Altered Hippocampal-Parahippocampal Function During Stimulus Encoding
A Potential Indicator of Genetic Liability for Schizophrenia

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IMPORTANCE Declarative memory—the ability to learn, store, and retrieve information—has been consistently reported to be altered in schizophrenia, and hippocampal-parahippocampal dysfunction has been implicated in this deficit. To elucidate the possible role of genetic risk factors in such findings, it is necessary to study healthy relatives of patients with schizophrenia who carry risk-associated genes but not the confounding factors related to the disorder.

OBJECTIVE To investigate whether altered brain responses, particularly in the hippocampus and parahippocampus, during the encoding phase of a simple declarative memory task are also observed in unaffected siblings who are at increased genetic risk for schizophrenia.

DESIGN, SETTING, AND PARTICIPANTS Functional magnetic resonance imaging was used with a simple visual declarative memory paradigm to test for differences in neural activation across normal control participants, patients with schizophrenia, and their healthy siblings. This study was conducted at a research center and included a total of 308 participants (181 normal control participants, 65 healthy siblings, and 62 patients with schizophrenia); all participants were white of European ancestry.

MAIN OUTCOMES AND MEASURES All participants completed a declarative memory task involving incidental encoding of neutral visual scenes interleaved with crosshair fixation while undergoing functional magnetic resonance imaging. Differences in hippocampus and parahippocampus activation and coupling across groups and correlations with accuracy were analyzed. Analyses were repeated in pairwise-matched samples.

RESULTS Both patients with schizophrenia and their healthy siblings showed reduced parahippocampal activation (bilaterally) and hippocampal-parietal (BA 40) coupling during the encoding of novel stimuli when compared with normal control participants. There was a significant positive correlation between parahippocampal activation during encoding and the visual-memory score.

CONCLUSIONS AND RELEVANCE These results suggest that altered hippocampal-parahippocampal function during encoding is an intermediate biologic phenotype related to increased genetic risk for schizophrenia. Therefore, measuring hippocampal-parahippocampal function with neuroimaging represents a potentially useful approach to understanding genetic mechanisms that confer risk for schizophrenia.

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Cognitive dysfunction is widely recognized as a consistent and critical component of schizophrenia. Many domains of cognitive function have been reported impaired in patients with schizophrenia including deficits in working memory/executive function, verbal/visual memory, language, attention, psychomotor skills, and general intelligence. The study of cognitive impairment in schizophrenia and its neural correlates is proving to be an informative approach to understanding genetic mechanisms of risk underlying schizophrenia. Evidence from several recent meta-analyses and from large family studies suggests that cognitive impairment is familial and is likely genetically related to the risk for schizophrenia. The evidence that cognitive impairment is familial and related to genetic risk argues that it is an intermediate phenotype on the pathogenic pathway from genetic variation to the clinical syndrome.

Declarative memory is among the several cognitive domains impaired in patients with schizophrenia. Human and animal lesion studies implicate the hippocampal formation (HF)—which includes the entorhinal cortex; the dentate gyrus; Ammon’s horn subfields CA1, CA2, and CA3; the subiculum; and the parahippocampal cortex—as a key structure in declarative memory, together with a network of other brain regions such as the prefrontal and parietal cortices. The role of these brain regions in declarative memory has been implicated through functional magnetic resonance imaging (fMRI) studies that have reported blood oxygenation level-dependent (BOLD) signal changes in the HF, dorsolateral prefrontal cortex (DLPFC), and parietal cortex, particularly the inferior parietal lobule (IPL). Specifically, the HF BOLD response has been reported to correlate with effective encoding and is purported to be linked to optimal integration of information coming from several cortical areas. The role of the DLPFC during declarative memory is related to the use of different search strategies, contextualization of events, and monitoring during retrieval. The IPL is thought to play a role in the modulation of an interaction between attention and memory for forming and retrieving memory traces.

Declarative memory deficits in patients with schizophrenia are thought to be due primarily to deficient encoding through a mechanism not fully attributable either to deficits in IQ or executive function. The deficit likely reflects dysfunction in the HF, prominently implicated in the pathophysiology of schizophrenia. Indeed, converging evidence from different lines of research, including postmortem, animal, and human neuroimaging studies, implicates abnormal HF as a consistent pathological feature of schizophrenia, although its role in memory dysfunction in schizophrenia is still under debate. Two recent meta-analyses also reported decreased BOLD response in the IPL regions in patients with schizophrenia during declarative memory tasks, suggesting a more widespread dysfunction of the declarative memory network.

Because many factors are likely to underlie declarative memory dysfunction in patients with schizophrenia (including severity and type of symptoms, dose and duration of neuroleptic treatment, and numerous other confounding factors associated with the experience of the disorder), the possibility that it is associated with genetic risk for schizophrenia cannot be determined in studies of patients alone. To approach this determination, it is crucial to define whether the impairment in declarative memory and in the underlying HF-related network is a trait or a state feature of schizophrenia. If an altered HF-related network is a trait feature, and likely heritable with involvement of risk-associated genes, then patients with schizophrenia and their first-degree biological relatives, who share on average 50% of the risk genes, should resemble one another more closely than they resemble unaffected individuals. To our knowledge, so far, only 2 studies have reported impairment of HF recruitment in siblings of patients with schizophrenia during a declarative memory task. However, it is uncertain whether the abnormality observed in these prior studies represents a pure trait phenomenon or is confounded by other state variables that could bias the results such as risk for conversion to psychosis or poor memory performance in these prior samples of siblings. It also is preferable to study siblings rather than parents because age is a state factor that affects memory and brain physiology. Nevertheless, more compelling evidence for a familial—likely genetic—impairment of declarative memory performance in patients with schizophrenia is suggested from behavioral studies, which report deficits in declarative memory tasks in healthy relatives particularly siblings.

In the present study, we tested whether altered function and connectivity of brain regions underlying declarative memory, particularly during the incidental encoding of complex visual scenes, fulfilled the characteristics of a potential intermediate phenotype related to the genetic risk for schizophrenia. Differences of BOLD response during a visual scenes memory task in the hippocampus-parahippocampus, parietal, and DLPFC regions, as well as in posterior hippocampal coupling with DLPFC and IPL, were explored in a large sample of patients with schizophrenia, unaffected siblings of patients with schizophrenia, and normal control participants.

Methods

Subjects

Whole Sample

Participants ( patients with schizophrenia [P Ts], their unaffected siblings [SIBs], and normal control [NC] individuals) were recruited through advertisements to participate in the Clinical Brain Disorders Branch Sibling Study of schizophrenia at the National Institute of Mental Health (D.R.W., principal investigator). The study was approved by the institutional review board of the Intramural Program of the National Institute of Mental Health, and written informed consent was obtained after complete description of the study was provided to the participants. Exclusion and inclusion criteria have been previously reported. A detailed description of the participants is reported in eAppendix 1 in Supplement. A total of 308 participants ( NCs: 181, SIBs: 65, and PTs: 62) with good-quality fMRI data and with recognition accuracy above chance ( > 50%) during the retrieval part of the task were included in the whole-sample analysis. Two different analyses were per-
formed: (1) an analysis included all 62 PTs, 65 SIBs and 181 NCs and in which the variables that were significantly different across groups were used as covariates of no interest (Table 1) and (2) an analysis in which groups were matched for demographic and performance variables (described here).

Matched Sample
To ensure that the neuroimaging results were not driven by differences in demographic and performance characteristics across groups, all the analyses were repeated in pairwise-matched samples (Table 2). Detailed description of the procedure and the participants are reported in eAppendix 1 in Supplement.

Neuropsychological Data
Each participant was administered a battery of psychological tests within a 1- to 2-day period of the fMRI scan. Factor scores for several cognitive domains were obtained from factor analysis of 23 standard neuropsychological test scores, as previously described.29 Because the fMRI protocol involves an encoding phase only because interpretation of the retrieval phase was treated as a baseline in the fMRI analyses. Participants were not told beforehand about the subsequent recognition phase, thus making the encoding incidental. During the encoding session, participants were instructed to determine whether each picture depicted an indoor or outdoor scene and were instructed to respond via a button press—left button for indoor and right button for outdoor—through a 2-second visual instruction (indoor/outdoor) on the screen preceding each block. The retrieval session began after a brief delay (about 2 minutes) following the encoding session. During the retrieval session, participants were instructed to determine whether the scene presented was seen during the encoding session and were instructed to respond via a button press (left button for new and right button for old, as indicated by the 2-second instruction on the screen at the beginning of each block). During each retrieval session, half the scenes were old (ie, presented during the encoding session), and half were new (ie, not presented during the encoding session).

In the current study, we limited our analyses to the encoding phase only because interpretation of the retrieval phase is particularly complex because it includes both encoding and retrieval processes (since subjects viewed 50% of new scenes during retrieval, likely engaging encoding processes). Furthermore, we also restricted our analyses to neutral scenes only to circumvent potential confounds related to differences across groups in processing affective stimuli.

Both accuracy and reaction time (RT) were recorded during the scan. Accuracy during retrieval was calculated as percentage of correct responses, \(d'\) and response bias (criterion \(C\)).
Each subject was scanned on a GE Signa 3-T scanner. Details on fMRI data acquisition are reported in eAppendix 1 in Supplement.

### Analysis

**Demographic and Behavioral Data**

Analyses of variance, 2-sample t tests, and $\chi^2$ tests were used to compare continuous and categorical variables (threshold for significance $P < .05$).

<table>
<thead>
<tr>
<th>PTs vs NCs</th>
<th>Mean (SD)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>NCs (n = 54)</td>
<td>PTs (n = 54)</td>
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<tr>
<td>Sex, M/F</td>
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<td>38/16</td>
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<td>34 (10.2)</td>
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<tr>
<td>Years of education</td>
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<tr>
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<td>62 (64)</td>
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<tr>
<td>WRAT score</td>
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<tr>
<td>Neutral encoding</td>
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<td></td>
</tr>
<tr>
<td>Accuracy, % correct</td>
<td>94 (4.96)</td>
<td>93 (5.3)</td>
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<tr>
<td>RT, ms</td>
<td>1202 (186)</td>
<td>1275 (218)</td>
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<tr>
<td>$d'$</td>
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<td>2.2 (0.8)</td>
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<tr>
<td>False alarm</td>
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<td>1.5 (1.5)</td>
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<tr>
<td>Hits</td>
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<td>9.3 (1.9)</td>
</tr>
<tr>
<td>Criterion C</td>
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<td>0.21 (0.46)</td>
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<table>
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<tr>
<th>SIBs vs NCs</th>
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<th>$P$ Value</th>
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<tr>
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<td>SIBs (n = 55)</td>
</tr>
<tr>
<td>Sex, M/F</td>
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<td>22/33</td>
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<tr>
<td>Age, y</td>
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<td>Years of education</td>
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<td>16 (2.4)</td>
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<td>Handedness$^c$</td>
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<td>WAIS score</td>
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<td>108 (11)</td>
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<tr>
<td>Neutral encoding</td>
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<tr>
<td>Accuracy, % correct</td>
<td>94 (4.3)</td>
<td>94 (4.5)</td>
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<tr>
<td>RT, ms</td>
<td>1146 (188)</td>
<td>1175 (175)</td>
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<tr>
<td>Neutral retrieval</td>
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<td>$d'$</td>
<td>2.4 (0.8)</td>
<td>2.4 (0.8)</td>
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<tr>
<td>False alarm</td>
<td>1.5 (1.3)</td>
<td>1.6 (1.5)</td>
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<tr>
<td>Hits</td>
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<td>10 (1.8)</td>
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<tr>
<td>Criterion C</td>
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<td>0.05 (0.05)</td>
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<table>
<thead>
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<th>PTs vs SIBs</th>
<th>Mean (SD)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
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<td>SIBs (n = 38)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>22/16</td>
<td>22/16</td>
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<tr>
<td>Age, y</td>
<td>34 (10)</td>
<td>34 (10)</td>
</tr>
<tr>
<td>Years of education</td>
<td>14 (2)</td>
<td>16 (2)</td>
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<tr>
<td>Handedness$^d$</td>
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<td>79 (47)</td>
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<tr>
<td>WRAT score</td>
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<td>107 (7)</td>
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<tr>
<td>Neutral encoding</td>
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<td></td>
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<tr>
<td>Accuracy, % correct</td>
<td>91 (12)</td>
<td>93 (5)</td>
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<tr>
<td>RT, ms</td>
<td>1253 (256)</td>
<td>1166 (187)</td>
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<td>Neutral retrieval</td>
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<tr>
<td>$d'$</td>
<td>2.2 (0.8)</td>
<td>2.3 (0.8)</td>
</tr>
<tr>
<td>False alarm</td>
<td>1.4 (1.4)</td>
<td>1.6 (1.5)</td>
</tr>
<tr>
<td>Hits</td>
<td>9.4 (1.6)</td>
<td>9.6 (2.1)</td>
</tr>
<tr>
<td>Criterion C</td>
<td>0.2 (0.4)</td>
<td>0.1 (0.5)</td>
</tr>
</tbody>
</table>

### Abbreviations:
- F, female; M, male;
- NCs, normal controls; PTs, patients with schizophrenia; RT, reaction time;
- SIBs, siblings (healthy siblings of PTs);
- WAIS, Wechsler Adult Intelligence Scale; WRAT, Wide Range Achievement Test.

- $^a$ Significant $P$ value.
- $^b$ Data not available in 11 NCs and 1 PT.
- $^c$ Data not available in 8 NCs.
- $^d$ Data not available in 15 PTs and in 9 SIBs.

### fMRI

First-Level Processing:
Images were processed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm). In the first-level analyses, linear contrasts were computed for each participant producing $t$-statistical parameter maps at each voxel for encoding of neutral visual stimulus relative to fixation. A detailed description of the preprocessing methods is included in eAppendix 1 in Supplement.
Psychophysiological Interaction | Psychophysiological interaction (PPI) analyses for encoding neutral greater than baseline conditions were run with seeds placed separately in the left and right posterior hippocampal regions. Specifically, we created an anatomical mask of the hippocampus. This anatomical mask was created using the Wake Forest University PickAtlas toolbox version 2.4 (http://fmri.wfubmc.edu/software/PickAtlas). We then divided the mask in 2 equal parts, and we used the posterior part as seed because the main effect of activation during incidental encoding of the visual scenes was predominantly in the posterior hippocampus (vide infra). This procedure was done separately for the left and right posterior hippocampus. A detailed description of the PPI analysis procedure is included in eAppendix 1 in Supplement.

Second-Level General Linear Model Analysis | For across-group differences in the whole sample (including all NCS, PTs, and SIBs with quality-control-screened fMRI and performance data, although not completely matched between groups [Table 1]), individual contrasts were entered into random-effects analyses of covariance (ANCOVAs), with sex, years of education, Wide Range Achievement Test score, and RT during encoding as covariates of no interest. For the 3 pairwise-matched group analyses (NCS vs PTs, NCS vs SIBs, and PTs vs SIBs), individual contrasts were entered into ANCOVA models in SPM5 (Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) using years of education, the variable the groups were not matched for, as a covariate of no interest. One-sample t tests were used as masks in the second-level analyses to identify brain responses associated with task conditions across all participants. A statistical threshold of \( P < .05 \) with false-discovery rate (FDR)\(^3\) at whole-brain level was used to identify significant differences in BOLD signal and coupling across groups. Given our a priori hypothesis for altered function of brain regions underlying declarative memory in PTs,\(^2\) a statistical threshold of \( P < .05 \), FDR corrected within regions of interest (ROIs), was also accepted to investigate BOLD-signal differences across groups in the HF (hippocampus and parahippocampus), DLPFC (BA 9 and 46), and IPL (BA 40, inferior parietal lobule). These ROIs were created using the Wake Forest University PickAtlas toolbox version 2.4. We reported results obtained using separate ROIs for these a priori brain regions, as well as those obtained using a combined large ROI that included all these a priori brain regions (HF-DLPFC-IPL).

Differences across groups in the functional coupling of HF with other regions in the brain, and specifically with the DLPFC and IPL, were explored. A statistical threshold of \( P < .05 \), FDR corrected at the whole-brain level and within the a priori-defined DLPFC and IPL ROIs, was used to signify differences in functional coupling of the HF across groups. We reported results obtained using separate ROIs for these a priori brain regions, as well as those obtained using a combined large ROI that included both DLPFC and IPL regions (DLPFC-IPL).

Correlation of Hippocampal-Parahippocampal Activity and Hippocampal Coupling With Behavioral Measures

To explore the relationship between hippocampal-parahippocampal activity and hippocampal coupling with behavioral measures, a simple regression analysis was performed within SPM5 entering single-subject first-level contrast maps for activation and PPI (for encoding neutral scenes > fixation cross), with \( d' \), visual memory, and working-memory factor scores as covariates of interest. Analyses with \( d' \) included all participants. Analyses with visual-memory factor scores and working-memory factor scores were limited to participants with available visual memory (NCS: 78, PTs: 50, and SIBs: 53) and working memory (NCS: 78, PTs: 37, and SIBs: 48) scores.

Relationship Between Altered Hippocampal-Parahippocampal Function During Declarative Memory and Other Cognitively Linked Physiological Intermediate Phenotypes

We aimed to explore whether the finding of decreased activity/coupling of HF during declarative memory, as tested during incidental encoding of visual scenes (vide infra), is a unique marker of increased genetic risk for schizophrenia distinct from the previously reported neuroimaging intermediate phenotypes, such as altered DLFPC activity\(^3\) and DLFPC-HF coupling\(^2\) during working memory and altered anterior cingulate cortex activity during response inhibition,\(^2\) or whether conversely it was mapping a redundant phenomenon. To determine this, we investigated a subgroup of NCS and SIBs who had fMRI data for both the working-memory task and the SDMT, and for both the response-inhibition task and the SDMT. Details are reported in eAppendix 1 in Supplement.

Results

Whole Sample

Demographic and Behavioral Results

Patients with schizophrenia differed from the other 2 groups in sex, Wide Range Achievement Test score, years of education, RT during encoding, and RT and accuracy during retrieval (Table 1). All these variables, except performance during retrieval (RT and accuracy), were included as covariates of no interest in analysis of the BOLD fMRI data.

Imaging Results: Activation

Consistent with previous studies, task-related activation included bilaterally the HF, the DLPFC, the ventrolateral prefrontal cortex, the medial frontal cortex, the visual cortices, the parietal cortex, the basal ganglia, the thalamus, and the right premotor/motor cortices\(^2\) (Figure 1A in Supplement). Analysis of covariance \( F \) test with diagnosis as a factor revealed BOLD-signal differences across groups in the bilateral parahippocampus, in the left inferior and superior parietal lobules, and in the left precentral and postcentral gyrus (all \( P_{\text{FDR-whole-brain}} < .05 \) [Figure 1A and Table 3]). Specifically, both PTs and SIBs showed decreased BOLD signal in the parahippocampus when compared with NCS (Table 3) (posthoc \( t \) test NCS > SIBs: left HF: \( x = −18, y = −36, z = −6 [Z = 4.02, P_{\text{FDR-HF-ROI}} = .003, \text{ and } P_{\text{FDR-HF-DLPFC-IPL-ROI}} = .009]; \) right HF: \( x = 30, y = −24, z = −15 \).
**Matched Samples**

**Demographic and Behavioral Results**

**PTs vs NCs** | A sample of NCs (n = 54) taken from the larger sample of 181 participants were strictly pairwise matched with PTs (n = 54) in age, sex, and accuracy in retrieval of neutral scenes (Table 2). Variables that differed between groups (years of education) were used as covariates of no interest in the neuroimaging analyses.

**SIBs vs NCs** | A subsample of NCs (n = 55) taken from the larger sample of 181 participants was strictly pairwise matched for age, sex, and accuracy with SIBs (n = 55). The 2 groups did not differ in the other demographic variables, except for years of education (P = .006), which were used as covariates of no interest in the neuroimaging analyses (Table 2).

**PTs vs SIBs** | A subsample of SIBs (n = 38) taken from the larger sample of 65 SIBs was strictly pairwise matched for age, sex, and accuracy, with a subsample of 38 PTs (derived by the larger...
sample of 55). The 2 groups still differed in years of education (P < .001), which was used as a covariate of no interest (Table 2).

**Imaging Results: Activation**

**PTs vs NCs** | Patients with schizophrenia showed lower activation in the parahippocampus bilaterally when compared with NCs (right HF: x = 24, y = −27, z = −9 [Z = 3.59, P_{FDR,HF,ROI} = .01, and P_{FDR,HF,DLPC,FPLC,ROI} = .06]; left HF: x = −24, y = −36, z = −9 [Z = 3.44, P_{FDR,HF,ROI} = .01, and P_{FDR,HF,DLPC,FPLC,ROI} = .06]), despite similar accuracy and using similar strategies as reflected by similar response bias (C, P = .62) (Figure 1B and Table 2). No other differences were observed across groups or with the opposite contrast (PTs > NCs).

**SIBs vs NCs** | Similar to PTs, SIBs showed lower activation in the parahippocampus compared with NCs (left HF: x = −18, y = −36, z = −9 [Z = 3.69 and P_{FDR,HF,ROI} = .06]), despite performing as well as NCs and using similar strategies (response bias C, P = .79) (Figure 1C and Table 2). No significant results were observed in other brain areas or with the opposite contrast (SIBs > NCs).

**PTs vs SIBs** | There was a trend toward significance (FDR corrected) for decreased parahippocampal and increased IPL BOLD signals in PTs when compared with SIBs (right HF: x = −21, y = −39, z = −12 [Z = 3.15 and P_{FDR,HF,ROI} = .12]; left IPL: x = −54, y = −33, z = 55 [Z = 3.30 and P_{FDR,IPL,ROI} = .06]; right IPL: x = 54, y = −26, z = 54 [Z = 2.82 and P_{FDR,IPL,ROI} = .06]). This was observed despite PTs performing and using strategies similar to SIBs, as assessed using the response bias measure (performance during encoding >91% in both groups and no difference in response bias, P = .26) (Table 2).

**Functional Coupling of Hippocampal Regions (PPI) Main Effect**

The effect of the encoding process on coupling between the left/right posterior hippocampal regions and the rest of the brain is shown in eFigure 2 in Supplement (detailed description in Appendix 2 in Supplement).
in posterior hippocampal coupling with other brain areas, including the DLPCF, or with the opposite contrast (PTs > NCs).

**SIBs vs NCs** | Siblings demonstrated decreased left hippocampal-bilateral IPL coupling when compared with NCs, similar to the pattern observed in PTs (left hippocampal-right IPL: x = 39, y = −48, z = 42 [Z = 3.66 and P_{FDR} = .02]; left IPL-right IPL: x = 39, y = −48, z = 42 [Z = 3.66 and P_{FDR} = .02]; graph in the middle: contrast estimates from the significant voxel). A similar decrease was observed in the left posterior hippocampus/right IPL functional coupling (left IPL: x = −60, y = −36, z = 45 [Z = 3.63 and P_{FDR} = .05]; graph on the right: contrast estimates from the significant voxel). Similar results were observed when seed was located in the left posterior hippocampus (see text for details).

**PTs vs SIBs** | No significant differences were observed between SIBs and PTs.

**Correlation of Hippocampal-Parahippocampal BOLD Signal During Encoding of Neutral Scenes and Behavioral Outcome**

Within each sample, there was a positive correlation between visual-memory factor scores and hippocampal-parahippocampal BOLD signal and a negative correlation between visual-memory factor scores and IPL BOLD signal (Table 4; Figure 3 represents the scatterplot of this correlation in the sample of NCs). Similarly, a negative correlation between *d*’ (accuracy during retrieval) and left IPL BOLD signal was observed within each sample (NCs: x = −36, y = −57, z = 48 [Z = 3.19 and P_{FDR} = .04]; trend in PTs: x = −48, y = −45, z = 57 [Z = 2.81 and P_{uncorrected} = .002]; trend in SIBs: x = −57, y = −54, z = 39 [Z = 2.79 and P_{uncorrected} = .003]).

Except for a trend for a positive correlation in the sample of SIBs (SIBs: x = 15, y = −30, z = −15 [Z = 2.70 and P_{uncorrected} = .004]), no significant correlations (positive or negative) were observed between hippocampal-parahippocampal activation and working-memory factor scores.

**Relationship Between Altered Hippocampal-Parahippocampal Function During Declarative Memory and Other Cognitively Linked Physiological Intermediate Phenotypes**

The data on the SDMT suggest that reduction in parahippocampal BOLD signal and hippocampal coupling with IPL could be a potential intermediate phenotype related to genetic risk for schizophrenia. We had previously reported parallel findings in the prefrontal cortex and its coupling with the hippocampus during a working-memory task28,33 and in the cingulate cortex and its link to the prefrontal cortex during a cognitive-control task.34 While these tasks engage relatively segregated neural systems, many of the participants were the same across these studies. This raises the possibility that the various findings may not be independent. To explore whether altered parahippocampal activation and hippocampal
coupling during the incidental encoding phase of SDMT is independent from altered DLPFC activation and DLPFC-hippocampal coupling observed during a working-memory task,28,33 we investigated a subgroup of NCs and SIBs that had good-quality imaging data for both the working-memory task and the SDMT and performed intraclass correlation (ICC) analyses. Specifically, within this group, we explored whether there were any correlations between the neuroimaging intermediate phenotypes observed with the working-memory task and the neuroimaging intermediate phenotype observed with the SDMT. Demographic and performance data of the 2 subgroups are reported in eTable 1 in Supplement. Briefly, the groups consisted of 56 NCs and 44 SIBs with similar demographic and performance (2-back for the working-memory task and \( d' \) for the SDMT) characteristics. Although to a less significant threshold given the small sample size, we observed differences in DLPFC BOLD response and DLPFC-HF coupling in the sample of SIBs compared with NCs during the working-memory task similar to the data previously reported (ie, SIBs showed increased BOLD signal in the DLPFC and decreased coupling between the right DLPFC-hippocampus compared with NCs; ANCOVA with years of education as the covariate of no interest; eTable 1 in Supplement), as well as differences in parahippocampal BOLD signal and hippocampal coupling in the sample of SIBs compared with NCs during the SDMT (ie, SIBs showed reduced HF BOLD signal and HF-IPL coupling explored with PPI compared with NCs; ANCOVA with years of education as the covariate of no interest; eTable 1 in Supplement) similar to the data observed in the bigger sample. We then performed correlation analyses (ICCs) on the signals

### Table 4. Correlation of BOLD Signal During Encoding of Neutral Scenes and Behavioral Outcome (Visual-Memory Factor Scores)

<table>
<thead>
<tr>
<th>Correlation</th>
<th>MNI Coordinates (X, Y, Z)</th>
<th>Z</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive correlation (contrast +1) for hippocampal formation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCs</td>
<td>−21, −15, −12</td>
<td>2.88</td>
<td>.002*</td>
</tr>
<tr>
<td>PTs</td>
<td>−12, −33, −18</td>
<td>2.78</td>
<td>.003*</td>
</tr>
<tr>
<td>SIBs</td>
<td>18, −15, −15</td>
<td>2.64</td>
<td>.004*</td>
</tr>
<tr>
<td></td>
<td>−18, −36, −6</td>
<td>2.37</td>
<td>.009*</td>
</tr>
<tr>
<td>Negative correlation (contrast −1) for inferior parietal lobule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCs</td>
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<td>4.00</td>
<td>.01b</td>
</tr>
<tr>
<td>PTs</td>
<td>51, −39, 51</td>
<td>3.11</td>
<td>.07 a and .001a</td>
</tr>
<tr>
<td>SIBs</td>
<td>36, −51, 42</td>
<td>1.97</td>
<td>.03a</td>
</tr>
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</table>

Abbreviations: BOLD, blood oxygen level–dependent; MNI, Montreal Neurological Institute; NCs, normal controls; PTs, patients with schizophrenia; SIBs, siblings (healthy siblings of PTs).

* Uncorrected P value.

b P value false-discovery rate–corrected within inferior parietal lobule regions of interest.

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**Figure 3. Scatterplot**

Scatterplot illustrates the positive correlation between hippocampal formation engagement and visual-memory scores in the sample of normal control participants. au indicates arbitrary unit. \( N = 78, r = 0.33, \) and \( P = .003. \)
extracted from these significant peak voxels to explore potential correlations between these intermediate phenotypes. We repeated a similar analysis also on the first eigenvalue obtained from the cluster. All ICCs (analyzed both with the signal extracted from the significant cluster and the most significant voxel within the cluster) were nonsignificant (all \( P > .40 \); ICC, 2 to 1; range, \(-0.4\) to \(0.4\)).

To explore whether altered parahippocampal activation and hippocampal coupling during the incidental encoding phase of SDMT is independent from altered anterior cingulate cortex activation observed during a cognitive-control task, 24 we investigated a subgroup of NCs and SIBs that had good-quality imaging data for both the cognitive-control task and the SDMT, and we performed ICC analyses. Demographic and performance data of the 2 subgroups are reported in eTable 2 in Supplement. The groups consisted of 113 NCs and 38 SIBs similar for demographic and performance (\% correct for the response-inhibition task and \( d'\) for the SDMT) characteristics. We observed differences in anterior cingulate BOLD response in the sample of SIBs compared with NCs during the response-inhibition task similar to the data previously reported (ie, SIBs showed decreased BOLD signal in the anterior cingulate compared with NCs; 2-sample \( t\) test; eTable 2 in Supplement), as well as differences in parahippocampal BOLD signal and hippocampal-IPL coupling in the sample of SIBs compared with NCs during the SDMT (ie, SIBs showed reduced parahippocampal BOLD signal and hippocampal-IPL coupling explored with PPI compared with NCs; 2-sample \( t\) test; eTable 2 in Supplement) similar to the data observed in the bigger sample. We then performed correlation analyses (ICCs) on the signals extracted from these significant peak voxels to explore potential correlations between these intermediate phenotypes. We repeated a similar analysis also on the first eigenvalue obtained from the cluster. All ICCs (analyzed both with the signal extracted from the significant cluster and the most significant voxel within the cluster) were nonsignificant (all \( P > .10 \); ICC, 2 to 1; range, \(-0.15\) to \(0.2\)).

### Discussion

In this study, we found that activation of the parahippocampus and hippocampal coupling with IPL during the incidental encoding phase of the SDMT are impaired both in PTs and their healthy SIBs. These results suggest that hippocampal-parahippocampal dysfunction in schizophrenia is a familial, likely heritable, trait.

#### Hippocampal-Parahippocampal Function and Visual-Memory Capacity

Consistent with the long-standing and well-accepted view of hippocampal-parahippocampal dysfunction in PTs, 16 we observed decreased posterior hippocampus-parahippocampus function, reflected as decreased parahippocampal activity and hippocampal coupling during the encoding of neutral visual stimuli in our patient sample. This is consistent with most fMRI studies reporting decreased hippocampal-parahippocampal recruitment during declarative memory tasks in schizophrenia (for a meta-analysis, see article by Achim and Lepage 22).

One crucial question is whether the hippocampal-parahippocampal dysregulation and memory deficits observed in schizophrenia are state or trait characteristics of the disorder. Our results suggest the latter. Indeed, our findings reported decreased hippocampal-parahippocampal function during encoding even in a sample of healthy SIBs of PTs well matched for potential confounding factors with the sample of NCs. To our knowledge, so far, only 2 studies have explored hippocampal-parahippocampal abnormalities during declarative memory with fMRI in genetically high-risk populations. 24,25 Both found abnormal hippocampal-parahippocampal recruitment similar to our results, although the differences in clinical status, 24 sex, and performance 25 of the high-risk participants vs the comparison group of NCs left unresolved whether the observed deficit was due to familial/genetic background or was due to these other confounding factors. Our findings are also consistent with the results of 2 magnetic resonance spectroscopic imaging studies that found reduced N-acetylaspartate—an in vivo marker for neuronal synaptic abundance—in the hippocampus both in PTs and their SIBs 37,38 and with those from behavioral studies that reported deficient performance on declarative memory tests in unaffected relatives. 5

Our consistent observation in each participant group of a positive correlation between the hippocampal-parahippocampal BOLD response during our declarative memory task and visual-memory ability measured with standard neuropsychological tests suggests that the visual-memory network identified with our task has real-world implications for declarative memory capacity. Our data also suggest that the hippocampal-parahippocampal function deficit is a discrete pathophysiological characteristic of the risk state independent of the risk associated with working-memory function. Studies of declarative memory and executive cognition in the same patients also suggest that these deficits are independent phenomena. 35,39 Our lack of finding a significant correlation between the BOLD response elicited during working memory and declarative memory, as well as during response-inhibition trials and declarative memory, support the conclusion that these are not redundant physiologic characteristics of the higher genetic risk state. As previously, we have shown that these phenotypes are largely independent, with any redundancy likely small, according to our ICC analyses. As we have argued previously, 28 this is not a surprising result because it is logical that the neural systems manifestations of increased genetic risk for schizophrenia will be diverse. This result also suggests that individual risk factors may show effects selectively on one or another of these phenotypes. 28 Additionally, while the hippocampal-parahippocampal region showed both reduced activity and reduced coupling with both IPL and DLPFC in PTs and their SIBs, these differences were observed in the context of 2 very different cognitive processes—the reduced activation and coupling with the IPL was observed during incidental encoding of complex scenes and the reduced coupling with the DLPFC was observed during a working-memory task—supporting the notion that diverse neurobiological mechanisms, presumably mediated by diverse genetic mechanisms, could be involved.
Abnormal Posterior Hippocampal Coupling With Inferior Parietal Lobule and Correlation With Cognitive Indices

Activation of the IPL was negatively correlated with visual-memory performance within each group (NCs, SIBs, and PTs), and both patients and healthy SIBs showed a significant decrease in the coupling between the hippocampus and IPL when compared with NCs. There are studies reporting an association between ventral parietal cortex (supramarginal and angular gyrus, i.e., IPL) function and encoding failure. During encoding of items that are subsequently forgotten, greater activation of the IPL has been reported. It has been hypothesized that this apparent increase in activity of the IPL during an attention-demanding task reflects a brief lapse in attention or mind wandering. This interpretation is consistent with our results. Within each group, we found that greater IPL activation was associated with worse visual-memory ability (and d’), suggesting a disruptive effect of IPL activation in the ability to effectively encode the visual information. While it may appear counterintuitive that IPL activation was negatively correlated with visual-memory performance in the presence of positive hippocampal-IPL coupling, it should be noted that PPI analysis measures the temporal coherence of the activity between brain regions across the time series irrespective of the amplitude of activity in these brain regions. Therefore, it is possible that while the averaged amplitude of IPL activity negatively correlates with visual-memory performance across participants, its activity across the time series could positively correlate with that of the hippocampus, reflecting positive functional coupling.

Interestingly, in PTs, there was increased activation in the left precentral and postcentral gyri when compared with SIBs and NCs. This is in keeping with the previously reported increased activation in the left sensorimotor cortex in PTs. It is likely the increased RT, reflecting the increased processing time in the PTs in the unmatched sample, may underlie this.

Limitations

One of the limitations in the current study was the block design of the SDMT, which limited our ability to disentangle the BOLD response related to successful encoding from that related to unsuccessful encoding. Future studies using an event-related design may help differentiate these 2 processes in greater detail. However, at the group level, our participants were well matched for overall performance. Another limitation was the relatively low spatial resolution of the fMRI data acquisition precluding us from clearly defining the origin of the BOLD signal—whether from the hippocampus or parahippocampal region—during the SDMT. Future studies with higher spatial resolution may help study the role of these regions, which are well known to be anatomically and functionally distinct, in the pathophysiology of schizophrenia. While decreased attentional and motivational factors could contribute to reduced hippocampal-parahippocampal engagement, it is a less likely explanation for our results as all participants, including patients and SIBs, performed above chance (>50% accuracy) during both the encoding and retrieval sessions and were matched for performance, suggesting that they were attending to the task as well as the healthy control participants. Finally, we cannot exclude the possibility that our functional results could be driven by structural differences in hippocampus/parahippocampus volumes across groups. Further studies using multimodal imaging techniques, including fMRI, high-resolution structural MRI, and diffusion tensor imaging, within the same individuals are required to disentangle this issue in greater detail. However, we would note that studies of hippocampal volume in healthy SIBs of PTs have not revealed volume differences in the hippocampus.

Conclusions

We reported evidence of impaired parahippocampal recruitment and impaired posterior hippocampal coupling with the parietal cortex during visual-memory encoding in PTs and in their healthy SIBs. This impairment is related to visual-memory ability and is not redundant with intermediate risk-associated phenotypes linked to working memory and prefrontal cortex and response inhibition and the cingulate cortex. Therefore, measuring hippocampal function through neuroimaging represents another potential intermediate phenotype that could prove useful in identifying neural system mechanisms for genetic susceptibility and as a potential biomarker for intervention.
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REFERENCES