40-Hz Auditory Steady-State Responses Characterize Circuit Dysfunctions and Predict Clinical Outcomes in Clinical High-Risk for Psychosis Participants: A Magnetoencephalography Study

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ABSTRACT

BACKGROUND: This study aimed to examine whether 40-Hz auditory steady-state responses (ASSRs) are impaired in participants at clinical high-risk for psychosis (CHR-P) and predict clinical outcomes.

METHODS: Magnetoencephalography data were collected during a 40-Hz ASSR paradigm for a group of 116 CHR-P participants, 33 patients with first-episode psychosis (15 antipsychotic-naïve), a psychosis risk–negative group (n = 38), and 49 healthy control subjects. Analysis of group differences of 40-Hz intertrial phase coherence and 40-Hz amplitude focused on right Heschl’s gyrus, superior temporal gyrus, hippocampus, and thalamus after establishing significant activations during 40-Hz ASSR stimulation. Linear regression and linear discriminant analyses were used to predict clinical outcomes in CHR-P participants, including transition to psychosis and persistence of attenuated psychotic symptoms (APSs).

RESULTS: CHR-P participants and patients with first-episode psychosis were impaired in 40-Hz amplitude in the right thalamus and hippocampus. In addition, patients with first-episode psychosis were impaired in 40-Hz amplitude in the right Heschl’s gyrus, and CHR-P participants in 40-Hz intertrial phase coherence in the right Heschl’s gyrus. The 40-Hz ASSR deficits were pronounced in CHR-P participants who later transitioned to psychosis (n = 13) or showed persistent APSs (n = 34). Importantly, both APS persistence and transition to psychosis were predicted by 40-Hz ASSR impairments, with ASSR activity in the right hippocampus, superior temporal gyrus, and middle temporal gyrus correctly classifying 69.2% individuals with nonpersistent APSs and 73.5% individuals with persistent APSs (area under the curve = 0.842), and right thalamus 40-Hz activity correctly classifying 76.9% transitioned and 53.6% nontransitioned CHR-P participants (area under the curve = 0.695).

CONCLUSIONS: Our data indicate that deficits in gamma-band entrainment in the primary auditory cortex and subcortical areas constitute a potential biomarker for predicting clinical outcomes in CHR-P participants.

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The brain’s endogenous rhythmic activity represents a fundamental feature of large-scale circuits that has been implicated in cognition and behavior as well as in psychiatric syndromes, such as schizophrenia (ScZ) (1). One way to probe neural oscillations is through entrainment by exogenous sources, such as visual or auditory stimuli or brain stimulation (2). Current theories suggest that exogenous entrainment interacts with ongoing oscillations of neural circuits that could provide possible treatment targets for brain disorders (3).

The auditory steady-state response (ASSR) is an oscillation that is entrained to the frequency and phase of temporally modulated auditory stimuli (4). ASSRs typically show a peak frequency at around 40 Hz, suggesting an auditory resonant frequency (5). Magnetoencephalography (MEG), positron emission tomography, intracranial recordings, and functional magnetic resonance imaging studies located the generators of the 40-Hz ASSR in medial areas of the primary auditory cortex (6) as well as in the hippocampus (7), thalamus, and brainstem regions (8–10).

One important application of the 40-Hz ASSR has been in ScZ because of the potential importance of gamma-band (30–200 Hz) oscillations in explaining cognitive deficits (10). Gamma-band oscillations have been hypothesized to establish communication between distributed neuronal ensembles (11) and their impairment to underlie the pronounced cognitive and perceptual dysfunctions in ScZ (12). This view is consistent with data indicating that rhythm-generating parvalbumin-
positive (PV+) GABA (gamma-aminobutyric acid) interneurons and NMDA receptors (NMDARs) are impaired in ScZ (13), highlighting the potential of gamma-band oscillations to provide insights into circuit abnormalities.

Currently, there is robust evidence that both amplitude and phase of 40-Hz ASSRs are impaired in ScZ (14,15) which have been linked to deficits in GABAergic neurotransmission (16). There is mixed evidence, however, for the presence of 40-Hz ASSR deficits in both individuals at a clinical high-risk for psychosis (CHR-P) (17–20) and patients with first-episode psychosis (FEP) (19,21). In addition, it is unclear whether 40-Hz ASSRs could constitute a possible biomarker for the early detection and diagnosis of emerging psychosis. This is important because CHR-P participants, who are identified on the basis of attenuated psychotic symptoms (APSs), brief limited intermittent psychotic symptoms, or functional decline with genetic risk (22) as well as self-experienced perceptual and cognitive disturbances known as basic symptoms (23), are characterized by cognitive deficits (24) and impairments in social and role functioning (25) that predict clinical outcomes (26,27). In addition, only a minority of CHR-P participants (~20% over a 3-year period) will develop a psychotic episode (28).

To address the potential of 40-Hz ASSRs as a biomarker for the early detection and diagnosis of emerging psychosis, we applied a state-of-the-art MEG approach to examine 40-Hz ASSRs in CHR-P participants as well as in patients with FEP. MEG is characterized by an improved signal-to-noise ratio for measurements of high-frequency oscillations compared with electroencephalography (EEG) (29) and is ideally suited for source reconstruction. Source-level estimation of 40-Hz ASSR has been shown to be higher at source level than at sensor level (30,31). Crucially, source estimation allows the unmixing of signal contributions from different source generators that are obscured at sensor level by individual differences in source orientation and field spread (32).

In this study, we investigated MEG-recorded virtual channel 40-Hz ASSRs in a group of 116 CHR-P participants and 33 patients with FEP as well as in 38 participants with substance abuse and affective disorders (clinical high-risk for psychosis–negative group [CHR-N]) and compared spectral power and phase-coherence measures with 49 healthy control (HC) subjects. We predicted that FEP and CHR-P groups would be characterized by a circumscribed dysfunction of 40-Hz ASSRs in the auditory cortex and subcortical brain regions, which would be closely linked to clinical outcomes. Specifically, we hypothesized that impaired 40-Hz ASSRs would predict persistence of APSs as well as transition to psychosis in CHR-P participants.

METHODS AND MATERIALS

Participants
A total of 236 participants were recruited from the Youth Mental Health Risk and Resilience Study (33) and divided into four groups: 1) 116 participants meeting CHR-P criteria; 2) 38 participants with nonpsychotic disorders, such as affective disorders (n = 11), anxiety disorders (n = 16), eating disorders (n = 1), and/or substance abuse (n = 10) (CHR-N); 3) 33 patients with FEP (15 antipsychotic [AP]-naïve); and 4) 49 HC participants without an Axis I diagnosis or family history of psychosis.

CHR-P status at baseline was established by ultra-high risk criteria according to the Comprehensive Assessment of At-Risk Mental States (CAARMS) Interview (22) and the Cognitive Disturbances and Cognitive-Perceptive Basic Symptoms criteria according to the Schizophrenia Proneness Instrument, Adult version (SPI-A) (23). Patients with FEP were assessed with the Structured Clinical Interview for DSM-5 (34) and with the Positive and Negative Syndrome Scale (35). For all groups except patients with FEP, cognition was assessed with the Brief Assessment of Cognition in Schizophrenia (BACS) (36). See Tables 1–3 for demographic and clinical data.

The study was approved by the ethical committees of the University of Glasgow and the National Health Service Research Ethics Committee Greater Glasgow & Clyde. All participants provided written informed consent.

Clinical Follow-up
Participants meeting CHR-P criteria were reassessed at intervals of 3, 6, 9, 12, 18, 24, 30, and 36 months to examine persistence of ultra-high risk criteria and transition to psychosis. Persistence of ultra-high risk criteria was operationalized by the continued presence of APSs up to 12 months in this study (see Supplement and Table S1).

Stimuli and Task
Auditory stimuli were 1000-Hz carrier tones (duration: 2 s), amplitude modulated (AM) at 40 Hz (31), presented binaurally through inner ear tubes, with an interstimulus interval of on average 2 seconds (jittered between 1.5 and 2.5 s, equal distribution). Participants were instructed to fixate a translucent screen (viewing distance: 75 cm). Participants received one block of 100 of 40-Hz AM tones (ripple tones) (Figure 1A). To control for potential attentional differences, 10 additional identical sounds with equal intensity levels over time (flat tones) were interspersed, serving as targets to respond to by button press. These target trials were not included in the analyses of the MEG data.

Neuroimaging
MEG data were acquired at baseline after clinical and neuropsychological assessments from a 248-channel 4D-BTI magnetometer system (MAGNES 3600 WH; 4D-Neuroimaging, San Diego, CA), recorded with 1017.25-Hz sampling rate and DC-400 Hz online filtered, T1 anatomical scans (3D magnetization prepared rapid acquisition gradient-echo sequences) were collected on a Siemens Trio Tim 3T scanner (Siemens Corp., Erlangen, Germany) (192 slices, voxel size 1 mm³, field of view = 256 × 256 × 176 mm³, repetition time = 2250 ms, echo time = 2.6 ms, flip angle = 9°) for subject-specific source localization of MEG activity.

Task Performance
Analysis of task data included percentage of correctly detected flat-tone targets, mean reaction times of correct responses, and false alarms.

40-Hz ASSRs During Emerging Psychosis
Table 1. Demographics, Clinical Data, and Task Performance

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HC</th>
<th>CHR-N</th>
<th>CHR-P</th>
<th>FEP</th>
<th>Group Effect*</th>
<th>Post Hoc Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Participants</td>
<td>49</td>
<td>38</td>
<td>116</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, Years, Mean (SD)</td>
<td>23 (3.6)</td>
<td>23 (4.7)</td>
<td>22 (4.5)</td>
<td>24 (4.5)</td>
<td>H3 = 10.1, p = .018</td>
<td>FEP &gt; CHR-P: p = .015</td>
</tr>
<tr>
<td>Sex, Male/Female, n (% Male)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education, Years, Mean (SD)</td>
<td>17 (3.0)</td>
<td>16 (3.5)</td>
<td>15 (3.2)</td>
<td>15 (2.8)</td>
<td>H3 = 9.9, p = .019</td>
<td>CHR-P &lt; HC: p = .032</td>
</tr>
<tr>
<td>BACS, Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal memory</td>
<td>52 (8.7)</td>
<td>0.01 (1.1)</td>
<td>-0.36 (1.3)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit sequencing</td>
<td>21 (2.1)</td>
<td>0.14 (1.2)</td>
<td>-0.16 (1.5)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Token motor</td>
<td>81 (11.6)</td>
<td>-0.66 (1.1)</td>
<td>-1.01 (1.3)</td>
<td>NA</td>
<td>H2 = 20.7, p &lt; .001</td>
<td>CHR-P &lt; HC: p &lt; .001</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>59 (13.9)</td>
<td>-0.22 (1.0)</td>
<td>0.05 (1.3)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symbol coding</td>
<td>74 (11.8)</td>
<td>0.00 (1.3)</td>
<td>-0.58 (1.1)</td>
<td>NA</td>
<td>H2 = 15.8, p &lt; .001</td>
<td>CHR-P &lt; HC: p &lt; .002</td>
</tr>
<tr>
<td>Tower of London</td>
<td>19 (1.7)</td>
<td>0.15 (1.3)</td>
<td>-0.15 (1.5)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite score</td>
<td>304 (24.2)</td>
<td>-0.15 (1.2)</td>
<td>-0.62 (1.4)</td>
<td>NA</td>
<td>H2 = 9.6, p = .008</td>
<td>CHR-P &lt; HC: p = .014</td>
</tr>
<tr>
<td>CAARMS, Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unusual thought content</td>
<td>0 (0.1)</td>
<td>1 (1.2)</td>
<td>2 (2.0)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonbizarre ideas</td>
<td>0 (0.4)</td>
<td>1 (1.1)</td>
<td>3 (1.8)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceptual abnormalities</td>
<td>0 (0.5)</td>
<td>1 (1.3)</td>
<td>3 (1.5)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disorganized speech</td>
<td>0 (0.1)</td>
<td>1 (0.9)</td>
<td>1 (1.4)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total severity score</td>
<td>1 (2.4)</td>
<td>6 (6.1)</td>
<td>30 (18.0)</td>
<td>NA</td>
<td>H2 = 125.2, p &lt; .001</td>
<td></td>
</tr>
<tr>
<td>GAF, Mean (SD)</td>
<td>88 (6.4)</td>
<td>70 (12.8)</td>
<td>58 (13.8)</td>
<td>39 (13.7)</td>
<td>H3 = 140.8, p &lt; .001</td>
<td></td>
</tr>
<tr>
<td>GF Role, Mean (SD)</td>
<td>8.6 (0.8)</td>
<td>8.1 (0.8)</td>
<td>7.4 (1.2)</td>
<td>NA</td>
<td>H2 = 50.5, p &lt; .001</td>
<td></td>
</tr>
<tr>
<td>GF Social, Mean (SD)</td>
<td>8.8 (0.4)</td>
<td>8.2 (0.8)</td>
<td>7.4 (1.3)</td>
<td>NA</td>
<td>H2 = 62.0, p &lt; .001</td>
<td></td>
</tr>
<tr>
<td>Medication, n (%)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>48 (98%)</td>
<td>27 (71%)</td>
<td>60 (52%)</td>
<td>14 (42%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>0</td>
<td>11 (29%)</td>
<td>46 (40%)</td>
<td>15 (45%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mood stabilizers</td>
<td>0</td>
<td>0</td>
<td>5 (4%)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>0</td>
<td>0</td>
<td>2 (2%)</td>
<td>18 (55%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 (2%)</td>
<td>2 (5%)</td>
<td>17 (15%)</td>
<td>7 (21%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEG Trials, Total Included, Mean (SD)</td>
<td>88 (5.4)</td>
<td>87 (6.6)</td>
<td>86 (5.8)</td>
<td>83 (7.7)</td>
<td>H3 = 16.3, p = .001</td>
<td>FEP &lt; HC: p = .019 FEP &lt; CHR-N: p = .019</td>
</tr>
</tbody>
</table>

Task Performance

| Hit rate, % correct (SD)         | 99.6% (2.0%) | 99.2% (2.7%) | 99.5% (2.2%) | 98.2% (7.3%) | H3 = 9.6, p = .048 | FEP < HC: p = .044 |
| False alarms, % errors (SD)      | 0.23% (1.03%) | 0.33% (0.79%) | 0.35% (0.82%) | 0.69% (1.68%) | H3 = 7.9, p = .048 |                      |
| Mean RT, ms (SD)                 | 479 (97.5) | 499 (122.9) | 503 (129.2) | 577 (190.8) |               |                      |

MEG Data Analysis

MEG data were analyzed with MATLAB (2013b; The MathWorks, Inc., Natick, MA) using the open-source FieldTrip Toolbox version 20161023 (http://www.fieldtriptoolbox.org/). Epochs of 4 seconds duration (1-s baseline), time-locked to sound onset, were extracted for the task-irrelevant 40-Hz AM tones only (excluding false alarm trials). Line noise contamination was attenuated with a discrete 50-Hz Fourier transform filter. Faulty sensors with large signal variance or flat signals were removed, and data were downsampled to 300 Hz. Artifact-free data were created by removing trials with excessive transient muscle activity, slow drift, or superconducting quantum interference device jumps using visual

BACS, Brief Assessment of Cognition in Schizophrenia; CAARMS, Comprehensive Assessment of At-Risk Mental States; CHR-N, clinical-high-risk negative; CHR-P, CHR positive; FEP, first-episode psychosis; GAF, Global Assessment of Functioning; GF, global functioning; HC, healthy control; MEG, magnetoencephalography; NA, not assessed; RT, reaction time.
*Except for sex statistical testing, which are based on χ² tests, all other tests are based on nonparametric Kruskal-Wallis H-tests: α = 0.05, two-sided, adjusted for ties, post hoc Bonferroni-corrected for multiple comparisons.
*3BACS scores for clinical groups were standardized to control group data, controlled for sex.
*Multiple categories possible.

40-Hz ASSRs During Emerging Psychosis
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Table 2. FEP-Specific Clinical Data

<table>
<thead>
<tr>
<th>Psychopathology</th>
<th>FEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANSS, Positive (Mean (SD))</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20 (8.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>16 (9.2)</td>
</tr>
<tr>
<td>Cognitive</td>
<td>21 (9.2)</td>
</tr>
<tr>
<td>Excitement</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>Depression</td>
<td>11 (5.6)</td>
</tr>
<tr>
<td>Total score</td>
<td>77 (28.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANSS, Positive (Mean (SD))</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>11 (5.8)</td>
</tr>
<tr>
<td>Cognitive</td>
<td>16 (9.2)</td>
</tr>
<tr>
<td>Excitement</td>
<td>21 (9.2)</td>
</tr>
<tr>
<td>Depression</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>Total score</td>
<td>42 (15.6)</td>
</tr>
</tbody>
</table>

**PANSS, Positive and Negative Syndrome Scale; SCID-IP, Structured Clinical Interview for DSM-5 Axis I Disorders-Patient Edition.**

**FEP, first-episode psychosis; NOS, not otherwise specified; PANSS, Positive and Negative Syndrome Scale; SCID-IP, Structured Clinical Interview for DSM-5 Axis I Disorders-Patient Edition.**

**Statistical Analyses**

**Main Effects of Stimulation and Group.** Main effects of stimulation were determined from dynamic imaging of coherent sources whole-brain activity, using Monte Carlo permutation-based dependent-sample t test (α = 0.05, one-sided, false discovery rate [FDR]-corrected) across all 236 participants, with baseline and stimulus-induced 40-Hz ASSR power as paired samples. Main effects of group included linear regression analysis (backward-selection method, α = 0.05, two-sided, 1000 sample bootstrapped) on 40-Hz amplitude and 40-Hz ITPC virtual channel data (250–2000 ms averaged relch data) from all regions significantly entrained to the 40-Hz stimulation.

**Group Differences.** Group differences in MEG-included trial numbers, behavioral task performance, and demographic and clinical as well as cognition data were assessed with nonparametric Kruskal-Wallis tests, α level 0.05, two-sided, with post hoc pairwise comparisons.

Table 3. CHR- and HC-Specific Clinical Data

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI-A, n (%) [COGDIS/COPER/both items]</td>
<td>0</td>
</tr>
<tr>
<td>CAARMS, n (%) [APS-/GRFD-criteria]</td>
<td>0</td>
</tr>
<tr>
<td>CAARMS+SPI-A, n (%) [COGDIS/COPER/both items]</td>
<td>0</td>
</tr>
<tr>
<td>SPI-A Severity, Mean (SD)</td>
<td></td>
</tr>
</tbody>
</table>

**MINI Categories, n (%)**

- Depressive/mood disorders 0 (0%) 11 (29%) 75 (85%)
- Anxiety disorders/PTSD/ODD 0 (0%) 16 (42%) 85 (73%)
- Drug/alcohol abuse/dependence 2 (4%) 10 (26%) 39 (33%)
- Eating disorders 0 (0%) 1 (2%) 9 (8%)

**APS, attenuated psychotic symptom; CAARMS, Comprehensive Assessment of At-Risk Mental States; CHR-N, clinical-high-risk negative; CHR-P, CHR positive; COGDIS, Cognitive Disturbances; COPER, Cognitive-Perceptive basic symptoms criterion; GRFD, genetic risk and functional decline; HC, healthy control; MINI, Mini-International Neuropsychiatric Interview; OCD, obsessive-compulsive disorder; PTSD, posttraumatic stress disorder; SPI-A, Schizophrenia Proneness Instrument, Adult version.**

**Multiple categories possible for comorbidities.**
Correlational and Classification Analyses of CHR-P Individuals. Correlations between 40-Hz ASSR data and clinical/cognitive baseline assessment data (CAARMS severity, CAARMS perceptual abnormality, SPI-A severity, SPI-A acoustic perceptual disturbances, Global Assessment of Functioning [GAF], global functioning [GF] role and social, BACS token motor, symbol coding, and composite scores) in the CHR-P group were investigated using linear regression.

In addition, linear discriminant analyses (LDAs) were conducted (SPSS version 26; IBM Corp., Armonk, NY) to assess classification accuracy of 40-Hz ASSR data for APS persistence and transition to psychosis in CHR-P participants. The analyses included the following contrasts: 1) APS-P (n = 34) versus APS-NT (n = 39) and 2) CHR-P-T (n = 13) versus CHR-P-NT (n = 97), using ITPC and amplitude measures from all ten 40-Hz entrained ROIs as dependent variables. The 40-Hz ASSR data were z-normalized to HC subjects. LDA results were cross-validated with a leave-one-out method. For both models, Wilks’ lambda/$\chi^2$ tests, standardized canonical discriminant function coefficients, and a receiver operating characteristic curve were evaluated.

RESULTS

Demographic/Clinical Data and Task Performance

The CHR-P group consisted of 30 participants who met SPI-A criteria, 31 participants with CAARMS criteria, and 55 participants who had a combination of SPI-A and CAARMS criteria (Table 3). The FEP group included significantly more male participants than the HC ($p = .004$), CHR-N ($p = .002$), and CHR-P groups ($p < .001$) (Table 1). Patients with FEP were...
Follow-up Outcomes

Follow-up data were available for 110 of the 116 CHR-P individuals (see Supplement). Thirty-four CHR-P participants continued to meet APS criteria at 12 months (APS-P), whereas 39 CHR-P participants did not (APS-NP). Compared with APS-NPs, the APS-P group scored significantly higher on CAARMS severity ($p < .001$) at baseline ($p = .028$) (Table S2). A total of 13 participants (11.2%) made a transition to psychosis after a mean follow-up period of 18 months (Table S3). Transitioned CHR-P-T participants had significantly lower GAF ($p = .034$) and GF social scores ($p = .023$) at baseline than the CHR-P-NT group.

Main Effect of 40-Hz ASSR Stimulation and Main Group Effects

Across all groups, 40-Hz AM tones induced increased sustained (250–2000 ms) 40-Hz power (Figure 1B, lower panel) in 10 brain regions ($F$ values = 2.5–4.6, $p = .002$, 95% CI = −0.042 to 0.005, FDR corrected), including RHES, RSTG, RMTG, right Rolandic operculum, right supramarginal gyrus, RHIP, right parahippocampal gyrus, RTHA, left Rolandic operculum, and left supramarginal gyrus. For these ROIs, virtual channel time-series data were computed using linearly constrained minimum variance beamformers (42), with normalized lead fields and a regularization parameter of 20% to attenuate leakage from nearby sources. Time series were computed separately for each voxel within each ROI and then combined into one time series per ROI using the first singular value decomposition component across the single-voxel data. Data were then submitted to time-frequency Fourier analysis (frequency resolution 0.25 Hz, sliding window of 500 ms, step size 25 ms, Hanning tapered), and ITPC was computed. In addition, 40-Hz ASSR-amplitude responses were computed by bandpass filtering single-trial virtual channel data with a sharp Butterworth filter (range 39.6–40.4 Hz, two-pass, roll-off = 4) (see Figure 1A). The filtered data were enveloped and averaged across trials. All virtual channel data were expressed as relch from baseline activity (−500 to 0 ms).

To reduce data dimensionality, linear regression analyses (backward selection) on 40-Hz amplitude and ITPC virtual channel data were used to select a limited set of ROIs from the 10 stimulus-entrained ROIs for post hoc analyses. For the 40-Hz ITPC data, a significant model was found including RHES activity only ($F_{1,234} = 5.15$, $p = .024$, adjusted $R^2 = 0.017$). For 40-Hz amplitude data, however, a combination of all ROIs except the LROL contributed significant variance to explaining main group differences ($F_{8,235} = 2.17$, $p = .031$, adjusted $R^2 = 0.045$). An additional Bonferroni correction was applied to these data ($\alpha$ level adjusted to 0.0055 [0.05/9 ROIs], which left a model for main group differences containing only RHES, RSTG, RTHA, and RHIP ($F_{4,231} = 3.95$, $p = .004$, adjusted $R^2 = 0.048$).

Post Hoc Group Differences at Sensor Level

Sensor-level data showed stimulus-induced 40-Hz power and ITPC increases over right and left frontal-temporal sensors (Figure S1). Compared with the HC group, both power and ITPC in the 40-Hz range were reduced over right temporal sensors (Figure S1) in CHR-P and FEP but not in the CHR-N group. However, these differences were not significant.

Post Hoc Group Differences at Source Level

At source level, post hoc estimation statistics (Figure 2A, B; Table S4) of 40-Hz amplitude data from RHES, RSTG, RTHA, and RHIP as well as 40-Hz ITPC data from RHES were used to evaluate differences between clinical groups (CHR-N, CHR-P, and FEP) and the HC group. Compared to the HC group, 40-Hz amplitude in RTHA and RHIP was significantly reduced in both FEP and CHR-P participants (RTHA-FEP: $p = .028$, uncorrected, $d = 0.52$; RHIP-FEP: $p = .037$, uncorrected, $d = 0.49$; RTHA-CHR-P: $p = .003$, FDR corrected, $d = 0.47$; RHIP-CHR-P: $p = 0.19$, uncorrected, $d = 0.37$). In addition, the FEP group was impaired in RHES amplitude ($p = .044$, uncorrected, $d = 0.48$), while CHR-P participants were impaired in RHES ITPC ($p = .043$, uncorrected, $d = 0.34$). No impairments were found for CHR-N participants.

Correlations Between MEG and Clinical/Neurocognitive Data in CHR-P Participants

RSTG amplitude was significantly correlated ($F_{3,112} = 3.8$, $p = .012$) with three predictors: CAARMS perceptual abnormalities ($\beta = −0.181$, $t = −1.8$, $p = .052$, 95% CI = −0.33 to 0.01), GAF scores ($\beta = −0.278$, $t = −2.6$, $p = .011$, 95% CI = −0.21 to −0.03), and GF social scores ($\beta = 0.241$, $t = 2.2$, $p = .026$, 95% CI = 0.13 to 2.08). There were no correlations in RSTG (or other MEG measures) with measures of psychosocial functioning and cognition, including BACS scores, GF role, SPI-A severity, SPI-A acoustic perceptual disturbances, or CAARMS severity.

40-Hz ASSR and Follow-up Outcomes in CHR-P Participants

Compared to the HC group, the APS-P group (Figure 3; Table S4) was impaired in 40-Hz amplitude in RTHA ($p = .014$, uncorrected, $d = 0.57$) and RHIP ($p = .002$, FDR corrected, $d = 0.72$), whereas the nonpersistent-APS-NP group was impaired only in RHES ITPC ($p = .012$, uncorrected, $d = 0.54$). Furthermore, APS-P and APS-NP groups differed significantly from each other in RHIP ($p = .003$, FDR corrected, $d = 0.70$) but not in RTHA ($p = .193$).

The transitioned CHR-P-T group also showed pronounced impairments in RTHA and RHIP, compared with the HC group (RTHA: $p = .008$, FDR corrected, $d = 1.02$; RHIP: $p = .011$, uncorrected, $d = 0.88$), with additional impairments in RHES-
ITPC signal ($p = .039$, uncorrected, $d = 0.76$). Weaker impairments were found in RTHA for the CHR-P-NT group ($p = .018$, uncorrected, $d = 0.40$). Compared with the CHR-P-NT individuals, transitioned CHR-P-T individuals were significantly impaired in RTHA ($p = .049$, uncorrected, $d = 0.72$), but not in RHIP ($p = .057$).

Figure 2. Main group differences in the 40-Hz auditory steady-state response (ASSR) signals. (A) Top row are group averaged 40-Hz amplitude data (relative change from baseline) from right hemisphere Heschl’s gyrus (RHES), right thalamus (RTHA), and right hippocampus (RHIP), with error bars representing SEM. Stimulus onset at time 0 and offset at 2000 ms. Below the respective Cumming estimation plots with data distribution swarm plots and group difference data distributions (compared with healthy control [HC] subjects). $^*$False discovery rate–corrected significant contrasts. (B) As panel (A) but for intertrial phase coherence (ITPC) data in RHES. In addition, time-frequency plots of ITPC data are shown for RHES for HC and the contrasts of HC with all three main clinical groups. Data represent relative change (relch) from baseline, similar to the amplitude data. (C) The main four regions of interest for which virtual channel data were computed and statistically examined for group differences. CHR-N, clinical-high-risk negative; CHR-P, CHR positive; FEP, first-episode psychosis; RSTG, right superior temporal gyrus.
Effects of AP Medication on 40-Hz ASSRs

We investigated potential effects of AP medication on 40-Hz ASSR amplitude in patients with FEP (non-AP-medicated FEPs: \(n = 15\); AP-medicated FEPs: \(n = 18\)). No significant differences were found between groups (RHES: \(p = .77\); RTHA \(p = .64\), and RHIP \(p = .28\)).

Classification of APS Persistence and Transition in CHR-P Participants

LDA with an embedded stepwise identification and removal of nonsignificantly contributing predictors was used to classify and predict clinical outcomes of CHR-P individuals based on MEG data.

LDA analyses of persistent (\(n = 34\)) vs nonpersistent-APS (\(n = 39\)) groups revealed a significant linear model \((F_{3,72} = 9.9, p < .001)\) including RHIP amplitude \((b = 20.347, t = 23.1, p = .002, 95\% \text{ CI} = -0.46 \text{ to } -0.10)\), RMTG amplitude \((b = -0.199, t = -1.7, p = .085, 95\% \text{ CI} = -0.22 \text{ to } -0.02)\), and RSTG ITPC \((b = 0.460, t = 4.3, p < .005, 95\% \text{ CI} = 0.15 \text{ to } 0.42)\). The three predictor-based classification model was significant (Wilks' lambda = 0.698, \(\chi^2_{3} = 25.0, p < .001\), area under the curve = 0.845), with standardized canonical discriminant function coefficients of 0.714 for RHIP amplitude, 0.426 for RMTG amplitude, and 20.948 for RSTG ITPC. A cross-validated total of 27 of 39 APS-NP (69.2%) and 25 of 34 APS-P (73.5%) participants were correctly classified.

For the 97 nontransitioned (CHR-P-NT) and 13 transitioned (CHR-P-T) participants, a significant linear regression model was found \((F_{1,108} = 4.0, p = .048)\), including only RTHA amplitude. The classification model was also significant (Wilks' lambda = 0.964, \(\chi^2_{1} = 3.9, p = .048\), area under the curve = 0.695). A cross-validated total of 52 of 97 CHR-P-NT (53.6%) and 10 of 13 CHR-P-T (76.9%) participants was correctly classified.

In summary, while persistence of APS is predicted by 40-Hz ASSR impairments in RHIP, RSTG, and RMTG, transitioning to FEP is predicted by RTHA impairments.

MEG Activity in Subcortical Regions

Estimation of subcortical activity potentially suffers from source leakage from cortical sources and/or effects of depth-bias corrections. To validate the RTHA and RHIP results, we therefore examined group differences also in left thalamus and left hippocampus. There were no main group differences in these ROIs (left thalamus: CHR-N, \(p = .31\), CHR-P, \(p = .41\), FEP, \(p = .08\); left hippocampus: CHR-N, \(p = .06\), CHR-P, \(p = .73\), FEP, \(p = .45\)).

Figure 3. 40-Hz auditory steady-state response amplitude impairments in clinical-high-risk–positive (CHR-P) subgroups. Cumming estimation plots of
DISCUSSION

Robust evidence exists for impairments in 40-Hz ASSRs in patients with ScZ (14), which is consistent with evidence for alterations in neural circuits that are involved in the generation of gamma-band rhythms, such as PV+ interneurons (13,16) and NMDAR-mediated neurotransmission (43). However, it is currently unclear whether 40-Hz ASSRs are impaired during emerging psychosis and can predict clinical outcomes. To address these fundamental issues, we implemented a state-of-the-art MEG approach to examine which brain regions contribute toward 40-Hz ASSR deficits in a large CHR-P sample and to determine whether such impairments predict persistence of APS-status and transition to psychosis.

Consistent with our hypothesis, we found overlapping reductions in 40-Hz ASSRs in FEP and CHR-P participants in RHES, RTHA, and RHIP, consistent with the prominent contribution of 40-Hz ASSR generators in the right hemisphere (44). Moreover, we found evidence for circumscribed correlations between 40-Hz ASSR amplitudes in the RSTG and severity of APS as well as impaired functioning in the CHR-P group. Deficits in RHES were in line with previous observations in ScZ that localized 40-Hz ASSR impairments to the primary auditory cortex and superior temporal cortex (45,46), although deficits have also been observed in the left hemisphere (14). These results contrast, however, with recent EEG studies that found intact 40-Hz ASSRs in both CHR-P (19,20) and FEP (19,47) groups.

One reason for our different findings may constitute the larger sample size in this study. We also implemented a novel analysis approach that involved narrow bandpass filtering of single-trial MEG activity, which, compared with more typically used time-frequency analyses, is less affected by trade-offs in time/frequency resolution. Importantly, the use of MEG allowed us to compute virtual channel source-level ASSRs from cortical and subcortical regions entrained to the stimulation frequency, thus increasing signal-to-noise ratio of ASSR estimates (31).

Interestingly, our whole-brain source-level approach revealed impairments beyond cortical auditory processing areas in CHR-P and FEP groups in RTHA and RHIP. The significant thalamic contribution toward 40-Hz ASSR deficits is consistent with several lines of evidence. Thalamic 40-Hz ASSRs have been observed with positron emission tomography, functional magnetic resonance imaging, and EEG/MEG (8,9,48). Furthermore, experimentally induced thalamic evoked gamma oscillations have been shown to have a direct effect on the auditory cortex ASSR responses to click trains (49). Similarly, the hippocampus exhibits an intrinsic 40-Hz rhythm that can be entrained by both internal network (50) and external signals such as ASSR stimulation (7,51).

Importantly, there is consistent evidence for circuit changes implicating deficits in GABAergic neurotransmission in ScZ in auditory as well as thalamic and hippocampal regions. PV+ interneurons are reduced in the hippocampus (52) and thalamic reticular nucleus (53) in ScZ, while in the auditory cortex, levels of the GABA-synthesizing enzyme GAD65 are decreased (54). Altered synaptic proteins implicating AMPA receptor subunits but not NMDARs have been shown to be altered in the auditory cortex in ScZ (55). Currently, the precise contributions of glutamatergic neurotransmission and GABAergic interneurons toward circuit deficits and aberrant gamma-band oscillations remain unclear, however. One possibility is that circuit deficits are due to a primary dysfunction in inhibitory interneurons in ScZ (56). In addition, evidence exists that impaired inhibition could be the result of NMDAR hypo-functioning on PV+ interneurons (57) or reduced NMDAR drive on pyramidal cells (58).

Crucially, our finding is that 40-Hz ASSR impairments in CHR-P participants represent a potential biomarker for predicting clinical outcomes. Specifically, we show that reductions in 40-Hz ASSRs predicted transition to psychosis in CHR-P participants with good accuracy similar to data from event-related potentials (59) or functional magnetic resonance imaging (60). In addition, MEG-recorded 40-Hz ASSRs distinguished CHR-P participants with persistence of APSs at 12-months from those who remitted with excellent accuracy. Predicting persistence of APSs is relevant because there is evidence to suggest that CHR-P participants who continue to experience APSs have poorer outcomes than CHR-P participants with transient APSs (61).

The finding that 40-Hz ASSR in RTHA predicts persistence of APSs is important, because only a minority of CHR-P participants will develop psychosis (62), and a large number of individuals will remit from CHR-P status (63). Moreover, this finding is consistent with previous findings that thalamic abnormalities are a potential biomarker for early detection and diagnosis (60). Finally, we did not find 40-Hz ASSR impairments in CHR-N individuals, suggesting that impaired gamma-band oscillations are specifically associated with the CHR-P and FEP phenotypes.

Strengths and Limitations

This study has several limitations. First, we localized MEG activity to the thalamus and hippocampus. While localization of subcortical generators remains challenging, we would like to note that reconstruction of MEG time courses in the thalamus and hippocampus have been demonstrated before by our group (64,65) and others (66,67). Moreover, our analyses revealed that there were no differences in similar depth regions in the opposite hemisphere, suggesting that signal leakage did not contribute to our observations. Second, the sample size of transitioned cases was relatively small (n = 13). Accordingly, further studies are required that replicate our findings in independent samples using nested cross-validation (68).

In conclusion, this study provides novel evidence on deficits in 40-Hz ASSRs in CHR-P participants and patients with FEP in auditory as well as thalamic and hippocampal regions. Crucially, the current findings highlight that 40-Hz ASSRs predict clinical outcomes in CHR-P participants, including transition to psychosis as well as persistence of APSs. Together, these findings highlight the potential of MEG as a novel approach to identify circuit dysfunctions and biomarker for clinical outcomes in psychosis.

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