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ARTICLE

Intrinsic connectivity of the prefrontal cortex and striato-limbic system respectively differentiate major depressive from generalized anxiety disorder

Xiaolei Xu[1], Jing Dai[1,2], Yuanshu Chen[1], Congcong Liu[1], Fei Xin[1], Xinqi Zhou[1], Feng Zhou[1], Emmanuel A. Stamatakis[3,4], Shuxia Yao[1], Lizhu Luo[1,2], Yulan Huang[5], Jinyu Wang[5], Zhili Zou[5], Deniz Vatansever[6], Keith M. Kendrick[1], Bo Zhou[5] and Benjamin Becker[1]

Major depressive disorder (MDD) and generalized anxiety disorder (GAD) are highly prevalent and debilitating disorders. The high overlap on the symptomatic and neurobiological level led to ongoing debates about their diagnostic and neurobiological uniqueness. The present study aims to identify common and disorder-specific neuropathological mechanisms and treatment targets in MDD and GAD. To this end we combined categorical and dimensional disorder models with a fully data-driven intrinsic network-level analysis (intrinsic connectivity contrast, ICC) to resting-state fMRI data acquired in 108 individuals ($n = 35$ and $n = 38$ unmedicated patients with first-episode GAD, MDD, respectively, and $n = 35$ healthy controls). Convergent evidence from categorical and dimensional analyses revealed MDD-specific decreased whole-brain connectivity profiles of the medial prefrontal and dorsolateral prefrontal cortex while GAD was specifically characterized by decreased whole-brain connectivity profiles of the putamen and decreased communication of this region with the amygdala. Together, findings from the present data-driven analysis suggest that intrinsic communication of frontal regions engaged in executive functions and emotion regulation represent depression-specific neurofunctional markers and treatment targets whereas dysregulated intrinsic communication of the striato-amygdala system engaged in reinforcement-based and emotional learning processes represent GAD-specific markers.

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INTRODUCTION

Major depressive disorder (MDD) and anxiety disorders are among the most prevalent and devastating disorders. Comorbidity represents the normative clinical course, with 70–90% lifetime co-morbidity between MDD and generalized anxiety disorder (GAD) [1, 2]. Overarching symptom-based approaches suggest both disorders share general affective distress accompanied by distinguishing features such as anhedonia (depression) and physiological hyperarousal (specific to anxiety disorders) [3]. Case control studies examining behavioral and neural dysregulations in MDD and GAD provided further evidence. Studies comparing either MDD or GAD patients with healthy controls revealed cognitive deficits in executive functions, reward processing and social cognition [4] while initial evidence from studies directly comparing MDD and GAD patients suggest distinguishable alterations in emotional processing bias and attributional style [5].

Functional magnetic resonance imaging (fMRI) studies suggest overlapping dysregulations in regional brain activation in MDD and GAD patients. Relative to healthy controls MDD patients exhibited increased responses in the amygdala, insula, and dorsal anterior cingulate cortex (ACC), in the context of decreased activity in the dorsal striatum, dorsolateral and medial prefrontal cortex (MPFC) during emotional and cognitive processing [6]. However, a recent meta-analysis failed to determine robust task-related regional activation alterations in MDD [7]. Several neuroimaging studies examining anxiety patients produced overwhelming, yet partly inconsistent results for dysfunctional processing in limbic and frontal regions [8], comparably few studies specifically focused on GAD. Two recent reviews covering task-based neuroimaging studies suggest exaggerated amygdala reactivity in the context of both decreased as well as increased frontal activation in GAD patients relative to healthy controls [2, 9].

Although meta-analyses of case control studies greatly advanced our knowledge of the pathological mechanisms underlying MDD and GAD the high convergence of behavioral and neurobiological signatures has led to a continuing debate about the degree to which the clinical diagnosis of MDD and GAD reflect distinct neurobiological mechanisms [2]. Recent meta-analyses revealed only few differences in brain structural and functional indices between distinct diagnostic categories [10]. These findings may reflect the limitation of traditional case control designs to determine disorder-specific neural biomarkers and emphasize the need for flanking dimensional and transdiagnostic studies that

[1]The Clinical Hospital of Chengdu Brain Science Institute, MOE Key Laboratory for NeuroInformation, University of Electronic Science and Technology of China, Chengdu 610054 Sichuan, China; [2]Chengdu Mental Health Center, Chengdu 610036 Sichuan, China; [3]Division of Anaesthesia, School of Clinical Medicine, Addenbrooke's Hospital, University of Cambridge, Hills Rd, Cambridge CB2 0SP, UK; [4]Department of Clinical Neurosciences, School of Clinical Medicine, Addenbrooke's Hospital, University of Cambridge, Hills Rd, Cambridge CB2 0SP, UK; [5]Department of Psychosomatic Medicine, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu 610072 Sichuan, China and [6]Institute of Science and Technology for Brain-Inspired Intelligence, Fudan University, 200433 Shanghai, China
Correspondence: Bo Zhou (tonyac7721@163.com) or Benjamin Becker (ben_becker@gmx.de)
These authors contributed equally: Xiaolei Xu, Jing Dai

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employ a strict control for important confounders such as medication and disorder duration [11].

To date only a few neuroimaging studies rigorously employed this approach and included MDD and GAD patients across diagnostic categories. Task-based neuroimaging studies revealed common and distinct alterations between MDD and GAD patients in amygdala, cingulate cortex and dorsomedial prefrontal cortex activation, yet separable alterations in ventrolateral prefrontal cortex and anterior insula activation and amygdala functional connectivity (FC) [12, 13]. However, the disorder-specific neural alterations identified in these studies varied according to the specific behavioral domain examined and the task-paradigm employed [12, 13]. Therefore task-independent assessments of intrinsic (resting state) brain function might represent a more general and robust neuroimaging-based biomarker for delinating disorder-specific neurofunctional alterations [14].

Two studies have combined a transdiagnostic and dimensional approach with resting-state fMRI to determine common and specific neural signatures of MDD and GAD. Both studies focused on the intrinsic organization of a priori defined regions or networks and demonstrated that subgenual ACC and ventral striatum connectivity exhibited opposite associations with MDD versus GAD-symptom load [15] and that resting-state connectivity between the limbic network and cortical regions specifically characterized patients with co-morbid MDD and GAD [16]. However, the interpretation of these findings is limited by an a priori focus on brain systems identified in case control studies and hypothesis-free data-driven approaches may promote a more unbiased determination of common and disorder-specific alterations to inform disorder-specific neuropathological models. Moreover, the assessment of intrinsic brain FC contributes to the identification of therapeutic targets for focal brain stimulation [17] which has been associated with changes that spread through the intrinsic networks of the brain and outlast the actual stimulation period [18]. Stimulating the dorsolateral prefrontal cortex (DLPFC) represents the primary target for noninvasive brain modulation in both MDD and GAD [19, 20]. In line with these findings the antidepressive treatment efficacy of noninvasive DLPFC stimulation has been associated with effects in distal brain regions and reorganization of multiple intrinsic brain networks, suggesting an important role of network-level effects for therapeutic efficacy [21]. The most efficient stimulation targets are linked by shared intrinsic networks, further emphasizing the therapeutic and mechanistic importance of network-level effects [22]. However, across focal brain stimulation approaches treatment response critically relies on the specificity and connectivity of the stimulation site emphasizing the need for the identification of disorder-specific targets.

To identify common and disorder-specific neuropathological markers and treatment targets the present study combined categorical and dimensional disorder models with a fully data-driven whole-brain intrinsic network-level analysis (Intrinsic Connectivity Contrast, ICC) to resting-state data acquired in 108 participants ($n = 35$ and $n = 38$ unmedicated patients with their first episode of GAD, MDD respectively, and $n = 35$ matched healthy controls). The ICC approach utilizes a network-level analysis without the need for a priori assumptions on regions of interest (ROIs) or arbitrary correlation thresholds to determine voxel-wise whole-brain connectivity patterns. Compared to conventional ROI-based approaches ICC therefore allows to determine global network-level alterations with a higher resolution and without a priori assumptions on disorder-relevant regions while controlling for threshold dependent connectivity variations between disorder groups [23, 24]. Together these advantages have led to an increasing use of the ICC in studies examining brain-based disorder markers and novel treatment approaches [25–27]. Given that the fully data-driven ICC approach is predominantly an exploratory strategy to examine the regions characterized by their global connectivity differences between groups a follow-up FC analysis with the regions exhibiting group differences in the ICC analysis was employed to further determine the associated networks contributing to these differences [similar approach see refs. [25, 26]].

MATERIALS AND METHODS

Participants

One hundred and eight participants were enrolled including 35 unmedicated, first-episode patients with GAD and 38 unmedicated patients with MDD recruited at the Sichuan Provincial People's Hospital and The Fourth People's Hospital of Chengdu (Chengdu, China) and 35 healthy controls (HC). GAD and MDD diagnosis were determined by an experienced psychiatrist according to DSM-IV criteria (Provincial People's Hospital) or ICD-10 criteria (Fourth People's Hospital) and further confirmed by an experienced psychologist using the Mini International Neuropsychiatric Interview (M.I.N.I.) for DSM-IV. Participants gave written informed consent after they were informed about the study procedures and informed that they were allowed to withdraw from this study at any time. During the experimental assessments all participants underwent brain functional and structural MRI assessments (see e.g., our previous publication reporting an empathy task-fMRI paradigm [12]), and were administered the Beck Depression Inventory II (BDI-II) and Penn State Worry Questionnaire (PSWQ) to determine MDD and GAD-symptom load, respectively. The Childhood Trauma Questionnaire (CTQ) was administered to control for potential effects of early life stress exposure on brain activation. To ensure data quality and reduce the burden for participants, all of them were explicitly asked whether their current status (e.g., exhaustion, emotional state) allowed to proceed with subsequent assessments (MRI, questionnaires). The study was fully approved by the local ethics committee (UESTC) and adhered to the latest revision of the Declaration of Helsinki.

All patients were unmedicated and had not previously received a diagnosis of or treatment for a psychiatric disorder. Diagnostic assessments were conducted during initial hospital admission by experienced psychiatrists and suitable patients underwent fMRI assessments during the period of further diagnostic clarification without receiving any treatment (<5 days after admission). The following exclusion criteria were applied to all participants: (1) history of or current episode of the following axis I disorders according to DSM criteria: post-traumatic stress disorder, feeding and eating disorders, substance use disorders, bipolar disorder, and mania, (2) history of or current clinically relevant medical or neurological disorder, (3) acute (within 6 weeks before the assessments) or chronic use of medication, (4) acute suicidal ideation, (5) contraindications for MRI, (6) left handedness, and, (7) excessive motion during MRI (head motion >3 mm).

According to the exclusion criteria ten subjects were excluded leading to a final sample of $n = 98$ (HC = 33, GAD = 31, MDD = 34) for fMRI analyses. Specific reasons for exclusion are displayed in Supplementary Fig. 1 and detailed diagnoses of GAD and MDD according to DSM criteria and M.I.N.I. are reported in Supplementary Information. To further account for subclinical co-morbid symptom load the categorical approach (comparing MDD, GAD, and HC) was flanked by a dimensional analysis strategy examining associations with MDD- and GAD-symptom load in the entire sample (pooling the data from MDD, GAD, and HC). In each case the influence of the other symptom dimension was controlled using FSL PALM-alpha110 toolbox (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM, Permutation Analysis of Linear Models, number of permutations = 10,000) including anxiety or depression symptom load as covariate respectively. The variance inflation factor (VIF) was used to assess collinearity between anxiety and depression symptom load. VIF = 2.4 indicated no problematic collinearity [28, 29].

All HC were without psychiatric disorders according to the M.I.N.I. interview. Several patients (and one HC) were too exhausted to continue with the questionnaires following the MRI assessments leading to participant numbers per group varying from 33 to 26 (BDI-II, PSWQ) and 32 to 23 (CTQ) respectively. Importantly, there was no significant difference in the number of participants remaining for analyses between groups ($\chi^2 = 0.14$, $p = 0.93$).

MRI data acquisition

Resting-state functional MRI (rsfMRI) data (195 volumes, acquisition duration 6.5 min) and brain structural MRI data were collected using a 3.0 Tesla GE MR750 system. Scanning parameters see Supplementary Information.

MRI data preprocessing

Functional time series were preprocessed using FMRIB software library (FSL, http://www.fmrib.ox.ac.uk/fsl) routines combined with advanced independent component analysis (ICA-AROMA) [30]. The first five functional volumes were discarded to achieve steady-state magnetization. Preprocessing for the remaining volumes included nonbrain tissue removal using BET, slice timing, realignment, intensity normalization, and smoothing with a Gaussian kernel of 6 mm full width at half maximum using FSL FEAT. Registration to high resolution structural images was carried out using FLIRT and further to standard space using FNIRT (nonlinear registration). Motion parameters, white matter and CSF signal regression and band pass filter (0.01–0.1 Hz) were performed using ICA-AROMA. Next, a strict noise reduction was conducted using the SPM12-based CONN fMRI connectivity toolbox (http://www.conn-toolbox.org) to further remove the first-order derivatives and apply linear detrending. Head motion for all participants were <3 mm and no significant differences were found between groups in mean frame-wise displacement ($F_{2,95} = 0.99$, $p = 0.38$).

Intrinsic connectivity contrast (ICC)

To allow an unbiased determination of voxel-based global connectivity the intrinsic global connectivity contrast was computed. This measure utilizes a graph-theoretical approach similar to the degree index in complex networks but without the need for a priori assumptions on regions of interest or arbitrary thresholds by weighing voxel-wise connections with their average r^2 [23]. The ICC index reflects the squared sum of mean connections of a given voxel with the rest of the brain, with higher values representing higher connectivity strength of a given voxel with every other voxel in the brain.

Follow-up seed-to-voxel functional connectivity (FC)

To further determine the specific networks contributing to the global connectivity differences revealed by the ICC approach a follow-up whole-brain seed-to-voxel FC analysis was further employed using the regions from ICC as seeds [similar approach see refs. [25, 26, 31]]. In addition, given that previous neuroimaging meta-analyses of case control studies reported amygdala alterations in both disorders [6, 9], while transdiagnostic studies reported distinguishable amygdala alterations in GAD and MDD [32, 33], and a limbic network especially the amygdala contributed to stratifying MDD and GAD patients [34] the follow-up FC analysis additionally focused on the amygdala in a more hypothesis-driven approach. To this end a small volume correction (svc) was applied to an atlas defined mask of the bilateral amygdala (combining the bilateral centromedial, basolateral, and superficial amygdala subregions in a single mask) which additionally allowed to further map the identified region to probabilistically defined amygdala subregions [35].

Both ICC and follow-up FC analyses were implemented within the CONN toolbox. Results are reported with a threshold of $p_{FDR} < 0.05$ (whole brain, cluster forming threshold $p_{uncorr} < 0.001$) for the ICC and $p_{FWE} < 0.05$ (svc) for amygdala-focused FC analyses.

Functional characterizations of the determined brain regions

To functionally characterize the identified regions meta-analytic decoding via the NeuroSynth (http://neurosynth.org/decode/) automated database was conducted. The top 20 terms associated with the identified regions in the large-scale database of functional imaging studies were visualized using word clouds. The size of the font reflects the correlation strength between the respective map and the meta-analytic determined terms.

RESULTS

Demographic data and dimensional symptom load

Participants in the MDD, GAD, and HC groups were matched for age ($p = 0.17$), gender ($p_{(\chi^2)} = 0.11$) and education level ($p = 0.54$). Symptom load and early life stress were analyzed using Univariate ANOVAs. Results revealed significant main effects of group for depressive symptom load (BDI-II, $F_{2,89} = 83.93$, $p < 0.001$, $\eta^2_p = 0.65$), GAD-symptom load (PSWQ, $F_{2,89} = 83.93$, $p < 0.001$, $\eta^2_p = 0.65$), and early life stress (CTQ, $F_{2,83} = 8.74$, $p < 0.001$, $\eta^2_p = 0.17$). Post hoc analyses indicated that depressive symptom load was higher in both GAD and MDD patients compared to HC, and in MDD compared to GAD patients. GAD symptom load and early life stress were significantly higher in both patient groups relative to HC, but not significantly different between the two patient groups (see Table 1 for details).

Intrinsic connectivity contrast

ICC functional connectivity maps were used to examine group differences in CONN (see Supplementary Fig. 2 for ICC maps in each group). The categorical analysis employed a one-way ANOVA with group as between-subject factor and revealed significant group differences in right medial prefrontal cortex (R_MPFC, *x/y/z*: 16/46/44, $k = 34$, $p_{FDR} = 0.035$, Fig. 1a), right putamen (*x/y/z*: 22/0/−4, $k = 28$, $p_{FDR} = 0.041$, Fig. 1b), and left dorsolateral prefrontal cortex (L_DLPFC, *x/y/z*: −40/24/28, $k = 41$, $p_{FDR} = 0.027$, Fig. 1c). To determine specific alterations between groups parameter estimates from the three clusters were extracted for post hoc analyses. Results indicated that specifically R_MPFC connectivity was decreased in MDD but not GAD patients compared to HC (Fig. 1d) and the right putamen connectivity was decreased in GAD patients compared to both MDD and HC groups (Fig. 1e). In addition, both GAD and MDD patients exhibited decreased L_DLPFC connectivity compared to HC (Fig. 1f).

Follow-up seed-to-voxel functional connectivity

To further determine common and disorder-specific network-level alterations between the three diagnostic groups, a whole-brain seed-to-voxel FC analysis was performed using seeds revealed by the ICC approach (R_MPFC, right putamen, L_DLPFC). Examing between-group differences by means of one-way ANOVAs with group as between-subject factor revealed no significant between-group differences on the whole-brain level (see Supplementary Fig. 3 for whole-brain FC maps in each group). In a more hypothesis-based analysis we specifically explored communications between the regions revealed by ICC and the amygdala using a small volume correction (svc) approach. For the putamen seed a main effect of group was observed in the right amygdala (*x/y/z*: 29/−12/−9, $p_{svc\text{-}FWE} < 0.05$, Fig. 2a), which probabilistically mapped into the centromedial subregion. Post hoc analysis indicated that both GAD and MDD patients exhibited decreased connectivity between putamen and amygdala compared to HC (Fig. 2b). No significant between-group differences in amygdala connectivity were found using the R_MPFC and L_DLPFC regions as seeds.

Dimensional analyses in the entire sample: associations between intrinsic network-level indices and symptom load

Results from the dimensional analyses confirmed that depressive symptom load was negatively associated with ICC of the R_MPFC

Table 1. Demographics, symptom load, and early life stress.

Group	HC	GAD	MDD	*F*	GAD vs. HC	MDD vs. HC	GAD vs. MDD
	$N = 33$	$N = 31$	$N = 34$				
male	$N = 12$ (36%)	$N = 15$ (48%)	$N = 8$ (24%)				
	Mean (SEM)	Mean (SEM)	Mean (SEM)				
Age (years)	26.79 (1.46)	30.74 (1.51)	28.18 (1.44)	$F_{2,95} = 1.82$	>0.18	>0.18	>0.18
Education (years)	14.15 (0.62) ($N = 33$)	14.32 (0.68) ($N = 28$)	13.38 (0.63) ($N = 32$)	$F_{2,90} = 0.62$	>0.92	>0.92	>0.92
PSWQ	39.49 (1.63) ($N = 33$)	58.08 (1.84) ($N = 26$)	63.00 (1.63) ($N = 33$)	$F_{2,89} = 56.94$	<0.001**	<0.001**	=0.144
BDI-II	5.21 (1.49) ($N = 33$)	23.15 (1.68) ($N = 26$)	32.18 (1.49) ($N = 33$)	$F_{2,89} = 83.93$	<0.001**	<0.001**	<0.001**
CTQ	43.00 (1.98) ($N = 32$)	50.30 (2.34) ($N = 23$)	54.71 (2.01) ($N = 31$)	$F_{2,83} = 8.74$	=0.058	<0.001**	=0.472**

All questionnaires were presented in Chinese version. Given that some participants did not completed all questionnaires (details see also: exclusion criteria, initial quality assessments and final sample) the number of subjects that indicated the respective analysis is reported for each measure.
PSWQ Penn State Worry Questionnaire, *BDI-II* Beck depression Inventory II, *CTQ* Childhood Trauma Questionnaire.
**$p < 0.005$.

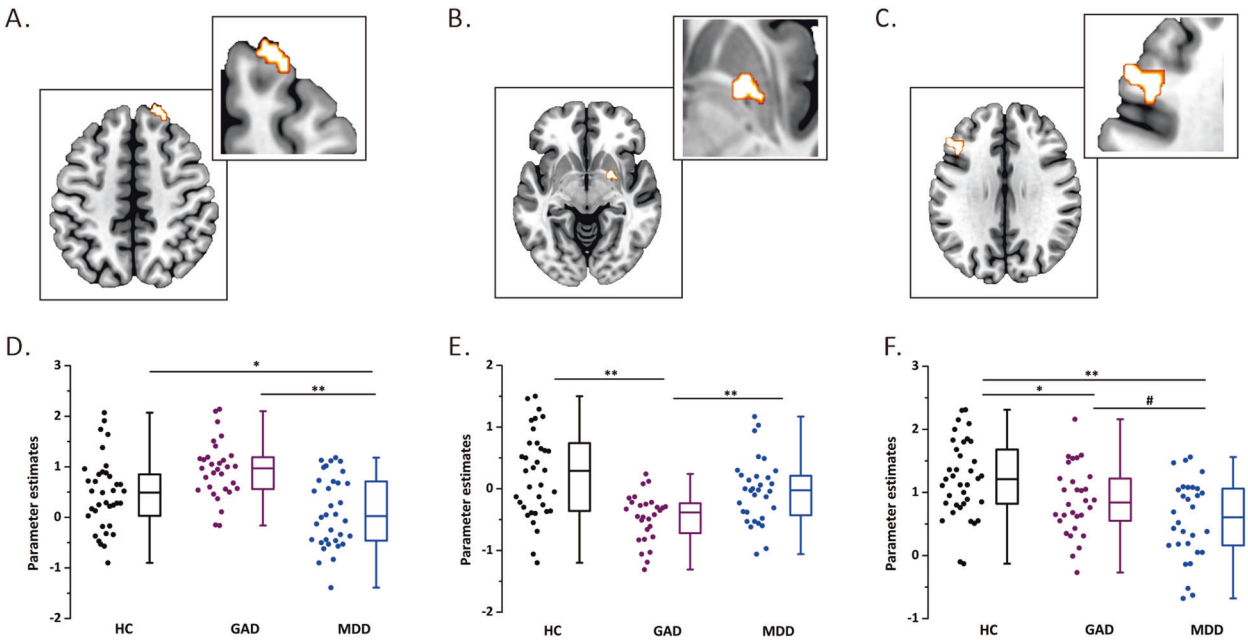

Fig. 1 Brain areas exhibited alterations in ICC analysis. a Right medial prefrontal cortex (R_MPFC); **b** right putamen; **c** left dorsolateral prefrontal cortex (L_DLPFC); **d** group differences in R_MPFC; **e** group differences in right putamen; **f** group differences in L_DLPFC. R_MPFC right medial prefrontal cortex, L_DLPFC left dorsolateral prefrontal cortex. For visualization, statistical maps are displayed with a threshold of $p < 0.005$ uncorrected.

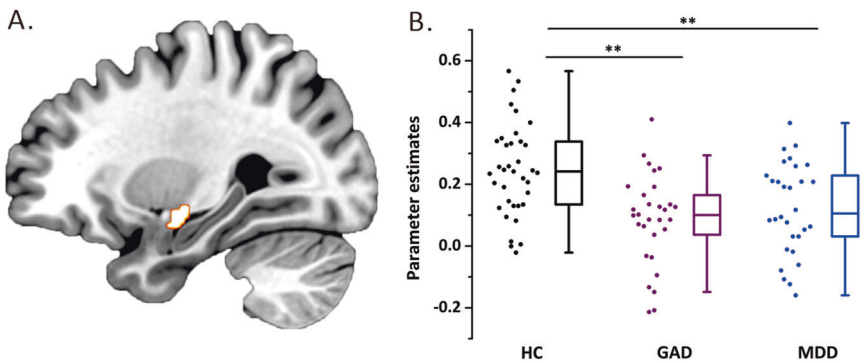

Fig. 2 Brain regions exhibiting aberrant functional connectivity with seeds from ICC. a Altered right putamen—right amygdala (centromedial) connectivity and **b** post hoc group differences. ICC intrinsic connectivity contrast.

($r = -0.184$, $p = 0.038$, Fig. 3a) and L_DLPFC ($r = -0.376$, $p = 0.0002$, Fig. 3b) while controlling for GAD symptom load, and that GAD symptom load was negatively associated with ICC of the right putamen ($r = -0.190$, $p = 0.034$, Fig. 3c) while controlling for depressive symptom load. On the FC level, connectivity strengths between putamen and amygdala were negatively correlated with GAD symptom load ($r = -0.216$, $p = 0.015$, Fig. 3d) after controlling for depressive symptom load.

Functional characterization of the identified brain regions

NeuroSynth decoding revealed that the highest correlated terms for R_MPFC were predominantly referring to cognitive processing and the default mode network (Fig. 4a). For the right putamen, the highest correlated terms were gains, losses, learning and reward (Fig. 4b). Major depression, executive control, and working memory were highly correlated terms for the L_DLPFC (Fig. 4c).

DISCUSSION

The present study applied a hypothesis-free fully data-driven approach combining a categorical and dimensional approach to determine common and disorder-specific alterations in whole-brain intrinsic network connectivity in unmedicated, first-episode MDD and GAD patients. The categorical analysis approach demonstrated that MDD patients specifically exhibited decreased whole-brain connectivity of the right MPFC compared to both HC and GAD, while GAD patients exhibited decreased right putamen whole-brain connectivity relative to both other groups suggesting

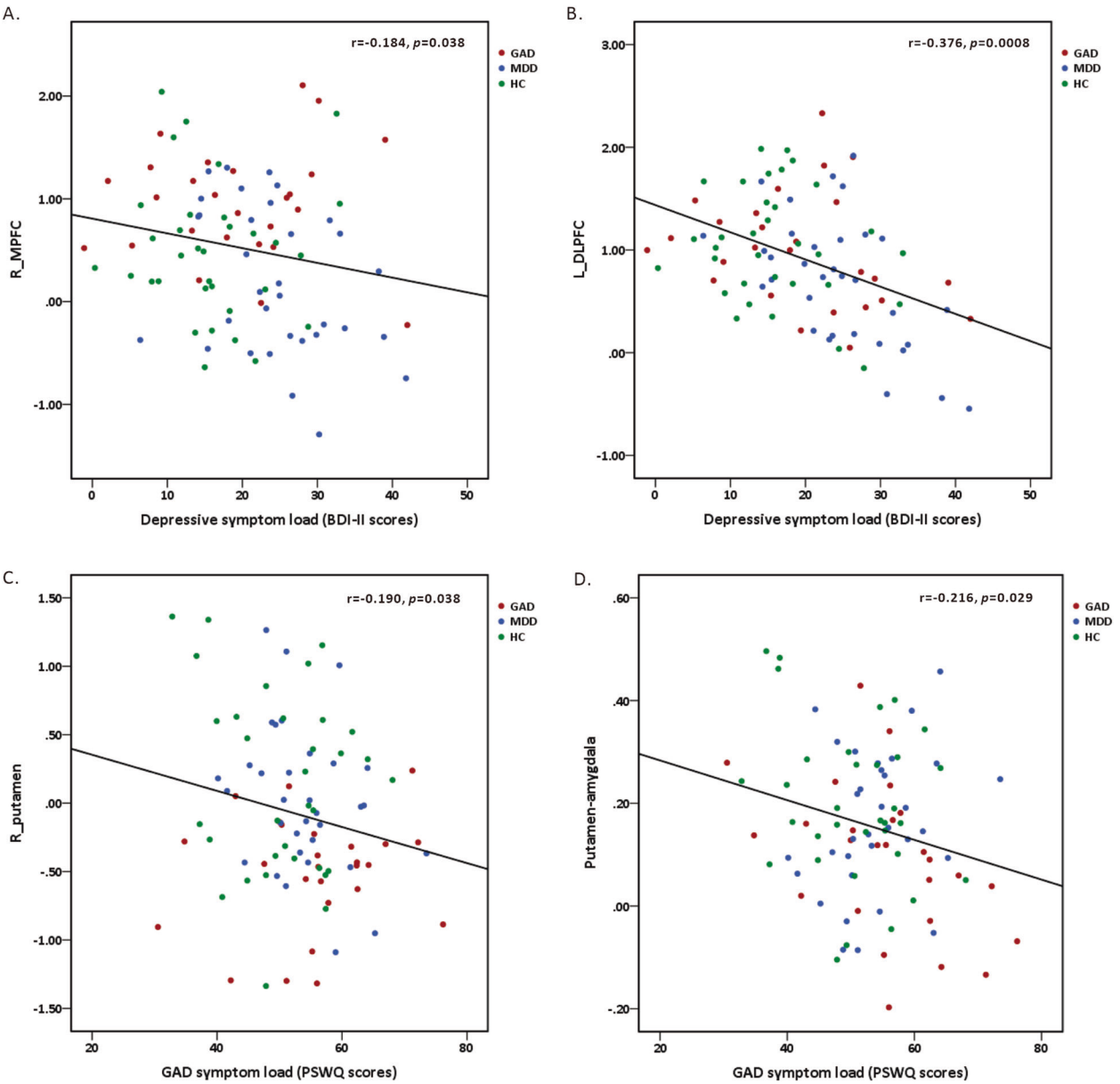

Fig. 3 Associations between neural indices and symptom load. Associations between **a** the right MPFC and depressive symptom-load; **b** the left DLPFC and depressive symptom load; **c** the right putamen and GAD symptom load; and **d** the right putamen—right amygdala connectivity and GAD symptom load. Scatter plots represented the entire sample pooling the data GAD, MDD, and HC. Diagnostic group membership is color-coded. Vertical axis reflects parameter estimates of corresponding brain areas. *$p < 0.05$; **$p < 0.005$, all p values FDR corrected for multiple comparisons. MPFC medial prefrontal cortex, DLPFC dorsolateral prefrontal cortex. Green = HC, blue = MDD, and red = GAD.

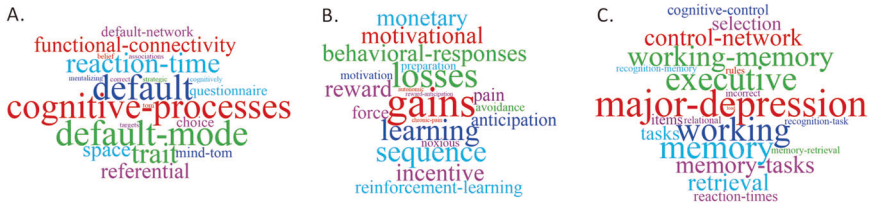

Fig. 4 Word clouds visualizing the functional characterization of the identified brain regions. NeuroSynth decoding of **a** right MPFC, **b** right putamen, and **c** left DLPFC. MPFC medial prefrontal cortex, DLPFC dorsolateral prefrontal cortex.

disorder-specific neurofunctional deficits. In contrast, both patient groups demonstrated decreased DLPFC whole-brain connectivity relative to HC, with pronounced deficits in MDD relative to GAD, and reduced putamen–amygdala connectivity indicating common neurofunctional deficits in MDD and GAD. Dimensional analyses further confirmed symptom-specific alterations such that the strengths of whole-brain connectivity alterations in the MPFC and DLPFC were associated with depressive symptom load whereas whole-brain putamen and putamen–amygdala communication were associated with GAD symptom load. Together these findings provide evidence for a separable neurofunctional basis of the disorders, with MDD being characterized by deficient whole-brain connectivity of frontal regions and GAD being characterized by deficits in dorsal striatum whole-brain connectivity and functional communication of this region with the amygdala.

Intrinsic connectivity deficits in frontal regions including the MPFC and DLPFC have been repeatedly reported in depressive disorders and previous studies employing similar data-driven approaches demonstrated reduced global brain connectivity within the MPFC, ventromedial prefrontal cortex, ACC, and DLPFC in MDD patients [36–38] relative to healthy control groups. In line with the functional characterization of the identified region, previous studies have demonstrated that the MPFC plays an important role in cognitive processes, including social cognition and emotion regulation [39], and represents a core hub of the brain's anterior default mode system involved in self-referential processing, autographic memory and social cognition [40]. Structural and task-based functional MRI studies have consistently shown reduced brain volume and attenuated engagement of the MPFC during cognitive processes and emotion regulation in depression [36, 41] and a prospective study in remitted MDD patients suggests that attenuated MPFC reactivity to mood provocation represents a risk factor for relapse [42]. Although previous meta-analytic approaches reported that impaired emotion regulation was associated with deficient MPFC engagement in anxiety disorders [43], studies in GAD revealed rather inconclusive results [2].

Numerous studies have reported deficient DLPFC activation and connectivity during cognitive and emotional processing in both depressive and anxiety disorders [44], with less consistent results in GAD [2]. Although the results from the categorical analysis revealed that both MDD and GAD exhibit reduced left DLPFC whole-brain connectivity relative to controls, deficits were more pronounced in MDD patients, and specifically associated with depressive symptom load in the entire sample. In line with the functional characterization of this region, the DLPFC is a key region for executive functions and explicit emotion regulation [45] which have been consistently found to be impaired in MDD. In addition, noninvasive stimulation of the left DLPFC has been successfully applied in the treatment of MDD [46].

In line with different functional characterization of the identified MPFC and DLPFC regions the subsequent FC analyses revealed that the two regions intrinsically connected to separable frontal networks (see Supplementary Fig. 3), suggesting that multiple frontal networks are disrupted in the disorders. On the other hand, the whole-brain FC maps of these regions were highly similar between the groups and not significantly different on the whole-brain level. This may reflect the higher sensitivity of data-driven methods that operate on the voxel level and may emphasize more global disruptions of communication of the identified regions with the entire brain.

In contrast to depression-specific alterations in frontal regions, whole-brain connectivity of the putamen and its communication with the amygdala were found to be specifically associated with GAD symptom load. Both the functional characterization of this region and previous studies indicate an important role of the putamen in reinforcement learning and alterations in its responses have been found in anxiety populations during processing of reward-related information including monetary gains or losses [47, 48]. GAD patients have repeatedly been shown to exhibit deficient reinforcement-based learning with the degree of deficit being associated with both anxiety symptoms as well as punishment-related putamen activation [47, 49]. The amygdala represent a structurally and functionally heterogenous region with at least two major functional subdivisions of the amygdala being commonly identified, the basolateral amygdala (BLA) and centromedial amygdala (CMA). Specifically, the BLA receives input from different brain regions and encodes aversive expectations whereas the CMA is a major output center for generating adaptive behavioral responses to aversive stimuli and is involved in the subjective experience of anxiety [50, 51]. The weakened connectivity between the CMA and putamen may therefore reflect an impaired integration of emotional experience with memory, or an inefficiency in utilizing recall of emotional memories as a tool to regulate or cope with emotional experience [39] promoting excessive and uncontrollable worry leading to impaired goal directed decision making in GAD [52].

Finally, exploratory analyses of the direction of the associations between neural indices and symptom load revealed exploratory evidence that—in contrast to GAD and HC—MDD patients exhibited a positive (non significant) association between GAD symptom load and putamen–amygdala connectivity (details see Supplementary Information). These findings align with previous results suggesting that amygdala connectivity may differentiate depression and anxiety, yet also emphasize the need to carefully examine the existence of opposite associations in subgroups as compared to pooled groups [53] in future transdiagnostic studies.

There are several limitations in the present study. First, with respect to the diagnostics it is noteworthy that, although the categorical diagnostics by experienced clinical investigators clearly differentiated between the MDD and GAD patients the groups did not differ in self-reported GAD (PSWQ), suggesting a limited sensitivity of the self-reported (dimensional) measure to differentiate between diagnostic categories. Second, although the strict inclusion criteria allowed us to control for important confounders such as medication only a minority of the patients in two large psychiatric hospitals met the strict enrollment criteria leading to a moderate sample size. Of note, the current sample size is larger compared to the previous transdiagnostic studies on GAD and MDD [13, 15]. Third, accumulating evidence suggests sex-differential neurofunctional alterations in depressive and anxiety disorders [54, 55], however, the current sample size did not allow

to further determine sex-differences. Therefore, sex-differences need to be further investigated in future studies with larger sample sizes. Fourth, results with respect to altered amygdala connectivity are based on a hypothesis-based amygdala-focused analysis. Although the findings resonate with previous reports on separable amygdala network alterations in the disorders the findings need to be interpreted cautiously. Finally, although the primary diagnosis of GAD and MDD was determined by experienced clinical psychiatrists several patients exhibited secondary co-morbid anxiety or depression in the M.I.N.I. interview (see Supplementary Information). Given co-morbidity rates as high as 70–90% between the disorders [1] the present study employed a dimensional approach to further validate the specificity of the neural dysregulations. Future studies should consider to include a co-morbid group to determine specific dysregulations that may arise from co-morbidity.

Together, findings from the present data-driven study suggest that deficient intrinsic communication of frontal regions, specifically the MPFC and DLPFC which are strongly engaged in executive functions and emotion regulation, represent disorder-specific neurofunctional markers and treatment targets for MDD. On the other hand, impaired intrinsic communication of subcortical regions, specifically the putamen and its connections with the amygdala which are strongly engaged in reinforcement-based learning and emotional memory integration, characterize GAD and might represent promising targets for the treatment of this disorder.

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AUTHOR CONTRIBUTIONS

XX, BB, and KMK designed the experiment. XX prepared the study protocols and procedures. BZ, JD, ZZ, YH, and JW performed the clinical assessments. XX, YC, CL, and FX acquired data. XX, FZ, XZ, EAS, LL, BB, and SY analyzed the data. XX, BB, KMK, DV, and EAS interpreted the data and drafted the paper. All authors commented on and gave final approval to the final version of the paper.

ADDITIONAL INFORMATION

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