Views on vulnerability

Cognitive and neurobiological vulnerability during remission of Major Depressive Disorder

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Decreased ability to access a clinically relevant control network in patients remitted from Major Depressive Disorder

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Neurobiological models to explain vulnerability of Major Depressive Disorder (MDD) are scarce and previous fMRI studies mostly examined ‘static’ functional connectivity (FC). Knowing that FC constantly evolves over time, it becomes important to assess how FC dynamically differs in remitted-MDD patients vulnerable for new depressive episodes. Using a recently developed method to examine dynamic FC, we characterized re-emerging FC-states during rest in 51 antidepressant-free MDD patients at high risk of recurrence (≥2 previous episodes), and 35 healthy controls. We examined differences in occurrence, duration and switching profiles of FC-states after neutral and sad mood-induction. Remitted MDD-patients showed a decreased probability of an FC-state ($p<0.005$) consisting of an extensive network connecting frontal areas -important for cognitive control-, with Default Mode, striatum and Salience areas, involved in emotional and self-referential processing. Even when this FC-state was observed in patients, it lasted shorter ($p<0.005$) and was less likely to switch to a smaller Prefrontal-Striatum network ($p<0.005$). The extent of differences between patients and controls decreased after sad mood-induction. Our findings suggest reduced ability of remitted-MDD patients to access a clinically relevant control network involved in the interplay between externally and internally-oriented attention. During sad mood, rrMDD might employ a compensatory mechanism to increase the activation of this FC-state. This study provides a novel neurobiological profile of MDD-vulnerability.
INTRODUCTION

Major Depressive Disorder (MDD) is a severe psychiatric disease which globally accounts for the greatest loss of years due to disability. This high impact of MDD is related to its high incidence and recurrence-rates, particularly in patients with multiple previous episodes. However, this vulnerability during MDD-remission has scarcely been studied from a level of intrinsic brain connectivity. Elucidating neural vulnerability factors in remitted recurrent MDD could facilitate the development of effective prediction tools or improve preventive treatments against recurrent MDD.

Previous studies have linked MDD to disrupted resting-state functional connectivity (FC) in several resting-state networks and systems, including frontal networks (FN), regulating cognitive control and attention, the Default Mode Network (DMN), involved in internal attention, the Salience Network (SN) and fronto-striatal pathways, both involved in salience-detection and emotion. During remission between episodes, MDD-vulnerability might specifically relate to a failure of control systems to down regulate DMN-activity, with the SN as switching-hub between the networks. However, the scarce research that has been conducted in remitted-MDD previously examined ‘static’ FC, representing mean connectivity over a period of scanning. Instead, growing evidence shows that brain activity at rest is not stable during the scan, but slowly wanders through a repertoire of time-varying, but reoccurring, states of coupling among brain regions. Dynamic-FC analysis allows characterizing these reoccurring FC-states.

Dynamic-FC changes during tasks and resting-state have been associated with psychiatric disorders and with reduced behavioral/cognitive performance in healthy subjects. For MDD specifically, findings from recent studies suggest aberrations in dynamic-FC involving DMN, frontal and SN-areas. However, it remains unclear whether this persists during MDD-remission.

The best way to characterize dynamic-FC remains under debate. Although the sliding-window analysis is most commonly used to calculate successive dynamic-FC matrices, the window size affects the temporal resolution, challenging its validity. In the current study, we instead use a recently developed method, the Leading Eigenvector Dynamics Analysis (LEiDA), which calculates dynamic-FC at the instantaneous level (for each recorded frame), and identifies dominant FC-states reoccurring over time. We compared differences in occurrence, duration and switching profiles of FC-states between antidepressant-free remitted recurrent MDD (rrMDD) and controls without personal and familial MDD-history, and we did so both after neutral and sad mood-induction. We hypothesized to find alterations in FC-states, particularly involving the Fronto-parietal network, DMN and SN, which would be influenced by sad mood-state.
METHODS AND MATERIALS

Participants
After approval by the local Medical Ethical Committee (METC) and written informed consent, 62 rrMDD patients with ≥2 depressive episodes as defined by the Structured Clinical Interview for DSM-disorders (SCID), in stable remission for ≥2 months according to DSM IV-criteria, and 41 healthy controls were scanned. Hamilton Depressive Rating Scale (HDRS-17) scores, an observer rated MDD-symptom scale to assess depression-severity, were ≤7. Patients were antidepressant-free for ≥8 weeks. Controls did not have a personal or familial history for psychiatric disease (assessed by SCID). All participants were aged 35-65 years. We excluded participants with alcohol/drug dependency; psychotic or bipolar disorder; predominant anxiety disorder; severe personality disorder; electroconvulsive therapy ≤2 months before scanning; history of severe head trauma; neurological disease; severe general physical illness and no Dutch/English proficiency. rrMDD patients and controls were matched for age, sex, educational level and working class. Participants were recruited through identical advertisements in freely available online and house-to-house papers, posters in public spaces and from previous studies in our and affiliated research centres.

Mood-induction paradigm
As described in more detail in previous work regarding this mood-induction, before the scan, participants described with as much detail as possible a memory which they regarded as neutral, (e.g. doing the dishes) and one which they regarded as being among the saddest in their life (e.g. losing a job, death of a relative). In addition, participants chose one neutral and one sad fragment of music from ten different fragments. Memories were scripted in key-sentences for display on the screen in the MRI-scanner. During memory display, we played the chosen neutral or sad music. Participants were asked to rate their current mood on a scale of 0 to 10 (0 being extremely sad; 10 extremely happy) after the neutral mood-induction, after the neutral resting-state scan and before and after the sad mood-induction. After the sad resting-state scan, the most extreme sadness was rated. The gap between the neutral and sad mood-induction, in which participants completed other fMRI tasks was ±125 minutes, including a 30 minutes break. We designed the sad mood-induction to be at the end of all fMRI scanning, as it might have been too stressful for participants to continue fMRI scanning and tasks after the sad mood-induction (Supplementary Figure 1).

Image acquisition and analyses
A 3 Tesla Philips Achieva XT scanner (Philips Medical Systems, Best, the Netherlands), equipped with a 32-channel SENSE head coil, was used to obtain the images. A high-resolution T1-weighted 3D structural image was acquired using fast-field echo (FFE) for anatomical reference (220 slices; TR: 8.3 ms; TE: 3.8 ms; FOV: 240 x 188; 240 x 240 matrix; voxel size: 1 x 1 x 1 mm³). Functional images were acquired with T2*-weighted gradient echo planar imaging (EPI) sequences. Participants were instructed to close their eyes without falling asleep. The scans comprised 210 volumes of 37
axial-slices (TR: 2000 ms; TE: 27.6 ms; FOV: 240 x 240; 80 x 80 matrix; voxel size = 3 x 3 x 3 mm). Slices were oriented parallel to the AC-PC transverse plane and acquired in ascending order with a gap of 0.3 mm.

**Preprocessing**

We preprocessed functional MRI data with MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) part of FSL (FMRIB’s Software Library, www.fmrib.ox.ac.uk/fsl). We used the default parameters of this imaging pre-processing pipeline on all participants: motion correction using MCFLIRT; non-brain removal using BET; spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor and temporal filtering. The EPI image was co-registered to the T1-weighted structural image, and the T1-weighted image was co-registered to standard MNI space. Mean BOLD time-series were then estimated in 90 brain areas of the Anatomical Automatic Labeling (AAL) atlas by averaging the BOLD signal over all voxels belonging to each brain area.

**Dynamic Functional Connectivity**

We used BOLD Phase Coherence Connectivity to obtain a time-resolved dynamic-FC matrix \(dFC\), with size \(N\times N\times T\), where \(N=90\) is the number of brain areas and \(T=210\) is the number of recording frames in each scan. We first estimated the phase of the BOLD signals in all areas \(n, q(n,t)\), using the Hilbert transform. Given the phases of the BOLD signals, the dynamic FC, \(dFC(n,p,t)\), between brain areas \(n\) and \(p\) at time \(t\), is obtained using the Equation:

\[
dFC(n,p,t) = \cos(\theta(n,t) - \theta(p,t))
\]

where \(\cos()\) is the cosine function. \(dFC(n,p,t)\) is 1 if two areas \(n\) and \(p\) have synchronized BOLD signals at time \(t\), and \(dFC(n,p,t)\) is 0 if the BOLD signals are orthogonal (with a phase difference of 90°).

**FC-states**

LEIDA considers only the leading eigenvector \(V_1(t)\) of each \(dFC(t)\), which captures the dominant FC-state of \(dFC(t)\) at time \(t\), instead of comparing the whole matrices. This vector contains \(N\) elements (each representing one brain area) and their sign (positive or negative) separates brain areas into communities according to their BOLD-phase relationship. Since \(V\) and \(-V\) represent the same state, we use a convention ensuring that most of the elements have negative values. When all elements of \(V_1(t)\) have the same sign, all BOLD signals are evolving in the same direction (within a range of \(\pi/2\), or 90°) and are hence considered to be following a single global mode. If \(V_1(t)\) has elements of different signs (i.e. positive and negative), the BOLD signals can be divided according to their phase into 2 modes/communities; following the global signal and following a smaller subset of brain areas with a phase shift of more than \(\pi/2\) with respect to the global mode.
The magnitude of each element in $V_1(t)$ indicates the ‘strength’ with which brain areas belong to the communities in which they are placed. Conveniently, eigenvectors can be represented in cortical space by representing each element as a sphere placed at the center of gravity of the corresponding brain area, coloring alike brain areas with the same sign (cyan/blue or yellow/red), and indicating their strength with a color scale, where lighter colors (cyan/yellow) indicate stronger contributions and darker colors (blue/red) weaker contributions. For example, Figure 1 (bottom right) shows an FC-state where the BOLD signals can be divided into 2 modes; the global signal (cyan areas) and a detached functional network (orange/red areas). The dominant FC-state can also be represented in matrix format (NxN) by computing the outer product $V_1V_1^T$ (Figure 1).

Figure 1. Time courses of recurrent FC-states obtained with LEiDA. The dominant connectivity patterns (captured by the leading eigenvector, $V_1(t)$) obtained at each time point from all fMRI scans are clustered into a discrete number of recurrent FC states. For illustration, the time courses of 3 different recurrent FC-states are plotted over a single fMRI session. The recurrent FC-state $\gamma$ is displayed as a 90x90 matrix (which is the eigenvector’s outer product) and in the cortical space, to indicate the brain areas involved in the FC-state. The FC-state consists of 2 orthogonal modes of BOLD signal coherence, one following the global signal: cyan and blue areas, and another forming a detached functional network: the yellow, orange and red areas. The colors represent the strength to which the brain area belongs to the detached functional network, with lighter colors showing the strongest contribution to the network and darker colors the weakest.

To detect recurrent FC-states, a $k$-means clustering was applied to all leading eigenvectors $V_1(t)$ across all subjects (rrMDD and controls in neutral and sad mood, resulting in 210x86x2=36120 leading eigenvectors). We varied $k$ (number of clusters) from 2 to 20, and for each $k$, obtained $k$
cluster centroids, each being an \( Nx1 \) vector representing a recurrent FC-state detected by the algorithm. We examined clustering solutions from \( k=2 \) to 20 instead of constricting analyses to a single clustering solution because we wanted to examine consistency in recurring FC-states independently of the number of clusters selected.

**Between group comparisons**

For each subject, condition and number of states \( k \), we calculated the probability of occurrence of each FC-state (fraction of epochs it occurred throughout the scan duration), the mean duration of each FC-state (mean number of consecutive epochs in the same state), the switching frequency (number of transitions per second (Hz)) and the switching profiles (probabilities of switching from a given FC-state to another). All values were compared between rrMDD and controls, after neutral and sad mood-induction using (non-parametric) permutation-based t-tests (5000 permutations) in matlab version R-2017b. We corrected for multiple comparisons by dividing the \( p \)-value by the number of \( k \) clusters considered (\( p<0.05/k \)). We examined group*mood interaction effects for the probability of occurrence or mean lifetime of the significant FC-patterns by means of a repeated measures ANOVA in SPSS version 25.\(^{39}\) We also report within-group differences for neutral vs. sad mood (rrMDD patients and controls separately) in the Supplementary Results.

**Code availability statement**
The LEiDA codes are publicly available at github.com/juanitacabral/LEiDA

**RESULTS**

**Sample characteristics**

Seventy-two rrMDD partients and 46 controls were initially eligible of which 62 and 41 were scanned, respectively. Of these participants, we excluded 9 rrMDD and 6 controls because of abnormal brain anatomy and 2 rrMDD due to technical difficulties. Fifty-one rrMDD patients and 35 controls were included in the final analysis (Supplementary Figure 2). No significant differences were observed between rrMDD patients and controls for sex, age, education, IQ, living situation, employment status and handedness. rrMDD showed higher levels of residual depressive symptoms (HDRS) \( p<0.001 \) (Table 1). Comparisons between rrMDD and controls did not change when restricted to the sample selected for the present fMRI analyses. The sad mood-induction significantly decreased mood-scores in both groups (\( p<0.001 \)), indicating that the mood-induction was effective, without a group*mood-induction interaction (\( p=0.95 \)). See Supplementary Table 1 for all mood ratings.
Relevant FC-state

Our analysis revealed an FC-state, which consistently appeared less and lasted shorter in rrMDD compared to controls in neutral mood (Figure 2; Supplementary Figure 3). This underrepresented FC-state consisted of an extensive network including frontal (dorsolateral prefrontal cortex (DLPFC) and fronto-orbital cortex), DMN (Posterior Cingulate Cortex, angular gyrus and medial prefrontal cortex (MPFC)), subcortical (the dorsal striatum (Str) (caudate, putamen) and pallidus) and SN areas (Anterior Cingulate Cortex, frontal operculum). We further indicate this FC-state as the ‘FN-DMN-Str-SN state’. See Supplementary Figure 4 for the overlap of this FN-DMN-Str-SN state with resting-state networks defined by previous whole brain parcellations.40,41 Of note, for another clustering solution (see Supplementary Figure 3), \( k=18 \), one subcortical FC-state (caudate, putamen, pallidus, thalamus and amygdala) lasted shorter between rrMDD patients and controls (2.74±0.26 vs. 4.55±0.63 seconds, \( p=0.0015 \), uncorrected).

In detail, of the 19 clustering solutions we considered (i.e. with \( k \) ranging from 2 to 20), 16 solutions revealed that this FN-DMN-Str-SN state significantly appeared less (lower probability).
in rrMDD compared to controls after correcting for multiple comparisons ($p<0.05/k$). Over the 16 clustering solutions, these FC-states highly correlated (Pearson’s $r>0.84$), which indicates that they likely refer to the same underlying FC-state, with differences arising from the number of output states constrained by $k$. Furthermore, the FN-DMN-Str-SN state lasted significantly shorter (lower mean lifetime) in seven clustering solutions, of which five had overlap with a significant lower probability (Supplementary Figure 3).

Combined, the FN-DMN-Str-SN state showed differences in probability of occurrence and/or duration between rrMDD and controls in 18 (out of 19) clustering solutions (Supplementary Figure 3). This indicates that the FC-state is consistently different between groups, largely independent of which clustering solution is chosen.

To display the FN-DMN-Str-SN state in more detail, we picked one clustering solution, $k=10$, which contained the most significant FC-state that showed between group differences in both probability and lifetime. For $k=10$, the FN-DMN-Str-SN state occurred less frequently (4.58±0.47% compared to 7.24±0.79% of the time, $p=0.0022$, uncorrected) and lasted shorter when dominant (3.78±0.11 vs. 5.10±0.18 seconds, $p=0.0020$, uncorrected) (Figure 2).

In Figure 3 we show the repertoire of functional networks that are returned by LEiDA when choosing $k=10$. This reveals different network configurations that appear, dissolve and reoccur in all subjects during the scan, with the global state of coherence showing the highest probability and variability. Notably, these networks overlap with resting-state networks and cognitive states based on a large-scale automatic synthesis of human functional neuroimaging data.

**Effect of sad mood-induction on FC-states**

Similar to neutral mood, during sad mood the FN-DMN-Str-SN state occurred less and lasted shorter in rrMDD compared to controls. However differences are only observed in higher clustering solutions, i.e. when more subdivisions in FC-states are made.

In detail, of the 19 clustering solutions considered, only three solutions ($k=17,18,19$) revealed that the FN-DMN-Str-SN state occurred less (lower probability) in rrMDD compared to controls after correction for multiple comparisons ($p<0.05/k$). Furthermore, this FC-state showed significantly shorter lifetimes in two of these three clustering solutions (Supplementary Figure 3).

In Figure 2D/E we show that, for $k=10$ (the clustering solution in which the FN-DMN-Str-SN state was highly significant in neutral mood) the probability of being in the FN-DMN-Str-SN state in sad mood was only significantly different between rrMDD and controls before correcting for multiple comparisons (6.0±0.51% compared to 7.80±0.93% of the time respectively, $p=0.049$, uncorrected) and that the duration was not significantly different (4.60±0.26 vs. 4.80±0.36 seconds respectively, $p=0.32$, uncorrected).
Figure 2. FC-state with a significant difference between rrMDD patients and controls (k=10) in probability of occurrence and lifetime during neutral mood. A. The dominant connectivity state is represented in the cortical space, where functionally connected brain areas (represented as spheres) are colored alike. The spheres colored in yellow, orange and red, represent areas in the frontal network, default mode network, striatum and salience network, which are all positively correlated between each other, but negatively correlated with the rest of the brain (cyan/blue colored spheres). The dominant state is also represented as the eigenvector’s outer product, which is a 90x90 matrix representing the number of brain areas and red or blue indicate positive or negative BOLD phase synchronization between them. B. Contribution of different brain areas to the dominant FC-state. Bars in yellow represent areas in the frontal network, default mode network, striatum and salience network and bars in light blue represent the rest of the brain. The magnitude of values indicates the ‘strength’ with which brain areas belong to the FC-state. C. The significant FC-state rendered on the cortex. D. Differences in probability of occurrence of this state between rrMDD patients and controls (4.58±0.47% vs. 7.24±0.79%, respectively, $p=0.0022$ in neutral mood and 6.0±0.51% vs. 7.80±0.93%, $p=0.049$ in sad mood). E. Differences in lifetime of this state between rrMDD patients and controls (3.78±0.11 vs. 5.10±0.18 seconds, $p=0.0020$ and 4.60±0.26 vs. 4.80±0.36, $p=0.32$ in sad mood). ** Significant group difference after correcting for multiple comparisons. *Significant group difference before correcting for multiple comparisons. Abbreviations: rrMDD: remitted-recurrent MDD; TR: repetition time
Mood*group interactions

We examined if there were significant group*mood interaction effects for the probability of occurrence or mean lifetime of the significant FC-pattern for k=10. We found no significant interaction effects for this FC-pattern for probability, F=0.634, p=0.425. However, there was a significant interaction effect for lifetime, F=4.32, p=0.041.
Within group differences in FC-states for neutral vs. sad mood
Remitted-MDD showed lower probabilities and lifetimes for sad vs. neutral mood in multiple FC-states (Supplementary Results/ Supplementary Figure 5). For controls, there were no within-group differences for neutral vs. sad mood.

Switching frequencies
Overall, mean switching frequencies (number of switches/second) did not differ between rrMDD and controls for all clustering solutions (from \( k=2 \) to 20) in neutral and in sad mood after correcting for multiple comparisons (all \( p \)-values>0.05/\( k \)).
**Switching probabilities**

We examined interactions for relevant FC-states in detail for \( k = 10 \) (the clustering solution that contained the FN-DMN-Str-SN state that was the most significant whilst different both in terms of probability and lifetime between groups). In Figure 4 we show the general switching pattern for the whole group for more common switches that exceed the threshold of a probability of 20% of switching to another state. On a whole group-level, the most common switches were those to the global FC-state: the most prevalent FC-state.

After correcting for \( k = 10 \) (\( p < 0.05 / k \)), in neutral mood, rrMDD patients showed a lower probability of switching from the FN-DMN-Str-SN state to the Prefrontal-Striatum state (16.5% vs. 23%, \( p = 0.004 \), uncorrected), and a higher probability to switch from the Prefrontal-Limbic state to the global state (6% vs. 1%, \( p = 0.003 \), uncorrected; Figure 4A). As shown in Figure 4, the Prefrontal-Striatum state shows a high probability of switching to the global state (on a group level), which indicates that the trajectory from the FN-DMN-Str-SN state to the global state (via the prefrontal-state) might be less flexible in rrMDD, whereas the trajectory from the Prefrontal-Limbic to the global state occurs more often.

**Effect of sad mood on switching profiles**

The overall switching pattern after sad mood-induction for the whole group was similar to the switching pattern during neutral mood (Figure 4B). During sad mood, for \( k = 10 \), we no longer identified differences in switching probabilities between groups after correcting for multiple comparisons. See the Supplementary Figure 6 for differences before correcting for multiple comparisons.

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**Figure 4 A/B (on the left). Switching probabilities for the whole group and differences between rrMDD and controls, for A. neutral and B. sad mood.** Switching probabilities for the whole group are shown above a threshold of 20% probability of switching to show more frequent switches. The whole group switching matrices (titled ‘whole group neutral’ and ‘whole group sad’) indicate the probability of, being in a given FC-state (rows), transitioning to any of the other states (columns) for the whole group. FC-states are represented in the cortical space, where functionally connected brain areas (represented as spheres) are colored alike. The spheres colored in yellow/red represent areas that are all positively correlated between them, but negatively correlated with the rest of the brain (cyan/blue colored spheres). The light blue arrows from and to the FC-states indicate the whole group switching probabilities, scaled to the magnitude of probability of switching. Significantly different transitions after correcting for multiple comparisons for \( k = 10 \) (\( p < 0.05 / 10 \)) for rrMDD compared to controls are illustrated in this figure, with the black arrow representing the transition that occurs with higher probability in rrMDD and in green the one that occurs with higher probability in controls (note these arrows have not been scaled according to magnitude of probability of switching). Transitioning differences between groups were calculated using a permutation based 2 sample T-test with 5000 permutations. rrMDD patients showed a lower probability of switching from the FN-DMN-Str-SN state to the Prefrontal-Striatum state (16.5% vs. 23%, \( p = 0.004 \)), and a higher probability to switch from the Prefrontal-Limbic state to the global state (6% vs. 1%, \( p = 0.003 \)). We identified no significant between group differences in sad mood after correction for \( k = 10 \) (\( p > 0.05 / k \)). Abbreviations: rrMDD: remitted-recurrent MDD; DMN: Default Mode Network; SN: Salience Network; Str: striatum; FN: Frontal Network
Within group differences for neutral vs. sad mood

Differences in switching probabilities were observed both for rrMDD and controls for neutral vs. sad mood, which we describe in the Supplementary Results and Supplementary Figure 6, 7A/B.

DISCUSSION

This study investigated differences in FC-states reoccurring over time during resting-state in remitted recurrent depressed patients (rrMDD) not taking antidepressants compared with never depressed controls. We identified decreased ability in rrMDD patients to access an FN-DMN-Str-SN state, consisting of frontal, DMN, striatum, and SN-areas. Our study provides a new framework for network abnormalities associated with vulnerability during MDD-remission.

The FN-DMN-Str-SN state, which consistently differed between rrMDD and controls consists of clinically relevant areas including important regions from the DMN (PCC and MPFC), Executive network (DLPFC) and Salience Network (ACC) (Supplementary Figure 4). These networks have been identified as affected in MDD before, and together form the ‘triple network’, a model employed for understanding affective and neurocognitive dysfunctions across multiple disorders. The extensive frontal network included in this FC-state consists of areas activated during cognitive control and flexible switching of attention from internal thought-processes to the external environment, with the DLPFC being particularly relevant.

Additionally, the FC-state includes a large DMN-component. Dominance of the DMN over networks involved in cognitive control has been associated with depressive symptoms and rumination. Further, it might be related to depressive recurrence, though this has not been empirically tested. Furthermore, the state included areas of the SN, a network that has been proposed to play a key role in switching brain activity between introspective, ruminative DMN functions and task-based executive networks functions. Last, the state included the dorsal striatum (caudate and putamen) and globus pallidus, structures involved in the focusing (and filtering) of cortical input. Abnormal functioning of fronto-striatal pathways might additionally lead to maladaptive regulation of emotions, contributing to anhedonia and rumination in MDD. Taken together, our results suggest decreased ability in rrMDD of brain areas regulating cognitive control (FN/SN) to diminish negative self-referential processes (DMN) and effectively regulate emotions (SN/cortico-striatal pathways).

Additionally, we observed a lower probability in rrMDD to switch from the FN-DMN-Str-SN state to a smaller prefrontal-striatum state. Interestingly, on a group level, the prefrontal-striatum state has a high probability of switching to the most prevalent state (global state). It has been suggested that the more frequently occurring global brain states allow for a greater range of either integration or segregation between neural networks and brain areas, i.e. more flexible switching to different brain states. This greater neural flexibility might facilitate cognitive flexibility.
As an additional measure of neural flexibility, in a post-hoc supplementary analysis we calculated the entropy\(^5\) for \(k=10\) and the FN-DMN-Str-SN state separately (Supplementary Discussion). We found that entropy associated to the FN-DMN-Str-SN state was significantly decreased in patients in neutral but not in sad mood. This indicates higher predictability of occurrence\(^5\) of this FC-state in rrMDD patients in neutral mood, supporting the idea that this brain state occurs in a less flexible manner.

 Unexpectedly, we observed smaller differences between rrMDD and controls for the FN-DMN-Str-SN state after sad mood-induction. After the sad mood-induction the duration of the FN-DMN-str-SN state increased in rrMDD but stayed similar in controls (significant mood*group interaction). This suggests that rrMDD have difficulties to access this state in neutral mood but are more able to recruit this state during sad mood (albeit slightly less than controls).

 We speculate that increased duration of this FC-state after sad mood-induction might reflect a compensatory mechanism in rrMDD to regulate brain activity. This could be an attempt (which might or might not be successful) to regulate negative self-referential and emotional processing when sad mood is induced. This differential increase supports our hypothesis that this FC-state is relevant for processes associated with affect and emotion in rrMDD. In controls, this FC-state is already more easily activated in neutral mood and therefore it might require less effort for this group to regulate brain activity during sad mood and in daily life. This is corroborated by the fact that this FC-state occurs more flexibly in controls during neutral mood than in remitted-MDD (but not during sad mood). Therefore, we hypothesize that inability to access this state during neutral mood might reflect increased effort for rrMDD to effectively and flexibly handle daily life stressors and changes in affect.\(^9,53,54\) This hypothesis needs to be tested by future research. Of note, we additionally observed within-group differences only in rrMDD for sad vs. neutral mood (Supplementary Results; Supplementary Figure 5).

 Results of this study are novel because our approach differs from common static-FC analyses, which previously identified aberrations in MDD in networks assumed to be temporally stable over the whole recording time, whereas we here show differences in networks that re-occur and dissolve over time. Of note, the FN-DMN-Str-SN state is only dominant during a small proportion of time. However, it consists of clinically relevant areas implicated in cognitive control and emotional/self-referential processing and occurred less, especially during neutral mood, in rrMDD-patients at high-risk for recurrence. Importantly, we found that this FC-state consistently differed between groups after multiple comparisons correction, largely independent of which clustering solution was chosen (Supplementary Figure 3). Further, using this approach we found that an FC-state of areas traditionally belonging to spatially defined resting-state networks derived from static-FC analysis, forms a separate network of increased coherence over time. This emphasizes transient dysfunctional interactions between multiple networks involved in psychological functioning, as opposed to uni-structural or single network abnormalities in the pathophysiology of MDD.

 Although this is still unclear, the more a functional network is accessed, the more stable it might become during rest because it reinforces underlying structural connections, perhaps trough
Hebbian learning mechanisms. For instance, it has been shown that cognitive training over time does not only alter FC but occurs alongside changes in the structural connectome. Here, healthy controls might have accessed the FN-DMN-Str-SN state more throughout life, which might be associated with a lower risk of occurrence of a depressive episode. If this hypothesis is true, it could be examined whether interventions such as cognitive control training or neurofeedback, which allows participants to regulate brain processes/states in real time, could increase the occurrence and duration of this FC-state.

Interestingly, two previous studies on dynamic-FC in acute MDD also found alterations in similar networks involving DMN/fronto-parietal and SN areas, albeit using different methods. Kaiser et al. used a sliding-window analysis, which has limitations related to window-size, and Demirtas et al. used instantaneous FC, more comparable to our study. Our approach of focusing on the dominant FC-state has the advantage of being more robust to high-frequency noise, as recurrences of the same pattern are more clearly detected. Merit for future studies lies in examining whether FC-states are altered when MDD-patients change from a remitted to a depressed state, and whether FC-alterations predict short- and long-term MDD-recurrence.

The state that occurred most in both groups was a state of global coherence of BOLD phases. This global state shows the greatest variability of all FC-states and might therefore allow for a greater range of correlations between areas to form, thereby functioning as a baseline state from which other FC-states are organized. However, this FC-state might relate to what is commonly described as the ‘global signal’, defined as the time series of signal intensity averaged across all brain voxels, composed of both neural and non-neural signals. It has recently been argued that including/removing this global signal might produce different complementary insights into the brain’s functional organization. The functional properties of this global state of coherence merit further examination.

Strengths and limitations
One of the strengths of this work is that we use a novel method (LEiDA) to determine and examine instantaneous dynamic-FC. Other important strengths of our study are that we have a relatively large sample, and that we study antidepressant-free patients, which excludes possible medication effects on FC-differences. A limitation of our study, related to using a novel method is the explorative nature of dynamic-FC states and their functional meaning. Further, although a strength of our method is that we examine FC-states instead of single regions, future work is needed to identify any specific area(s) that might drive the activation of the FN-DMN-Str-SN state and may serve as target for intervention.

Conclusion
Using the novel LEiDA-approach to examine instantaneous dynamic FC, this study provides new insights on aberrations in dynamic brain network connectivity in remitted MDD patients. This new framework for exploring dynamic FC could potentially be extended to other diseases that have been related to pathological resting-state connectivity. Overall, our findings suggest reduced
neural ability and flexibility of patients remitted from MDD, but at high risk for recurrence, to access a clinically relevant control network involved in the interplay between emotional and attentional processing and (negatively) internally oriented attention.

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Role of the Funder/sponsor: None of the supporting organizations had any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript; and decision to submit the manuscript for submission.

Additional contributions
First, we would like to thank the study subjects that participated in this research. Second, we acknowledge the thoughtful comments of Prof. Z. Segal¹ and Prof. C.L. Bockting² regarding our in-scanner mood-induction procedure.

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Conflicts of Interest
The authors declare no conflicts of interest
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Supplementary Figure 1. Mood-induction paradigm

Figure S1. In scanner mood-induction paradigm. The resting-state fMRI scans after neutral and sad mood-induction were used for analysis; *Information regarding other fMRI tasks and break is described in the methodological paper of this study (Mocking et al. 2016) **Rating of most sad moment during the sad mood resting-state scan
Supplementary Figure 2. Disposition of participants

Abbreviations: AD: antidepressants; fMRI: functional Magnetic Resonance imaging; HDRS: Hamilton Depressive Rating Scale; MDD: Major Depressive Disorder

Supplementary Table 1. Mood-ratings

<table>
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<tr>
<th></th>
<th>rrMDD</th>
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<th>Between-group statistics</th>
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<td>SD</td>
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<td>- after scan</td>
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<td>- difference¹</td>
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<tr>
<td>Sad Mood</td>
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<td>6.32</td>
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<td>1.88</td>
<td>5.94</td>
<td>1.39</td>
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</table>

HC: healthy control; MIP: Mood-induction Procedure; rrMDD: remitted recurrent major depressive disorder; U: Mann-Whitney U non-parametric test statistic; F: F-statistic from repeated measures analysis; p: p-value; SD: Standard Deviation; ¹Scores significantly decreased during neutral resting-state in both groups (p=0.001). ²Scores significantly decreased during sad resting-state in both groups (p<0.001)
*Subjects rated their lowest mood during the sad resting-state scan retrospectively.
Supplementary Figure 3. Dominant FC-states emerging in brain activity over time

We ran the \( k \)-means clustering algorithm with \( k \) ranging from 2 to 20 (vertical axis) and for each \( k \) detected \( k \) recurrent FC-states, over all time points, over all subjects and both mood-states. Depending on \( k \), a more distinct subdivision in different FC-states is identified from the entire sample. A permutation-based 2-sample t-test (with 5000 permutations) was used to identify significant differences between groups (rrMDD patients versus controls) and a permutation based paired T-test was used to identify differences between mood-states (neutral versus sad mood). Results are corrected for multiple comparisons with \( p<0.05/k \). FC-states that differ significantly in terms of probability between rrMDD and controls are indicated left of the FC-state with *P in neutral mood (16 states), and in +P, in sad mood (3 states). FC-states that differ in terms of lifetime (duration) are indicated with *L in neutral mood (7 states) +L in sad mood (2 states). In rrMDD, FC-states that differ significantly in terms of probability between neutral vs. sad are indicated with #P for probability (23 states) and #L for mean lifetime (7 states). In controls, no states differed in terms of probability or mean lifetime for neutral vs. sad mood. In red we indicate the FN-DMN-Str-SN state that significantly differs between rrMDD patients and controls in probability of occurrence or duration. Furthermore, with a red arrow we indicate in which clustering solutions the FN-DMN-Str-SN state is significantly different between groups for probability of occurrence and/or lifetime. As shown in the figure, the FN-DMN-Str-SN state consistently differs between groups, largely independent of which clustering solution is chosen. Abbreviations: rrMDD: remitted-recurrent MDD; DMN: Default Mode Network; SN: Salience Network; Str: striatum; FN: Frontal Network; TR: repetition time.
**SUPPLEMENTARY RESULTS**

**FC-state analysis for remitted-MDD and controls separately**

**Probability of occurrence**

In rrMDD, out of the 19 clustering solutions we considered (with $k$ from 2 to 20), in 16 clustering solutions 23 FC-states occurred less (lower probability) in sad mood compared to neutral mood after correcting for multiple comparisons (thus in some clustering solutions, multiple FC-states differed for neutral vs. sad mood in rrMDD). Thirteen of these FC-states consisted of the Insula-Auditory State (**Supplementary Figure 5A**), 9 FC-states consisted of the Somatosensory-Insula state (**Supplementary Figure 5B**), and one FC-state consisted of the Visual-Hippocampus state (**Supplementary Figure 5C**). For illustrative purposes, we display the states derived from the clustering solution $k=10$ (also see **Figure 3** in the main document for these FC-states for $k=10$).

**Duration**

In rrMDD, out of 19 clustering solutions we considered (with $k$ from 2 to 20), 7 FC-states lasted significantly shorter (lower mean lifetime), in sad mood compared to neutral mood after correcting for multiple comparisons. One FC-state consisted of the Insula-Auditory state (**Supplementary Figure 5A**), two FC-states consisted of a Visual-Hippocampus state (**Supplementary Figure 5C**), one FC-state consisted of the FN-DMN-Str-SN state (**Supplementary Figure 5D**), and three consisted of a Somatosensory-Visual State (**Supplementary Figure 5E**). For illustrative purposes, we display the states derived from the clustering solution $k=10$ (also see **Figure 3** in the main document for these FC-states for $k=10$).

**Supplementary Figure 4.** Overlap of resting-state networks with the FN-DMN-Str-SN state

The FN-DMN-Str-SN state is projected on top of 4 resting-state networks, defined according to a cortical 7-network parcellation based on 1000 participants by Yeo et al.\(^40\) and a striatum parcellation by Choi et al.\(^41\) to show overlap of this FC-state with traditional resting-state networks. Abbreviations: DMN: Default Mode Network; SN: Salience Network; Str: striatum; FN: Frontal Network.
In controls, there were no differences for probability or mean lifetime for neutral vs. sad mood.

Supplementary Figure 5. FC-states showing differences in probability/duration in rrMDD

- **S5A Insula-Auditory**: Lower probability of occurrence for 13 FC-states and shorter duration for 1 FC-state in sad vs. neutral mood in rrMDD.

- **S5B Somatosensory-Insula**: Lower probability of occurrence for 9 FC-states in sad vs. neutral mood in rrMDD.

- **S5C Visual-Hippocampus**: Lower probability of occurrence for 1 FC-state and shorter duration for 2 FC-states in sad vs. neutral mood in rrMDD.

- **S5D FN-DMN-Str-SN**: Shorter duration for 1 FC-state in sad vs. neutral mood in rrMDD.

- **S5E Somatosensory-Visual**: Shorter duration for 3 FC-states in sad vs. neutral mood in rrMDD.

Supplementary Figure 5. FC-states showing differences in probability and/or duration for neutral vs. sad mood in rrMDD. For illustrative purposes, FC-states are displayed as derived from the clustering solution k=10. Differences were not only observed for k=10, but in multiple different clustering solutions (from k=2 to 20; Supplementary Figure 3). FC-states are represented in the cortical space, where functionally connected brain areas (represented as spheres) are colored alike. The spheres colored in yellow/red represent areas that are all positively correlated between them, but negatively correlated with the rest of the brain (blue colored spheres). FC-states are also represented as the eigenvector’s outer product, which is a 90x90 matrix representing the number of brain areas and red or blue indicate positive or negative BOLD phase synchronization between them. Within group differences were calculated using a permutation based paired T-test with 5000 permutations and we corrected for multiple comparisons (p>0.05/k). Abbreviations: rrMDD: remitted-recurrent MDD; DMN: Default Mode Network; SN: Salience Network; Str: striatum; FN: Frontal Network.
Switching profiles for $k=10$

For sad compared to neutral mood, rrMDD showed decreased probability to switch from the Somatosensory-Insula state to the Visual-Hippocampus state in the $k=10$ solution, (28% vs. 19%, $p=0.0023$; after correcting for multiple comparisons; Supplementary Figure 7A). Of note, these two FC-states also showed a lower probability of occurrence and duration during sad versus neutral mood in MDD. This might explain the lower switching probabilities between these states in sad compared to neutral mood.

For sad versus neutral mood, controls showed increased probability to switch from the Prefrontal-Limbic state to the global state (8% vs. 4%, $p=0.0018$; after correcting for multiple comparisons; Supplementary Figure 7B). For controls this switching probability difference was the only difference for sad compared to neutral mood in our analyses.

See Supplementary Figure 6 for all between and within group differences uncorrected for multiple comparisons and 7A/B for all within group differences in switching probabilities for neutral versus sad mood corrected for multiple comparisons.
Supplementary Figure 6. Switching probability differences; matrices

Supplementary Figure 6. Switching probability matrices, showing differences between rrMDD and controls in neutral and sad mood for \( k=10 \), not corrected for multiple comparisons. Matrices indicate the probability of, given being in a FC state (rows), transitioning to any of the other states (columns). Values indicate number of switches (relative to all switches during the time series) and were estimated for each participant. Transitioning differences between groups were calculated using a permutation based T-tests (paired T-test or 2 sample T-tests with 5000 permutations). All differences are not corrected for multiple comparisons.* differences \((p<0.05)\) in probability of transitioning to another state for rrMDD vs controls in neutral mood, * differences \((p<0.05)\) in probability of transitioning to another state for rrMDD vs. controls in sad mood + differences in probability of transitioning to another state for neutral vs. sad mood in controls, + differences in probability of transitioning to another state for neutral vs. sad mood in rrMDD. Abbreviations: rrMDD: remitted-recurrent MDD
Supplementary Figure 7. Differences in probabilities of switching between FC-states for $k=10$, for A. rrMDD in neutral vs. sad mood. B. Controls in neutral vs. sad mood. Switching probabilities averaged for neutral and sad for groups separately are shown above a threshold of 20% probability of switching to show more frequent switches. The switching matrices (titled ‘rrMDD mean mood’ and ‘control mean mood’) indicate the probability of, being in a given FC-state (rows), transitioning to any of the other states (columns). FC-states are represented in the cortical space, where functionally connected brain areas (represented as spheres) are colored alike. The spheres colored in yellow/red represent areas that are all positively correlated between them, but negatively correlated with the rest of the brain (cyan/blue colored spheres). The light blue arrows from and to the FC-states indicate the switching probabilities averaged over mood state, scaled to the magnitude of probability of switching. Significantly different transitions ($p<0.05/10$) are illustrated in this figure, with black arrows representing the transitions that occur with higher probability in neutral mood and in greens the ones that occur with higher probability in sad mood. Values were estimated for each subject and then a permutation-based paired t-test (5000 permutations) was applied to test for the between-group or within-group significance. Abbreviations: rrMDD: remitted-recurrent MDD; DMN: Default Mode Network; SN: Salience Network; Str: striatum; FN: Frontal Network;
SUPPLEMENTARY DISCUSSION

Entropy for k=10 and for the FN-DMN-Str-SN state separately

We tested if there was a significant difference in entropy\(^{51}\) between groups for \(k=10\), by computing for each subject \(s\), the overall entropy as:

\[
H(s) = - \sum P_s \log_2 P_s
\]

where \(P_s\) is a vector containing the probabilities of each state for subject \(s\). We tested differences in entropy for \(k=10\). Entropy is a measure of the average amount of uncertainty or predictability present in a given probability distribution.\(^{52}\) The higher the homogeneity of probability values across states, the higher the entropy for that subject will be, and the lower the predictability of the occurrence of states. To the extreme, if only one state has 100% probability and the others have null probability, then the entropy is zero. Thus, lower entropy indicates more restricted dynamical repertoire.\(^{52}\)

Additionally, we investigated the entropy associated with the FN-DMN-Str-SN state, given by

\[
H_c(s) = -P_s(c) \log_2 P_s(c)
\]

In the case of one state, if a state has high entropy there is high uncertainty/low predictability of occurrence of that state.

We found that, the overall entropy of the system was statistically similar in MDD patients in remission compared to controls for the clustering solution of \(k=10\) in neutral mood (mean \(H(s)=2.59\) vs. \(2.61\) respectively, \(p=0.63\)) and in sad mood (mean \(H(s)=2.48\) vs. \(2.51\) respectively, \(p=0.26\)). This indicates that, despite the significant decrease in the probability of the FN-DMN-Str-SN state in remitted patients, the distribution of probabilities is not affected, which suggests a regulation by intrinsic homeostatic processes. Yet, we found that the entropy associated with the FN-DMN-Str-SN state was significantly decreased in rrMDD patients compared to controls (mean \(H(c)=0.19\) vs. \(0.25\) respectively, \(p=0.006\)) in neutral mood. In sad mood however, we found no significant differences between rrMDD and controls in entropy of this FC-state (mean \(H(c)=0.23\) vs. \(0.26\) respectively, \(p=0.16\)).

This finding indicates high predictability/less uncertainty of probability of occurrence values of this FC state in remitted MDD patients in neutral mood. This might be associated with the flexibility with which this state occurs and thus supports the idea that the flexibility of brain dynamics is reduced in psychiatric disorders, including MDD.\(^{16}\)