Amygdala functional connectivity in major depression – disentangling markers of pathology, risk and resilience

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Abstract

Background. Limbic-cortical imbalance is an established model for the neurobiology of major depressive disorder (MDD), but imaging genetics studies have been contradicting regarding potential risk and resilience mechanisms. Here, we re-assessed previously reported limbic-cortical alterations between MDD relatives and controls in combination with a newly acquired sample of MDD patients and controls, to disentangle pathology, risk, and resilience.

Methods. We analyzed functional magnetic resonance imaging data and negative affectivity (NA) of MDD patients ($n = 48$), unaffected first-degree relatives of MDD patients ($n = 49$) and controls ($n = 109$) who performed a faces matching task. Brain response and task-dependent amygdala functional connectivity (FC) were compared between groups and assessed for associations with NA.

Results. Groups did not differ in task-related brain activation but activation in the superior frontal gyrus (SFG) was inversely correlated with NA in patients and controls. Pathology was associated with task-independent decreases of amygdala FC with regions of the default mode network (DMN) and decreased amygdala FC with the medial frontal gyrus during faces matching, potentially reflecting a task-independent DMN predominance and a limbic-cortical disintegration during faces processing in MDD. Risk was associated with task-independent decreases of amygdala-FC with fronto-parietal regions and reduced faces-associated amygdala-fusiform gyrus FC. Resilience corresponded to task-independent increases in amygdala FC with the perigenual anterior cingulate cortex (pgACC) and increased FC between amygdala, pgACC, and SFG during faces matching.

Conclusion. Our results encourage a refinement of the limbic-cortical imbalance model of depression. The validity of proposed risk and resilience markers needs to be tested in prospective studies. Further limitations are discussed.

Introduction

Major depressive disorder (MDD) is a large contributor to the global burden of disease (GBD 2016 DALYs and HALE Collaborators, 2017) and many patients do not benefit from available treatment options (Trevino *et al.*, 2014). Meta-analyses of neuroimaging studies support a limbic-cortical imbalance (Fitzgerald *et al.*, 2008; Sacher *et al.*, 2012; Graham *et al.*, 2013; Lai, 2014) and a default mode network (DMN) predominance (Graham *et al.*, 2013; Kaiser *et al.*, 2015) as neural mechanisms of negative affectivity (NA) (Mayberg, 1997; Disner *et al.*, 2011) and rumination (Marchetti *et al.*, 2012). To integrate these findings into a model of the pathogenesis of MDD, it is critical to determine whether they form causal factors, correlates, or consequences of the disorder (Kessler *et al.*, 2011).

Numerous imaging genetics studies aimed to discover the pathogenesis of heterogeneous, polygenic mental disorders by identifying intermediate phenotypes (IPs), also referred to as endophenotypes. IPs are conceptualized to mediate between the genotype of a disorder and its behavioral phenotype. Per definition, they are to be found in non-affected family members at a higher rate than in the general population, to be quantifiable, heritable, state-independent, and co-segregating with the illness within families (Gottesman and Gould, 2003; Meyer-Lindenberg and Weinberger, 2006).

Amygdala hyperactivity and aberrant amygdala–prefrontal functional connectivity (FC) during emotion processing have been proposed as IPs for depression as they have been observed in healthy carriers of risk variants of candidate genotypes for depression (Hariri

et al., 2002; Heinz et al., 2005; Pezawas et al., 2005; Schardt et al., 2010; Fornito and Bullmore, 2012) and are key assumptions of the limbic-cortical imbalance model of negatively biased emotion processing (Disner et al., 2011).

In a recent study, we tested one of the validity criteria of these putative IPs, their higher presence in first-degree relatives of MDD patients (Wackerhagen et al., 2017). Considering the limited interpretability of FC for causal effects in amygdala–prefrontal interactions and contradicting findings in imaging genetics studies [decreased connectivity might reflect dysregulation (Pezawas et al., 2005); increased connectivity might reflect compensation (Heinz et al., 2005; Schardt et al., 2010)], we used the identical paradigm as in the imaging genetics studies of reference (Hariri et al., 2002; Pezawas et al., 2005), and assessed amygdala FC across as well as between conditions using generalized psychophysiological interactions [gPPI (McLaren et al., 2012)] to explore the influence of emotional stimuli on amygdala–prefrontal FC. We found that in relatives compared to controls, amygdala FC across conditions was increased with the perigenual anterior cingulate cortex (pgACC) and decreased with the medial frontal cortex (MFG); amygdala FC with both pgACC and MFG was inversely correlated with NA. Considering that cortical regulation of limbic activity is mediated by the pgACC (Disner et al., 2011), we discussed reduced amygdala-MFG in relatives to reflect a potential risk marker and increased amygdala-pgACC FC to serve a compensatory function, potentially promoting resilience.

Here, we re-assessed the previously reported data in combination with a newly acquired group of unrelated MDD patients and additional controls, aiming to disentangle neural mechanisms of pathology, risk, and compensation/resilience. Through mechanisms of familial transmission, including genetic as well as environmental factors, first-degree kinship with a depressed relative is one of the strongest predictors of depression onset and multiplies depression risk by the factor 2–3 (Sullivan et al., 2000; Li et al., 2008; Klein et al., 2013; Wilde et al., 2014, p. 20). We assumed that features unique in patients compared to both unaffected groups would reflect markers of pathology that were not related to familial risk for depression. We further assumed that features shared by both patients and relatives that differed from controls would indicate markers of risk for depression. Further, we assumed that features distinguishing relatives from both controls (who were not at familial risk) and patients (who, in the majority, had first-degree relatives affected by depression or other psychiatric disorders), might be associated with compensation for familial risk and resilience, since relatives were never affected of mental disorders despite their increased risk load. Other studies comparing familial risk, patient and control groups have used the same or similar approaches (Amico, 2011; Doucet et al., 2017).

Using identical methods as in our previous study (Wackerhagen et al., 2017), we assessed brain responses and task-dependent amygdala FC during a faces matching task. We further investigated associations of the brain activity and FC measures with NA, and performed further exploratory analyses of the results.

Methods

Participants

The data were taken from a larger dataset, which were acquired in two independent multicenter studies of the neuro-genetic causes of depression, schizophrenia, and bipolar disorder. The first one was conducted in Berlin, Bonn, and Mannheim, Germany, from 2009 to 2013 and included healthy controls and first-degree relatives of patients affected by one of the disorders (Erk et al., 2014). The second one was conducted in Berlin and Mannheim and included patients affected by one of the disorders and an additional control group. Participants were recruited via local hospitals and advertisements and received small monetary expense allowances.

Inclusion criteria for the group of relatives were to have one or more first-degree relative(s) with a diagnosis of MDD, and no familial history of bipolar disorder, schizophrenia, or substance dependence (except for tobacco), as confirmed in structured clinical interviews or by medical reports. Except for familial history of MDD in relatives, relatives and controls had no personal or familial histories of lifetime axis I disorders, as confirmed by structured clinical interviews (Wittchen et al., 1997). MDD patients were included if currently diagnosed with recurrent depressive disorder or a depressive episode that was either severe or had lasted for at least two years (see online Supplementary Section S8 for clinical characteristics). All participants were native German speakers; for the purpose of genetic homogeneity, only subjects with German/European ancestry up to the second generation were included. Participants gave written informed consent before participation. The study was approved by local ethics committees.

We excluded controls and relatives with scores higher than 18 in the Beck Depression Inventory (BDI-I) (Hautzinger et al., 1994), any participants with neuroradiological findings indicating severe neurological disorders (e.g. cerebral tumors, multiple sclerosis, stroke) as well as with missing data, strong image artifacts, excessive movement (>3 mm translation and/or 1° rotation) during the task scan, or insufficient signal (<10 voxels activation in the faces > shapes contrast a $T > 0.1$) in the right or left amygdala, as determined during PPI analyses. After matching for age and sex, the sample for group comparisons included $n = 203$ participants (Fig. 1, online Supplementary Fig. S2).

Psychological measures

We assessed NA using the BDI-I (Hautzinger et al., 1994), the depression scale of the Symptom Checklist-90-Revised (SCL-90-R, Franke, 2002), the state-trait anxiety inventory (STAI, Laux et al., 1981), and the neuroticism scale of the NEO-FFI (Costa and McCrae, 1992). Consistent with study 1 (Wackerhagen et al., 2017), to reduce the alpha error probability due to multiple testing when assessing brain-behavior correlations, we computed a condensed NA score from these highly correlated measures using factor analysis in SPSS (prerequisites were met, online Supplementary Table S3). We used the multiple choice vocabulary test (MWT-B, Lehrl et al., 1995) as an indicator of general intelligence.

Emotional processing task

In both studies, functional magnetic resonance imaging (fMRI) data were taken from the identical protocol, which included six tasks (total duration 1 h). The implicit emotion processing paradigm was an adaptation of the face matching task (Hariri et al., 2000), in which participants were instructed to identify matching pairs in trios of stimuli, containing angry or fearful faces in the emotion condition, and geometric shapes in the control condition. The task (274 s total duration) was composed of four blocks
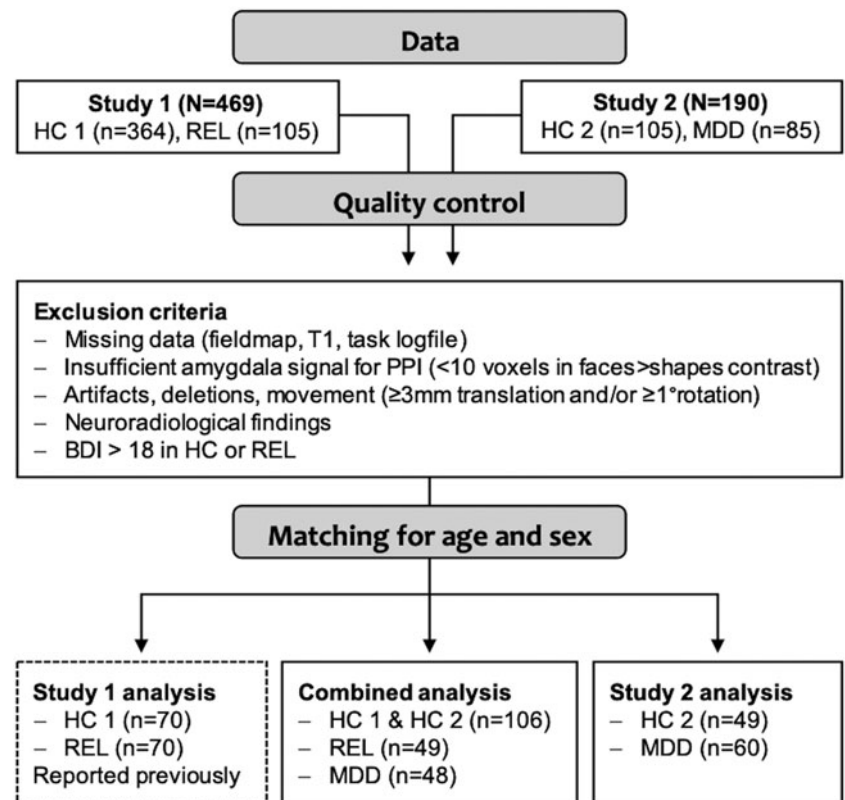
Fig. 1. Overview of the sample and analyses. The sample was acquired in two consecutive studies, study 1 (Wackerhagen et al., 2017) and study 2. After excluding cases that did not meet quality criteria, subsamples matched for age and sex were generated for subsequent analyses. In the main analysis, brain response and task-dependent amygdala FC were compared between controls (HC, from study 1 and study 2), first-degree relatives of MDD patients (REL) and MDD patients (MDD). In the sample of HC2 and MDD from study 2, we assessed the task effects reported in study 1 as well as group effects (results are provided in online Supplementary Material).

per condition, each of which consisted of one instruction (2 s) and six trials (5 s each) (online Supplementary Section S3).

MRI parameters

Functional and structural MRI were performed at 3T Siemens Trio scanners (Erlangen, Germany) using 12-channel head coils and identical scanning protocols. In total, 135 whole-head gradient echo-echo planar imaging (GE-EPI) volumes were acquired (28 slices, matrix size = 64×64, slice thickness = 3 mm, interslice gap = 1 mm, field of view = 192 mm, voxel size = $3 \times 3 \times 3$ mm^3, TR = 2 s, TE = 30 ms, flip angle = 80°) during the task, as well as a field inhomogeneity map and a T1-weighted anatomical 3D image (for registration and normalization, spatial resolution = 1 mm^3, field of view = 192, TR = 1.57 s, TE = 2.74 ms, flip angle = 15°).

Image processing

Preprocessing was done using statistical parametric mapping methods as implemented in SPM12 for MATLAB (www.fil.ion.ucl.ac.uk/spm/software/spm12/). Images underwent correction for slice acquisition delay and head motion, unwarping, registration of the EPI to the individual T1 image, normalization into standard space (Montreal Neurological Institute, $3 \times 3 \times 3$ mm^3 voxels, 4th Degree B-Spline interpolation), spatial smoothing (8 mm FWHM), and high-pass filtering (cut-off 128 s).

Analyses of task-related brain activity

Brain responses were estimated in SPM12, using a generalized linear modeling framework with a model including regressors for the faces and shapes condition, instructions, button presses, and six head motion parameters. Temporal dependencies were removed using a first order autoregressive model, and model parameters were estimated using a restricted maximum likelihood algorithm. Linear contrast images for each task condition ('faces'; 'shapes') were entered into group analyses.

Analyses of task-related functional connectivity

Amygdala FC was assessed for each amygdala separately, both *across* and *between* conditions, using a gPPI framework (online Supplementary Section S4). While, like FC, PPI does not provide information about the causal relations and directions of effects between functionally connected regions (region A could cause B to increase/decrease; B could cause A to increase/decrease, and other factors could influence A and B to increase/decrease simultaneously), it does provide insight about the degree to which the temporal correlation (= FC) of BOLD signal between brain regions (physiological factor) is modulated by the experimental context (psychological factor) (Friston et al., 1997). As physiological term, the time-series was extracted from region of interest (ROI) masks of the right and left amygdala separately, masked by the T-contrast image of the task effect (faces > shapes; $t \geqslant 0.1$; $k \geqslant 10$ voxels). Locations of extracted time-series were predominantly (87% and 89%) in the laterobasal amygdala. As psychological terms, all regressors modeling the task were included. As PPI terms, each psychological term was multiplied by the physiological term (right or left amygdala time-series, respectively) and convolved with the canonical hemodynamic response function implemented in SPM (McLaren et al., 2012). Head motion parameters were included as regressors of no interest. For each individual and each amygdala separately, linear contrast images were computed for (1) the effect *across* conditions ('PPI faces

and PPI shapes'), and (2) the differential effect *between* conditions ('PPI faces > PPI shapes'), which were then entered into group analyses.

Group analyses

Consistent with study 1, in order to use equivalent statistical methods for brain response and FC, we performed analyses of variance for repeated measures (rmANOVAs) for all group level analyses. For brain activation, one rmANOVA with the between-subject factor, 'group' (controls/relatives/patients) and the within-subject factor 'condition' (faces/shapes) was conducted. For FC, two rmANOVAs were conducted to assess the FC effects across and between conditions. Both analyses included the between-subject factor 'group' and the within-subject factor 'seed location' (right amygdala/left amygdala). For the across-conditions analysis, linear contrast images 'PPI faces and PPI shapes' were used as dependent variables. For FC effects between conditions, the linear contrast 'PPI faces > PPI shapes' was used. To eliminate task-unspecific between-subject variance, in all rmANOVAs, the individual mean over conditions or seed locations were modeled additionally. Potentially confounding effects of site were assessed by including site as covariates of no interest in *post-hoc* analyses.

Effects of NA on brain response and amygdala FC (right and left amygdala separately, across conditions and faces > shapes) were assessed in ANOVA analyses in SPM, including NA as covariate of interest as well as the factor *group*, and age, sex, and site as covariates of no interest. Group effects on amygdala FC were investigated in a subsample including only unmedicated patients.

Regions of interest and statistical thresholds

We used identical ROI masks as in study 1. The amygdala masks, which were generated in a literature-based approach (Beck *et al.*, 2012), as combined maps of coordinates of peak effects in the amygdala reported in 19 studies of the faces matching task, and anatomical atlases of the amygdala (Zilles *et al.*, 2002; Amunts *et al.*, 2005). A ROI mask of the PFC for hypothesis-driven tests was created of the combined anatomical boundaries of the lateral, medial, and orbital surfaces of the frontal lobe as provided by the automated anatomical labeling atlas as implemented in the WFU pickatlas for SPM (see online Supplementary Section S5).

To decrease the alpha error probability, we performed a Bonferroni-correction for nine tests (three group contrast, respectively, for activity, FC across, and FC between conditions) and applied a statistical threshold of $p < 0.0055$ to voxel-wise whole-brain FWE-corrected results of the main analyses. Anatomical labeling of coordinates of group effects was performed using the Anatomy toolbox for SPM, version 2.1 (Eickhoff *et al.*, 2005). We further used the intrinsic connectivity network (ICN) atlas (Kozák *et al.*, 2017) to identify the spatial involvement of group effects with functional networks (online Supplementary Sections S6 and S13). ROI masks and statistical images of group and task effects are provided at Neurovault (Gorgolewski *et al.*, 2015; https://neurovault.org/collections/DGADFDYM/).

Exploratory analysis

We explored group effects on task-dependent FC of the left superior frontal gyrus (SFG), where we found activation to be negatively correlated with NA in controls and patients, but not in relatives.

Results

Sample characteristics

Clinical and demographic sample characteristics are provided in Table 1. Groups did not significantly differ in age, sex, years of education, type of school education, and vocabulary test scores. Patients had significantly higher NA scores than relatives and controls, while relatives did not significantly differ from controls (online Supplementary Table S6). On average, participants responded faster in the shapes condition than in the faces condition (mean response time shapes = 1.12 s, s.d. = 0.23; mean response time faces = 1.21 s, s.d. = 0.25; $F = 55.10$; $p < 0.0001$). There were no statistically significant group differences or group-by-condition interactions in response time.

Task-related brain activity

Brain response across groups was increased during faces compared to shapes matching in the visual cortex, subcortical structures (thalamus, hippocampus, and amygdala), pre- and postcentral regions, the dorsal prefrontal cortex, and temporal areas (online Supplementary Section S9).

Groups did not differ in voxel-wise analyses of brain activation. ROI-analyses of group effects on amygdala response revealed marginal differences (patients > relatives > controls) on a descriptive level, which were not statistically significant (online Supplementary Section S10). In controls and patients, but not in relatives, NA was inversely correlated with brain response (faces > shapes) in a cluster in the left SFG (BA32; $T = 4.37$; $p = 0.029$, FWE-corrected for the PFC ROI; Fig. 2). Additionally, in patients, activation in this cluster was inversely correlated with the Hamilton Depression score and the global clinical impression scale (online Supplementary Section S11).

Task-related amygdala functional connectivity

Main effects of condition

During faces compared to shapes matching, amygdala FC was increased with visual regions including the fusiform gyrus and the right inferior frontal gyrus, while it was decreased with midline structures (precuneus, cuneus, posterior and mid cingulate gyrus, anterior cingulate cortex) and temporal regions (online Supplementary Section S9).

Group effects on amygdala FC

In the pathology contrast (controls and relatives > patients), across conditions, patients showed decreased amygdala FC compared to controls and relatives in midline structures including the right medial prefrontal cortex, the posterior cingulum and the parietal cortex. ICN atlas labeling of these regions indicated the highest spatial involvement with the DMN (Fig. 3a, online Supplementary Table S12, Fig. S10).

Analyses of group-by-condition interactions revealed a cluster in the right middle frontal gyrus (BA6, BA9) with reduced amygdala FC in the faces condition in patients compared to controls and relatives (Fig. 3b, online Supplementary Table S12). We observed similar results assessing the pathology contrast including unmedicated patients only (online Supplementary Section S15).

In the risk contrast (controls > relatives and patients), across conditions, patients and relatives showed decreased amygdala FC to the superior parietal cortex and right SFG (BA6), clusters showing strongest spatial involvement with fronto-parietal

Table 1. Sample characteristics (*N* = 203)

	Controls (*n* = 106)	Relatives (*n* = 49)	Patients (*n* = 48)	df	F χ² H	p
Age *mean* ± s.d., F	31.0 ± 9.3	29.0 ± 8.1	31.9 ± 9.0	200	1.97	0.143
Sex *n (%)*, χ²						
Male	43 (40.6)	16 (32.7)	14 (29.2)	2	2.17	0.338
Female	63 (59.4)	33 (67.3)	34 (70.8)			
Study site *n (%)*, χ²						
Charité Berlin	62 (58.5)	18 (36.7)	23 (47.9)	4	26.79	<0.0001
ZI Mannheim	30 (28.3)	15 (30.6)	25 (52.1)			
University of Bonn	14 (13.2)	16 (32.7)	–			
Secondary school degree *n (%)*, H						
Hauptschule (9 years)	66 (5.7)	4 (8.2)	2 (4.3)	2	3.25	0.197
Realschule (10 years)	27 (25.5)	5 (10.2)	15 (32.6)			
Abitur (12 years)	73 (68.9)	40 (81.6)	29 (63.0)			
Years of education *mean* ± s.d., F	14.2 ± 2.9	14.5 ± 2.8	14.8 ± 2.2	198	0.900	0.408
Psychological measures *mean* ± s.d., F						
Multiple choice vocabulary test	30.8 ± 3.3	30.8 ± 3.2	30.7 ± 3.1	195	0.019	0.981
SCL-90-R depression	0.18 ± 0.23	0.29 ± 0.36	1.55 ± 0.822	197	147.51	<0.0001
BDI	2.47 ± 3.12	4.36 ± 3.56	22.10 ± 11.93	197	158.74	<0.0001
STAI-S	31.78 ± 6.62	31.85 ± 5.31	49.72 ± 10.88	165	88.31	<0.0001
STAI-T	33.87 ± 8.84	35.76 ± 8.10	56.53 ± 10.65	194	105.05	<0.0001
NEO neuroticism	14.53 ± 6.58	17.50 ± 6.99	32.53 ± 8.05	196	108.57	<0.0001
Negative affect score	−0.52 ± 0.45	−0.30 ± 45	1.44 ± 0.90	202	194.00	<0.0001
Task response time *mean* ± s.d., F						
Faces	1.21 ± 0.24	1.25 ± 0.30	1.18 ± 0.19	200	0.784	0.458
Shapes	1.11 ± 0.21	1.12 ± 0.29	1.11 ± 0.20	200	0.004	0.996
MDD severity measures						
HAM-D			13.31 ± 7.26			
Psychotropic medication *n (%)*			33 (69)			
Age of onset *mean* ± s.d. *(range)*			22.3 ± 8.9 (8–45)			
CGI *mean* ± s.d. *(range)*			3.87 ± 1.3 (1–6)			
GAF *mean* ± s.d. *(range)*			62.28 ± 14.4 (30–95)			

BDI, Beck's Depression Inventory; CGI, Clinical Global Impression; GAF, Global Assessment of Functioning; H, Kruskal–Wallis-*H*; HAM-D, Hamilton Depression Inventory; MDD, Major Depressive Disorder; NEO, NEO Five Factor Inventory; SCL-90-R, Symptom Checklist-90-Revised; STAI-S, State Trait Anxiety Inventory-State Anxiety; STAI-T, State Trait Anxiety Inventory – Trait Anxiety; ZI, Central Institute of Mental Health.
P was assessed two-sided.

components (Fig. 4*a*, online Supplementary Table S13, Fig. S11). Analyses of group-by-condition interactions revealed a cluster in the right fusiform gyrus, in which amygdala FC during faces matching was reduced in relatives and patients compared to controls (Fig. 4*b*, online Supplementary Table S13).

In the resilience contrast (relatives > controls and patients), across conditions, relatives showed increased amygdala FC with the pgACC (BA32) (Fig. 5*a*, online Supplementary Table S14). Analyses of group-by-condition interactions revealed clusters in the right middle frontal cortex (BA 9) and bilateral temporal cortices, in which amygdala FC was increased during the faces condition in relatives compared to controls and patients (Fig. 5*b*, online Supplementary Table S14).

The NA score did not show significant effects on amygdala FC in voxel-wise analyses at uncorrected $p < 0.0001$. A hypothesis-driven investigation of the previously observed negative correlation of NA with amygdala-pgACC FC (Wackerhagen *et al.*, 2017) revealed a significant effect across groups ($r = −0.144$; $p = 0.020$) and in controls ($r = −0.164$; $p = 0.046$), but did not reach significance in relatives ($r = −0.124$; $p = 0.197$) and patients ($r = 0.010$; $p = 0.474$).

Exploratory findings of group differences in SFG FC

Exploratory analyses of FC of the left SFG revealed a significant group by task interaction in the resilience contrast, with an increase of SFG FC in relatives with the pgACC during faces
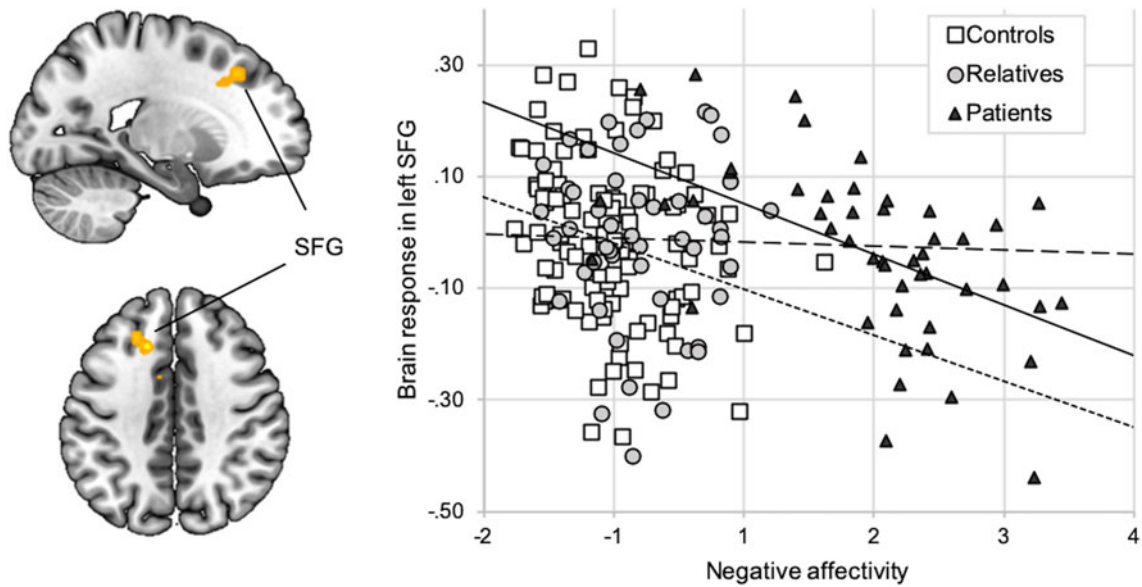
Fig. 2. Correlation between NA and brain response (faces > shapes). Activation during faces compared to shapes (*y*-axis) in the left SFG ($x = -15$, $y = 23$, $z = 41$; $k = 49$) was inversely correlated with the NA factor score (*x*-axis). The correlation was significant across groups ($r = -0.22$; $p < 0.01$) as well as in patients ($r = -0.53$; $p < 0.01$, regular line) and in controls ($r = -0.22$; $p < 0.01$; short-dotted line), but not in relatives ($r = -0.02$; $p = 0.88$; long-dotted line).
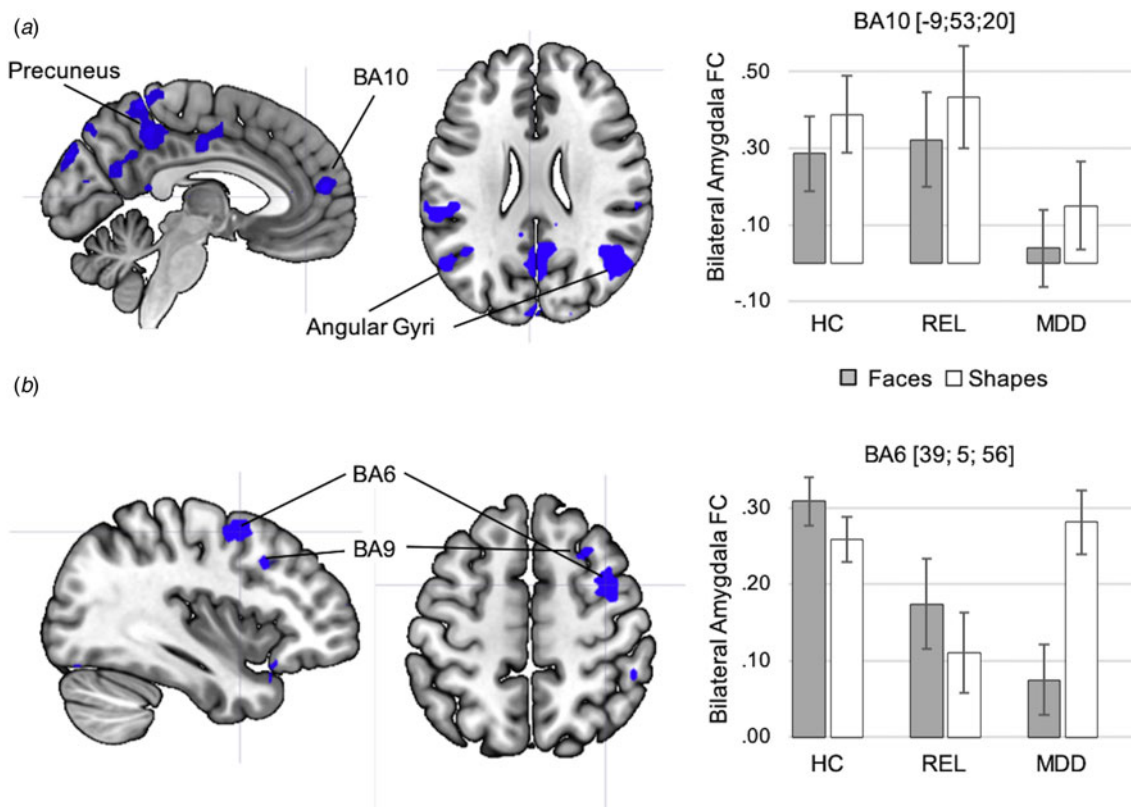

Fig. 3. Effects of pathology (controls and relatives > patients) on amygdala FC are depicted across conditions (*a*) and between conditions (*b*). Across conditions, MDD patients showed reduced amygdala FC with the middle frontal gyrus (BA10), the mid cingulate cortex, precuneus, and bilateral angular gyrus. Statistically significant group-by-condition interactions were observed in the right prefrontal cortex (BA6, BA9), showing reduced amygdala FC during faces compared to shapes processing in MDD patients. The significance-threshold is $p < 0.05$ family-wise error corrected for the whole-brain, cluster size $k = 10$ voxels, error bars indicate +1 standard error of the mean. BA, Brodmann area; HC, healthy controls; FC, functional connectivity; MDD, major depressive disorder; REL, relatives.
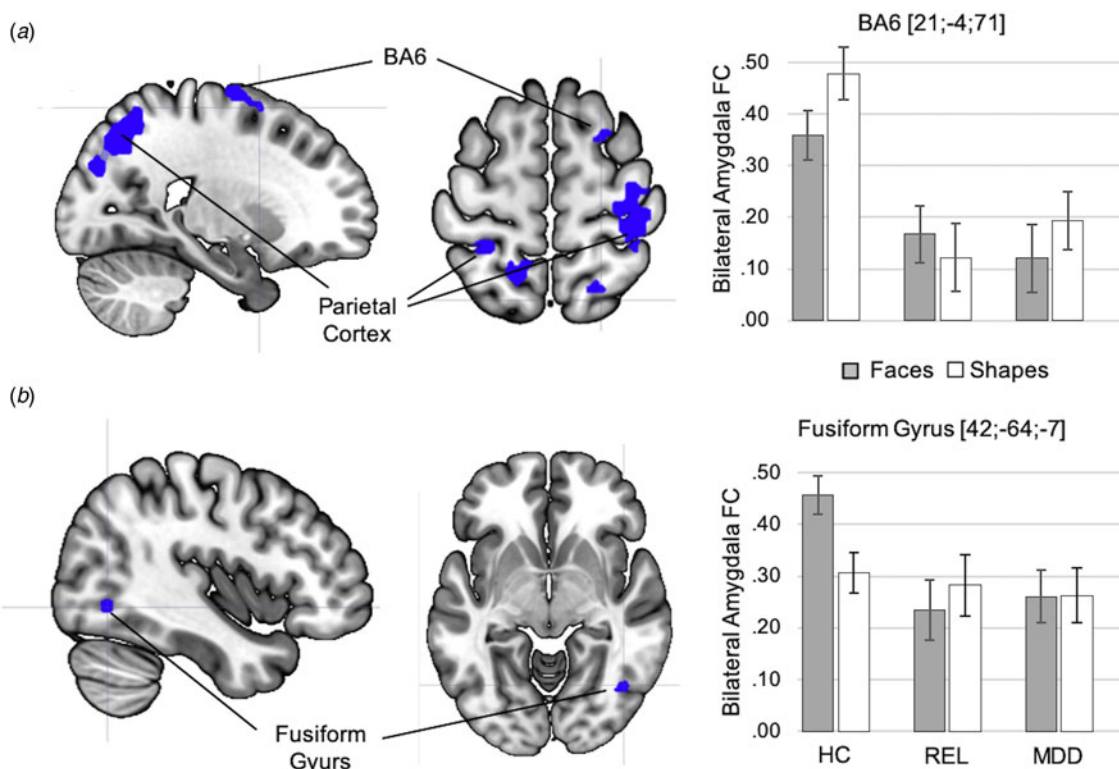
Fig. 4. Effects of risk (controls > relatives and patients) on amygdala FC are depicted across conditions (*a*) and between conditions (*b*). Across conditions, relatives and MDD patients showed reduced amygdala FC with frontal and parietal regions. Statistically significant group-by-condition interactions of amygdala FC were observed in the right fusiform gyrus, showing significantly stronger amygdala FC in controls in the faces compared to the shapes conditions, while amygdala FC did not significantly differ between condition in relatives and MDD patients. The significance-threshold is $p < 0.05$ family-wise error corrected for the whole-brain, cluster size $k = 10$ voxels, error bars indicate +1 standard error of the mean. BA, Brodmann area; HC, healthy controls; FC, functional connectivity; MDD, major depressive disorder; REL, relatives.

matching compared to the two other groups ($p < .05$, FWE-corrected for PFC-ROI; Fig. 5*c*, online Supplementary Section S17).

Including site as a covariate did not significantly change the results.

Discussion

We compared brain response and amygdala FC between MDD patients, controls, and first-degree relatives of MDD patients to disentangle markers of pathology, risk, and resilience capacity.

Task-dependent changes of amygdala FC

We observed comparable effects to our previously reported findings of increased amygdala FC with the fusiform gyrus and right inferior frontal gyrus, and decreased amygdala FC with midline structures including pgACC, precuneus and angular gyrus, in the faces compared to the shapes condition in study two. We suggest that increased amygdala FC with the fusiform and frontal gyrus reflects an increased involvement of visual and central executive structures with salience processing regions during angry and fearful faces processing that likely corresponds to salience-induced attention. We previously discussed reduced amygdala FC during faces matching with the pgACC to result from an inverse co-activation of these regions, which might correspond to an inhibitory control process of prefrontal structures over the amygdala (Etkin *et al.*, 2011; Bzdok *et al.*, 2013) as a neural mechanism of automatic attentional regulation (Phillips

et al., 2008). This pattern could also be explained by a relative uncoupling of amygdala and the DMN during faces matching, as the more salient faces condition might suppress the task-negative DMN and enhance exteroceptive processing circuits, which the visual and frontal regions, that increased in amygdala FC during faces processing, belong to.

Pathology

While limbic-prefrontal activation did not significantly differ between groups, activation in the faces > shapes contrast in the left SFG (BA32) was inversely correlated with NA, which is in line with the limbic-cortical imbalance model, proposing negatively biased emotion processing to correspond to a hypoactivation of dorsal prefrontal structures. However, as meta-analyses reporting limbic-prefrontal imbalance in MDD mainly included studies of emotional regulation (Graham *et al.*, 2013), processing of sad and happy faces (Fitzgerald *et al.*, 2008; Lai, 2014), or glucose metabolism at rest (Sacher *et al.*, 2012), our null-findings in group comparisons of task activation could indicate that the limbic-cortical imbalance model is not completely generalizable to implicit processing of angry and fearful faces. Given the marginal trend of increased amygdala response in relatives and patients, the effects might not be large enough to be detected in our sample size (Munafò *et al.*, 2008).

Compared to the unaffected groups, patients showed a decrease of amygdala FC with midline structures including key regions of the DMN, which also decreased in amygdala FC in
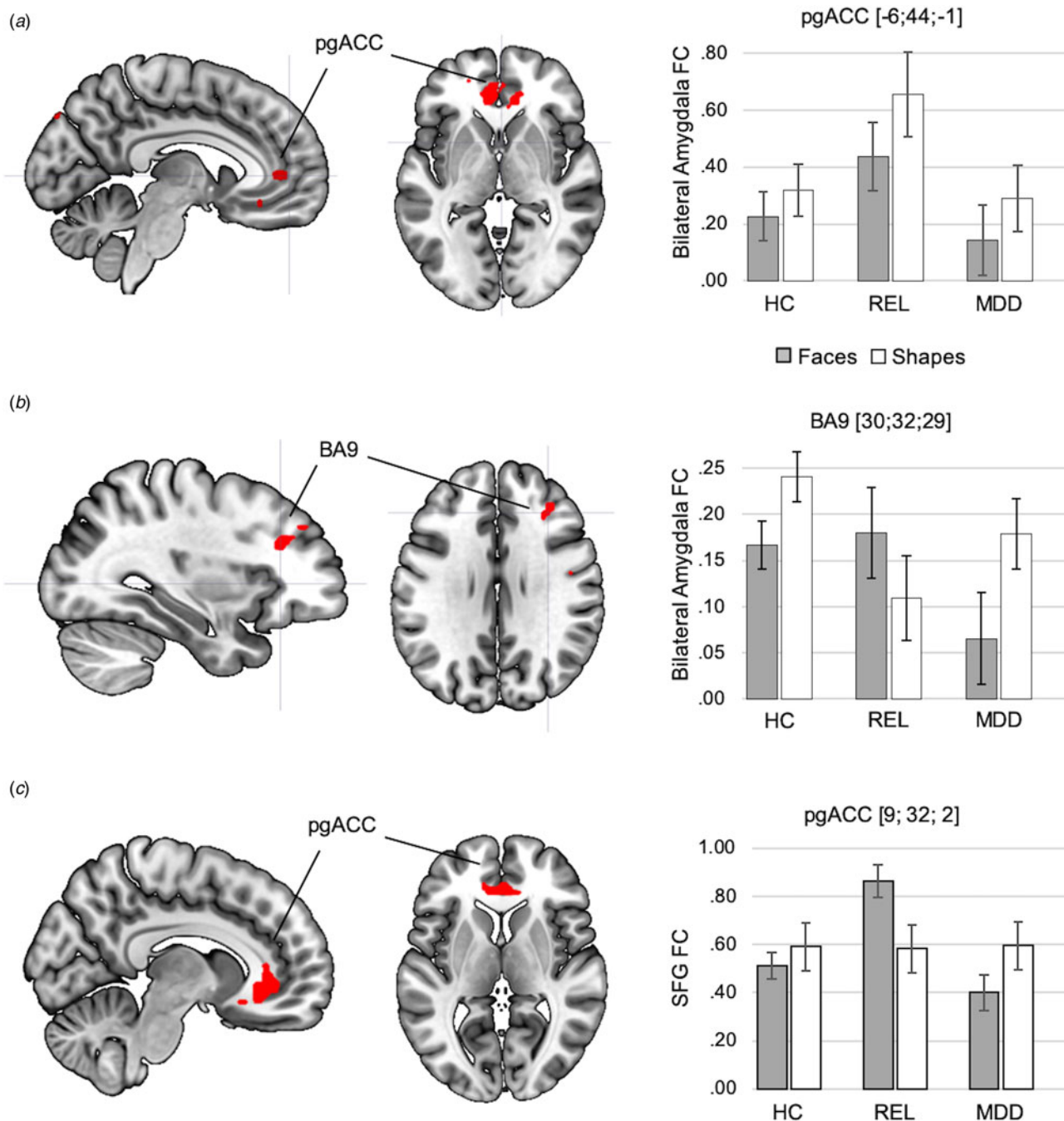
Fig. 5. Effects of resilience capacity (relatives > controls and patients) on amygdala FC are depicted across conditions (*a*) and between conditions (*b*). Across conditions, relatives showed increased amygdala FC with the pgACC compared to controls and MDD patients. Statistically significant group-by-condition interactions of amygdala FC were observed in the right prefrontal cortex (BA9), showing stronger amygdala FC in relatives in the faces compared to the shapes conditions, while amygdala FC in controls and MDD patients was significantly lower during faces compared to shapes matching in controls and MDD patients. The significance-threshold is $p < 0.05$ family-wise error corrected for the whole-brain, cluster size $k = 10$ voxels. (c) Result of exploratory analysis: the cluster of the SFC, in which activation was inversely correlated with NA in controls and patients, showed a significant group-by-task interaction in FC with the pgACC, which was driven by an increase in SFG-pgACC FC in relatives during faces matching compared to shapes matching. The significance threshold for this result is $p < .05$ family-wise-error corrected for the PFC ROI. Error bars indicate +1 standard error of the mean. BA, Brodmann area; HC, healthy controls; FC, functional connectivity; MDD, major depressive disorder; pgACC, perigenual anterior cingulate cortex; REL, relatives; SFG, Superior Frontal Gyrus.

the faces > shapes contrast across groups. While the condition-dependent changes in amygdala FC in these regions did not differ in patients, their connectivity was decreased across conditions. Increased activation and connectivity of the DMN have been attributed to rumination in MDD (Marchetti *et al.*, 2012; Graham *et al.*, 2013; Kaiser *et al.*, 2015), and it has been suggested

that the amygdala, as part of the salience network, acts as a switch between the 'task-negative' DMN and the 'task-positive' central executive network (CEN) (Havlík *et al.*, 2017). It could be speculated that a reduced task-independent temporal correlation of amygdala and DMN corresponds to a relative disintegration of the salience network with the DMN, which might contribute

to the DMN-predominance and enhanced interoceptive processing in MDD in the absence of external stimulation. This hypothesis should be further investigated in resting state studies.

Furthermore, we observed a group by condition interaction in the middle frontal gyrus (BA6, BA9): while amygdala FC increased during faces matching in controls and relatives, patients showed significantly lower amygdala FC in the faces condition, which might reflect an insufficient integration of executive control and salience processing structures in context of emotional cues as proposed in the limbic-cortical imbalance model (Disner *et al.*, 2011).

Risk

Both relatives and patients showed decreased amygdala FC with the lateral parietal and SFG across conditions. These regions overlapped with the faces > shapes contrast of amygdala FC at uncorrected $p < 0.0001$ and compose key components of the CEN. The CEN has been reported to show reduced connectivity in MDD patients in a meta-analysis of resting state studies (Kaiser *et al.*, 2015). Reduced resting-state amygdala FC with the right frontal cortex has been shown to be shared by adolescents with depression and at high risk for depression (Singh *et al.*, 2018*b*). Together, this suggests a reduced task-independent integration of amygdala and executive control structures as risk mechanism for depression and should be investigated in longitudinal resting state studies.

In group by condition analyses, relatives and patients showed reduced modulation of amygdala FC with the right fusiform gyrus, a region involved in the detection of faces, which overlapped with the faces > shapes contrast of amygdala FC across groups. Aberrations of emotion processing in the early visual stream have been reported in familial risk (Zhong *et al.*, 2016; Singh *et al.*, 2018*a*) as well as in depression (Ho *et al.*, 2016). Alterations in the stimulus-dependent integration of visual input regions with the amygdala might thus play a role in the etiology of affective disorders and should be investigated in prospective studies.

Resilience capacity

Relatives showed increased amygdala FC with the pgACC across conditions, not only compared to controls, as previously reported (Wackerhagen *et al.*, 2017), but also compared to patients. We further observed a group-by-condition interaction of amygdala FC in the right SFG, showing an increase of amygdala FC during faces matching in relatives, and a decrease during faces matching in controls and patients. Exploratory analyses of FC of the left SFG, in which lower task response was associated with higher NA in controls and patients, revealed task-dependent increases in amygdala-pgACC FC in relatives during faces processing. Given the mediating role of the pgACC for cortical regulation of the amygdala during facial emotion recognition (Wolf *et al.*, 2014; Motzkin *et al.*, 2015), our findings might reflect an intensified integration of executive and salience signals through a stronger involvement of the pgACC, both during faces processing and independent of task conditions, in relatives. This altered neural mechanism of emotional faces processing might play a compensatory role in relatives and might contribute to the lack of an association between SFG task response and NA in relatives.

Our previously reported negative association of amygdala-pgACC FC and NA was present across groups and in the control sample including controls from study 2, but did not reach significance in the smaller relatives group and was not present in patients. A false negative result due to a lack of statistical power is to be considered. Altogether, our findings suggest a protective role of pgACC involvement in the limbic-cortical integration during emotional processing, which should be investigated in prospective studies.

Limitations

We assessed differences of brain activity and amygdala FC between faces and shapes matching, which we assumed to elicit neural processes relevant for emotional processing. However, a comparison with neutral faces instead of shapes and with a resting condition would have allowed to distinguish effects of emotional valence/arousal and of the matching task itself, which would have provided a better understanding of the psychological processes underlying the imaging measures that were elicited during our task.

Since our cross-sectional approach cannot identify causal relationships, the validity of reported putative risk and resilience markers needs to be proven in prospective studies conceptualizing resilience as an absence of psychopathology or quick recovery after stress (Kalisch *et al.*, 2017).

Our assumption of resilience/compensation as the contrast in which relatives showed stronger amygdala FC than controls and patients is highly simplified and may not be the only possible interpretation. Unique features in relatives might as well present risk mechanisms that occur before the onset of depression and recede in the course of the illness or its medication.

Furthermore, it is possible that patients who had first-degree relatives with MDD had compensatory or resilience mechanisms but were faced with a larger familial risk load due to a larger number of affected relatives. Future studies should document the number of affected relatives, which we did not do in our relatives sample, in order to allow for an investigation of this matter.

As FC is a correlative measure and cannot be interpreted with respect to causal effects and directions between the involved regions, we have taken care to interpret it as the degree of involvement in the same process in favor of speculating about inhibitory or excitatory signaling. Complementary measures such as dynamic causal modeling would be of high interest to reveal causal relationships between the regions with altered FC that were observed here.

While our focus on amygdala FC builds on an established body of literature, it might lead to a reductionist view of brain function and should be complemented by network approaches. Although our *post-hoc* analysis revealed comparable effects in unmedicated patients, medication effects should be systematically assessed in future studies. Finally, although our findings of shared aberrant amygdala FC in the patient and the relatives group compared to controls provide evidence of one validity criterion of an IP, other important validity criteria, e.g. its heritability, need to be proven.

Conclusion

Our results suggest a reduced SFG response to faces *v.* shapes processing, reduced task-independent amygdala FC with DMN regions, and diminished amygdala FC with the medial frontal gyrus during faces compared to shapes matching as markers of depressive pathology. Task-independent reductions of amygdala FC with fronto-parietal regions and a reduced faces-dependent

modulation of amygdala-fusiform FC might present risk markers and putative IPs for depression, while a false negative finding of amygdala hyperreactivity due to a limited sample size is to be considered. Last, increased task-independent amygdala-pgACC FC, and increased faces-modulated amygdala-pgACC-SFG FC might reflect an intensified integration of executive and salience signals in unaffected participants at increased familial risk for depression, which might serve as compensatory mechanism, potentially contributing to resilience capacity. Provided that the considered pathogenic implications of our findings can be confirmed in prospective studies, these results can contribute to a person-tailored clinical prognosis and therapeutic prescription in the future, which might improve treatment efficacy and economic efficiency in health care and help to reduce the burden of depression.

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