treatments such as SSRI's (Harmer, 2008; Korb et al., 2009; MacQueen, 2009; Lee et al., 2011; Pizzagalli, 2011; Furey et al., 2012), cognitive behavior therapy (Siegle et al., 2006), and transcranial magnetic stimulation (Gershon et al., 2003) as well as more experimental approaches that produce a rapid-onset of antidepressant action, including ketamine (Salvadore et al., 2009, 2010) and scopolamine (Furey et al., 2012). The identification of pretreatment biological measures that correlate with treatment response may prove particularly useful for guiding treatment decisions and hastening clinical response. In addition to predicting treatment response to specific or experimental agents, identified biomarkers carry the potential to be applicable to conventional treatments as well.

Several limitations to the current findings should be considered; those associated with the clinical findings (side effects, carry-over effects, unblinding and cognitive effects) have been discussed previously (archives 2006; BP 2010). Limitations associated with our primary results include a small sample size, although such sample sizes are not uncommon for placebo controlled pharmacoinaging studies with multiple groups (Surguladze et al., 2005; Furey et al., 2013). A second limitation is related to the concern around the reproducibility of BOLD

response to emotional faces. Importantly, the primary findings are based only on data acquired in the first session, and thus multiple session practice effects are not of concern. The possibility that habituation effects influence BOLD measures acquired post-scopolamine should be considered. The literature reflecting the impact of task repetition across imaging sessions is complicated and suggests that BOLD response is unchanged in some brain regions, while in other areas BOLD activity increases or decreases across sessions (Tegeler et al., 1999; Miki et al., 2001; Qin et al., 2003; Kirschen et al., 2005). Reproducibility of activation is relatively good in the visual cortex, with between 75% and 90% overlap in significant voxels (Rombouts et al., 1998; Miki et al., 2001). The literature does report suppression of the BOLD response with repeated presentations of the same stimuli (Weigelt et al., 2008), which prompted us to use unique stimuli in each session. Importantly, BOLD signal did not change in a systematic manner post-scopolamine in the current study, as the changes that did occur were related to treatment outcome. A final limitation is related to the correlation between the change in the BOLD signal bias following scopolamine and treatment outcome. As the change score inherently retains information regarding the pretreatment BOLD estimate, this analysis is not independent from the primary analysis.

The need to improve upon current treatment options is clear, and the identification of biomarkers of response, particularly in a human biomarker of rapid antidepressant effect paradigm (Zarate et al., 2013), will facilitate this goal. Functional brain imaging has been used successfully to discriminate between treatment responders and nonresponders (Korb et al., 2009; Salvadore et al., 2010; Furey et al., 2013) and to begin to characterize pretreatment, brain-based differences among patients that reflect the potential to respond to specific treatments. These findings indicate that biological variables underlie the clinical variability associated with treatment response, supporting the concept that a more personalized approach to treatment has the potential to be successful. Our findings further demonstrate that brain function per se is not sufficient to predict treatment response, but rather task- and/or stimulus-specific neural activity in key brain regions is necessary to successfully predict treatment outcome.

The findings reported here show that baseline, pretreatment neural activity and the rapid manipulation of this activity predict clinical response to scopolamine, indicating that baseline brain function inherently reflects the potential to respond to treatment. While others have observed correlations between response to various antidepressants and neural activity in both visual cortex and sgACC, those effects were observed 2 weeks into treatment, and thus the predictive nature was unrelated to baseline function. Importantly, our findings replicate earlier results indicating that differential neural response to emotional stimuli in visual cortex might provide a useful biomarker for identifying a subgroup of patients who will respond favorably to scopolamine. While these findings may not offer immediate utility to clinicians, they do indicate that biologically based measures can inform us about the potential for patients to respond to treatment and further may guide the development of novel antidepressants.

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## Interest Statement

The NIMH has filed a use-patent for the use of scopolamine in the treatment of depression, and Drs. Furey and Drevets are identified as co-inventors on this pending patent application in the US and an existing patent in Europe. Dr. Zarate is listed as a co-inventor on a patent application for the use of ketamine and its metabolites in major depression. Dr. Zarate has assigned his rights in the patent to the US government but will share a percentage of any royalties that may be received by the government. Dr. Drevets has consulted for Johnson & Johnson Pharmaceuticals, Inc. and RBM/Myriad, Inc. and currently is an employee of Johnson & Johnson, Inc. All other authors report no biomedical financial interests or potential conflicts of interest.

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