Successful Pharmacologic Treatment of Major Depressive Disorder Attenuates Amygdala Activation to Negative Facial Expressions: A Functional Magnetic Resonance Imaging Study

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ABSTRACT

Objective: Studies of the effects of pharmacotherapy for major depressive disorder (MDD) on limbic-subcortical-prefrontal brain networks show variable results. We quantified functional changes in the amygdala and the related limbic-subcortical-prefrontal structures after paroxetine treatment with functional magnetic resonance imaging relative to clinical responder status.

Method: We scanned 22 patients with unipolar, *DSM-IV*–defined MDD (men and women aged 25–55 years; 17-item Hamilton Depression Rating Scale [$HDRS_{17}$] score > 18) at study entry and after 6 (T0) and 12 (T1) weeks of paroxetine treatment. Our paradigm contrasted negative (fearful, angry), happy, and neutral faces relative to scrambled faces. Twenty-one age-matched (± 2.5 y) and sex-matched controls were scanned once. Patients received open-label paroxetine 20 mg/d for 6 weeks (T0). Nonresponders at T0 were randomly assigned to receive double-blind true dose escalation (paroxetine 30–50 mg/d) or placebo dose escalation for another 6 weeks (T1). The study was conducted from July 2005 to February 2007.

Results: At study entry, MDD patients showed increased ventral/limbic and decreased dorsal prefrontal activations to negative faces. At T0 and T1, respectively, 5/20 and 13/20 patients responded to paroxetine. After 12 weeks (at T1), overall amygdala activations remained unchanged relative to study entry. However, amygdala activations were significantly lower in treatment responders versus nonresponders ($P = .001$). Amygdala activations correlated with $HDRS_{17}$ scores ($P < .04$). Left amygdala activation correlated inversely with pregenual anterior cingulate cortex activation ($P = .001$). Dorsal cingulate gyrus and dorsolateral prefrontal activations increased after 6 and 12 weeks of treatment, regardless of clinical response.

Conclusions: Successful paroxetine treatment decreases amygdala activation, presumably by improved frontolimbic control, in line with selective serotonin reuptake inhibitor–induced increased functional connectivity between the pregenual anterior cingulated cortex, prefrontal cortex, and amygdala. Changes in amygdala activation when processing negative faces might serve as an indicator for improved frontolimbic control, which is required for clinical response.

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Major depressive disorder (MDD) is a highly prevalent and disabling disease[1] often treated with selective serotonin reuptake inhibitors (SSRIs). Unfortunately, response and remission rates are only modest (30%–50%). Noninvasive neuroimaging techniques may aid in clarifying the neurobiological mechanism of antidepressant response.

Previous studies on the etiopathogenesis of MDD provided evidence for a dysfunctional limbic-subcortical-prefrontal network in MDD.[2,3] The "ventral" or limbic compartment of this network (amygdala, insula, ventral striatum, ventral anterior cingulate gyrus, and prefrontal cortex) has been suggested to be responsible for the identification of the emotional significance of stimuli and the production of affective states.[3] In MDD, increased activation of the ventral system has been observed. When compared with controls, increased activation of the (left) amygdala to negative (sad/angry/fearful) facial expressions in those with MDD has been consistently reported.[4–8] Therefore, amygdala activation is considered to represent emotional responsiveness in MDD,[9] associated with MDD severity. Consequently, successful treatment of MDD with SSRIs is expected to normalize ventral hyperactivation.

Effects of open-label treatment with antidepressants on blood-oxygen-level-dependent (BOLD) activation in MDD patients have been explored in 4 functional magnetic resonance imaging (fMRI) studies after 8 weeks of treatment[6,10–12]; another study investigated patients who had remitted after 22 weeks.[13] These studies reported increased BOLD activations in neocortical regions, including the cingulate gyrus (dorsal and anterior parts) after treatment with fluoxetine[6] and venlafaxine.[11] Decreased amygdala activation following antidepressant therapy was first reported in a sertraline study by Sheline et al,[10] and then in studies with fluoxetine (sad faces)[6] and bupropion (emotional oddball task),[12] but not in a study with venlafaxine (negative, positive, and neutral International Affective Picture System pictures).[11] Because all of the above treatment studies reported high response rates (one study explicitly excluded nonresponders[11]), it remains unclear whether observed changes over time are driven by treatment or by clinical response. This issue could be addressed by comparisons of treatment responders versus nonresponders, which have not yet been reported.

Therefore, the aim of this study was to evaluate changes in the amygdala and the limbic-subcortical-prefrontal network in response to (negative) facial expressions after paroxetine treatment of MDD relative to responder status. We expected to observe increased activation of the amygdala (MDD vs controls) at study entry, followed by attenuation of amygdala hyperactivity, together with increased activations in neocortical areas after treatment. We hypothesized that these changes would be associated with clinical response.

Clinical Points

- Only *successful* paroxetine treatment decreases amygdala activation.
- This amygdala signal is inversely correlated with the activation of the pregenual anterior cingulate cortex.
- These findings might change the interpretation of decreased amygdala activities after SSRI treatment in previous studies and suggest improved frontolimbic control as a requisite for clinical response.

METHOD

Participants

After obtaining approval from the institutional ethical committee and written informed consent, we recruited 22 patients (aged 25–55 years) from our outpatient department as a part of a larger study (ISRCTN44111488).[14] Inclusion criteria were MDD as determined by the Structured Clinical Interview for *DSM-IV* Axis I Disorders (SCID)[15] and a 17-item Hamilton Depression Rating Scale ($HDRS_{17}$)[16] score > 18. Patients were drug free (> 4 weeks and ≥ 5 half-lives after a previous antidepressant). Patients could have used 1 or more antidepressants (except paroxetine) at an effective dose for ≥ 6 weeks during the current MDD episode. Exclusion criteria were pregnancy (or wish to become pregnant), standard fMRI contraindications, bipolar disorder, psychotic features, neurologic impairments, primary anxiety or substance abuse disorders, and acute, severe suicidal ideation.

We individually matched each patient by gender and age (± 2.5 years) with a control subject in good mental and physical health and without lifetime use of psychotropics. We excluded subjects with a lifetime psychiatric disorder (according to the SCID; including abuse or addiction disorders), a Beck Depression Inventory[17] score > 9, average alcohol use > 4 units per day (preceding month), or a first-degree relative with psychiatric disorder(s). The study was conducted from July 2005 to February 2007.

Treatment Schedule

After assessment at study entry, patients received open-label treatment with paroxetine 20 mg/d for 6 weeks. At 6 weeks (T0), nonresponders (< 50% decrease in $HDRS_{17}$ score) were randomized (stratified for age).[14] They received either a true paroxetine dose escalation (+10 to +30 mg/d according to adverse effects) or a placebo dose escalation *added to* paroxetine 20 mg/d. Staff and patients were blinded. No additional antidepressants/antipsychotics or psychotherapy was allowed. Dosages remained unchanged the last 3 weeks of the study. Adherence was checked by self-report,[18] pill counts, and serum paroxetine concentrations.[14]

Measurements

Depression severity was measured with the $HDRS_{17}$ at study entry, at randomization (end of the open-label treatment phase; T0), and 6 weeks after randomization (T1). Agreement between trained raters was good (intraclass correlation coefficient = 0.98). Primary clinical outcome was the proportion of responders (≥ 50% decrease in $HDRS_{17}$ score). At study entry, T0, and T1, we planned fMRI sessions (including the facial expression task, a cognitive task [reported elsewhere],[19] and a structural scan).

fMRI

For description of the fMRI paradigm and settings, preprocessing, and first-level analyses, see online supplementary material (PSYCHIATRIST.COM). Contrast images (all faces, negative faces, happy faces) from individual analyses were entered into second-level (random-effects) analyses for between-group comparisons and changes over time. Main effects were identified at $P < .01$, false discovery rate (FDR)–corrected for multiple comparisons (extent threshold: 10 voxels).[20] Planned contrasts were patients versus controls, changes over time (study entry–T0–T1), and responders versus nonresponders. For comparison of responders versus nonresponders, we used a 2-way analysis of variance with time (6 or 12 weeks) and response (yes/no) as factors. This way, we estimated effects of response versus nonresponse. Thus, the T0 scan of a nonresponder at week 6 would be classified as such, while at week 12, after improvement ≥ 50%, the T1 scan would be classified as a responder. Because of small cells and lack of clinical efficacy of dose escalation,[14] we did not model the randomized paroxetine/placebo dose escalation, but only accounted for potential effects of dosage by its inclusion as a regressor of no interest. All interactive effects of group/time/response × stimulus (masked with the relevant main effect) were identified at $P < .001$ ($z > 3.09$) uncorrected (extent threshold: 10 voxels), masked with the relevant main effect at $P < .05$, and at $P < .005$ ($z > 2.57$) in the amygdala. We performed post hoc analyses to investigate whether observed mean amygdala activation (negative faces) at weeks 6 and 12 correlated with activations in other brain regions. We therefore extracted the mean activation of left and right amygdala per subject from the T0 and T1 scans and used these parameters as regressors in new second-level models. We used the Duvernoy atlas as anatomic reference.[21] We report the happy faces results in the online supplementary material.

Statistics

We compared study-entry characteristics of patients and healthy controls with independent-samples *t* tests for continuous variables and χ^2 or Fisher exact test for categorical variables. We used linear mixed models (compound symmetry variance/covariance structure) to assess differences in scan performance (reaction times and errors) between patients and healthy controls and in patients over time. Single voxel SPM5 (Statistical Parametric Mapping; Wellcome Trust Centre for Neuroimaging, London, United Kingdom [www.fil.icon.ucl.ac.uk/spm/], operated under Matlab version 7.3.0.267 [2006; MathWorks, Natick, Massachusetts]) parameter estimates for maximum left and right amygdala signal of all patients at study entry, T0, and T1 were extracted (negative faces) and used for graphical representations. We

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Table 1. Characteristics of Study Sample

Characteristic	Patients (n = 22)	Controls (n = 22)	*P*
Age, mean ± SD, y	43.3 ± 7.93	43.7 ± 7.99	
Male, n (%)	14 (63.6)	14 (63.6)	
Handedness, n, right/left	20/2	21/0[a]	.488
Educational level, n (%)[b]			
< High	17 (77.3)	6 (27.3)	.001
High	5 (22.7)	16 (72.7)	
MDD severity, mean ± SD score			
$HDRS_{17}$	23.1 ± 3.61	NA	
$IDS\text{-}SR_{30}$	42.9 ± 7.69	4.6 ± 3.87[a]	< .001
Anxiety level			
STAI I (state)	58.5 ± 8.29	29.4 ± 7.74[a]	< .001
STAI II (trait)	62.0 ± 9.07	30.9 ± 9.17[a]	< .001
Comorbidity, n (%)			
Anxiety	3 (13.6)	NA	
Alcohol dependence	2 (9.1)	NA	
Cannabis dependence	1 (4.5)	NA	
Scan task performance[a]			
Reaction time, mean ± SD, ms	954.7 ± 238.3	830.0 ± 143.3	.045
Correct response, mean ± SD, %	95.5 ± 3.24	97.5 ± 2.66	.032

[a]One female control did not attend; no questionnaire or scan performance data available.
[b]Education level: high = university level or equivalent.
Abbreviations: $HDRS_{17}$ = 17-Item Hamilton Depression Rating Scale, $IDS\text{-}SR_{30}$ = Inventory of Depressive Symptomatology-Self Rated, MDD = major depressive disorder, NA = not applicable, STAI = State-Trait Anxiety Inventory.

quantified the association of these estimates with $HDRS_{17}$ scores with linear regression models. We used SPSS v15.0.1 (SPSS; Chicago, Illinois) and GraphPad Prism v5.00 (GraphPad; La Jolla, California) for additional analyses.

RESULTS

Patients, Controls, Behavioral Data, and Patient Disposition

We recruited 22 patients and 22 controls. One control did not attend her visit and could not be replaced. Controls had significantly higher education levels. MDD patients had significantly higher state and trait anxiety than controls ($P < .001$), slower reaction times ($P = .045$), and more gender-judgment errors ($P = .032$) (Table 1). Reaction times and errors were not related to education level.

Twenty patients completed the study: 5/20 (25%) and 13/20 (65%) were responders at weeks 6 and 12, respectively. No week 6 responders deteriorated afterward. State anxiety (State-Trait Anxiety Inventory scores) decreased over time (study entry to T1; paired *t* test, $P = .03$) (Table 2). After 6 weeks of initial paroxetine treatment, 15 nonresponders received a randomized dose escalation; for 11 of them, repeated scans were analyzable (true dose escalation: n = 4). All subjects had serum paroxetine concentrations > 5 μg/L at T1 except for 1 patient (considered nonadherent at T0 [serum paroxetine concentration = 1.5 μg/L]). Clinical outcomes were not significantly different between true and placebo dose escalation, as in the larger cohort.[14] Reaction times and error rates did not change over time and were not significantly different between responders and nonresponders or associated with $HDRS_{17}$ scores.

Table 2. Clinical Response and Task Performance of Patients During Follow-Up (n = 20)[a,b]

Measure	T0 (randomization)	T1 (endpoint)
$HDRS_{17}$ score, mean ± SD		
All	15.7 ± 5.14	11.1 ± 5.31
T0 responders	9.8 ± 3.11	7.2 ± 1.48
True dose escalation	18.0 ± 5.13	13.5 ± 6.70
Placebo dose escalation	17.3 ± 2.75	11.0 ± 3.87
% Decrease in $HDRS_{17}$ score, mean ± SD		
All	30.9 ± 26.67	51.6 ± 25.66
T0 responders	59.9 ± 9.53	70.5 ± 2.24
True dose escalation	20.5 ± 26.36	39.4 ± 34.07
Placebo dose escalation	22.0 ± 21.01	52.0 ± 15.09
Response ($HDRS_{17}$ decrease ≥ 50%), n (%)		
All	5 (25)	13 (65)
T0 responders	5 (100)	5 (100)
True dose escalation	…	4 (50)
Placebo dose escalation	…	4 (57)
Remission ($HDRS_{17} \leq 7$), n (%)		
All	2 (10)	5 (25)
T0 responders	2 (40)	3 (60)
True dose escalation	…	1 (13)
Placebo dose escalation	…	1 (14)
$IDS\text{-}SR_{30}$ score, mean ± SD		
All	30.6 ± 8.80	27.2 ± 11.46
T0 responders	19.8 ± 6.46	21.6 ± 4.72
True dose escalation	35.3 ± 6.71	33.5 ± 14.92
Placebo dose escalation	33.0 ± 5.66	23.9 ± 7.03
STAI I (state) score, mean ± SD		
All	54.1 ± 7.20	51.3 ± 8.05
T0 responders	49.8 ± 7.27	51.5 ± 6.03
True dose escalation	56.8 ± 7.36	51.2 ± 11.55
Placebo dose escalation	54.3 ± 6.82	51.2 ± 5.72
Scan task performance, mean ± SD		
Reaction time, ms	936.8 ± 242.8	921.7 ± 252.2
Correct response, %	96.3 ± 3.74	95.6 ± 6.26

[a]All differences between true dose escalation and placebo dose escalation were nonsignificant (analysis of variance; $P > .14$).
[b]Ns were as follows. For $HDRS_{17}$ and $IDS\text{-}SR_{30}$ results: T0 responders, n = 5; true dose escalation, n = 8; placebo dose escalation, n = 7; for STAI I results: T0 responders, n = 4; true dose escalation, n = 6; placebo dose escalation, n = 7; for scan task performance: at T0, n = 18; at T1, n = 17.
Abbreviations: $HDRS_{17}$ = 17-item Hamilton Depression Rating Scale, $IDS\text{-}SR_{30}$ = Inventory of Depressive Symptomatology-Self Rated, STAI = State-Trait Anxiety Inventory.

One patient refused fMRI scans during follow-up. We discarded 2 study-entry scans, 2 T0 scans, and 3 T1 scans due to excessive movements, leaving 41 study-entry scans (20 patients), 17 T0 scans, and 16 T1 scans analyzable.

Main effects of task (all faces vs baseline) showed robust activation of bilateral amygdala, fusiform gyrus, insula, dorsolateral prefrontal cortex (DLPFC), right orbitofrontal cortex (OFC), and right dorsomedial prefrontal cortex (DMPFC) (see online supplementary material).

Amygdala Activation

Study-entry scans: MDD patients versus controls. The all faces contrast showed higher activations bilaterally in the extended amygdala[22–25] in MDD patients (vs controls). Higher activations were also found in the right amygdala of patients with the negative faces contrast, but not in the left amygdala (Table 3A). Amygdala activations at study entry did not differ in final treatment responders and nonresponders (post hoc analyses; all contrasts).

Table 3. Activation of the Amygdala[a]

Contrast	Brain Region	Side	x, y, z (MNI mm)	Cluster Size (k)	Maximum Voxel *z*	*P*
A. MDD patients vs controls (at study entry)						
MDD patients > controls						
All faces	Amygdala (extended)[b]	R	18, 2, −9	12	3.04	.001
		L	−16, −4, −9	39	2.63	.004
Negative faces	Amygdala (extended)[b]	R	18, 2, −9	13	2.99	.001
		L	−16, −4, −9	16	2.46	.007[c]
B. Study entry vs 6 wk of treatment (T0)						
Study entry > 6 wk of treatment (T0)						
All faces	Amygdala	L	−16, 0, −15	12	1.97	.024[c]
Negative faces	Amygdala	L	−14, 2, −12	11	2.25	.012[c]
6 wk of treatment (T0) > study entry						
All faces	Amygdala	R	28, −2, −15	28	3.22	.001
Negative faces	Amygdala	R	28, 0, −15	47	3.47	< .001
C. Study entry vs 12 wk of treatment (T1)						
All faces	…[d]					
Negative faces	…[d]					
D. 6 wk (T0) vs 12 wk (T1) of treatment						
6 wk > 12 wk						
All faces	Amygdala	R	26, −2, −15	53	3.4	< .001
Negative faces	Amygdala	R	26, −2, −15	16	2.94	.002
E. Nonresponders (T0 and T1[e]) vs responders (T0 and T1[e])						
Nonresponders > responders						
All faces	Amygdala	R	18, −2, −18	98[f]	3.24	.001
Negative faces	Amygdala	R	18, −2, −18	30	3.20	.001
		L	−22, 2, −18	24	3.11	.001
Nonresponders > responders with dosage as covariate						
All faces	Amygdala	R	18, −2, −18	95	3.56	< .001
Negative faces	Amygdala	R	18, −2, −18	39	3.47	< .001
		L	−24, 4, −21	15	3.01	.001

[a]Shaded text describes the comparison; italicized text reports the direction of the findings (eg, part A shows significantly greater activation in MDD patients relative to controls on both sides for the all faces contrast and on the right for the negative faces contrast).
[b]*Extended amygdala* describes a subset of neurons involved in the mediation of aversive emotional responses among others in the substantia innominata. This extended amygdala is characterized as a region with a functional and anatomic continuum of the traditionally defined amygdala, based on similarities in cytoarchitecture as well as neurotransmitter and projection systems.[22–25]
[c]Below threshold for significance ($P < .005$).
[d]No decrease in activation relative to study entry.
[e]Combined in a full-factorial model.
[f]In 1 cluster with orbitofrontal cortex.
Abbreviations: L = left, MDD = major depressive disorder, MNI = Montreal Neurologic Institute, R = right.

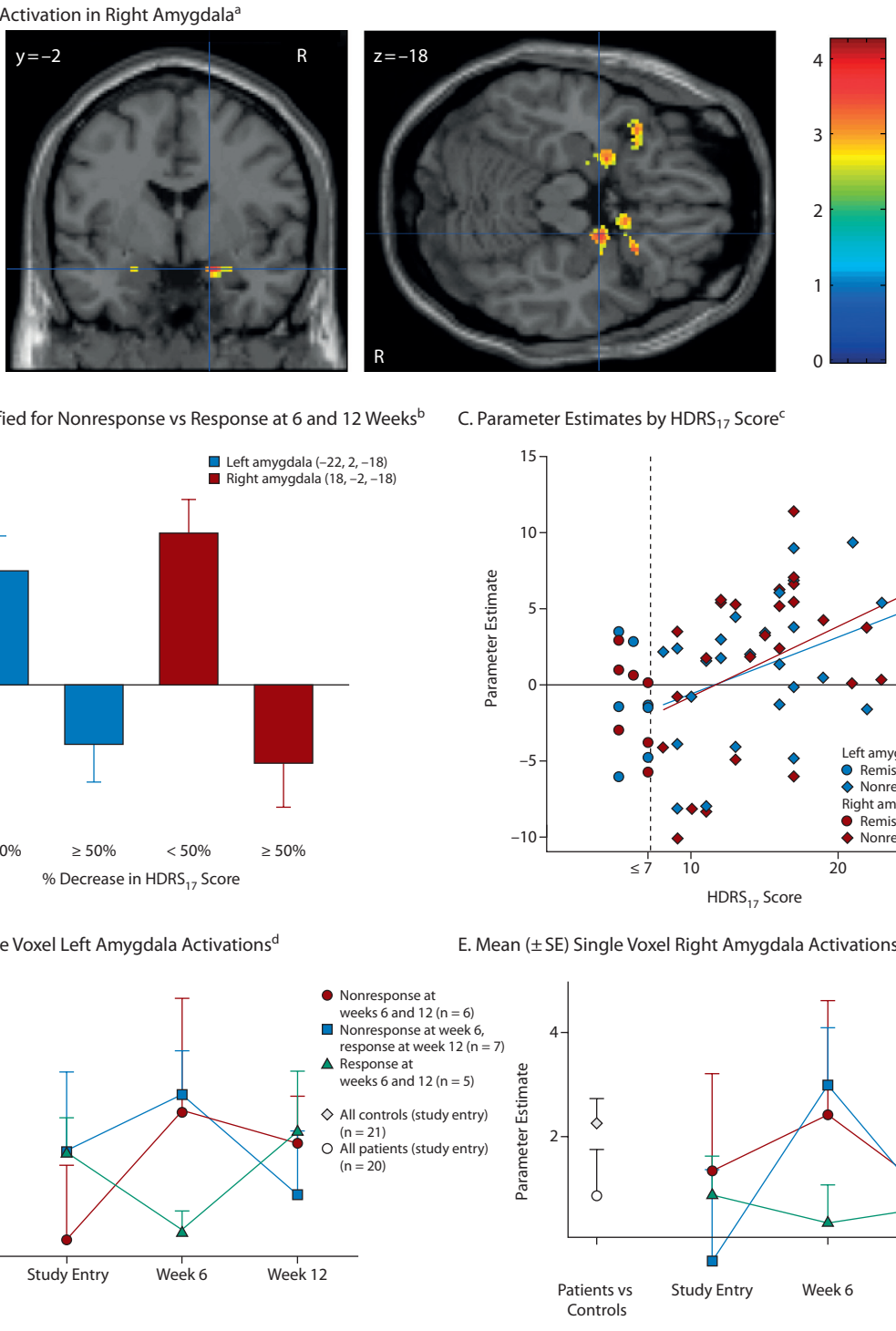
Changes in amygdala activations after 6 and 12 weeks of paroxetine treatment. After 6 weeks of treatment, activations in left amygdala regions decreased, although at a subthreshold level only, relative to the study-entry scan (all and negative faces; Table 3B). The right lateral amygdala showed significantly increased activations after 6 weeks of treatment (all and negative faces; Table 3B). Week 6 treatment nonresponders (n = 12) had increased amygdala activation for negative faces (left, $z = 3.22$, $P = .001$; right, $z = 2.87$, $P = .002$) relative to week 6 responders.

After 12 weeks of treatment, both all and negative faces contrasts revealed no decreases in activation relative to study entry (Table 3C). When we compared week 6 with week 12, there was a significant decrease in activation in the right amygdala in the same region that showed increased activation at week 6 relative to study entry (all and negative faces; Table 3D).

Activations in responders and nonresponders (T0 and T1 scans combined). When comparing nonresponders and responders at 6 and 12 weeks in the full-factorial model with response and time as factors, we found higher bilateral amygdala activations in nonresponders relative to responders (negative faces; $P = .001$; Figure 1; Table 3E). There was no significant time × response interaction ($P > .001$). Activations in bilateral amygdala were significantly correlated with $HDRS_{17}$ scores in patients who did not reach remission ($HDRS \leq 7$; negative faces; $r = 0.37$ [left] and $r = 0.45$ [right]; $P < .04$), but not in patients who remitted ($P > .2$). For the all faces contrast, higher activations in nonresponders were significantly different from responders in the right amygdala only.

We investigated whether observed effects were driven by anxiety levels by including State-Trait Anxiety Inventory scores as a covariate. Significance levels and cluster sizes of amygdala activations in all contrasts were more robust (data available from the authors on request), indicating that results were not confounded by reduced anxiety levels in responders. When we accounted for differences in paroxetine dosage at T0 and T1 (dosage as a regressor), significance levels and cluster sizes of right amygdala activation became more robust, and slightly reduced the activation in the left amygdala (negative faces; Table 3E). Therefore, activations might have been reduced by higher dosages of paroxetine in the right amygdala only.

Figure 1. Amygdala Activation Relative to Clinical Response ($HDRS_{17}$ ≥ 50% decrease) After 6 and 12 Weeks of Treatment With Paroxetine



Negative faces contrast. For parts A–C, week 6 and week 12 scans and response status were analyzed in a full-factorial model with 2 factors: time (week 6/week 12) and response (no/yes). Responder/nonresponder rates were 5/12 at week 6 and 11/5 at week 12.

[a]MNI 18, –2, –18. T-contrast: nonresponders (weeks 6 and 12) > responders (weeks 6 and 12) masked for effect in nonresponders.

[b]Significant difference between nonresponders and responders ($F_{1,32} = 4.591$; $P < .0001$); no significant lateralization ($F_{1,32} = 0.1517$; $P = .70$).

[c]Datapoints represent 33 observations (17 observations at T0 and 16 observations at T1) in 17 patients for left and right amygdala activity (total of 66 datapoints). Estimates centralized to mean. Significant positive correlations for left (0.37 ± 0.17 [SE]; $F_{1,24} = 4.764$; $P = .039$; $r^2 = 0.17$; shown in blue) and right (0.45 ± 0.20 [SE]; $F_{1,24} = 4.964$; $P = .036$; $r^2 = 0.17$; shown in red) amygdala in patients who did not reach remission ($HDRS_{17}$ score ≤ 7). Regression lines for left and right amygdala are not significantly different ($F_{1,48} = 0.097$; $P = .76$).

[d]Activations of left (MNI –20, –6, –15; part D) and right (MNI 22, –6, –15; part E) amygdala. Mixed models showed significant effects of response only ($F_{1,95.741} = 4.784$; $P = .031$), but not of time ($P > .05$), without significant 2- and 3-way interactions of response, time, and side ($P > .05$). No significant differences ($P > .05$) between groups without response or with onset of response in week 6 or week 12.

Abbreviations: $HDRS_{17}$ = 17-item Hamilton Depression Rating Scale, MNI = Montreal Neurologic Institute.

Table 4. Positive and Negative Correlations With Second-Level Amygdala Activations (negative faces)

Contrast	Brain Region	Side	x, y, z (MNI mm)	Cluster Size (k)	Maximum Voxel *z*	*P*
Left amygdala (MNI −22, 2, −18) activation						
Positive[a]	Hippocampus	R	26, −18, −12	102	4.24	<.001
	Amygdala	R	22, 4, −18	127	5.09	<.001
	Orbitofrontal cortex	R	24, 14–21	…[b]	4.34	<.001
		L	−38, 18, −21	33	3.77	<.001
	Parahippocampal gyrus	L	−30, −34, −18	98	4.25	<.001
	Insula	R	38, −6, 12	18	3.60	<.001
	Subgenual anterior cingulate cortex	R	6, 14, −12	10	3.59	<.001
	Parietal	R	52, −54, −15	37	3.88	<.001
Negative[c]	Pregenual anterior cingulate cortex	R	4, 50, 0	14	2.73	.001
Right amygdala (MNI 18, −2, −18) activation						
Positive[a]	Hippocampus	R	24, −22, −12	…[b]	4.56	<.001
		R	30, −32, −6	17	4.51	<.001
		L	−16, −30, −6	57	5.07	<.001
	Amygdala	L	−18, −4, −21	65	4.49	<.001
		L	−18, −10, −12	42	4.20	<.001
	Orbitofrontal cortex	L	−38, 16, −21	40	4.01	<.001
		L	−34, 34, −12	18	3.77	<.001
		R	40, 14, −21	13	3.48	<.001
	Dorsolateral prefrontal cortex	R	58, 14, 36	14	3.70	<.001
Negative[c]	…					

[a]Activations for $P < .001$ with extend voxel size 10.
[b]In 1 cluster with right amygdala.
[c]Activations for $P < .01$ with extend voxel size 10.
Abbreviations: L = left, MNI = Montreal Neurologic Institute, R = right.

Left amygdala activations correlated positively with the contralateral amygdala, bilateral OFC, and right subgenual anterior cingulate cortex (sgACC) (Table 4). Right amygdala activation correlated positively with the contralateral amygdala, bilateral OFC, and right DLPFC (middle frontal gyrus). Left amygdala signal was inversely correlated with the pregenual anterior cingulate cortex (pgACC). Only in patients whose $HDRS_{17}$ score *decreased* were the SPM estimates of this pgACC-amygdala coupling significantly correlated with the relative decrease in $HDRS_{17}$ score (eFigure 1).

Activations in Other Brain Regions

Study-entry scans: MDD patients versus controls. We found lower activations in MDD patients relative to controls in bilateral ventrolateral prefrontal cortex, left posterior cingulate gyrus, left DMPFC, right DLPFC, and left fusiform gyrus (all and negative faces; eTable 1).

Changes in activations after 6 and 12 weeks. After 6 weeks of treatment (eTable 2A and 2B), we found increased activations in the left posterior and right pgACC and left DMPFC (all faces; eTable 2B). For negative faces, activations of right anterior, right pgACC and bilateral anterior cingulate gyrus, left DMPFC, bilateral DLPFC, and left nucleus accumbens were increased.

After 12 weeks of treatment (eTable 2C and 2D), we found increased activations in bilateral DLPFC (middle frontal gyrus) (all and negative faces). We found increased activations in the posterior cingulate gyrus (negative faces).

Activations in responders and nonresponders (T0 and T1 scans combined). Treatment responders showed higher activations in right DLPFC (all faces; posterior superior frontal gyrus/premotor cortex; Montreal Neurologic Institute [MNI] 20, 20, 63; $z = 3.53$; $P < .001$) and left nucleus accumbens (all and negative faces; MNI −8, 12, −9; $z > 3.28$; $P < .002$) relative to nonresponders. Controlling for anxiety levels and dosage did not alter these effects.

DISCUSSION

The present fMRI study in MDD patients evaluated the changes in amygdala activation in response to (negative) facial expressions after 6 and 12 weeks of paroxetine treatment. We found that at study entry patients had higher bilateral (extended) amygdala activation and that changes in amygdala activation were related to treatment response induced by SSRI therapy. Finally, treatment responders had significantly lower amygdala activations than nonresponders. This result confirms and extends the previous findings of a decrease in amygdala activation after SSRI treatment. In particular, our findings suggest that decreases in amygdala activation are a result of not just SSRI exposure but also treatment response. Additionally, we observed changes in other brain regions during paroxetine treatment that were indicative of increased dorsal control. At study entry, we found lower dorsal (left cingulate cortex, left DMPFC, and right DLPFC) activations in MDD patients relative to controls, which were increased after 6 and 12 weeks of treatment, regardless of clinical response. These effects remained when we controlled for effects of dosage and state anxiety.

Brain Activations in MDD and Changes by Antidepressants

Our study confirms findings of an increased reactivity of ventral "limbic" structures (eg, amygdala and insula) in MDD patients, and decreased activations in dorsal prefrontal

areas (PFC and cingulate),[4–7,26,27] during viewing of emotional pictures. The extended amygdala was previously shown to be involved in negative affective states[23] and processing of emotional stimuli.[22] Furthermore, paroxetine treatment increased dorsal prefrontal activation (middle frontal gyrus),[6,8,11–13] but did not reduce activations in the amygdala per se, as shown in direct comparisons over time.

In previous reports, sertraline, fluoxetine, and bupropion were found to reduce abnormal amygdala activations in response to negative stimuli in MDD patients,[6,10,12] but no differential effects between treatment responders and nonresponders were reported, presumably because response rates were high: 10/11 (91%),[10] 13/19 (68%),[6,28] and 6/8 (75%).[12] Our data suggest an alternative interpretation of previous findings: increased activation of the middle frontal gyrus might be a direct effect of paroxetine, in contrast to decreased amygdala activation, which was associated with treatment response. The observations that the middle frontal gyrus modulates emotional responses[29] and is dysfunctional in MDD[2,8,30] corroborate this hypothesis.

In healthy volunteers, single doses of citalopram reduced[31] and increased[32] amygdala activations. Reduced amygdala activation was also reported after 1 week of citalopram treatment.[33] Harmer and colleagues proposed that reductions in amygdala activation represent decreased negative attentional bias induced by SSRIs (or reboxetine[34]), providing a platform for subsequent cognitive and psychological reconsolidation.[35] Although a reduction of attentional bias in MDD patients was recently shown after a single dose of reboxetine,[36] it remains unclear whether this hypothesis is true for MDD patients treated with SSRIs. Our finding of increased activation of the amygdala in response to negative faces among nonresponders (despite 6 weeks of paroxetine treatment) might suggest that decreases in attentional bias with SSRIs occur only in treatment responders.

Amygdala activations at weeks 6 and 12 were correlated with activity of other limbic structures (contralateral amygdala, OFC, sgACC) and correlated inversely with pgACC activity. These findings corroborate the hypothesis of an inhibitory connection between the amygdala and the pgACC, in which the pgACC has a critical role in the communication between dorsal and ventral compartments.[2,37–39] Although increased pgACC-amygdala coupling remains controversial because findings are potentially influenced by patient selection[39,40] and confounders,[39,41,42] Chen and colleagues[39] reported increased functional coupling of the amygdala with the prefrontal, pgACC, striatum, and thalamus after 8 weeks of fluoxetine treatment. Using a different method, we also found associations of amygdala activation with these brain regions.

The observed increase in amygdala activations after 6 weeks in nonresponders, followed by a decrease when patients responded afterward (Figure 1D, 1E), may seem at odds with our hypothesis that amygdala activations are associated with clinical response. However, two complementary observations might reconcile these findings. First, amygdala activation is inversely related to serotonin-1A (5-HT_{1A}) receptor density.[43] Because SSRIs are known to decrease 5-HT_{1A} receptor density[44] and desensitize 5-HT_{1A} receptors,[45] our observations might represent a pharmacologic effect. In addition, our data show that in treatment responders (with larger decreases in $HDRS_{17}$ scores) higher pgACC-amygdala coupling is achieved (eFigure 1), which is hypothesized to result in a net improved regulation of amygdala activation, most prominently in responders, as seen in Figure 1D and 1E. These tentative explanations of biphasic amygdala responses should be further explored in future multimodality imaging studies.

Taken together, the decreased amygdala activation in responders, the inverse association of the pgACC and DLPFC activity with amygdala activation, the correlation of increased pgACC-amygdala association with relative $HDRS_{17}$ score decrease, and the increase in DLPFC function after paroxetine exposure all suggest that paroxetine exposure over time improves dorsal prefrontal regulation of abnormal limbic activity.[8,39,40,46] This effect might exist in addition to the SSRI effects on limbic and subcortical brain function, with subsequent bottom-up effects on cognition.[2,47] Whether the magnitude of such improved control and/or functional connectivity is related to the achievement of treatment response and remission remains to be elucidated.[39,40] If so, nonresponse could be perceived as a failure of improvement of cognitive control. Attenuation of amygdala activation during SSRI treatment might then indicate increased dorsal prefrontal regulation.

Additional Findings

Including dosages made the effects in the right amygdala more robust (negative faces) and hardly affected activations in the left amygdala. The absence of unambiguous effects on bilateral amygdala activation might be interpreted as in line with the evidence that dose escalation of SSRIs has no clinical efficacy in MDD.[14,48–50]

Previous pretreatment, resting state, 18F-fluorodeoxyglucose positron emission tomography (PET) studies have shown that increased metabolism in the pgACC predicted better treatment response.[37] This finding was replicated in 2 fMRI studies with negative faces/pictures[6,11,51] and in our post hoc comparisons of study-entry scans (see online supplementary material). Moreover, we also found increased (study-entry) activations in the sgACC in final treatment nonresponders.[52] Therefore, pretreatment activations of the pgACC and sgACC may serve as predictors for (future) treatment resistance, which merits further investigation.

Limitations

Similar to previous studies,[6,10,11] we did not include a placebo group because our primary aim was to compare responders versus nonresponders. A full placebo group, as in previous PET studies,[2] might have controlled for placebo effects and differentiated unambiguously between true drug effects and placebo-response effects. Therefore, future fMRI studies should preferably include a full placebo group.[51]

Our secondary randomization of treatment nonresponders after 6 weeks in 15 patients yielded only 11 pairs of repeated scans, clearly insufficient to model this placebo-controlled dose escalation. Instead, we included dosage as a covariate.

Finally, we scanned healthy controls once, so habituation effects were not assessed.[6,11] However, the biphasic response in MDD patients, with the initial increase in amygdala activity being associated with a high nonresponse rate at T0 and a clear distinction between treatment responders and nonresponders, suggests that changes in responsiveness over time cannot be explained by learning effects.

CONCLUSION

This fMRI study in MDD patients investigated changes in amygdala activation and the limbic-subcortical-prefrontal network after paroxetine treatment. Bilateral amygdala activation decreased in treatment responders only, while DLPFC activations increased during treatment with paroxetine irrespective of response status. Decreased amygdala activations correlated with increased right pgACC and DLPFC activations. Together with a previous report of increased functional connectivity between amygdala, pgACC, and prefrontal cortex after fluoxetine exposure, we hypothesize that paroxetine increases frontolimbic control in treatment responders. Changes in amygdala activation as measured by fMRI might be an indicator for this increased connectivity and treatment response. Future studies are needed to clarify whether spontaneous response or response after psychotherapy or placebo reveal a similar attenuation in amygdala activation during processing of negative faces.

Drug names: bupropion (Wellbutrin, Aplenzin, and others), citalopram (Celexa and others), fluoxetine (Prozac and others), paroxetine (Paxil, Pexeva, and others), sertraline (Zoloft and others), venlafaxine (Effexor and others).

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Potential conflicts of interest: None reported.

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Supplementary material: Available at PSYCHIATRIST.COM.

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Supplementary Material

Article Title: Successful Pharmacologic Treatment of Major Depressive Disorder Attenuates Amygdala Activation to Negative Facial Expressions: A Functional Magnetic Resonance Imaging Study

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List of Supplementary Material for the article

1. **Methods and Results** — Supplementary description of the study methods and results.
2. **eTable 1** — Activations in other brain regions: MDD patients vs. controls (study entry scans)
3. **eTable 2** — Activations in other brain regions: changes after 6 (T0) and 12 weeks (T1) of treatment relative to study entry
4. **eFigure 1** — Inverse pregenual anterior cingulate coupling with left amygdala and decrease in HDRS score

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Supplemental material Methods and Results

Methods:

For a figure with the design of the study with fMRI instead of SPECT-scans, see Ruhe et al.[14]

Facial expression task paradigm

We used an event-related emotional faces paradigm, which reliably activates the anterior medial temporal lobe including the amygdala.[S1] We presented four human face stimuli: angry, fearful, happy, and neutral human faces [S2] and scrambled faces (with centred arrows) as baseline condition. Each face stimulus condition consisted of 10 pictures; each picture was presented three times. Stimuli were randomized once and presented in identical order to all subjects, using the same task for each session. Stimuli were displayed for 2500ms with a variable interstimulus interval (400-600ms), to increase experimental power and to decrease expectancy effects. To control for overflow effects, we displayed a baseline stimulus after each one or two face pictures. Subjects were instructed to make gender judgements during presentation of face stimuli, no feedback was provided. To familiarize participants, the task was explained outside the scanner.

fMRI imaging

We acquired fMRI scans in the afternoon/early evening using a 3Tesla Intera MRI scanner (Philips, Eindhoven, Netherlands). We used a 6-channel head-coil, the head was fixated by foam pads. Stimuli were generated by a Pentium PC and projected on a screen at the patient's feet, visible through a mirror on the coil. Stimulus onset was triggered by a pulse from the scanner. We recorded subject's performance and reaction times (RTs) with 2 magnet

compatible response boxes.

Each session, we obtained a volumetric T1-weighted coronal scan (TE/TR=4.6/9.63 msec, field of view=24×24 cm, flip angle=8°, number of excitations=1, matrix= 256×256, 182 slices, slice thickness= 1.2 mm, interslice gap= 0 mm, scan time=7 min) covering the entire brain volume, and 260 T2*-weighted axial echoplanar imaging (EPI) images sensitive to blood oxygen level dependent (BOLD) contrast (TE/TR= 35/2530.4 msec, field of view=24×24 cm, flip angle=90°, number of excitations=1, matrix=128×128, 36 ascending slices, slice thickness= 3 mm, interslice gap = 0.3 mm, scan time=10 min).

Individual analysis

For all fMRI data-analyses we used SPM5 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/), operated under Matlab version 7.3.0.267 (2006b; the Mathworks, Natick, Massachusetts, USA). Standard preprocessing of scans consisted of correcting for slice-timing differences, head movements, coregistration to the structural scan, normalization to SPM/MNI standard space (voxelsize 2*2*3 mm), and smoothing (8 mm full-width half-maximum Gaussian filter). Next, BOLD responses were modeled to affective facial expressions and baseline conditions for each voxel. For each subject, weighted contrasts were computed for simple main effects across all stimulus types combined (angry/fearful, happy, and neutral faces vs. baseline = 'all faces'), and within stimulus type contrasts (angry/fearful vs. baseline = 'negative faces'; happy vs. baseline = 'happy faces').

Results:

Main effects at study-entry in patients and healthy controls (Table available on request)

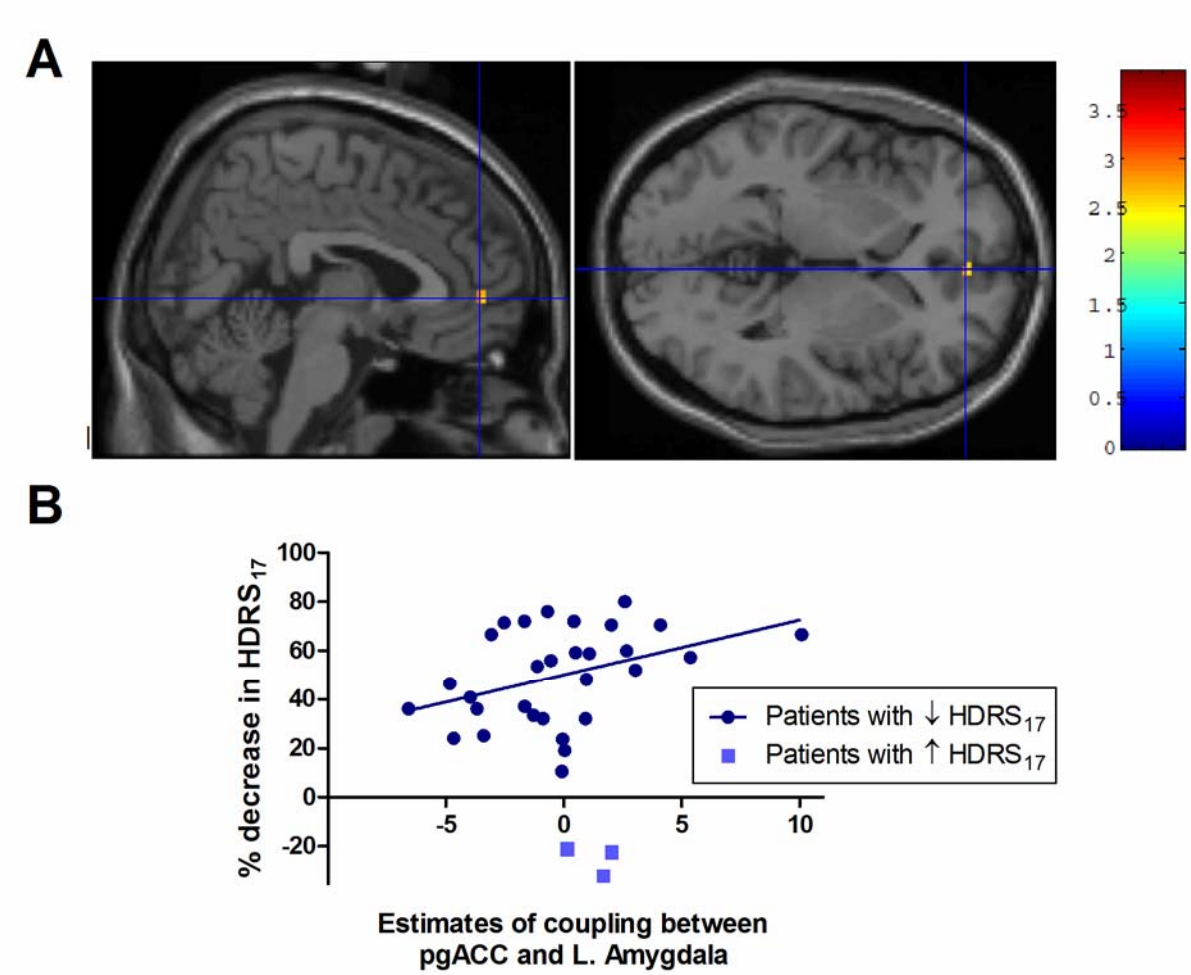
Combining the study-entry scans of patients and controls (all faces contrast) showed robust activation of bilateral amygdala, fusiform gyrus, dorsolateral prefrontal cortex (DLPFC), (anterior) insula, occipital cortices, and right orbitofrontal cortex (OFC; extending into the right anterior insula), parietal cortex and dorsomedial prefrontal cortex (DMPFC). These effects were also found for negative faces, except for the right amygdala, left insula, and left DLPFC, which were not activated above threshold. With the happy faces contrast, we found main effects for bilateral fusiform gyrus, insula, occipital cortices, and right DLPFC, OFC (extended from insula), thalamus and parietal cortex.

Activation of the amygdala by happy faces

At study-entry, when compared with controls, MDD-patients had no higher amygdala activations when contrasting happy faces. After 6 (T0) and 12 weeks (T1) of treatment, we found no significant changes in bilateral amygdala activations at our threshold relative to study-entry (happy faces contrast).

When we compared non-responders and responders after 6 weeks and 12 weeks (full factorial model), we found higher right amygdala activations in non-responders relative to responders (happy faces: MNI 12, 2, -18; k=45; z=2.85; p=0.002). Controlling for anxiety and dosage by including these variables as covariates revealed that activations in the right amygdala by happy faces were not related to state-anxiety, but might have been reduced by higher dosages paroxetine (MNI 16, 4, -18; k=63; z=3.01; p=0.001).

Figure S1. Inverse pregenual Anterior Cingulate coupling with left amygdala and decrease in HDRS-score.



A. Pregenual Anterior Cingulate Cortex (pgACC; MNI 4,50,0) correlated inversely with left amygdala activation (scans at T0 and T1 combined, n= 17 and n= 16, respectively).

B. Estimates of the coupling between the pgACC and left amygdala plotted against the relative decrease in $HDRS_{17}$-scores per subject. Significant positive correlation with the % decrease in $HDRS_{17}$ (2.25 ±1.00 [SE]; $F_{1,28}$=5.082; p=0.032; r^2=0.15) for patients who improve only (circles), but not for those who do not improve (squares).

Activation in other brain regions

Study-entry scans: MDD-patients versus controls

Contrasting all faces versus baseline showed higher activations in the left insula in MDD-patients compared with controls (Table S1A). For happy faces MDD-patients showed higher activation in the left subthalamic nucleus.

With the all faces contrast, we found lower activations in MDD-patients relative to controls in bilateral ventrolateral prefrontal cortex (VLPFC), left posterior and anterior cingulate cortex, left DMPFC, bilateral DLPFC and fusiform gyrus (Table S1B). For negative faces, we found lower activations in MDD-patients in bilateral VLPFC, left posterior cingulate cortex, and bilateral fusiform gyrus. With happy faces, we found lower activations in right VLPFC, right premotor cortex, and left fusiform gyrus in MDD-patients relative to controls.

In post-hoc analyses, final treatment responders showed higher activations at study-entry in the right pregenual (rostral) cingulate (MNI 14, 44, 3; k=4; Z=2.77; p=0.003; negative faces), relative to final non-responders. In contrast, non-responders showed higher study-entry activations in the subgenual cingulate (MNI 0, 26, -3; k=11; Z=3.90; p<0.001; negative faces).

Changes in activations after 6 and 12 weeks of paroxetine treatment

After 6 weeks of treatment (T0), relative to study-entry, we found decreased activations in the right posterior hippocampus (all faces; Table S2A) and left cuneus (all and negative faces). Increased activations were found in - amongst other regions - the left posterior and right pregenual cingulate cortex and left DMPFC (all faces; Table S2B). For negative faces, activations of bilateral anterior cingulate cortex, left DMPFC and bilateral DLPFC were increased.

Table S1. Activations in other brain regions. MDD-patients vs. controls (study-entry scans)

Contrast	Brain region	L/ R	x,y,z (MNI mm)	Cluster size (k)	Max. voxel Z	p
A. MDD > controls						
All Faces	Insula	L	-26 4 12	30	3.13	0.001
Neg. Faces	Insula	L	-28 6 15	32	3.26	0.001
Hap. Faces	Subthalamic nucleus	L	-12 -6 -6	14	3.35	<0.001
B. Controls > MDD						
All Faces	VLPFC	R	50 20 -6	222	3.95	<0.001
		L	-34 22 -6	23	3.20	<0.001
	DLPFC	R	42 16 27	61	3.73	<0.001
		R	48 -2 51	16	3.31	<0.001
		L	-54 16 0	25	3.48	<0.001
	DMFPC	L	-4 10 63	76	3.49	<0.001
	Fusiform gyrus	L	-42 -54 -21	61	3.61	<0.001
	Cingulate cortex, Anterior	L	-8 26 42	58	3.36	<0.001
	Posterior	L	-6 -20 51	23	3.59	<0.001
Neg. Faces	DMFPC	L	-4 10 63	383	4.37	<0.001
	VLPFC	R	50 20 -6	241	3.74	<0.001
		L	-34 22 -6	41	3.09	0.001
	DLPFC	R	42 16 27	76	4.25	<0.001
	Fusiform gyrus	L	-42 -54 -21	67	3.78	<0.001
		R	46 -40 -24	49	3.10	0.001
	Cerebellum	L	-16 -38 -21	13	3.28	0.001
	Sup. temporal gyrus	R	58 -6 -12	37	3.45	<0.001
	Cingulate cortex, posterior	L	-12 -22 48	39	3.41	<0.001
Hap. Faces	VLPFC	R	54 32 3	81	3.51	<0.001
	Precentral gyrus	R	44 2 48	27	3.37	<0.001
	Sup. Temporal gyrus	R	54 -48 12	34	3.51	<0.001
		R	48 -36 6	27	3.20	0.001
	Fusiform gyrus	L	-40 -54 -18	50	3.13	0.001

Abbreviations: see also Table 4. DMPFC= dorsomedial prefrontal cortex, VLPFC= ventrolateral prefrontal cortex;

Table S2. Activations in other brain regions. Changes after 6 (T0) and 12 weeks (T1) of treatment relative to study-entry.

Contrast	Brain region	L/R	x,y,z (MNI mm)	Cluster size (k)	Max. voxel Z	p
<u>After 6 weeks of treatment</u>						
A. Study-entry > T_0						
All Faces	Cuneus	LR	0 -66 12	67	4.27	<0.001
	Hippocampus, posterior	R	28 -36 0	31	3.27	0.001
Neg. Faces	Cuneus	L	-2 -66 12	51	3.97	<0.001
Hap. Faces	Sup.temporal sulcus	L	-48 -46 6	11	3.48	<0.001
	Insula	L	-36 16 -18	31	3.36	<0.001
B. T_0 > study-entry						
All Faces	Cingulate cortex, posterior	L	-12 -18 48	21	4.13	<0.001
	anterior	L	2 4 33	70	3.37	<0.001
	pregenual	R	4 36 3	15	3.41	<0.001
	Hippocampus, dorsal	R	28 -28 -12	31	3.74	<0.001
	DMFPC	L	-10 22 60	32	3.39	<0.001
	Inf. temporal gyrus	L	-44 -4 -39	14	3.28	0.001
Neg. Faces	DMFPC	L	-4 22 60	288	4.56	<0.001
	Cingulate cortex, anterior	R	2 30 33	133	3.21	0.001
	anterior	LR	2 2 36	69	3.27	0.001
	VLPFC	L	-24 58 30	82	3.89	<0.001
	DLPFC	L	-50 26 -3	16	3.65	<0.001
		L	-28 44 42	55	3.51	<0.001
		L	-30 22 54	14	3.28	0.001
		R	32 -2 54	12	3.22	0.001
		R	24 42 48	136	3.09	0.001
	Hippocampus, dorsal	R	30 -28 -9	48	3.53	<0.001
	Cerebellum	L	-22 -34 -27	15	3.21	0.001
Hap. Faces	Cerebellum	L	-24 -32 -24	23	3.78	<0.001
<u>After 12 weeks of treatment</u>						
C. Study entry > T_1						
All Faces	Sup.Temporal Gyrus	R	50 -48 18	101	3.67	<0.001
	Hippocampus posterior	R	22 -36 0	34	3.30	<0.001
Neg. Faces	-					
Hap. Faces	OFC	L	-34 30 -9	140	3.91	<0.001
	Hippocampus, dorsal	R	20 -36 0	34	3.80	<0.001

	Insula	L	36 12 -3	12	3.33	<0.001
D. T_1 > Study entry						
All Faces	DLPFC, middle frontal gyrus	R	28 6 48	194	6.02	<0.001
		R	10 -12 63	16	3.25	0.001
		L	-48 22 18	32	3.58	<0.001
		L	-28 4 51	21	4.15	<0.001
		L	-4 0 60	97	3.19	0.001
	DLPFC	L	-38 2 48	23	3.62	<0.001
Neg. Faces	DLPFC, middle frontal gyrus	R	28 6 48	487	5.14	<0.001
		R	12 -4 66	20	3.31	0.001
		L	-28 4 51	107	4.82	<0.001
		L	-46 20 24	114	3.82	<0.001
		R	42 20 21	30	3.66	<0.001
	DLPFC	R	60 -20 30	65	3.63	<0.001
		L	-46 8 30	35	3.44	<0.001
	Precentral gyrus	L	-26 -16 57	39	3.67	<0.001
	Cingulate cortex, posterior	R	6 -12 30	106	3.53	<0.001
Hap. Faces	DLPFC	L	-26 -6 51	30	3.84	<0.001
	Hippocampus	R	24 -16 -18	19	3.23	0.001

Abbreviations: see Table 4 and S1.

After 12 weeks of treatment (T1), relative to study-entry, we found decreased activations in the right posterior hippocampus for the all faces contrast (Table S2C). We found no significant decreases for negative faces, and decreased activation in the left insula and right dorsal hippocampus for happy faces. At T1, we found increased activations in left DLPFC for all three contrasts. Furthermore, increased activations were found in bilateral premotor and motor cortices (all faces and negative faces contrasts), posterior cingulate cortex (negative faces) and in the right hippocampus (happy faces contrast; Table S2D).

Activations in responders and non-responders (T0 and T1 scans combined; Table available on request)

Non-responders showed significant ($p \leq 0.001$) higher activations in right OFC, right insula and right dorsal hippocampus (all faces and negative faces), brainstem (all faces), relative to non-responders after 6 and/or 12 weeks of treatment. In contrast, treatment

responders showed higher activations in right DLPFC (all faces) and left nucleus accumbens (all and negative faces). Furthermore, with the happy faces contrast, responders had higher activations in the left dorsal hippocampus, bilateral cingulate cortex, left insula and right mediodorsal thalamus. Controlling for anxiety and dosage by including these variables as covariates did not alter these effects.

Supplemental references:

S1. Wolfensberger SPA, Veltman DJ, Hoogendijk WJG, et al. Amygdala responses to emotional faces in twins discordant or concordant for the risk for anxiety and depression. Neuroimage 2008;41:544-552.

S2. Ekman P, Friesen W. Pictures of facial affect. Palo Alto, CA: Consulting Psychologists; 1976.