

Altered DLPFC–Hippocampus Connectivity During Working Memory: Independent Replication and Disorder Specificity of a Putative Genetic Risk Phenotype for Schizophrenia

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Altered connectivity of dorsolateral prefrontal cortex (DLPFC) and hippocampus during working memory is considered an intermediate phenotype for schizophrenia (SCZ), but the relevance for other mental disorders with shared genetic background remains unknown. Here we investigated its presence in unaffected first-degree relatives of patients with bipolar disorder (BD) or major depressive disorder (MDD). Furthermore, we aimed to provide an independent replication of this phenotype in first-degree relatives of SCZ patients. We acquired functional magnetic resonance imaging (fMRI) data from 309 healthy controls and 218 healthy first-degree relatives of index patients with SCZ ($n = 62$), BD ($n = 66$) and MDD ($n = 90$), who completed the n -back working memory paradigm. We observed a significant group effect on DLPFC–hippocampus coupling ($P_{\text{FWE}} = .031$, all P -values region of interest [ROI] corrected). Post hoc comparisons revealed that this effect was driven by the SCZ relatives, who showed a significant increase in the negative functional connectivity of the DLPFC and right hippocampus compared to controls ($P_{\text{FWE}} = .001$), BD relatives ($P_{\text{FWE}} = .015$) and trend-wise also MDD relatives ($P_{\text{FWE}} = .082$). Comparison of BD and MDD relatives to the controls revealed no difference (P_{FWE} -values $> .451$). Supplementary analyses suggested that the SCZ relatives finding is robust to a range of potential confounds, including structural differences. Our data further support altered DLPFC–hippocampus connectivity during working memory as an intermediate phenotype for SCZ. This suggests that this phenotype is relatively specific to

SCZ and does not translate to other genetically related disorders in the mood-psychosis spectrum.

Key words: schizophrenia/working memory/imaging genetics/intermediate phenotype/fMRI

Introduction

Schizophrenia (SCZ) is a severe brain disorder with a heritability of up to 80% and a complex genetic architecture.^{1,2} Etiological models of SCZ propose that clinical symptoms emerge as a result of a complex interplay of genetic and environmental risk factors which impact neural trajectories and functional integration during development.^{3–6} A popular strategy for dissecting the complex pathophysiology of SCZ is to investigate genetic influences on “brain-based” imaging phenotypes, which should, in principle, be closer to the genetic mechanisms of a disorder than the clinical phenotype itself.^{6,7} In particular, considerable research efforts have focused on the neuroimaging investigation of working memory deficits, which are a heritable part of the disorder and linked to poorer long-term outcomes.^{8–10}

A critical prerequisite for an imaging intermediate phenotype is that the link to the increased genetic risk for the illness is convincingly established. One line of evidence is the demonstration that the phenotype is present in an intermediate form in healthy first-degree relatives of patients.^{11,12} Within the domain of working

memory research, a particularly promising finding is that of altered functional connectivity of the dorsolateral prefrontal cortex (DLPFC) and the hippocampus in patients with SCZ. First demonstrated in 2001,¹³ this phenotype has been reported in prodromal, first-episode and chronic SCZ patients,^{13–15} their unaffected siblings,¹⁵ healthy carriers of genome-wide supported psychosis risk variants,^{15–19} and animal genetic models.²⁰ More recent investigations using transcranial magnetic stimulation (TMS) methods have provided evidence of a link of this genetic risk-related phenotype to neural plasticity.²¹

Taken together, these data suggest that DLPFC–hippocampus coupling is a phenotype suitable for the dissection of the genetic contributions to SCZ. In this work, we aimed to add to this evidence in 2 ways. First, we performed an independent replication of a link to SCZ familial risk, which is crucial in supporting the establishment of any intermediate phenotype. To this end, we aimed to replicate the presence of abnormalities in DLPFC–hippocampus connectivity in unaffected first-degree relatives of SCZ patients, as previously reported.¹⁵ Since the reliable measurement of quantitative trait markers is of crucial importance in imaging genetics,²² we closely followed the methodological approach of this group but restricted our analysis to a “seeded connectivity” approach, which showed superior reliability (intraclass correlation coefficient [ICC] (2,1): 0.50) as compared to psychophysiological interaction analysis (ICC (2,1): 0.05) based on earlier work.²¹

Furthermore, despite increasing evidence for shared genetic variance between SCZ and bipolar disorder (BD),²³ and to a lesser degree with major depressive disorder (MDD),²⁴ the question to which degree altered DLPFC–hippocampus coupling is specific to the diagnosis of SCZ has yet to be addressed. On the one hand, shared genetic variance could support a presence of this intermediate phenotype across diagnostic boundaries and would argue for a relationship of clinical features shared between these disorders, such as psychosis.²⁵ If, on the other hand, the phenotype is present only in relatives of patients with SCZ, this could indicate a relationship, on the level of genetics, to a unique portion of the genetic variance, and on the level of behavior to symptoms that are not as prominent in BD and MDD, such as cognitive symptoms. This would stimulate further transdiagnostic inquiries and inform conceptual considerations. Therefore, we tested the question of disorder specificity by extending our analysis in SCZ relatives to the assessment of unaffected first-degree relatives of patients with BD and MDD.

Methods and Materials

Subjects

We studied 527 subjects comprising 309 healthy volunteers and 218 unaffected first-degree relatives (ie, parents, siblings and children) of index patients with SCZ ($n = 62$), BD ($n = 66$) and MDD ($n = 90$). All subjects were between

18 and 63 years of age, of European ancestry and were recruited from the cities of Mannheim, Berlin and Bonn (see [table 1](#) for demographic details). None of the healthy volunteers had a first-degree relative with a history of mental illness. For the relatives groups, a trained psychiatrist or psychologist established ICD-10 and DSM-IV diagnoses of the index patients. None of the subjects fulfilled any exclusion criteria such as a lifetime history of significant general medical, psychiatric, or neurological illness, current or past psychotropic pharmacological treatment, or head trauma. Clinical assessments included the Symptom Checklist-90 (SCL-90)²⁶ and the schizotypal personality questionnaire (SPQ).²⁷ Intelligence was assessed with the German multiple-choice vocabulary intelligence test (Mehrfachwahl-Wortschatz-Test-B [MWTB]).²⁸ Trail Making Tests (TMT-A, TMT-B) were used for the assessment of processing speed and executive functioning.^{29,30} All participants provided written informed consent for a protocol approved by the local ethics committees of the Universities of Heidelberg, Bonn and Berlin.

Working Memory Task, Imaging Data Acquisition and Preprocessing

We studied brain function during working memory using the well-established n -back functional magnetic resonance imaging (fMRI) paradigm^{15,16,22,31} (supplementary materials). Whole-brain fMRI was performed on three 3T MR systems (Siemens Trio) using identical scanner protocols (supplementary materials). Functional data were acquired using echo planar imaging sequences with the following specifications: 28 axial slices, 4 mm slice thickness, 1 mm gap, repetition time (TR)/echo time (TE) = 2000/30 ms, 80° flip angle, 192 × 192 mm field of view (FOV), 64 × 64 matrix. Images were preprocessed following previously published procedures^{15,16} using standard processing routines in SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>). Briefly, all images were realigned to the first image, slice-time corrected and spatially normalized into a standard stereotactic space (Montreal Neurological Institute [MNI] template) with volume units (voxels) of 3 × 3 × 3 mm, and smoothed with a 9 mm full width at half maximum (FWHM) Gaussian kernel. Quality control analyses suggested stable scanner performance and no systematic differences of image quality between groups (supplementary materials). Head motion parameters were quantified as previously detailed,^{32,33} including frame-wise displacement according to Power et al.³⁴ Furthermore, we acquired structural data, which were analyzed using voxel-based morphometry (supplementary materials).

First-Level Modeling of DLPFC Functional Connectivity

We extracted the first eigenvariate of the seed time series from 6 mm spheres centered on the individual activation maximum in the “2-back > 0-back” contrast in the

Table 1. Sample Characteristics of Healthy Controls and First-Degree Relatives of Schizophrenia (SCZ), Bipolar Disorder (BD), and Major Depressive Disorder Patients

	Controls (<i>n</i> = 309)	SCZ Relatives (<i>n</i> = 62)	BD Relatives (<i>n</i> = 66)	MDD Relatives (<i>n</i> = 90)	<i>P</i>
Demographics and MRI-related characteristics					
Sex (female/male)	159/150	40/22	40/26	58/32	.056
Site (Bonn/Mannheim/Berlin)	137/87/85	18/22/22	33/15/18	36/30/24	.266
Handedness (right/left/both)	279/23/6	51/7/3	60/5/1	82/6/2	.713
Smoking (never/former/current)	119/73/93	28/9/18	33/13/12	41/17/18	.166
Age (y)	33.2 (9.9)	32.7 (12.4)	32.2 (12.2)	28.3 (9)	<.001*
Education (y)	15.5 (2.6)	15.2 (2.5)	16 (2.7)	15.4 (2.4)	.325
Kinship ^a (parent/child/sibling)		8/23/29	1/10/50	2/21/62	<.001*
2-back accuracy (percent correct)	78 (19)	71 (21)	76 (20)	78 (18)	.078
2-back response time (ms)	472.3 (292.2)	506.9 (287.5)	459.9 (262)	427.6 (258.5)	.372
Movement: abs. translation (mm)	5.23 (3.66)	6.56 (5.48)	5.69 (3.82)	4.84 (3.11)	.039*
Movement: abs. rotation (degrees)	4.43 (4.15)	5.44 (4.99)	5.00 (5.69)	4.19 (3.51)	.252
Movement: power's FD	0.14 (0.06)	0.16 (0.09)	0.15 (0.08)	0.13 (0.06)	.046*
Signal-to-noise-ratio ^b	94.95 (16.70)	90.24 (18.97)	93.63 (17.14)	96.23 (17.31)	.219
Signal-to-fluctuation-noise-ratio ^b	323.58 (89.95)	305.63 (109.80)	323.61 (88.75)	336.08 (90.23)	.264
(Neuro-)psychology (<i>n</i> per group)					
MWTB (300/59/64/82)	59.55 (7.40)	58.25 (8.09)	62.14 (8.44)	60.68 (7.41)	.021*
SPQ (300/61/63/84)	8.98 (7.86)	11.08 (10.80)	9.68 (6.96)	10.93 (9.51)	.137
SCL-90-R Somatization (298/61/61/82)	45.55 (7.76)	48.39 (9.44)	45.82 (8.42)	47.38 (7.48)	.039*
SCL-90-R Obsessive-compulsive (298/61/61/82)	47.14 (7.81)	47.74 (10.08)	46.00 (8.77)	47.22 (8.30)	.694
SCL-90-R Interpersonal sensitivity (298/61/61/82)	45.32 (7.40)	46.34 (9.40)	45.82 (8.97)	46.70 (8.23)	.502
SCL-90-R Depression (295/61/61/82)	45.33 (7.75)	47.11 (10.09)	45.44 (9.28)	47.00 (8.19)	.236
SCL-90-R Anxiety (295/61/61/82)	45.72 (6.90)	47.57 (8.98)	45.66 (8.12)	46.34 (6.51)	.311
SCL-90-R Hostility (291/61/61/84)	45.90 (6.85)	46.75 (8.20)	46.56 (7.19)	44.82 (10.25)	.421
SCL-90-R Phobic anxiety (297/61/61/85)	46.25 (4.67)	46.54 (5.64)	45.75 (4.41)	43.82 (9.39)	.006*
SCL-90-R Paranoid ideation (298/61/61/83)	44.81 (8.64)	45.90 (8.71)	45.44 (7.38)	43.80 (8.97)	.480
SCL-90-R Psychoticism (295/61/61/82)	45.44 (5.83)	47.44 (8.63)	46.02 (7.03)	45.55 (6.62)	.176
TMT-A (301/61/64/87)	24.48 (7.78)	25.38 (8.57)	26.89 (9.08)	23.49 (8.75)	.071
TMT-B (301/61/64/87)	52.37 (17.25)	58.91 (20.78)	54.38 (17.78)	50.99 (23.40)	.057
Volumetric data (<i>n</i> per group)					
Hippocampus volume ^c (299/58/56/78)	0.64 (0.06)	0.65 (0.04)	0.64 (0.04)	0.65 (0.05)	.135
DLPFC volume ^c (299/58/56/78)	0.50 (0.07)	0.51 (0.07)	0.53 (0.07)	0.50 (0.06)	.018*

Note: DLPFC, dorsolateral prefrontal cortex; FD, frame-wise displacement; MWTB, multiple-choice vocabulary test (T-transformed); SPQ, schizotypal personality questionnaire; SCL-90, symptom checklist 90 (T-transformed); TMT, trail making test. Categorical variables are reported as numbers of cases (note that not all categorical variables were available for all cases). Continuous variables are reported as mean and SD, and the numbers of available datasets per group were added in case of missing data. *P* values are based on an analyses of variance model with group as factor of interest. In case of significant group differences (denoted with *), we performed post hoc *t* tests and reported the results in the main text.

^aSee supplementary materials for details on kinship.

^bSee supplementary materials for details on scanner performance metrics.

^cAverage grey matter volume equivalents, see supplementary materials for details on methods.

right DLPFC. A predefined anatomical mask (BA 46 and lateral BA 9) from the Wake Forest University (WFU) PickAtlas (www.fmri.wfubmc.edu/downloads) served as a spatial constraint. Individual first-level models were defined with the subject-specific DLPFC time series as regressors of interest, and the following regressors of no interest: (1) movement parameters from the realignment step, (2) first eigenvariates derived from cerebral spinal fluid and white matter masks,^{16,19} and (3) regressors encoding for the experimental conditions. The model estimation step included a high pass filter of 128 seconds and an adjustment for the global brain signal.

Association of DLPFC–Hippocampus Connectivity With Familial Risk for Psychiatric Disorders

Group differences in the calculated seeded DLPFC functional connectivity maps were examined using random-effects group statistics at the second level. Since we aimed at the independent and regionally specific replication of the previously reported link between DLPFC–hippocampus connectivity and SCZ genetic risk,¹⁵ we defined an a priori hypothesized region of interest (ROI) within the anterior hippocampus based on the significant regional finding reported in Rasetti et al.¹⁵ To this end, we defined

a 15 mm sphere at the peak voxel coordinate of the Rasetti et al intermediate phenotype finding ($x = 15$, $y = -6$, $z = -15$), and intersected this region with an anatomical mask of the hippocampus derived from the Automated Anatomical Labeling Atlas,³⁵ resulting in an ROI comprising 49 voxels. We evaluated both research questions, ie, the replication of the link to SCZ genetic risk and the diagnostic specificity of the previously reported finding within this ROI using small volume correction at $P_{\text{FWE}} < .05$ family-wise error (FWE) corrected for multiple comparisons.

Statistical analysis was performed using an Analysis of Covariance (ANCOVA) model with group as between-subject factor, and age, sex, and site as nuisance covariates. In a first step, we assessed the main effect of group (F test: controls vs SCZ relatives vs BD relatives vs MDD relatives). Given that this analysis uncovered a significant

group effect (reported below), we further performed specific post hoc tests to (1) replicate the finding of stronger negative connectivity (ie, anti-correlation of time-courses between hippocampus and DLPFC) in SCZ relatives compared to the controls (t test: controls > SCZ relatives), and to (2) assess the specificity of this finding relative to the other diagnostic groups (t test: controls > BD relatives, controls > MDD relatives, BD relatives > SCZ relatives, MDD relatives > SCZ relatives).

Confirmatory Analyses

Since we observed significant differences in DLPFC–hippocampus coupling between controls and SCZ relatives (see results section), we further wanted to rule out the possibility that this finding related to differences in

Table 2. Sample Characteristics of the Confirmatory Analysis With Algorithmically Matched Healthy Controls and First-Degree Relatives of Schizophrenia Patients

	Controls ($n = 186$)	SCZ Relatives ($n = 62$)	P
Demographics and MRI-related characteristics			
Sex (female/male)	117/69	40/22	.939
Site (Bonn/Mannheim/Berlin)	54/66/66	18/22/22	1
Handedness (right/left/both)	165/17/4	51/7/3	.441
Smoking (never/former/current)	70/47/48	28/9/18	.199
Age (y)	32.78 (9.88)	32.69 (12.37)	.956
Education (y)	15.55 (2.53)	15.22 (2.48)	.375
2-back accuracy (percent correct)	0.72 (0.20)	0.71 (0.21)	.74
2-back response time (ms)	506.59 (295.62)	506.98 (287.56)	.993
Movement: abs. translation (mm)	5.99 (4.15)	6.56 (5.48)	.389
Movement: abs. rotation (degrees)	4.97 (4.53)	5.44 (4.99)	.487
Movement: power's FD	0.15 (0.07)	0.16 (0.09)	.208
Signal-to-noise-ratio ^a	94.69 (15.92)	90.24 (18.97)	.071
Signal-to-fluctuation-noise-ratio ^a	313.26 (92.42)	305.63 (109.80)	.592
(Neuro-)psychology (n per group)			
MWTB (178/59)	59.56 (7.66)	58.25 (8.09)	.264
SPQ (178/61)	8.72 (7.74)	11.08 (10.80)	.067
SCL-90-R Somatization (177/61)	45.32 (8.10)	48.39 (9.44)	.015*
SCL-90-R Obsessive-compulsive (177/61)	46.76 (7.59)	47.74 (10.08)	.429
SCL-90-R Interpersonal sensitivity (177/61)	44.50 (7.22)	46.34 (9.40)	.114
SCL-90-R Depression (177/61)	45.01 (7.80)	47.11 (10.09)	.094
SCL-90-R Anxiety (174/61)	45.05 (6.90)	47.57 (8.98)	.024*
SCL-90-R Hostility (172/61)	45.42 (6.74)	46.75 (8.20)	.211
SCL-90-R Phobic anxiety (177/61)	45.95 (4.19)	46.54 (5.64)	.392
SCL-90-R Paranoid ideation (177/61)	44.25 (7.83)	45.90 (8.71)	.170
SCL-90-R Psychoticism (176/61)	44.87 (5.64)	47.44 (8.63)	.009*
TMT-A (179/61)	24.35 (8.17)	25.38 (8.57)	.403
TMT-B (179/61)	52.52 (17.63)	58.91 (20.78)	.021*
Volumetric data (n per group)			
Hippocampus volume ^b (174/58)	0.65 (0.05)	0.65 (0.04)	.376
DLPFC volume ^b (174/58)	0.50 (0.07)	0.51 (0.07)	.724

Note: DLPFC, dorsolateral prefrontal cortex; FD, frame-wise displacement; MWTB, multiple-choice vocabulary test (T-transformed); SCZ, schizophrenia; SPQ, schizotypal personality questionnaire; SCL-90, symptom checklist 90 (T-transformed); TMT, trail making test. Categorical variables are reported as numbers of cases (note that not all categorical variables were available for all cases). Continuous variables are reported as mean and SD, and the numbers of available datasets per group were added in case of missing data. P values are based on 2 sample t tests.

^aSee supplementary materials for details on scanner performance metrics.

^bAverage grey matter volume equivalents, see supplementary materials for details on methods.

*Significant group differences.

the demographics, task performance or head movement between groups. To this end, we performed additional, confirmatory SPM analyses in which we compared the 62 SCZ first-degree relatives with a subset of 186 healthy controls that were matched based on age, sex, task performance, and movement (table 2), using the “optimal” matching algorithm from the MatchIt package (version 2.4, <https://cran.r-project.org/web/packages/MatchIt/MatchIt.pdf>) in R (version 3.1.3). The “optimal” matching algorithm requires a case-control ratio as an input variable and only accepts integers. We decided to match 3 controls for every SCZ relative because this gave us the most controls while also providing sufficient degrees of freedom to match all of the above-described covariates. The confirmatory analysis was calculated as ANCOVA, with group as between-subject factor and age, sex, and site as nuisance covariates. In a second confirmatory analysis, we aimed at assessing whether our result related to differences in neuropsychological variables that could not be matched between groups (table 2). We thus estimated another second-level SPM model that also controlled for these variables in addition to the standard age, sex and site covariates (complete data were available for 61 SCZ relatives and 183 healthy controls). For both confirmatory analyses, results were evaluated within the hippocampus ROI using small volume correction and were considered significant at a FWE corrected P value $< .05$.

For consistency reasons and in order to substantiate the null findings for BD and MDD relatives (see Results section), we performed the same confirmatory

analyses for BD and MDD relatives (supplementary materials).

Results

Comparison of Sample Characteristics

With respect to demographic variables, age ($P < .001$) and kinship status ($P < .001$) were significantly different between groups (tables 1 and 2). Regarding (neuro)psychological variables, we observed group differences for intelligence as measured by the MWTB as well as the somatization and phobic anxiety subscale of the Symptom Checklist questionnaire. Post hoc t tests revealed that SCZ relatives showed significantly higher somatization ($P = .012$) than controls and BD relatives performed significantly better on the MWTB intelligence test compared to controls ($P = .014$). Lastly, MDD relatives exhibited a lower level of phobic anxiety than controls ($P < .001$). With respect to measurement-related variables, SCZ relatives showed higher translational movement during the scan ($P = .018$). With respect to regional hippocampal volume, we detected no significant differences between study groups at the replication site of the functional connectivity intermediate phenotype (all P values $> .13$, tables 1 and 2). However, DLPFC volume was significantly different between groups ($P = .018$), with BD relatives showing higher values (table 1). For the samples of the first confirmatory analysis in SCZ relatives ($n = 62$) and the algorithmically matched healthy controls ($n = 186$), significantly higher scores for the SCZ relatives were detected for the SCL somatization ($P = .015$), anxiety ($P = .024$) and psychoticism ($P = .009$) subscales. The

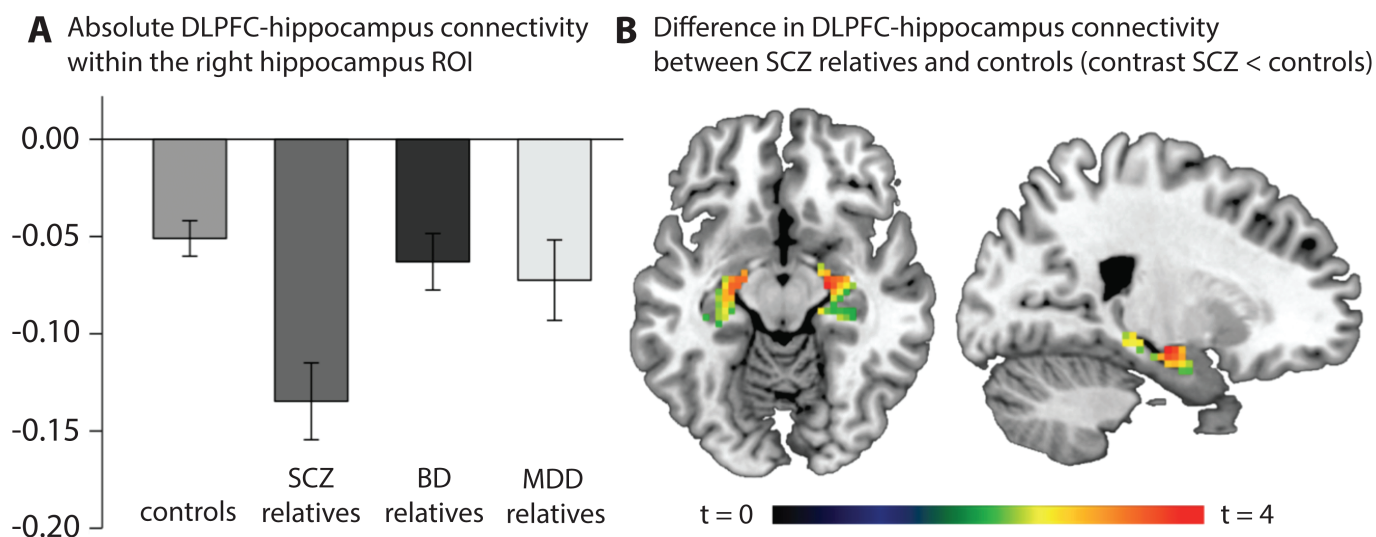


Fig. 1. Alterations in DLPFC–hippocampus functional connectivity in first-degree relatives. Panel A: Bar-plot shows mean absolute connectivity values and SEs for all 4 study groups at the voxel with the maximum group difference (ANCOVA main effect of group; $x = 21$, $y = -13$, $z = -12$, $P_{\text{FWE}} = .037$, ROI corrected). A value of zero indicates no correlation, while negative values indicate a negative correlation between compared time courses. Panel B: Difference in DLPFC–hippocampus coupling between SCZ relatives and controls (post hoc t test SCZ relatives < controls on functional connectivity between right DLPFC and hippocampus; $x = 21$, $y = -13$, $z = -12$; $Z = 3.89$, $P_{\text{FWE}} = .001$, ROI corrected). Images are thresholded at $t > 2$ for illustrative purposes. Color bars represent t values. DLPFC, dorsolateral prefrontal cortex; ROI, region of interest; SCZ, schizophrenia.

relatives group also performed worse on the TMT-B test ($P = .021$).

Alterations in DLPFC Seeded Functional Connectivity in First-Degree Relatives

The ANCOVA model revealed a significant main effect of group within the a priori defined hippocampus ROI ($x = 21, y = -13, z = -12$; $Z = 3.02, P_{\text{FWE}} = .031$, ROI corrected). The follow-up t-tests suggested that this effect was driven by the SCZ relatives group (figure 1A). Specifically, replicating the initial report of the Weinberger group,¹⁵ we detected a significantly stronger negative functional connectivity of the DLPFC with the right hippocampus ROI in SCZ relatives compared to the controls (controls > SCZ: $x = 21, y = -13, z = -12$; $Z = 3.89, P_{\text{FWE}} = .001$, ROI corrected, figures 1A and B). Notably, no similar effect was observed when the other 2 relatives groups were compared to the control group (controls > BD, controls > MDD; all Z values < 1.51, all P_{FWE} -values > .452, ROI corrected). In addition, we detected a significant difference between the SCZ and BD relatives (BD > SCZ: $x = 18, y = -13, z = -15$; $Z = 3.19, P_{\text{FWE}} = .015$, ROI corrected) and a similar trend-level difference between the SCZ and MDD relatives (MDD > SCZ: $x = 24, y = -10, z = -15$; $Z = 2.55, P_{\text{FWE}} = .082$, ROI corrected). Finally, we observed no significant differences between BD and MDD relatives (BD > MDD: $x = 18, y = -13, z = -18$; $Z = 1.24, P_{\text{FWE}} = .561$, ROI corrected). Supplementary analyses suggest that the main effect of group was unlikely influenced by potential confounds, such as kinship status (ie, parent vs sibling vs child), DLPFC and hippocampus volume, DLPFC activation, task performance, intelligence, and subject motion (supplementary materials). These observations indicate that the previously reported connectivity abnormality in SCZ first-degree relatives is indeed replicable and relatively specific to the genetic risk for SCZ.

Confirmatory Analyses

Our confirmatory analyses of the algorithmically matched SCZ and control samples (table 2) revealed an even stronger difference between the SCZ relatives and controls in the functional connectivity between the DLPFC and the right hippocampus ROI ($x = 21, y = -13, z = -12$; $Z = 4.37, P_{\text{FWE}} < .001$, ROI corrected). Moreover, in the second confirmatory analysis, we additionally controlled for all psychological variables for which group differences were observed (ie, TMT-B, SCL subscales for somatization, anxiety and psychoticism). The observed connectivity difference remained stable in this analysis as well ($x = 21, y = -13, z = -12$; $Z = 4.09; P_{\text{FWE}} = .001$, ROI corrected). These observations confirm the outcome of our initial analysis and argue against a confounding influence of these variables on the detected group difference.

Results of the analogously performed analyses in the other 2 relatives groups similarly confirmed the findings obtained by the full sample, ie, no difference in functional DLPFC–hippocampus coupling between BD or MDD relatives and the algorithmically matched controls (supplementary materials).

Discussion

The first objective of this study was to replicate the association of altered DLPFC–hippocampus connectivity in healthy first-degree relatives of SCZ patients. Demonstration of an association in healthy first-degree relatives is a core principle of the intermediate phenotype concept, since the relatives carry an enriched set of SCZ susceptibility variants³⁶ but lack many of the confounding factors that complicate the interpretation of patient data (eg, medication). The influence of these risk variants may emerge at the level of “intermediate” neural circuits that result in altered brain function without overt symptoms observed in affected individuals. In the second part of the study, we further explored a hitherto unaddressed question for this phenotype, namely whether altered DLPFC–hippocampus coupling is specific to the genetic predisposition to SCZ or, alternatively, is indicative of vulnerability to a broader spectrum of genetic disorders in the mood-psychosis spectrum.

In the first part of this study, we report significant alterations in the functional connectivity of the DLPFC with the right hippocampus in unaffected first-degree relatives of SCZ patients. This observation provides an independent replication of Rasetti et al¹⁵ and supports the contention that this phenotype is indeed related to genetic risk for the disorder. Our finding is supported by a confirmatory analysis in an algorithmically matched group of 62 first-degree relatives and 186 healthy controls controlling for influence of other between-group differences (eg, in site or task performance) in this analysis.^{34,37} The balanced study groups in this analysis, which accounted for several motion parameters, including the sum of rotational and translational movement and frame-wise displacement,³⁴ yielded even stronger statistical results than our initial analysis with unmatched groups, suggesting that our findings in SCZ relatives is unlikely explained by subject motion. In addition, we could show that the effect was not influenced by grey matter volume in the hippocampus or DLPFC, suggesting that the detected functional group differences are unlikely due to differences in local brain structure. Moreover, our result was also robust to correlation with DLPFC activation, several psychopathological variables and measurable deficits in executive functioning as defined by the TMT-B.

Taken together, these observations support the idea that alterations in DLPFC–hippocampus connectivity indeed reflect a physiological network deficit and are not an epiphenomenon of differences in overt task behavior,

measurable neuropsychological differences or pre-existing structural alterations. Finally, the localization and directionality of our finding is highly consistent with that of the healthy siblings of SCZ patients in a prior study using the same fMRI task and analogous data processing routines.¹⁵ Our findings thus provide an independent replication of this prior work, thereby corroborating the notion that altered DLPFC–hippocampus seeded connectivity reflects a functionally relevant aspect of SCZ familial risk, and further highlight the value of this imaging phenotype for the dissection of the genetic basis of the disorder.

In the second, transdiagnostic part of our study we showed that the phenotype of altered DLPFC–hippocampus coupling in SCZ relatives is not detectible in healthy first-degree relatives of patients with BD or MDD. This observation suggests that altered DLPFC–hippocampus connectivity is relatively specific to the genetic risk for SCZ and less related to the part of the genetic risk variance that is shared with the other 2 diagnostic conditions of the mood-psychosis spectrum. The lack of phenotype association with BD familial risk, in particular, is somewhat surprising given the reported association of this phenotype with common genetic variants that confer risk to both SCZ and BD,^{16–19} the high shared genetic variance between these disorders,²³ and the evidence for a mutual phenotype in an episodic memory task.²⁵ On the clinical level, this observation argues against the notion that altered DLPFC–hippocampus connectivity is more generally related to the risk for psychosis, the most prominent shared symptom between BD and SCZ. Rather, cognitive domains prominent in SCZ but not in BD and MDD should be studied. In this context recent evidence suggests an association of altered DLPFC–hippocampus coupling with the genome-wide supported risk variant for Alzheimer’s disease (AD) in the bridging integrator 1 gene (BIN1).³⁸ While speculative, this might suggest that the phenotype rather relates to persistent cognitive impairment, a core feature of both SCZ and AD but not BD and MDD. Further transdiagnostic intermediate phenotype research is needed to substantiate or refute this hypothesis.

Several limitations of our study merit discussion. Firstly, functional connectivity is correlative and thus should not be interpreted to show group differences in the causal interaction of regions, or actual anatomical connections. Secondly, while we went to great length to ensure our samples were balanced for a range of demographic, psychological, task and data quality-related confounds, given the large number of variables considered, not all variables could be properly matched between groups (ie, SCL subscale “phobic anxiety”). However, our confirmatory analyses revealed no direct association between these variables and our outcome measure. More importantly, we cannot fully exclude that our results were influenced by variables that we did not account for in our sample matching or modeling procedures. Specifically, although prior genetic studies^{15,16} suggest that this

phenotype indeed reflects a trait phenomenon linked to the genetic (rather than familial) risk for SCZ, we cannot fully rule out that our connectivity finding in SCZ relatives was influenced by the effects of a shared environment. Moreover, the slightly lower heritability for BD^{39–41} and the considerably lower heritability for MDD^{41–43} compared to SCZ^{23,40,41} could have led to a reduction in statistical power, which might in turn have contributed to the observed null finding in BD and MDD relatives. Finally, although the relatives groups were comparable in age, they might not be comparable in terms of genetic risk or resilience. More precisely, since the age at onset for SCZ and BD is earlier than for MDD,^{44,45} the findings in our SCZ and BD relatives (mean age > 30 y) might reflect effects of resilience to a higher degree than in MDD relatives (mean age < 30 y). Conversely, effects of genetic risk might be more pronounced in the MDD relatives group.

In conclusion, we replicate prior data¹⁵ in an independent sample by showing that DLPFC–hippocampus functional connectivity during working memory is compromised in healthy first-degree relatives of SCZ patients. This observation further corroborates the notion that altered DLPFC–hippocampus coupling is indeed an intermediate connectivity phenotype signaling a specific neural system mechanism of the neurogenetic risk architecture of SCZ. We further extend prior knowledge by showing that this phenotype appears to be rather specific to SCZ genetic risk, and does not appear to reflect a trait phenomenon linked to the genetic risk for psychosis in general but to persistent cognitive impairment.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin* online.

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