THE JOURNAL OF CLINICAL PSYCHIATRY

Supplementary Material

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

Author(s): Henricus G. Ruhé, MD, PhD; Jan Booij, MD, PhD; Dick J. Veltman, MD, PhD; Martin C. Michel, MD, PhD; and Aart H. Schene, MD, PhD

DOI Number: 10.4088/JCP.10m06584

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

Disclaimer

This Supplementary Material has been provided by the author(s) as an enhancement to the published article. It has been approved by peer review; however, it has undergone neither editing nor formatting by in-house editorial staff. The material is presented in the manner supplied by the author.

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)

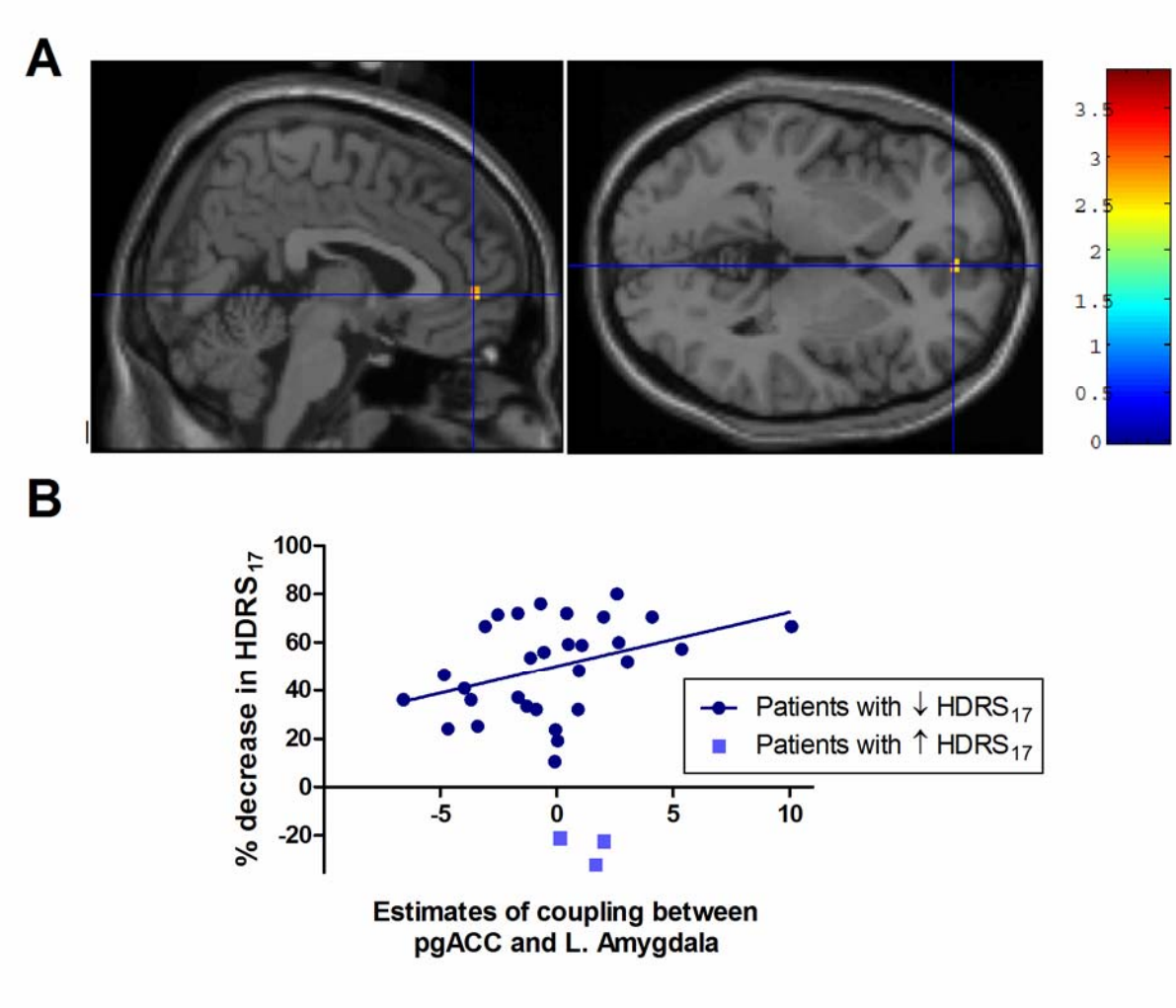
Combining the study-entry scans of patients and controls (all faces contrast) showed robust activation of bilateral amygdala, fusiform gyrus, dorsolateral prefrontal cortex (DLPFC), (anterior) insula, occipital cortices, and right orbitofrontal cortex (OFC; extending into the right anterior insula), 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.