Acta Psychiatrica Scandinavica

Acta Psychiatr Scand 2016: 134: 339–349

DOI: 10.1111/acps.12596

Abnormal functional–structural cingulum connectivity in mania: combined functional magnetic resonance imaging-diffusion tensor imaging investigation in different phases of bipolar disorder

Martino M, Magioncalda P, Saiote C, Conio B, Escelsior A, Rocchi G, Piaggio N, Marozzi V, Huang Z, Ferri F, Amore M, Inglese M, Northoff G. Abnormal functional—structural cingulum connectivity in mania: combined functional magnetic resonance imaging-diffusion tensor imaging investigation in different phases of bipolar disorder.

Objective: The objective of the study was to investigate the relationship between structural connectivity (SC) and functional connectivity (FC) in the cingulum in bipolar disorder (BD) and its various phases.
Method: We combined resting-state functional magnetic resonance imaging and probabilistic tractographic diffusion tensor imaging to investigate FC and SC of the cingulum and its portions, the SC–FC relationship, and their correlations with clinical and neurocognitive measures on sustained attention in manic (n = 21), depressed (n = 20), and euthymic (n = 20) bipolar patients and healthy controls (HC) (n = 42).
Results: First, we found decreased FC between the anterior and posterior parts of the cingulum in manic patients when compared to depressed patients and HC. Second, we observed decreased SC of the cingulum bundle, particularly in its anterior part, in manic patients when compared to HC. Finally, alterations in the cingulum FC (but not SC) correlated with clinical severity scores while changes in the cingulum SC (but not FC) were related with neurocognitive deficits in sustained attention in BD.
Conclusion: We demonstrate for the first time a reduction in FC and concomitantly in SC of the cingulum in mania, which correlated with psychopathological and neurocognitive parameters, respectively, in BD. This supports the central role of cingulum connectivity specifically in mania.

M. Martino[1,*], P. Magioncalda[1,*], C. Saiote[2], B. Conio[1], A. Escelsior[1], G. Rocchi[1], N. Piaggio[3], V. Marozzi[1], Z. Huang[4], F. Ferri[4], M. Amore[1], M. Inglese[2,5,6], G. Northoff[4,7,8,9,10,11,12]

[1]Section of Psychiatry, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Health, University of Genoa, Genoa, Italy, [2]Department of Neurology, Radiology and Neuroscience, Mount Sinai School of Medicine, New York, NY, USA, [3]Section of Neuroradiology, Department of Radiology, University of Genoa, Genoa, Italy, [4]Mind, Brain Imaging, and Neuroethics, Royal's Institute of Mental Health Research, University of Ottawa, Ottawa, ON, Canada, [5]Magnetic Resonance Research Center on Nervous System Diseases, University of Genoa, Genoa, [6]Section of Neurology, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Health, University of Genoa, Genoa, Italy, [7]University of Ottawa Brain and Mind Research Institute, Centre for Neural Dynamics, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada, [8]Graduate Institute of Humanities in Medicine, Taipei Medical University, Taipei, [9]Taipei Medical University-Shuang Ho Hospital, Brain and Consciousness Research Center, New Taipei City, [10]National Chengchi University, Research Center for Mind, Brain and Learning, Taipei, Taiwan, [11]Centre for Cognition and Brain Disorders (CCBD), Normal University Hangzhou, Hangzhou, China and [12]ITAB, University of Chieti, Chieti, Italy

Key words: affective disorders; bipolar disorder; magnetic resonance imaging; neuroimaging

Paola Magioncalda, Ospedale San Martino, Largo Rosanna Benzi n.10, ZIP 16100, Genova, Italy.
E-mail: paola.magioncalda@gmail.com

*These authors have contributed equally to this work.

Accepted for publication May 3, 2016

Significant outcomes

- The manic phase of bipolar disorder (but not the depressive and euthymic phases) shows a decrease in both functional connectivity and structural connectivity within the cingulum.
- Cingulum changes in functional connectivity correlate with clinical severity scores, while changes in structural connectivity correlate with neurocognitive deficits in bipolar patients.
- Functional connectivity and structural connectivity in the cingulum seem to show dynamic changes across the phases of bipolar disorder and may have clinical relevance.

Limitations

- Patients under treatment
- Patients with different illness duration
- The cross-sectional design of the study

Introduction

In recent years, a growing number of anatomo-functional brain's alterations have been detected in patients affected by bipolar disorder (BD) (1–3). Among the investigated structures, the cingulum, a core component of the limbic system, is a central candidate for abnormality in psychiatric illnesses in general (4), and in BD in particular (5). Indeed, the cingulum shows alterations in functional connectivity (FC) and structural connectivity (SC) in BD, especially in its anterior part, and these abnormalities could be central in the pathophysiology of this illness (6–8). However, it remains unclear whether the structural and functional cingulum changes are related to specific phases or states within BD, that is, manic or depressive phases, or are rather trait-dependent as related to BD independently of its specific phases.

Previous resting-state functional magnetic resonance imaging (fMRI) studies found differences in the FC patterns of the prefrontal cortex (PFC), and especially the anterior cingulate cortex (ACC), with other cortical and subcortical areas in BD patients in general (regardless of the phase of illness) (7, 9). In particular, a hypoconnection was especially observed in the anterior midline regions of the default mode network (DMN) (10–12), of which the perigenual ACC (PACC) and posterior cingulate cortex (PCC) constitute the core midline structures (13). However, it is still unclear whether specific FC alterations of the cingulum manifest in specific phases of BD (manic, depressed, and euthymic) and whether the FC of specific portions of the cingulum (anterior, middle, and posterior) are differently affected.

Concerning SC, recent meta-analyses on whole-brain diffusion tensor imaging (DTI) studies including BD patients in the various phases of illness found widespread white matter (WM) alterations, and especially the anterior and posterior cingulum and some of the connected areas (genus of corpus callosum and WM fibers close to the parahippocampal gyrus) were found as the most constantly altered tracts in BD (14, 15). These studies mostly reported a decrease in fractional anisotropy (FA) as well as an increase in mean diffusivity (MD) and radial diffusivity (RD) and, in few cases, changes in axial diffusivity (AD) (14, 15). However, previous tractography studies that specifically reconstructed the cingulum bundle and included only euthymic or remitted patients only partially confirmed the DTI changes in the cingulum that were found in whole-brain studies including BD patients in all phases (4, 16–22). Therefore, structural alterations in the cingulum were mainly found in BD samples overall (regardless of the phase of illness) with respect to euthymic/remitted patients; this inconsistency may suggest that structural abnormalities are dynamic rather than static and change across the different phases of illness. However, studies investigating the cingulum and its different portions in the various phases of BD are still lacking. Furthermore, although it was already shown in healthy subjects that SC could underlie FC in the cingulum (as part of the DMN) (23), to date, to the best of our knowledge, the relationship between the cingulum FC and the underlying SC remains to be investigated in BD in general and in its various phases (see though (24) for a combined investigation of the hippocampal FC and SC in remitted BD patients).

Moreover, abnormalities in FC and SC may play a role at a clinical level. In addition to the specific psychopathological symptomatology, BD was found to be associated with various cognitive

deficits, with profile changes across the different phases of illness (25). Interestingly, in recent studies in BD patients, neurocognitive alterations and clinical severity scores were found to be related to WM microstructural abnormalities and functional disconnectivity, respectively (10, 26, 27). In particular, sustained attention is one of the most affected cognitive domains in BD (25, 28), showing differences across the active phases—for example, more commission errors during mania while more omission errors during depression (25). Importantly, sustained attention was found to be mediated by the cingulum in healthy subjects (29). However, the impact of the functional and structural disconnectivity of the cingulum on clinical and neurocognitive (e.g., sustained attention) dysfunction in patients in the various phases of type I BD needs to be further investigated.

Aims of the study

The general aim is to investigate the functional connectivity and structural connectivity of the cingulum as well as their role in psychopathology and cognition in the various phases of bipolar disorder type I—mania, depression, and euthymia—and healthy controls.

Our specific aims are to investigate the following:

i) The cingulum functional connectivity between perigenual anterior cingulate cortex and posterior cingulate cortex, as well as the functional connectivity of the different portions of the cingulum, in the various phases of illness. As previous functional magnetic resonance imaging data in bipolar disorder suggested a disconnection within the cingulum as well as within default mode network (of which perigenual anterior cingulate cortex and posterior cingulate cortex represent the main midline regions) (see above), we hypothesized reduced functional connectivity in the cingulum between perigenual anterior cingulate cortex and posterior cingulate cortex, but no functional connectivity alterations in the other portions of the cingulum, possibly in specific phases of illness.
ii) The cingulum structural connectivity between perigenual anterior cingulate cortex and posterior cingulate cortex, as well as the structural connectivity of the different portions of the cingulum, in the various phases of illness, and its correlation with the functional connectivity. As previous diffusion tensor imaging findings in bipolar disorder showed white matter alterations mainly localized in the anterior and posterior parts of the cingulum, we hypothesized reduced structural connectivity —that is, reduced fractional anisotropy and increased mean diffusivity and radial diffusivity—in the cingulum (between perigenual anterior cingulate cortex and posterior cingulate cortex), possibly mainly in the anterior part. As structural connectivity was found to be related with functional connectivity in healthy controls (see above), structural connectivity reduction may also correlate with potential functional connectivity alterations.
iii) The correlations of cingulum structural connectivity and functional connectivity with neurocognitive and psychopathological–clinical measures. As in bipolar disorder patients, white matter microstructural abnormalities and functional disconnectivity were found to be related to neurocognitive alterations and clinical severity scores, respectively (see above), we hypothesized possible differential correlations of neurocognitive and clinical parameters with structural connectivity and functional connectivity.

Methods and material

Participants were recruited from the in-patient and out-patient service of the Psychiatric Clinic at the University of Genoa (IRCCS AOU San Martino —IST, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Health, Section of Psychiatry). The Ethical Committee of San Martino Hospital approved the study, and a written informed consent was obtained from all the participants.

The study was conducted on 61 bipolar patients —21 in manic, 20 in depressive, and 20 in euthymic phases—and 42 healthy controls (HC). All patients met DSM-IV criteria for current BD type I without major comorbidities, as assessed by the Structured Clinical Interview for Axis-I Disorders/ Patient edition (SCID-I/P) (30), and depressive and manic symptom severity was measured using Hamilton Depression Scale (HAM-D) (31) and Young Mania Rating Scale (YMRS) (32) respectively. Other comorbidities consisted of panic disorder ($n = 4$), social phobia ($n = 3$), generalized anxiety disorder ($n = 2$), obsessive–compulsive disorder ($n = 5$), somatoform disorders ($n = 3$), and bulimia ($n = 5$). Finally, almost all the bipolar patients were under medication with mood stabilizers (lithium: $n = 17$; valproate: $n = 26$; other antiepileptic drugs: $n = 23$), antipsychotics (atypical antipsychotics: $n = 30$; typical antipsychotics: $n = 7$), antidepressants (serotonin reuptake inhibitors: $n = 10$; tricyclic antidepressants: $n = 6$;

duals and others antidepressants: $n = 10$), and benzodiazepines ($n = 21$) (Table S1). Moreover, all the participants were administered the continuous performance test (CPT), a computerized test used to evaluate distractibility and impulsivity in the study of sustained attention (33).

All images were acquired at 1.5-T with a GE scanner, that is, resting-state fMRI, DTI, and structural sequences.

Functional connectivity

After standard resting-state fMRI preprocessing, a FC analysis with a ROI-to-ROI approach was performed using the PACC and PCC as ROIs of the midline structures of the DMN (13), according to our *a priori* hypothesis on the disconnectivity of the anterior region with the posterior region of the cingulate cortex. To first detect differences between the whole bipolar group and control group, the PACC-PCC FC was entered into a two-sample *t*-test between BD and HC. After obtaining significant differences between BD and HC, the PACC-PCC FC was entered in an analysis of variance (ANOVA) followed by *post hoc* Games-Howell test to detect differences between the various subgroups, that is, manic, depressed, and euthymic patients, and HC. All results were thresholded at a corrected $P < 0.05$.

Subsequently, a whole-brain voxel-wise FC analysis using the PACC as seed region was performed to further confirm the results of the ROI-to-ROI approach and the specific role of PACC. The same procedure was performed using the PCC, as well as the supragenual ACC (SACC) and middle cingulate cortex (MCC) as seed regions of control.

Finally, the FC within the anterior, middle, and posterior parts of the cingulum were entered in ANOVAS and *post hoc* tests to explore eventual FC alterations in specific portions of the cingulum and control the specificity of the PACC-PCC FC results.

Structural connectivity

First, after standard DTI preprocessing, probabilistic tractography of the cingulum was performed by adapting the ROIs used in the FC analysis. The mean FA, MD, RD, and AD of the cingulum were calculated and entered into a 4 (DTI parameters) × 4 (subgroups) ANOVA and *post hoc* test to detect differences in the cingulum SC between the various subgroups. Then, each of the DTI parameters hypothesized to be altered (FA, MD, and RD, as well as AD values for control analysis) was entered into an ANOVA and *post hoc* test to control which DTI parameter is specifically altered. All results were thresholded at a corrected $P < 0.05$.

Second, we obtained the anterior, middle, and posterior parts of the cingulum and the corresponding DTI parameters were calculated. The altered DTI parameters were entered into separate ANOVAS and *post hoc* tests for each portion of the cingulum to explore which of them is mainly affected. Then, each of the DTI parameters of each altered portion of the cingulum was entered into an ANOVA and *post hoc* test to control which DTI parameter is specifically altered in the different cingulum portions.

Finally, the inferior frontal occipital fasciculus (IFOF) was reconstructed and analyzed in the same manner as a control tract.

Furthermore, within the entire sample, partial correlations controlling for subgroup status were performed between the altered DTI parameters and PACC-PCC FC to detect potential relationships between functional and structural disconnections. All correlation results were considered significant at the Bonferroni-corrected $P < 0.05$.

Cognitive–clinical and neuroimaging correlations

Finally, within the entire sample, partial correlation analyses controlling for subgroup status were performed between the neurocognitive variables on sustained attention as well as for YMRS and HAM-D total scores on the one hand, with the altered parameters of cingulum connectivity, that is, the altered DTI parameters and PACC-PCC FC, on the other. Furthermore, correlation analyses were performed for medication variables and illness duration with the same neuroimaging parameters. All correlation results were considered significant at the Bonferroni-corrected $P < 0.05$.

See the supporting information (Data S1) for a detailed description of sample, acquisition parameters, imaging processing, and statistical analyses.

Results

Functional connectivity

First, in the ROI-to-ROI analysis, the two-sample *t*-test of the PACC-PCC FC between BD and HC showed a significant decrease in FC in bipolar patients when compared to controls ($t = -2.076$; $P = 0.041$). The ANOVA and *post hoc* analysis of the PACC-PCC FC detected a significant difference between subgroups ($F = 4.224$; $P = 0.008$) with manic patients showing decreased PACC-PCC FC

when compared to both HC ($P = 0.003$) and depressed patients ($P = 0.019$) (Fig. 1 and Table S2).

Second, in the voxel-wise control analyses, BD patients showed a significant decrease in FC between the PACC (as seed) and PCC, as well as SACC/area eight right, insula right, temporal–parietal junction left and middle temporal gyrus left (Table S3a). Among subgroups, manic patients showed decreased FC between the PACC (as seed) and PCC (and insula right) when compared to HC (Figure S1a and Table S3a). Euthymic patients showed decreased FC between the PACC (as seed) and temporo-parietal junction left (Table S3a). Furthermore, a decrease in FC between the PCC (as seed) and PACC/medial PFC was found in manic patients when compared to both HC and depressed patients (Table S3b). In contrast, no significant difference was detected in FC between subgroups when the SACC and MCC were taken as seed regions.

Finally, no significant difference was found in any of the portions of the cingulum between the various subgroups (PACC-SACC FC: $F = 0.979$ $P = 0.406$; SACC-MCC FC: $F = 0.076$ $P = 0.973$; MCC-PCC FC: $F = 0.685$ $P = 0.563$) (Figure S1b).

Structural connectivity

First, the 4 (DTI parameters) × 4 (subgroups) ANOVA and *post hoc* analysis of the cingulum SC showed a significant main effect between the various subgroups ($F = 2.790$; $P = 0.045$) with manic patients showing decreased values when compared to HC ($P = 0.036$) (interaction between DTI parameters and subgroups: $F = 1.511$; $P = 0.145$). Then, the ANOVAs for each DTI parameter of the whole cingulum showed a significant difference between subgroups in mean FA ($F = 2.829$; $P = 0.042$), MD ($F = 2.958$; $P = 0.036$), and RD ($F = 3.581$; $P = 0.017$), but not in AD ($F = 0.584$; $P = 0.651$). Among subgroups, the *post hoc* analysis showed decreased FA ($P = 0.034$) as well as increased MD ($P = 0.035$) and RD ($P = 0.012$) in manic patients when compared to HC (Fig. 2, Table S2 and Figure S2a). Furthermore, we performed explorative analyses on the SC of the left and right cingulum, separately. A significant difference between subgroups was found in the right cingulum bundle in FA ($F = 3.205$; $P = 0.027$), MD ($F = 2.890$; $P = 0.039$), and RD values ($F = 3.557$; $P = 0.017$). Specifically, among subgroups, manic patients showed increased MD ($P = 0.044$) and RD values ($P = 0.022$) as well as marginally decreased FA values ($P = 0.055$), when compared to HC, and decreased FA values ($P = 0.032$) when compared to euthymic patients. By contrast, no significant differences were found in the SC of the left cingulum.

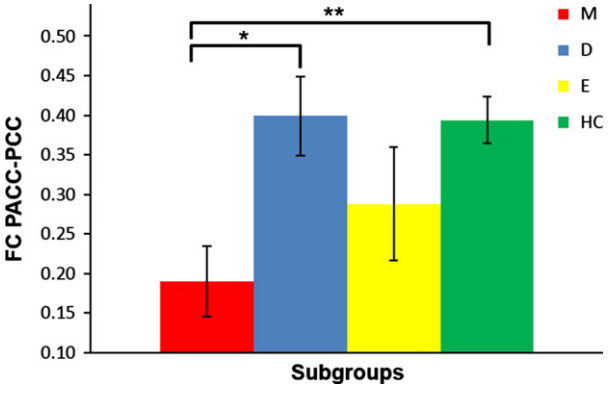

Fig. 1. Differences in functional connectivity of the cingulum in mania, depression, euthymia, and controls. ANOVA with Games-Howell *post hoc* test of the PACC-PCC FC between the various subgroups. Corrected $P < 0.05$*; $P < 0.01$**. FC, functional connectivity; PACC, perigenual anterior cingulate cortex; PCC, posterior cingulate cortex; M, manic patients; D, depressed patients; E, euthymic patients; HC, healthy controls.

Second, the 3 (altered DTI parameters: FA, MD, RD) × 4 (subgroups) ANOVA and *post hoc* analysis of the anterior part of the cingulum SC showed a significant main effect between the various subgroups ($F = 2.780$; $P = 0.045$) with manic patients showing decreased values when compared to HC ($P = 0.012$) (interaction between DTI parameters and subgroups: $F = 2.981$; $P = 0.008$). Then, the ANOVAs for each DTI parameter of the anterior part of the cingulum showed a significant difference between subgroups in mean FA ($F = 2.783$; $P = 0.045$), MD ($F = 3.838$; $P = 0.012$), and RD ($F = 3.918$; $P = 0.011$). Among subgroups, the *post hoc* analysis showed decreased FA ($P = 0.011$) as well as increased MD ($P = 0.039$) and RD ($P = 0.011$) in manic patients when compared to HC (Figure S2b). In contrast, no significant differences were found in the DTI parameters in the middle part (main effect: $F = 2.156$ $P = 0.098$; interaction between DTI parameters and subgroups: $F = 1.306$ $P = 0.256$) and in the posterior part of the cingulum (main effect: $F = 1.010$ $P = 0.392$; interaction between DTI parameters and subgroups: $F = 1.009$ $P = 0.420$).

Finally, the 4 (DTI parameters) × 4 (subgroups) ANOVA and *post hoc* analysis of the IFOF SC showed a marginal difference between subgroups (main effect: $F = 2.479$ $P = 0.066$; interaction between DTI parameters and subgroups: $F = 1.867$ $P = 0.058$). A difference in this control tract was found between subgroups in mean RD ($F = 3.214$; $P = 0.026$) with only depressed

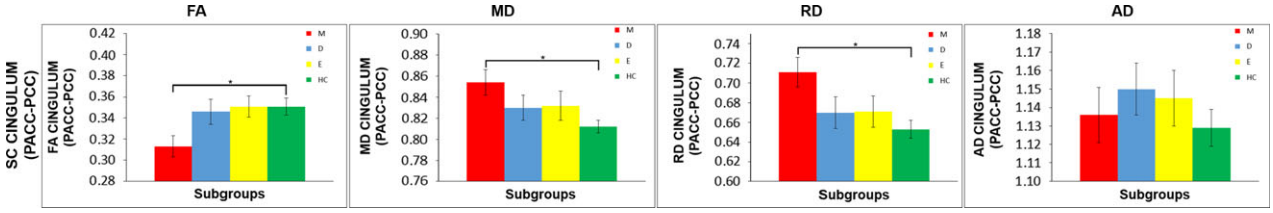

Fig. 2. Differences in structural connectivity of the cingulum in mania, depression, euthymia, and controls. ANOVA with Games-Howell *post hoc* test of the mean FA, mean MD, mean RD, and mean AD values of the cingulum (from PACC to PCC) between the various subgroups. MD, RD, and AD values are multiplied per 10^3. Corrected $P < 0.05$*. SC, structural connectivity; PACC; perigenual anterior cingulate cortex; PCC, posterior cingulate cortex; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AD, axial diffusivity; M, manic patients; D, depressed patients; E, euthymic patients; HC, healthy controls.

patients showing increased RD ($P = 0.038$) when compared to HC (Figure S2c).

Furthermore, the PACC-PCC FC showed a significant direct correlation with FA ($r = 0.253$; $P = 0.013$; CI:0.062–0.425) and a significant inverse correlation with MD ($r = -0.269$; $P = 0.008$; CI:−0.436 to −0.087) and RD ($r = -0.302$; $P = 0.003$; CI:−0.466 to −0.124) of the cingulum (Figure S2d).

Cognitive–clinical and neuroimaging correlations

With regard to neurocognitive evaluation on sustained attention, significant differences between the various subgroups were found in all the CPT parameters: total hits ($F = 9.219$; $P = 0.000$), omission errors ($F = 8.573$; $P = 0.000$), and commission errors ($F = 5.414$; $P = 0.002$). Among subgroups, manic patients showed decreased total hits ($P = 0.001$) and increased omission ($P = 0.000$) and commission errors ($P = 0.046$), when compared to HC; furthermore, depressed patients showed increased omission errors ($P = 0.030$) when compared to HC (Figure S3). The CPT total hits showed a significant inverse correlation with MD ($r = -0.313$; $P = 0.002$; CI:−0.454 to −0.150) and RD ($r = -0.324$; $P = 0.001$; CI:−0.468 to −0.142), as well as a tendency toward a significant direct correlation with FA ($r = 0.241$; $P = 0.016$; CI:0.030–0.404) of the cingulum (Fig. 3a). The CPT omission errors showed a significant direct correlation with MD ($r = 0.314$; $P = 0.002$; CI:0.141–0.468) and RD ($r = 0.312$; $P = 0.002$; CI:0.129–0.469), as well as a tendency toward a significant inverse correlation with FA ($r = -0.216$; $P = 0.033$; CI:−0.389 to −0.032), of the cingulum (Fig. 3a). The CPT commission errors did not correlate with the SC measures. In contrast to the correlation with SC, neither CPT measure correlated with PACC-PCC FC.

Finally, with regard to clinical correlations, the YMRS total score showed a significant inverse correlation with the PACC-PCC FC ($r = -0.287$; $P = 0.004$; CI:−0.437 to −0.130) (Fig. 3b). The HAM-D total score did not correlate with the FC measure. In contrast to their correlation with FC, the clinical scores did not correlate with the DTI parameters of the cingulum.

Discussion

Main findings

The main findings are the following: (i) the PACC-PCC FC was decreased in manic patients when compared to both depressed patients and HC; (ii) the SC of the cingulum, especially its anterior part, was decreased in manic patients when compared to HC; (iii) the clinical symptom severity scores correlated with the PACC-PCC FC (but not with SC), while the neurocognitive scores correlated with the measures of cingulum SC (but not with FC).

Functional connectivity

At the functional level, we found reduced FC between PACC and PCC, in particular in the manic phase of BD. In the subsequent voxel-wise analyses using the PACC as seed region, the functional hypoconnection with PCC was confirmed in mania specifically. Further confirmation was obtained using the PCC as seed, which again yielded a decrease in the PACC-PCC FC in manic patients. Additionally, our data show regional specificity of changes for the anterior and posterior portions of the cingulum, because no significant FC differences were found between subgroups in any other portion. Our results confirm previous findings that showed the central role of the functional hypoconnectivity of PACC in BD (10–12). Importantly, our results extend these earlier observations by demonstrating the abnormal relationship specifically between the PACC and PCC in the manic (rather than depressive or euthymic) phase of BD. This is possibly related to the roles of the PACC and PCC as central nodes within the

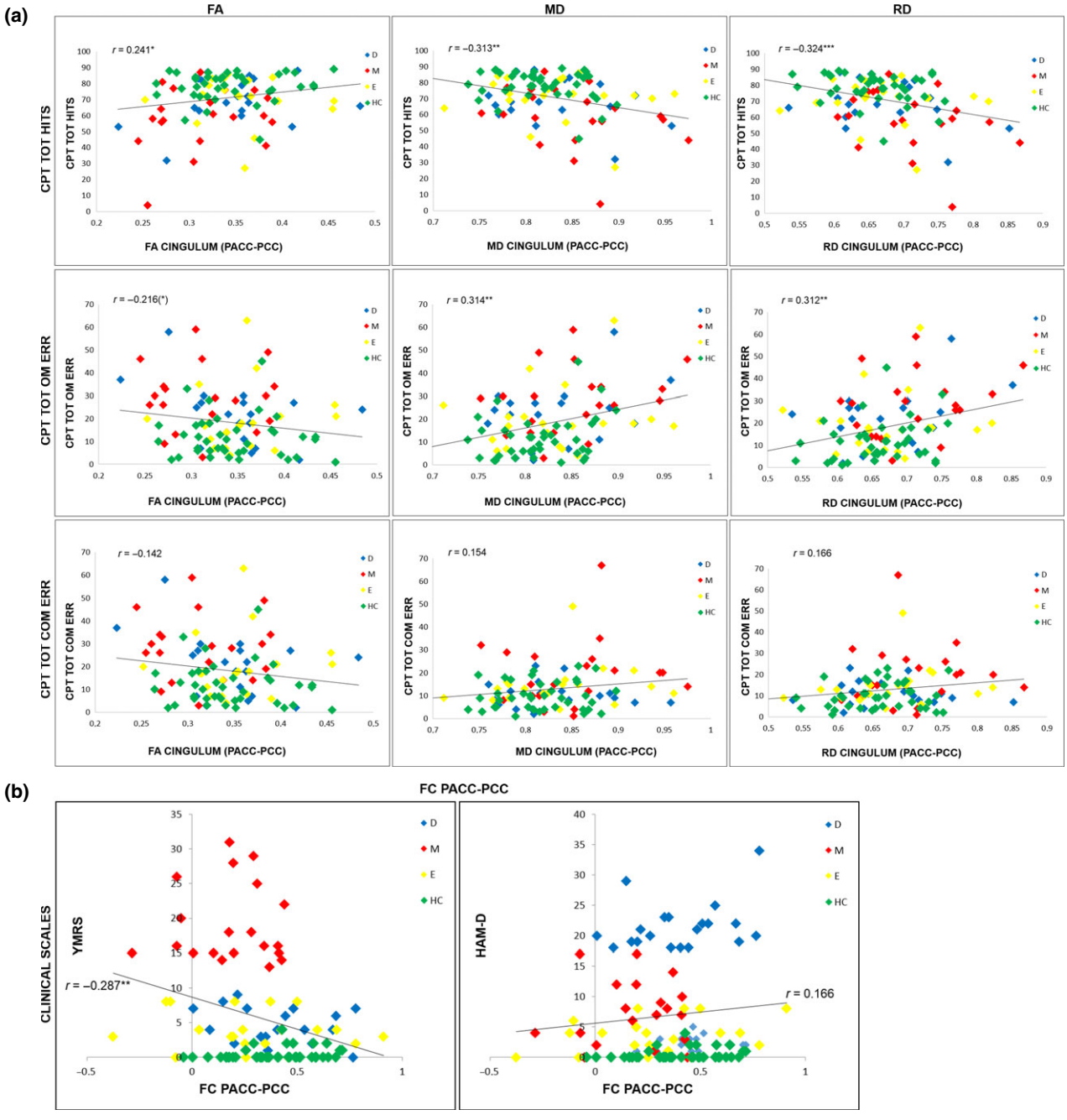

Fig. 3. (a) Correlations between neurocognitive deficits in sustained attention and structural connectivity. Partial correlations controlling for subgroup status (r) between the CPT parameters and the altered DTI parameters—mean FA, mean MD, and mean RD values—of the cingulum. MD and RD values are multiplied per 10^3. Bootstrap correction was carried out to detect outliers. All correlation results were considered significant at the Bonferroni-corrected $P < 0.05$ level. Corrected $P < 0.05$*; $P < 0.01$**; $P < 0.001$ ***. FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; CPT TOT HITS, continuous performance test—total hits; CPT OMM ERR, continuous performance test—total omission errors; CPT COM ERR, continuous performance test—total commission errors. (b) Correlations between clinical parameters and functional connectivity. Partial correlations controlling for subgroup status (r) between the clinical severity scores—YMRS and HAM-D—and the PACC-PCC FC of the cingulum. Bootstrap correction was carried out to detect outliers. All correlation results were considered significant at the Bonferroni-corrected $P < 0.05$ level. Corrected $P < 0.01$**. FC, functional connectivity; PACC, perigenual anterior cingulate cortex; PCC, posterior cingulate cortex; YMRS, Young Mania Rating Scale; HAM-D, Hamilton Depression Scale; M, manic patients; D, depressed patients; E, euthymic patients; HC, healthy controls.

cingulate midline structures of the DMN (also supported by the fact that both ROIs were taken from a DMN template), rather than between the other portions of the cingulum (that are not part of the DMN but are included in other networks such as the salience network).

Structural connectivity

Previous studies that investigated bipolar patients, without differentiation between phases of illness, clearly reported WM alterations of the cingulum (14, 15). However, previous DTI and tractography studies that mainly investigated BD patients in euthymic or remitted phases showed inconsistent results in the cingulum (4, 16–22). Some found significant differences between BD patients and HC [e.g., (20, 22, 34, 35)], while others did not [e.g., (4, 16)]. By contrast, none of the previous DTI studies included and directly compared the various phases of BD. Therefore our findings may shed some light on these inconsistencies due to the investigation of the various phases of BD. Moreover, it could address the issue of regional specificity of WM changes by including the IFOF as a control tract. We found reduced SC in the cingulum (from PACC to PCC), especially in its anterior part, in mania as distinguished from depressed and euthymic patients when compared to HC. By contrast, no significant difference was found in the IFOF SC in manic patients (but rather in depressed patients), thus confirming the specific role of the cingulum in mania. Our results are consistent with previous findings in BD in general on SC alterations in the cingulum (14, 15) and specifically in its anterior part (4, 5, 20, 36). At the same time, our findings go beyond by showing cingulum-specific structural changes specifically in mania (rather than in depressed and euthymic patients, when compared to HC). Tentatively, these findings suggest that structural changes in the cingulum are specifically associated with mania and are thus dynamic, that is, state-dependent (as related to the manic rather than depressive phase) rather than static, that is, trait-dependent (as related to BD itself rather than its specific phases). The assumption of state dependence may be further supported by the fact that we observed no structural changes in the euthymic phase as well as by the correlation of SC with state-dependent FC. This is also in accordance with previous findings that did indeed suggest acute mood states to be associated with acute state-dependent microstructural WM changes (37). On the other hand, previous evidences of structural alterations in the cingulum in euthymia are inconstant (see above), and they could also depend on the preceding phase of illness [e.g., in BD patients who remitted from mania (35)] or on a psychotic history [which can be related to more severe WM alterations (20–22, 38)], which is almost absent in our sample.

Cognitive–clinical and neuroimaging correlations

Finally, we investigated the sustained attention with CPT, which has been shown to be affected in BD, mainly in its acute phases (25). Our findings confirm sustained attention deficits in mania (rather than euthymia and possibly depression) (39) and show for the first time that they are related to structural changes in the cingulum. The more severe the structural changes in the cingulum, the higher the degree of deficits in sustained attention in mania. This result is further supported by previous findings in healthy subjects showing that the cingulum does indeed mediate sustained attention tasks (29). In contrast to cingulum SC, PACC-PCC FC did not correlate with sustained attention deficits but rather with psychopathological symptoms as measured with the YMRS. This underlines the psychopathological, that is, clinical relevance, of PACC-PCC FC changes. The more decreased the PACC-PCC FC, the stronger the manic symptoms. This correlation further points out the central role of the anterior–posterior midline FC specifically in mania, with the PACC-PCC functional disconnectivity being related to the psychopathological symptoms.

Functional–structural disconnectivity of the cingulum in mania and its cognitive–clinical relevance

What are the pathophysiological features underlying the apparently state-dependent structural changes in the cingulum in the manic phase? Acute stress and/or inflammation, which could trigger the active manic phases (40–42), can lead to alterations in oligodendroglial and myelin microstructure (43–45), which are reflected in reduced FA and concomitant increased RD (8, 18). Interestingly, the anterior cingulate was found to be altered in BD (see above) and dis/hyperconnected with stress-related regions such as the amygdala and hippocampus in euthymia (46, 47). What remains unclear at this point in time is why the manic phase specifically (rather than the depressive phase) shows structural alterations in the cingulum. Future investigations focusing on the relationship between acute stress/inflammation and cingulum connectivity in both manic and depressive phases are thus needed. In turn, alterations in SC with concomitant alterations in FC in the cingulum in mania seem to lead to cognitive disturbances and psychopathological symptoms (Fig. 4). Showing reduced FC for the anterior–posterior cingulate underlines the central role of the midline regions and the associated DMN in mania. The PACC-PCC FC is the core part of the DMN that

shows particularly high resting-state activity levels (48), high degrees of metabolism (49), and high levels of variability (50). Our findings suggest that the PACC-PCC FC is reduced entailing altered or decreased connectivity within the DMN. At the psychological level, the DMN has been associated with mind wandering (51, 52) and self-relatedness (53, 54), with the latter showing a strong neural overlap with the resting-state activity (13, 55–57). One could consequently suggest in a tentative way that manic patients suffer from alterations in mind wandering and self-relatedness: Changes in mind wandering and self-relatedness may be well manifested in psychopathological symptoms such as flight of ideas and distractibility as related to an attention pattern that is excessively focused on external stimuli at the expense of the self and its internal stimuli in mind wandering (52). Our finding of the PACC-PCC FC decrease correlating with manic symptoms as well as the cingulum SC decrease correlating with attention disturbances may lend some initial support to this tentative hypothesis, which, however, needs to be investigated on separate grounds in the future, for instance, by testing mind wandering and self-relatedness in fMRI in BD.

Limitations

The main limitation of the study is the medication confound, because almost all the patients in our sample were undergoing pharmacotherapy. However, when investigated, the medication load—that is, the number and dosage of the various medications—and each different medication class (mood stabilizers, antidepressants, antipsychotics, and benzodiazepines) did not correlate with the PACC-PCC FC or with DTI parameters of the cingulum in BD (all $P > 0.05$). Moreover, for each medication class, no significant difference in these neuroimaging parameters was found between patients who were in treatment with the respective drug and patients who were not (all $P > 0.05$). Analogously, no differences were found between lithium users and non-users. Thus, although some influence of treatment on our findings cannot be excluded, these results suggest that the changes in FC and SC, even if potentially associated with pharmacotherapy, are not the mere consequence of drug treatment in our sample (58).

Furthermore, our sample consisted of patients at different age and stages of disease. However, age and illness duration were not significantly different between subgroups and did not correlate with PACC-PCC FC or with DTI parameters of the cingulum (all $P > 0.05$).

Finally, our findings need to be confirmed in future longitudinal studies.

See the supporting information (Data S1) for a detailed description of methodology and statistical results regarding the discussion of limitations.

In conclusion, we demonstrate for the first time a concomitant reduction in the structural and functional connectivity in the cingulum in bipolar mania when compared to HC, as distinguished from depressed and euthymic patients. Moreover, cognitive deficits in sustained attention were related to structural deficits while psychopathological symptoms correlated with functional deficits in the cingulum. We tentatively hypothesize that stress and/or inflammation could trigger SC damage in the cingulum and concomitant FC changes between the PACC and PCC in mania, which seems to at least predispose the manic

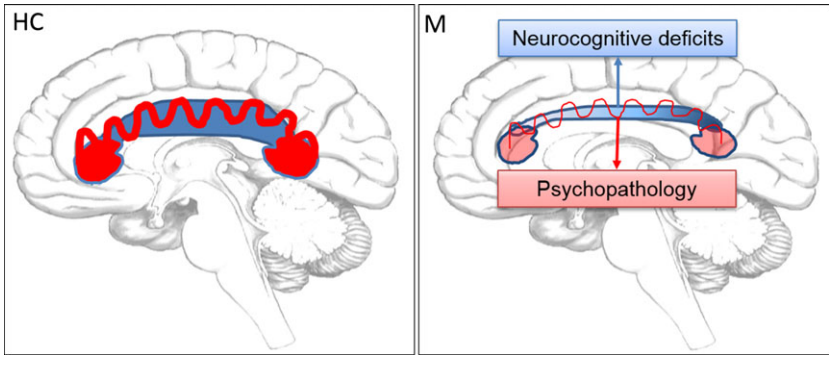

Fig. 4. Schema—Functional and structural disconnectivity in the cingulum in mania vs. controls. Schematic representation of the main findings in mania when compared to healthy controls. The PACC (anterior red sphere) shows a functional hypoconnectivity (thinner red wave) with PCC (posterior red sphere); the functional hypoconnectivity correlates with symptoms. The cingulum bundle shows a structural hypoconnectivity (thinner blue structure) mainly in its anterior part (in lighter blue); the structural hypoconnectivity correlates with neurocognitive deficits in sustained attention. PACC, perigenual anterior cingulate cortex; PCC, posterior cingulate cortex; M, manic patients; HC, healthy controls.

psychopathological symptoms. If extended and confirmed, our findings may open a novel door for a more complex pathogenetic model of mania which may have clinical, diagnostic, and therapeutic ramifications.

Acknowledgements

GN is grateful for financial support from EJLB-CIHR, the Michael Smith Foundation, and CIHR. GN is CRC Tier1 Chair University of Ottawa. MI is grateful for grant support from the Noto Foundation. The authors thank Professor Giovanni Luigi Mancardi for the access to the Magnetic Resonance Research Center of the Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Health, University of Genoa, Genoa, Italy. The authors are also grateful to Ubaldo Fanti for his technical help in imaging acquisitions.

Declaration of interest

Authors have no conflict of interest to declare.

The authors did not receive any financial support for this paper.

References

1. Soares JC, Mann JJ. The functional neuroanatomy of mood disorders. J Psychiatr Res 1997;**31**:393–432.
2. Frangou S. A systems neuroscience perspective of schizophrenia and bipolar disorder. Schizophr Bull 2014;**40**:523–531.
3. Savitz JB, Rauch SL, Drevets WC. Clinical application of brain imaging for the diagnosis of mood disorders: the current state of play. Mol Psychiatry 2013;**18**:528–539.
4. Emsell L, Leemans A, Langan C et al. Limbic and callosal white matter changes in euthymic bipolar I disorder: an advanced diffusion magnetic resonance imaging tractography study. Biol Psychiatry 2013;**73**:194–201.
5. Fountoulakis KN, Giannakopoulos P, Kovari E, Bouras C. Assessing the role of cingulate cortex in bipolar disorder: neuropathological, structural and functional imaging data. Brain Res Rev 2008;**59**:9–21.
6. Margulies DS, Kelly AM, Uddin LQ, Biswal BB, Castellanos FX, Milham MP. Mapping the functional connectivity of anterior cingulate cortex. NeuroImage 2007;**37**:579–588.
7. Vargas C, Lopez-Jaramillo C, Vieta E. A systematic literature review of resting state network-functional MRI in bipolar disorder. J Affect Disord 2013;**150**:727–735.
8. Heng S, Song AW, Sim K. White matter abnormalities in bipolar disorder: insights from diffusion tensor imaging studies. J Neural Transm 2010;**117**:639–654.
9. Strakowski SM, Delbello MP, Adler CM. The functional neuroanatomy of bipolar disorder: a review of neuroimaging findings. Mol Psychiatry 2005;**10**:105–116.
10. Ongur D, Lundy M, Greenhouse I et al. Default mode network abnormalities in bipolar disorder and schizophrenia. Psychiatry Res 2010;**183**:59–68.
11. Meda SA, Ruano G, Windemuth A et al. Multivariate analysis reveals genetic associations of the resting default mode network in psychotic bipolar disorder and schizophrenia. Proc Natl Acad Sci USA 2014;**111**:E2066–E2075.
12. Magioncalda P, Martino M, Conio B et al. Functional connectivity and neuronal variability of resting state activity in bipolar disorder-reduction and decoupling in anterior cortical midline structures. Hum Brain Mapp 2015;**36**:666–682.
13. Qin P, Northoff G. How is our self related to midline regions and the default-mode network? NeuroImage 2011;**57**:1221–1233.
14. Wise T, Radua J, Nortje G, Cleare AJ, Young AH, Arnone D. Voxel-based meta-analytical evidence of structural disconnectivity in major depression and bipolar disorder. Biol Psychiatry 2015;**79**:293–302.
15. Vederine FE, Wessa M, Leboyer M, Houenou J. A meta-analysis of whole-brain diffusion tensor imaging studies in bipolar disorder. Prog Neuropsychopharmacol Biol Psychiatry 2011;**35**:1820–1826.
16. Lin F, Weng S, Xie B, Wu G, Lei H. Abnormal frontal cortex white matter connections in bipolar disorder: a DTI tractography study. J Affect Disord 2011;**131**:299–306.
17. Benedetti F, Absinta M, Rocca MA et al. Tract-specific white matter structural disruption in patients with bipolar disorder. Bipolar Disord 2011;**13**:414–424.
18. Versace A, Andreazza AC, Young LT et al. Elevated serum measures of lipid peroxidation and abnormal prefrontal white matter in euthymic bipolar adults: toward peripheral biomarkers of bipolar disorder. Mol Psychiatry 2014;**19**:200–208.
19. Leow A, Ajilore O, Zhan L et al. Impaired inter-hemispheric integration in bipolar disorder revealed with brain network analyses. Biol Psychiatry 2013;**73**:183–193.
20. Wang F, Jackowski M, Kalmar JH et al. Abnormal anterior cingulum integrity in bipolar disorder determined through diffusion tensor imaging. Br J Psychiatry 2008;**193**:126–129.
21. Kumar J, Iwabuchi S, Oowise S, Balain V, Palaniyappan L, Liddle PF. Shared white-matter dysconnectivity in schizophrenia and bipolar disorder with psychosis. Psychol Med 2015;**45**:759–770.
22. Sarrazin S, Poupon C, Linke J et al. A multicenter tractography study of deep white matter tracts in bipolar I disorder: psychotic features and interhemispheric disconnectivity. JAMA Psychiatry 2014;**71**:388–396.
23. van Oort ES, van Cappellen, van Walsum AM, Norris DG. An investigation into the functional and structural connectivity of the Default Mode Network. NeuroImage 2014;**90**:381–389.
24. Knochel C, Stablein M, Storchak H et al. Multimodal assessments of the hippocampal formation in schizophrenia and bipolar disorder: evidences from neurobehavioral measures and functional and structural MRI. Neuroimage Clin 2014;**6**:134–144.
25. Quraishi S, Frangou S. Neuropsychology of bipolar disorder: a review. J Affect Disord 2002;**72**:209–226.
26. Poletti S, Bollettini I, Mazza E et al. Cognitive performances associate with measures of white matter integrity in bipolar disorder. J Affect Disord 2015;**174**:342–352.
27. Oertel-Knochel V, Reinke B, Alves G et al. Frontal white matter alterations are associated with executive cognitive function in euthymic bipolar patients. J Affect Disord 2014;**155**:223–233.
28. Malhi GS, Ivanovski B, Hadzi-Pavlovic D, Mitchell PB, Vieta E, Sachdev P. Neuropsychological deficits and functional impairment in bipolar depression, hypomania and euthymia. Bipolar Disord 2007;**9**:114–125.
29. Takahashi M, Iwamoto K, Fukatsu H, Naganawa S, Iidaka T, Ozaki N. White matter microstructure of the cingulum

and cerebellar peduncle is related to sustained attention and working memory: a diffusion tensor imaging study. Neurosci Lett 2010;**477**:72–76.
30. Ventura J, Liberman RP, Green MF, Shaner A, Mintz J. Training and quality assurance with the Structured Clinical Interview for DSM-IV (SCID-I/P). Psychiatry Res 1998;**79**:163–173.
31. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960;**23**:56–62.
32. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry 1978;**133**:429–435.
33. Conners CK. EPSTEIN, JN, ANGOLD, A, KLARIC, J. Continuous performance test performance in a normative epidemiological sample. J Abnorm Child Psychol 2003;**31**:555–562.
34. Canales-Rodriguez EJ, Pomarol-Clotet E, Radua J et al. Structural abnormalities in bipolar euthymia: a multicontrast molecular diffusion imaging study. Biol Psychiatry 2014;**76**:239–248.
35. Chan WY, Yang GL, Chia MY et al. Cortical and subcortical white matter abnormalities in adults with remitted first-episode mania revealed by Tract-Based Spatial Statistics. Bipolar Disord 2010;**12**:383–389.
36. Yatham LN, Lyoo IK, Liddle P et al. A magnetic resonance imaging study of mood stabilizer- and neuroleptic-naive first-episode mania. Bipolar Disord 2007;**9**:693–697.
37. Zanetti MV, Jackowski MP, Versace A et al. State-dependent microstructural white matter changes in bipolar I depression. Eur Arch Psychiatry Clin Neurosci 2009;**259**:316–328.
38. Lu LH, Zhou XJ, Keedy SK, Reilly JL, Sweeney JA. White matter microstructure in untreated first episode bipolar disorder with psychosis: comparison with schizophrenia. Bipolar Disord 2011;**13**:604–613.
39. Clark L, Goodwin GM. State- and trait-related deficits in sustained attention in bipolar disorder. Eur Arch Psychiatry Clin Neurosci 2004;**254**:61–68.
40. Bidzinska EJ. Stress factors in affective diseases. Br J Psychiatry 1984;**144**:161–166.
41. Proudfoot J, Doran J, Manicavasagar V, Parker G. The precipitants of manic/hypomanic episodes in the context of bipolar disorder: a review. J Affect Disord 2011;**133**: 381–387.
42. Hamdani N, Tamouza R, Leboyer M. Immuno- inflammatory markers of bipolar disorder: a review of evidence. Front Biosci (Elite Ed) 2012;**4**:2170–2182.
43. Chen L, Lui S, Wu QZ et al. Impact of acute stress on human brain microstructure: an MR diffusion study of earthquake survivors. Hum Brain Mapp 2013;**34**:367–373.
44. Miller VM, Lawrence DA, Mondal TK, Seegal RF. Reduced glutathione is highly expressed in white matter and neurons in the unperturbed mouse brain–implications for oxidative stress associated with neurodegeneration. Brain Res 2009;**1276**:22–30.
45. Miralbell J, Soriano JJ, Spulber G et al. Structural brain changes and cognition in relation to markers of vascular dysfunction. Neurobiol Aging 2012;**33**:1003 e9–1003 e17.
46. Wang F, Kalmar JH, He Y et al. Functional and structural connectivity between the perigenual anterior cingulate and amygdala in bipolar disorder. Biol Psychiatry 2009;**66**: 516–521.
47. Houenou J, Wessa M, Douaud G et al. Increased white matter connectivity in euthymic bipolar patients: diffusion tensor tractography between the subgenual cingulate and the amygdalo-hippocampal complex. Mol Psychiatry 2007;**12**: 1001–1010.
48. Northoff G. Unlocking the brain. New York: Oxford University Press, 2014
49. Raichle ME, Macleod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci USA 2001;**98**:676–682.
50. Lee TW, Northoff G, Wu YT. Resting network is composed of more than one neural pattern: an fMRI study. Neuroscience 2014;**274**:198–208.
51. Mason MF, Norton MI, van Horn JD, Wegner DM, Grafton ST, Macrae CN. Wandering minds: the default network and stimulus-independent thought. Science 2007; **315**:393–395.
52. Smallwood J, Schooler JW. The science of mind wandering: empirically navigating the stream of consciousness. Annu Rev Psychol 2015;**66**:487–518.
53. Northoff G, Heinzel A, de Greck M, Bermpohl F, Dobrowolny H, Panksepp J. Self-referential processing in our brain–a meta-analysis of imaging studies on the self. NeuroImage 2006;**31**:440–457.
54. Northoff G, Qin P, Feinberg TE. Brain imaging of the self–conceptual, anatomical and methodological issues. Conscious Cogn 2011;**20**:52–63.
55. Whitfield-Gabrieli S, Moran JM, Nieto-Castanon A, Triantafyllou C, Saxe R, Gabrieli JD. Associations and dissociations between default and self-reference networks in the human brain. NeuroImage 2011;**55**:225–232.
56. D'Argembeau A, Collette F, van der Linden M et al. Self-referential reflective activity and its relationship with rest: a PET study. NeuroImage 2005;**25**:616–624.
57. Schneider F, Bermpohl F, Heinzel A et al. The resting brain and our self: self-relatedness modulates resting state neural activity in cortical midline structures. Neuroscience 2008;**157**:120–131.
58. Phillips ML, Travis MJ, Fagiolini A, Kupfer DJ. Medication effects in neuroimaging studies of bipolar disorder. Am J Psychiatry 2008;**165**:313–320.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (a) Functional connectivity map: mania vs. controls. (b) Differences in functional connectivity of the various portions of the cingulum in mania, depression, euthymia and controls.

Figure S2. (a) Probabilistic tractography reconstruction of the cingulum and IFOF. (b) Differences in structural connectivity of the various portions of the cingulum in mania, depression, euthymia and controls. (c) Differences in structural connectivity of the IFOF in mania, depression, euthymia and controls. (d) Correlations between structural and functional connectivity.

Figure S3. Differences in neurocognitive tasks in mania, depression, euthymia and controls.

Table S1. Subject Demographic and Clinical Information.

Table S2. FC and SC of the Cingulum.

Table S3. (a) FC results with PACC as seed region ($x = 0$, $y = 45$, $z = 0$). (b) FC results with PCC as seed region ($x = -4$, $y = -49$, $z = 25$).

Data S1. Supplemental Methods and Material and Supplemental Limitations. A detailed description of sample, acquisition parameters, imaging processing, statistical analyses and limitations.