# Supplementary information

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# 1. Supplementary Methods

## 1.1 Medication load index

The medication load index was computed according to the procedure described by Hassel et al. (1). Therefore, each psychotropic medication was coded as absent = 0, low = 1 (equal or lower average dose), or high = 2 (greater than average dose), relative to the midpoint of the daily dose range recommended by Physician’s-Desk-Reference (2). Then, all medication codes per participant and time point were summed, which finally yielded a composite measure of total medication exposure for each subject and time point (medication load index).

## 1.2 Paradigm

Each face-processing block of the paradigm consisted of six trios of faces. A face trio contained a target face on the top and two faces on the bottom (right and left), of which one was identical to the target face. In contrast to the face processing paradigm by Hariri et al. (3), all faces within a trio expressed the same emotion (either anger or fear, also see **Supplementary Fig. 2A**). Within a trio, one of the bottom faces was completely identical to the target face, while the other bottom face presented a different person with the same emotion. Participants had to select the bottom face that was completely identical to the target face. The face trios within one face-processing block were balanced for gender and emotion (anger or fear).

During the sensorimotor control blocks, participants viewed a trio of geometric shapes (circles and/or ellipses, also see **Supplementary Fig. 2B**). A shape trio contained a target shape on the top and two shapes on the bottom (right and left). Participants were instructed to select the shape on the bottom that was identical to the target shape. Each sensorimotor control block consisted of six different shape trios.

Face and shape blocks were presented alternately (sequence: shapes – faces – shapes – faces – shapes – faces – shapes – faces – shapes). All face- and shape-processing blocks of the fMRI paradigm were preceded by an instruction (“Match faces” or “Match shapes” in German) that lasted 2 seconds. In the face-processing blocks, each of the six face trios was presented for 4 seconds with a variable interstimulus interval of 2 seconds to 6 seconds (mean=4 seconds), for a total block length of 48 seconds. In the sensorimotor control blocks, each of the six shape trios was presented for 4 seconds with a fixed interstimulus interval of 2 seconds, for a total block length of 36 seconds. The total task time was 390 seconds.

## 1.3 Acquisition of fMRI data

T2\* functional data were acquired using a single-shot echoplanar (EPI) sequence, with parameters selected to minimize distortion in the region of central interest, while retaining an adequate signal-to-noise ratio (S/N) and T2\* sensitivity: 34 slices, matrix 64 x 64, resolution 3.6 × 3.6 × 3.6 mm; repetition time = 2.1 s, echo time = 30 ms, flip angle = 90°. The slices were acquired in an interleaved mode (first odd, then even), image numbering transversal F>>H. The slices were tilted 25° from the anterior and posterior commissure line in order to minimize dropout artifacts in the orbitofrontal and mediotemporal regions. The presentation of the stimuli was projected to the rear end of the scanner (Sharp XG-PC10XE with additional high frequency shielding; Osaka, Japan). During the experiment, subjects lay supine in the MRI scanner.

## 1.4 Preprocessing of fMRI data

Preprocessing and first-level analyses of functional imaging data were performed using Statistical Parametric Mapping software (SPM8; https://www.fil.ion.ucl.ac.uk/spm/). Functional images were motion-corrected (using a set of six rigid body transformations determined for each image), spatially normalized to the standard Montreal Neurological Institute (MNI) space and smoothed with a Gaussian kernel of 8 mm full-width at half-maximum.

Six participants (*n*=4 patients with depression, *n*=2 healthy controls) had to be excluded due to excessive head movements at either baseline or follow-up scan (exclusion criterion movements > 3mm/3°), resulting in the final study sample.

## 1.5 Statistical analyses

### 1.5.1 Effects of medication dose and psychotherapy on changes in brain function

To account for potential treatment effects on changes in brain function, we computed a subsequent second SPM model, including only patients with depression (2x2 relapse x time ANCOVA). In this model, we additionally included as covariates: a) the medication load index, and b) psychotherapeutic treatment during study interval (dummy-coded: 1, “yes” ≥ 12 sessions, corresponding to a short-term therapy according to the German guidelines for psychotherapy [Psychotherapie-Richtlinie]; 2, “no” < 12 sessions). Again, relapse x time interaction analyses were performed, as well as regression analyses for medication load index and psychotherapeutic treatment in order to test whether the relapse x time interaction remained stable also under controlling for medical and psychotherapeutic treatments and in order to investigate potential associations of treatments with brain function. These analyses were conducted for all three ROIs (bilateral amygdala, bilateral insula and bilateral hippocampus) as well as at the whole-brain level.

### 1.5.2 Relapse prediction based on baseline brain data vs. clinical data

Additionally, we aimed to investigate whether differences in baseline brain activity have predictive value additionally to clinical variables for relapse prediction. Therefore, we performed three different models (only within the MDD patient group) with stepwise logistic regression analyses (forward selection) as described in the following, each with relapse (1=yes / 0=no) as dependent variable:

#### 1.5.2.1 Clinical data

The first model should investigate the predictive value of clinical data for predicting relapse. Therefore, in the first stepwise logistic regression model, only clinical variables describing previous course of illness were entered (stepwise forward selection) as independent variables, which were: number of depressive episodes before baseline, number of inpatient treatments before baseline, duration of inpatient treatment before baseline, cumulative duration of depression before baseline and baseline Hamilton Depression Rating Scale (HDRS) score.

#### 1.5.2.2 Baseline brain functional data

The second model should investigate the predictive value of baseline brain functional data for predicting relapse. Therefore, in the second stepwise logistic regression model, only variables describing baseline brain function were entered (stepwise forward selection) as independent variables, which were: left and right baseline amygdala function, left and right baseline hippocampus function and left and right baseline insula function (all obtained by extracting mean cluster contrast values out of SPM, using the eigenvariate function, of the significant clusters resulting from the post-hoc *t*-test at baseline no-relapse < relapse from the amygdala, hippocampus and insula ROI analyses).

#### 1.5.2.3 Clinical data + baseline brain functional data

The third model should investigate the predictive value of baseline brain functional data in addition and compared to clinical data for predicting relapse. Therefore, in the third stepwise logistic regression model, all clinical data from model 1.5.2.1 additionally to all baseline brain functional data from model 1.5.2.2 were entered (stepwise forward selection) as independent variables, which were in summary: number of depressive episodes before baseline, number of inpatient treatments before baseline, duration of inpatient treatment before baseline, cumulative duration of depression before baseline and baseline Hamilton Depression Rating Scale (HDRS) score, left and right baseline amygdala function, left and right baseline hippocampus function and left and right baseline insula function.

### 1.5.3 Effects of remission status on follow-up brain activity

To exploratively investigate influences of remission status on follow-up brain activity, the relapse group was divided into two subgroups according to the remission status at follow-up (relapse in full remission vs. relapse in current depression). Then, a subsequent one-way ANOVA was computed in SPM, including only the images (faces > shapes) of the follow-up study time point as dependent variable and the factor subgroup as independent variable (healthy controls, no-relapse in full remission, relapse in full remission, relapse in current depression). The main effect of subgroup was tested by an *F*-test, and in case of a significant main effect of group, subsequent post-hoc *t*-tests were conducted to compare subgroups. This analysis was performed for all three ROIs (bilateral amygdala, bilateral insula and bilateral hippocampus) as well as at the whole-brain level.

# 2. Supplementary Results

## 2.1 Effects of medication dose and psychotherapy on changes in brain function

Two thirds (*n*=48; 67%) of the patients with depression were under psychopharmacologic medication at both study time points, while around a quarter (*n*=20; 28%) of the patients received medication at baseline but stopped medication intake during the study interval. A minor part either took no medication at any study time point (*n*=2; 3%) or started medication during the study interval (*n*=2; 3%). The majority of patients with MDD (*n*=49; 68%) was under psychotherapeutic treatment between baseline and follow-up.

### 2.1.1 ROI analyses

**Supplementary Table 6** shows the results of the subsequent second SPM model with patients only (relapse x time ANCOVA) and including medical and psychotherapeutic treatments as covariates. The relapse x time interaction in the amygdala and insula remained significant also under the inclusion of medical and psychotherapeutic treatments. Neither medication load, nor psychotherapy during study interval was significantly associated with activity in any of the three ROIs.

### 2.1.2 Whole-brain analysis

Under inclusion of medical and psychotherapeutic treatments as covariates, the relapse x time interaction was no longer significant at the whole-brain level (*p*TFCE-FWE=.061). Neither medication load, nor psychotherapy during study interval were significantly associated with activity at the whole-brain level (all *p*TFCE-FWE’s>.053).

## 2.2 Relapse prediction based on baseline brain data vs. clinical data

The results of the three logistic regression analyses predicting relapse (yes=1 / no=0) can be found in detail in **Supplementary Table 7**. In summary, the models revealed the following results:

### 2.2.1 Clinical data

The model with only clinical data revealed a model with “cumulative duration of depression before baseline” as the only significant predictor. This model predicted relapse significantly (*Χ*²(1)=15.814, *p*<.001, Nagelkerke’s *R*²=.272).

### 2.2.2 Baseline brain functional data

The model with only baseline brain functional data significantly predicted relapse as well (*Χ*²(1)=11.043, *p*=.001, Nagelkerke’s *R*²=.196), however with a lower goodness of fit compared to the model including clinical data only. The stepwise regression revealed “baseline left insula activity” as the only significant predictor.

### 2.2.3 Clinical data + baseline brain functional data

The stepwise logistic regression with both clinical data and baseline brain functional data predicted relapse significantly (*Χ*²(2)=24.137, *p*<.001, Nagelkerke’s *R*²=.393) and even showed a better goodness of fit compared to the models with clinical data or baseline brain functional data alone. The stepwise regression revealed the variables “cumulative duration of depression before baseline” and “baseline left insula activity” as significant predictors.

## 2.3 Effects of remission status on follow-up brain activity

### 2.3.1 ROI analyses

The ROI analyses of the one-way ANOVAs investigating differences in brain activity at follow-up between subgroups divided by remission status revealed a significant main effect of subgroup only for the insula ROI (x=40, y=26, z=-6, *F*(3,110)=7.00, *k*=11, *p*TFCE-FWE=.043).

Post-hoc *t*-tests of the insula ROI revealed that the relapse group in current depression had significantly lower bilateral insula activity at follow-up compared to HC (right: x=48, y=0, z=-2, *t*(110)=3.90, *k*=714, *p*TFCE-FWE=.004; left: x=-46, y=-6, z=-2, *t*(110)=3.56, *k*=213, *p*TFCE-FWE=.023) as well as lower right insula activity compared to both the relapse group in full remission (x=40, y=26, z=-4, *t*(110)=4.01, *k*=409, *p*TFCE-FWE=.009) and to the no-relapse group (x=36, y=28, z=4, *t*(110)=3.16, *k*=14, *p*TFCE-FWE=.046). All other subgroup comparisons for the insula ROI were not significant (all *p*TFCE-FEW’s> .136).

For the hippocampus (*p*TFCE-FWE>.99) and amygdala (*p*TFCE-FWE>.99) ROIs, there was no significant main effect of subgroup.

### 2.3.2 Whole-brain analysis

At the whole-brain level, no significant main effect of subgroup emerged (*p*TFCE-FWE=.307).

# 3. Supplementary Tables

## Supplementary Table 1 Psychopharmacological medication intake at baseline and follow-up

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Relapse group**  ***n*=47** | | **No-relapse group**  ***n*=25** | |
|  | **Baseline** | **Follow-up** | **Baseline** | **Follow-up** |
| **Drug class** | *n* | *n* | *n* | *n* |
| Selective serotonin reuptake inhibitor | 20 | 14 | 9 | 4 |
| Selective serotonin noradrenaline reuptake inhibitor | 17 | 18 | 9 | 9 |
| Antipsychotics | 13 | 9 | 6 | 3 |
| Noradrenergic and specific serotonergic antidepressant | 6 | 4 | 5 | 2 |
| Tricyclic antidepressants | 3 | 1 | 2 | 0 |
| Mood-stabilizers | 2 | 6 | 0 | 1 |
| Norepinephrine-dopamine reuptake inhibitor | 1 | 3 | 0 | 0 |
| Monoamine oxidase inhibitor | 0 | 1 | 0 | 0 |
| Others | 7 | 4 | 5 | 2 |

*Notes*: Multiple entries per patient possible.

## Supplementary Table 2 Longitudinal within-group changes in brain function from baseline to follow-up for the amygdala and insula regions of interesta

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | **Region of interest** | **Hemisphere** | **Subregions** | **MNI-Coordinates (x,y,z)** | ***t*-valueb** | **Cluster size *k*c** | ***p*TFCE-FWE value** |
| **No-relapse group** | **Baseline < Follow-up** | | | | | | |
| Amygdala | Right | Laterobasal | 34, 2, -26 | 2.69 | 28 | **.037** |
| Insula | Left | Posterior  (Ig2, Id1) | -44, 0, -2 | 3.19 | 194 | **.029** |
|  | Right | – | 40, 0, 0 | 2.86 | 62 | **.042** |
| **Baseline > Follow-up** | | | | | | |
| Amygdala | – | – | – | – | – | >.999 |
| Insula | – | – | – | – | – | .732 |
| **Relapse group** | **Baseline < Follow-up** | | | | | | |
| Amygdala | – | – | – | – | – | >.999 |
| Insula | – | – | – | – | – | >.999 |
| **Baseline > Follow-up** | | | | | | |
| Amygdala | – | – | – | – | – | .066 |
| Insula | Left | Posterior  (Id1) | -42, 6, -12 | 3.77 | 200 | **.017** |
|  | Right | Posterior  (Id1) | 44, 2, -10 | 3.92 | 175 | **.020** |
|  | Left | – | -36, 22, -4 | 3.13 | 126 | **.035** |
| **Healthy control group** | **Baseline < Follow-up** | | | | | | |
| Amygdala | – | – | – | – | – | .226 |
| Insula | – | – | – | – | – | .078 |
| **Baseline > Follow-up** | | | | | | |
| Amygdala | – | – | – | – | – | >.999 |
| Insula | – | – | – | – | – | >.999 |

*Abbreviations*: FWE = Family-wise error corrected, Id = Insular lobe, dysgranular area, Ig = Insular lobe, granular area, MNI = Coordinates of the peak-voxel of the significant cluster according to the standard Montreal Neurological Institute space, TFCE = Threshold-free cluster enhancement.

aResults for post-hoc *t*-tets are only presented for the amygdala and insula regions of interest, as for the hippocampus, the interaction effect did not reach significance (*p*FWE=.055).

bDegrees of freedom for all *t*-values were df=220.

cOnly significant clusters (*p*FWE<.05) are reported.

## Supplementary Table 3 Cross-sectional differences in baseline brain function between groups for region of interest analyses

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Comparison** | **Region of interest** | **Hemi-sphere** | **Subregions** | **MNI-Coordinates (x,y,z)** | ***F-* / *t*-value** | **Cluster size *ka*** | ***p*TFCE-FWE value** |
| **Main effect of group**  **at baseline  (*F*-test)** | **Main effect of group at baseline (*F*-Test)b** | | | | | | |
| Amygdala | Right | Laterobasal | 34, -2, -22 | 6.09 | 4 | **.047** |
|  | Right | Laterobasal | 32, -4, -20 | 5.61 | 1 | **.049** |
| Hippocampus | Left | Subiculum,  CA1-3, DG, entorhinal cortex, HATA | -16, -16, -26 | 9.26 | 351 | **.020** |
|  | Right | Subiculum, DG, CA1-3 | 32, -24, -26 | 9.02 | 338 | **.020** |
|  | Right | CA1 | 34, -4, -22 | 5.99 | 26 | **.045** |
| Insula | Left | Posterior (Ig1-2, Id1) | -40, 0, -10 | 9.64 | 690 | **.008** |
|  | Right | Posterior (Ig2) | 42, 0, -4 | 7.72 | 447 | **.020** |
|  | Right | Posterior (Ig1-2) | 34, -18, 14 | 6.42 | 55 | **.042** |
|  | Right | – | 34, 18, -12 | 5.83 | 11 | **.049** |
| **No-relapse vs. Healthy controls** | **No-relapse < Healthy controlsc** | | | | | | |
| Amygdala | Left | – | -26, -2, -28 | 2.84 | 1 | **.049** |
| Hippocampus | Right | Subiculum, DG, CA1-3, HATA, entorhinal cortex | 24, -36, -6 | 3.12 | 499 | **.025** |
|  | Left | Subiculum, CA1, entorhinal cortex | -20, -16, -28 | 3.23 | 75 | **.035** |
|  | Left | Subiculum, DG, CA1, CA2 | -20, -42, -6 | 3.09 | 79 | **.041** |
|  | Left | Entorhinal cortex | -26, -2, -30 | 2.91 | 25 | **.045** |
| Insula | Right | Posterior (Ig1-2) | 42, -6, 6 | 3.51 | 659 | **.015** |
|  | Left | – | -46, 2, 0 | 3.41 | 14 | **.045** |
| **No-relapse > Healthy controls** | | | | | | |
| Amygdala | – | – | – | – | – | >.999 |
| Hippocampus | – | – | – | – | – | >.999 |
| Insula | – | – | – | – | – | .864 |
| **No-relapse vs. Relapse** | **No-relapse < Relapsec** | | | | | | |
| Amygdala | Right | Laterobasal, centromedian, amygdalostriatal, superficial | 34, -2, -22 | 3.49 | 104 | **.013** |
|  | Left | Laterobasal, amygdalostriatal, centromedian | -30, 0, -28 | 3.12 | 109 | **.020** |
| Hippocampus | Right | CA1-3, subiculum, DG, HATA, entorhinal cortex | 32, -24, -26 | 4.25 | 1330 | **.004** |
|  | Left | Subiculum, DG, CA1-3, HATA, entorhinal cortex | -16, -16, -26 | 4.30 | 1062 | **.005** |
| Insula | Left | Posterior (Ig1-2, Id1) | -44, 2, -4 | 4.29 | 1532 | **.002** |
|  | Right | Posterior (Ig1-2, Id1) | 42, 0, -4 | 3.90 | 1471 | **.004** |
| **No-relapse > Relapse** | | | | | | |
| Amygdala | – | – | – | – | – | >.999 |
| Hippocampus | – | – | – | – | – | >.999 |
| Insula | – | – | – | – | – | >.999 |
| **Relapse vs.**  **Healthy controls** | **Relapse < Healthy controls** | | | | | | |
| Amygdala | – | – | – | – | – | >.999 |
| Hippocampus | – | – | – | – | – | >.999 |
| Insula | – | – | – | – | – | >.999 |
| **Relapse > Healthy controls** | | | | | | |
| Amygdala | – | – | – | – | – | .248 |
| Hippocampus | – | – | – | – | – | .367 |
| Insula | – | – | – | – | – | .064 |

*Abbreviations*: CA = Cornu ammonis, DG = Dentate gyrus, FWE = Family-wise error corrected, HATA = Hippocampal-amygdaloid transition area, Id = Insular lobe, dysgranular area, Ig = Insular lobe, granular area, MNI = Coordinates of the peak-voxel of the significant cluster according to the standard Montreal Neurological Institute space, TFCE = Threshold-free cluster enhancement.

aOnly significant clusters (*p*FWE<.05) are reported.

bDegrees of freedom of all *F*-values were df=2,220.

cDegrees of freedom of all *t*-values were df=220.

## Supplementary Table 4 Longitudinal relapse x time interaction (one-tailed, assuming activity increases in the no-relapse group and decreases in the relapse group) at the whole-brain level

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Anatomical regiona** | **Hemis-phere** | **MNI-Coordinates (x,y,z)** | ***t*-valueb** | **Cluster size *k*c** | ***p*TFCE-FWE-value** |
| Insula / Superior temporal gyrus / Inferior frontal gyrus, triangular part and opercular part / Temporal pole: Superior temporal gyrus / Rolandic operculum | Left | -44, 2, -10 | 4.32 | 2190 | **.027** |
| Insula / Inferior frontal gyrus, opercular part / Rolandic operculum / Posterior orbital gyrus / Temporal pole: Superior temporal gyrus / Superior temporal gyrus / Inferior frontal gyrus, orbital part / Amygdala | Right | 44, 2, -8 | 3.81 | 1256 | **.037** |
| Inferior frontal gyrus, triangular part / Middle frontal gyrus / Superior frontal gyrus | Left | -34, 44, 20 | 3.56 | 299 | **.043** |
| Anterior cingulate gyrus, supracallosal | Left / Right | 2, 20, 16 | 3.88 | 166 | **.044** |
| Substantia nigra, pars reticulata / Thalamus, ventral lateral, ventral posterolateral | Right | 18, -14, -6 | 3.44 | 102 | **.047** |
| Middle frontal gyrus | Right | 48, 48, 8 | 3.49 | 14 | **.049** |
| Middle frontal gyrus / Superior frontal gyrus | Right | 34, 48, 28 | 3.38 | 26 | **.049** |

*Abbreviations*: FWE = Family-wise error corrected, MNI = Coordinates according to the standard Montreal Neurological Institute space, TFCE = Threshold-free cluster enhancement.

aOnly the regions with at least 2% participation in the significant cluster are reported.

bDegrees of freedom of all *t*-values were df=220.

cOnly significant clusters (*pTFCE-*FWE <.05) with cluster size *k*>10 are reported.

## Supplementary Table 5 Cross-sectional differences in baseline brain function between groups for whole-brain analysis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Anatomical regiona** | **Hemi-sphere** | **MNI-Coordinates (x,y,z)** | ***F-* / *t*-value** | **Cluster size *k*b** | ***p*TFCE-FWE-value** |
| **Main effect of group**  **at baseline (*F*-test)** | **Main effect of group at baseline (*F*-Test)c** | | | | | |
| Rolandic operculum / Insula / Inferior frontal gyrus, opercular part / Precentral gyrus | Right | 46, 4, 12 | 9.45 | 328 | **.040** |
| Red nucleus / Substantia nigra, pars compacta / Raphe nucleus, dorsal / Substantia nigra, pars reticulata | Right / Left | 18, -12, -6 | 9.89 | 228 | **.042** |
| Fusiform gyrus / Lobule IV, V of cerebellar hemisphere / Parahippocampal gyrus | Left | -26, -34, -24 | 9.19 | 163 | **.043** |
| Parahippocampal gyrus | Left | -14, -16, -26 | 9.61 | 61 | **.044** |
| Insula / Superior temporal gyrus | Left | -40, 0, -10 | 9.64 | 97 | **.046** |
| Thalamus, ventral posterolateral, pulvinar medial / Hippocampus / Thalamus, pulvinar lateral, ventral lateral, pulvinar anterior, pulvinar inferior | Right | 18, -32, 4 | 8.21 | 147 | **.047** |
| Fusiform gyrus, Lobule IV, V and VI of cerebellar hemisphere | Right | 20, -54, -16 | 8.82 | 25 | **.048** |
| **No-relapse vs.**  **Relapse** | **No-relapse < Relapsed,e** |  |  |  |  |  |
| Insula / Fusiform gyrus / Rolandic operculum / Inferior frontal gyrus, opercular part / Parahippocampal gyrus / Thalamus, ventral posterolateral / Lobule IV, V of cerebellar hemisphere / Precentral gyrus / Thalamus, ventral lateral | Right / Left | 18, -12, -6 | 4.41 | 3640 | **.008** |
| Fusiform gyrus / Lobule IV, V and VI of cerebellar hemisphere / Lingual gyrus / Parahippocampal gyrus | Right | 20, -54, -16 | 4.19 | 643 | **.008** |
| Insula / Superior temporal gyrus / Rolandic operculum / Inferior frontal gyrus, opercular part | Left | -44, 2, -4 | 4.29 | 464 | **.008** |
| **No-relapse > Relapse** | – | – | – | – | >.999 |
| **No-relapse vs.**  **Healthy controls** | **No-relapse < Healthy controls´d** | | | | | |
| Precentral gyrus / Inferior frontal gyrus, triangular part / Insula / Rolandic operculum / Postcentral gyrus / Precuneus / Putamen / Inferior frontal gyrus, opercular part / Supramarginal gyrus / Middle frontal gyrus / Fusiform gyrus / Middle cingulate & paracingulate gyri / Lingual gyrus | Right / Left | 54, 36, 10 | 4.57 | 15697 | **.026** |
| Superior frontal gyrus / Middle frontal gyrus / Middle cingulate & paracingulate gyri / Superior frontal gyrus, medial | Right | 22, 38, 42 | 3.59 | 1530 | **.037** |
| Middle cingulate & paracingulate gyri / Precuneus / Posterior cingulate gyrus | Right / Left | 6, -36, 36 | 3.31 | 709 | **.044** |
| Caudate nucleus / Thalamus, ventral lateral | Right | 20, -8, 20 | 2.84 | 63 | **.045** |
| Middle temporal gyrus | Right | 48, -66, 12 | 3.94 | 107 | **.047** |
| Inferior parietal gyrus / Supramarginal gyrus | Left | -56, -32, 40 | 3.25 | 178 | **.047** |
| Postcentral gyrus | Left | -38, -30, 48 | 3.31 | 139 | **.049** |
| Putamen / Pallidum | Left | -20, 10, 4 | 3.07 | 63 | **.049** |
| Postcentral gyrus / Inferior parietal gyrus | Left | -48, -38, 58 | 2.89 | 19 | **.049** |
| **No-relapse > Healthy controls** | – | – | – | – | .736 |
| **Relapse**  **vs.  Healthy controls** | **Relapse < Healthy controls** | – | – | – | – | .648 |
| **Relapse > Healthy controls** | – | – | – | – | .267 |

*Abbreviations*: FWE = Family-wise error corrected, MNI = Coordinates according to the standard Montreal Neurological Institute space, TFCE = Threshold-free cluster enhancement.

aOnly the regions with at least 2% participation in the significant cluster are reported.

bOnly significant clusters (*pTFCE-*FWE <.05) with cluster size *k*>10 are reported.

cDegrees of freedom of all *F*-values were df=2,220.

dDegrees of freedom of all *t*-values were df=220.

eOnly significant clusters with *pTFCE-*FEW<.01 are reported.

## Supplementary Table 6 Results of the relapse x time ANCOVA including medication and psychotherapy as covariates for region of interest analyses

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Contrast** | **Anatomical region** | **Hemisphere** | **MNI-Coordinates (x,y,z)** | ***t*-valuea** | **Cluster size *k*** | ***p*TFCE-FWE-value** |
| **Relapse x time interaction including medication and psychotherapy as covariatesb** | Amygdala | Left | -28, 2, -22 | 3.58 | 72 | **.010** |
| Hippocampus | – | – | – | – | .095 |
| Insula | Left | -44, 6, -10 | 4.08 | 917 | **.004** |
| Insula | Right | 36, 18, -16 | 3.59 | 681 | **.014** |
| **Main effect of medicationc** | Amygdala | – | – | – | – | .183 |
| Hippocampus | – | – | – | – | .083 |
| Insula | – | – | – | – | .071 |
| **Main effect of psychotherapyc** | Amygdala | – | – | – | – | .547 |
| Hippocampus | – | – | – | – | .422 |
| Insula | – | – | – | – | .518 |

*Abbreviations*: FWE = Family-wise error corrected, MNI = Coordinates according to the standard Montreal Neurological Institute space, TFCE = Threshold-free cluster enhancement.   
aDegrees of freedom of all *t*-values were df=134.  
bTest was conducted one-tailed, assuming activity increases in the no-relapse group and activity decreases in the relapse group  
cTest was conducted one-tailed, assuming a negative association.

## Supplementary Table 7 Results of logistic regression models predicting relapse (yes = 1, no = 0) with clinical data and brain functional data

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Model** | **Predictor variable** | **B** | **SE** | ***p*** | **Odds ratio** | **95% CI for  Odds Ratio** | |
| **Lower bound** | **Upper bound** |
| **Model 1 Clinical data only** | Cumulative duration of depression before baseline | 0.066 | 0.023 | **.004** | 1.068 | 1.021 | 1.117 |
| Constant | -0.661 | 0.446 | .138 | 0.516 |  |  |
| **Model 2**  **Brain functional data only** | Baseline left insula activity | 4.110 | 1.656 | **.013** | 60.923 | 2.374 | 1563.620 |
| Constant | 0.561 | 0.267 | .036 | 1.752 |  |  |
| **Model 3 Clinical data + brain functional data** | Baseline left insula activity | 3.847 | 1.567 | **.014** | 46.834 | 2.170 | 1010.810 |
| Cumulative duration of depression before baseline | 0.065 | 0.024 | **.007** | 1.067 | 1.018 | 1.119 |
| Constant | -0.705 | 0.483 | .144 | 0.494 |  |  |

*Notes:* All three models were performed separately, with the following independent variables in a stepwise (forward selection) approach: Model 1: number of depressive episodes before baseline, number of inpatient treatments before baseline, duration of inpatient treatment before baseline, cumulative duration of depression before baseline, baseline HDRS score, Model 2: left and right baseline amygdala function, left and right baseline hippocampus function, left and right baseline insula function, Model 3: all variables of Model 1 and Model 2.

# 4. Supplementary Figures

## Supplementary Fig. 1. Flow diagram visualizing the exclusion process from the Muenster Neuroimaging Cohort.

Subjects with fMRI paradigm   
and SCID-I   
at baseline and after two years

*n* = 52 HC, n = 86 MDD

Subjects with complete data   
at baseline and follow-up   
meeting inclusion criteria

*n =* 44 HC*, n =* 76 MDD

Final study sample

*n* = 42 HC, *n* = 72 MDD

Excluded because of  
  
- ECT between baseline   
and follow-up (*n* = 10 MDD)  
  
- HC with depressive episode between baseline and   
follow up (*n* = 8 HC)

Excluded because of   
excessive head movement   
(> 3mm / 3°)

*n =* 2 HC*, n =* 4MDD

*Abbreviations*: ECT = electroconvulsive therapy; fMRI = functional magnetic resonance imaging; HC = healthy controls; MDD = patients with major depressive disorder;   
SCID-I = structured clinical interview for DSM-IV.

## Supplementary Fig. 2. Example trial of fMRI paradigm.

**(A)** During the face-processing task, participants viewed a trio of faces (all expressing anger or fear) and had to match one of two faces on the bottom identical to the target face at the top. The descriptions “Fearful Face 1” and “Fearful Face 2” in this figure are placeholders for the original faces that were derived from the Ekman and Friesen stimulus set (4). In the original paradigm, participants viewed the original faces of the Ekman and Friesen stimulus set (4). **(B)** During the sensorimotor control blocks, participants viewed a trio of geometric shapes (circles and/or ellipses). Participants had to match the shape on the bottom that was identical to the target shape.



## Supplementary Fig. 3. Typical brain functional responses (faces > shapes) at baseline for regions of interest comparing patients with and without future relapse.

**(A)** Typical amygdala responses at baseline for patients with major depressive disorder with future relapse (MDD relapse) and patients without future relapse (MDD no-relapse). fMRI contrast values were computed by extracting the first eigenvariate of the significant clusters (left: x=-30, y=0, z=-28, *t*(220)=3.12, *k*=109, *p*TFCE-FWE=.020; right: x=34, y=-2, z=-22, *t*(220)=3.49, *k*=104, *p*TFCE-FWE=.013) resulting from the amygdala ROI analysis of the no-relapse < relapse post-hoc *t*-test at baseline. Error bars indicate 1 SEM.

**(B)** Typical hippocampus responses at baseline for patients with major depressive disorder with future relapse (MDD relapse) and patients without future relapse (MDD no-relapse). fMRI contrast values were computed by extracting the first eigenvariate of the significant clusters (left: x=-16, y=-16, z=-26, *t*(220)=4.30, *k*=1062, *p*TFCE-FWE=.005; right: x=32, y=-24, z=-26, *t*(220)=4.25, *k*=1330, *p*TFCE-FWE=.004) resulting from the hippocampus ROI analysis of the no-relapse < relapse post-hoc *t*-test at baseline. Error bars indicate 1 SEM.

**(C)** Typical insula responses at baseline for patients with major depressive disorder with future relapse (MDD relapse) and patients without future relapse (MDD no-relapse). fMRI contrast values were computed by xtracting the first eigenvariate of the significant clusters (left: x=-44, y=2, z=-4, *t*(220)=4.29, *k*=1532, *p*TFCE-FWE=.002; right: x=42, y=0, z=-4, *t*(220)=3.90, *k*=1471, *p*TFCE-FWE=.004) resulting from the insula ROI analysis of the no-relapse < relapse post-hoc *t*-test at baseline. Error bars indicate 1 SEM.

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## Supplementary Fig. 4. Results of whole-brain analysis at baseline comparing patients with and without future relapse (no-relapse < relapse).

Significant clusters of the whole-brain analysis for the no-relapse < relapse group at baseline. The figure displays clusters significant at *p*TFCE-FWE<.01. Color bar indicates *t*-values.



# References

1. Hassel S, Almeida JRC, Kerr N, Nau S, Ladouceur CD, Fissell K, et al. Elevated striatal and decreased dorsolateral prefrontal cortical activity in response to emotional stimuli in euthymic bipolar disorder: no associations with psychotropic medication load. *Bipolar Disord*. 2008;**10**(8):916–27.

2. Reynolds CR. Physician’s Desk Reference. In *Encyclopedia of Special Education* (eds CR Reynolds & E Fletcher-Janzen). John Wiley & Sons, Inc., 2008.

3. Hariri AR. Serotonin Transporter Genetic Variation and the Response of the Human Amygdala. *Science*. 2002;**297**(5580):400–3.

4. Ekman P, Friesen W V. *Pictures of Facial Affect*. Consulting Psychologists Press, 1976.