Development of Sensory Gamma Oscillations and Cross-Frequency Coupling from Childhood to Early Adulthood

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Given the importance of gamma oscillations in normal and disturbed cognition, there has been growing interest in their developmental trajectory. In the current study, age-related changes in sensory cortical gamma were studied using the auditory steady-state response (ASSR), indexing cortical activity entrained to a periodic auditory stimulus. A large sample (n = 188) aged 8–22 years had electroencephalography recording of ASSR during 20-, 30-, and 40-Hz click trains, analyzed for evoked amplitude, phase-locking factor (PLF) and cross-frequency coupling (CFC) with lower frequency oscillations. Both 40-Hz evoked power and PLF increased monotonically from 8 through 16 years, and subsequently decreased toward ages 20–22 years. CFC followed a similar pattern, with strongest age-related modulation of 40-Hz amplitude by the phase of delta oscillations. In contrast, the evoked power, PLF and CFC for the 20- and 30-Hz stimulation were distinct from the 40-Hz condition, with flat or decreasing profiles from childhood to early adulthood. The inverted U-shaped developmental trajectory of gamma oscillations may be consistent with deviations from this trajectory.

Keywords: cross-frequency coupling, development, GABA, gamma oscillations, phase-locking factor, synaptic pruning

Introduction

Synchronous gamma-band (30–80 Hz) oscillations are an important mechanism for coordinating neural activity in the service of cognitive and sensory processing. Altered gamma oscillations have also been implicated in the pathophysiology of neuropsychiatric disorders such as schizophrenia and autism. Accordingly, investigating the normal developmental trajectory of gamma oscillations is of vital importance to understanding the neurophysiologic underpinnings of normal cognitive development and how pathophysiologic disturbances in gamma activity in neurodevelopmental disorders can give rise to deviations from this trajectory.

Gamma oscillations have been most studied in humans using electroencephalography (EEG), which records at the scalp the summed effect of the synchronous postsynaptic activity in a large number of cortical pyramidal neurons (Nunez and Srinivasan 2005). As various structural and functional processes that support gamma oscillations involve a protracted neurodevelopmental course from childhood through to adulthood, the development of gamma oscillations would be expected to follow a similarly extended trajectory before reaching the mature state. For instance, structural magnetic resonance imaging (MRI) studies find that gray matter thickness in auditory cortical areas (posterior superior temporal gyrus) decreases linearly from childhood into early adulthood (Gogtay et al. 2004). Such macro-level observations of gray matter changes are thought to reflect synaptic pruning over development. Early studies by Huttenlocher and Dabholkar (1997) suggested that auditory and visual regions completed pruning processes in early adolescence while prefrontal cortex had a more extended course into mid-adolescence. However, these interpretations were limited by sparse sampling over the adolescence and adulthood periods, and more recent studies with denser sampling over this range have indicated that synaptic elimination continues beyond adolescence into the third decade of life (Petanjek et al. 2011). Such structural changes are accompanied by extensive changes in components of GABA neurotransmission (Beneyto and Lewis 2011) that could critically change the functional capacity to produce gamma oscillations. Parvalbumin (PV) fast-spiking cells are a subclass of GABA interneurons that, given their nonadapting firing and postsynaptic GABA-A receptors that possess fast kinetics, can support sustained, high-frequency firing. These properties allow PV fast-spiking interneurons to play their critical role in the temporal regulation of gamma oscillations through feedback inhibitory coupling with pyramidal cells (Bartos et al. 2007; Buzsáki and Wang 2012). Interestingly, during development, GABA-A receptors shift their subunit composition from alpha-2 to alpha-1 subtypes, resulting in a shift from slower to faster inhibitory decay kinetics (Hashimoto et al. 2009). Since the frequency of gamma oscillations depends critically on the decay time course for inhibition (Bartos et al. 2007), such a shift could increasingly provide the means to support higher frequency oscillations. This developmental shift also follows a protracted temporal course, beginning postnatally and continuing through to adulthood (Hashimoto et al. 2009). Thus, both structural refinements and molecular changes occur with a protracted course but exactly how these interact and give rise to physiologic changes over development in the form of gamma oscillations requires explicit investigation.

Steady-state responses to trains of periodic stimuli have been extensively used to examine oscillations in auditory, visual, and somatosensory modalities (Colon et al. 2012) and have the advantage of a high signal-to-noise ratio (Vialatte et al. 2010). Auditory steady-state responses (ASSRs) have been used to study the development of gamma oscillations...
over a wide range of ages (Maurizi et al. 1990; Rojas et al. 2006; Poulsen et al. 2009) and are sensitive to selective gamma oscillatory disturbances in schizophrenia (Kwon et al. 1999; Light et al. 2006; Spencer et al. 2008; Brenner et al. 2009; Krishnan et al. 2009; Kömek et al. 2012) and pharmacologic modulation (Vohs et al. 2010; Kömek et al. 2012). Developmental studies of gamma oscillations have found that infants and children (3 months to 6 years) exhibit poor ASSR (Stapells et al. 1988), whereas older children (5–8 years) sustain better gamma responses (Maurizi et al. 1990), with 10-year olds progressing to show comparatively higher gamma responses at 11.5 years (Poulsen, Picton, and Paus 2009). A study spanning a much broader age range (5–52 years), found increasing ASSR from childhood through adolescence, with plateauing from early adulthood (Rojas et al. 2006). However, the relatively sparse sampling at the younger ages (30 subjects younger than 20 years old) precluded a detailed characterization beyond the first-order trend of increases toward adulthood. In the current study, we employed the ASSR paradigm with a dense sampling of the 8–22 years age range to investigate the precise trajectory by which gamma activity emerges and evolves over this critical period of development.

Prior developmental studies using ASSR have focused on magnitude measures (amplitude/power) of gamma oscillations. Another important measure of oscillatory activity is phase-locking factor (PLF), which is a measure of the consistency with which neural activity is phase locked to the stimulus across trials and is analytically independent of the oscillation magnitude. Another important aspect of gamma oscillatory dynamics that has gained increasing attention is cross-frequency coupling (CFC), commonly indexed as the modulation of gamma amplitude by the phase of slower rhythms. Modulation by theta rhythms is most often reported, with critical implications for cognitive processing (Jensen and Colgin 2007) including working memory (Sauseng et al. 2009; Axmacher et al. 2010; Fujisawa and Buzsáki 2011) and sensory selection (Schroeder and Lakatos 2009). Interestingly, theta–gamma CFC in hippocampus is dependent on fast inhibition onto PV interneurons (Wulff et al. 2009) suggesting that the developmental trajectory CFC may track that of gamma oscillatory power which also depends on fast inhibitory kinetics (Bartos et al. 2007). The phases at other lower frequency oscillations, including at delta (1–3 Hz) and alpha (8–12 Hz) bands, have also been noted to modulate gamma band amplitudes (Canolty and Knight 2010).

The current study investigated the developmental trajectory of gamma oscillations using the ASSR. It is the first study with sufficiently dense sampling to permit a detailed characterization of ASSR gamma over the critical developmental age range. We evaluated ASSR gamma amplitude in 188 subjects spanning the age range 8–22 years old, binned in increments of 3 years [late-childhood; 8–10 years, early-adolescence: 11–13 years, mid-adolescence: 14–16 years, late-adolescence: 17–19 years, early adulthood (The age bin definitions correspond to ranges that are commonly used in the literature. However, the particular age limits are arbitrary and definitions can vary widely, in particular, in distinguishing late-adolescence from early adulthood given the extended maturational course of some cognitive and neural processes.): 20–22 years]. This is also the first study to examine the maturation process of PLF and CFC of gamma oscillations.

### Materials and Methods

#### Participants

One hundred eighty-eight participants (age range 8–22 years, M = 14.5, SD = 4.4) were recruited from the greater Allegheny County to participate in this study. Of these, 7 participants were excluded based on participant withdrawal, ineligibility, or technical issues. Thus, data from 181 participants were entered into the analyses (age range 8–22 years, M = 14.5, SD = 4.4). Written informed consent was obtained prior to testing in accordance with the Institutional Review Board at the University of Pittsburgh. Participants and accompanying legal guardians were monetarily compensated for their participation. Recruitment numbers were well balanced across the age range but with greater sampling for the 8–10-year-old subjects (Table 1) in order to facilitate future longitudinal follow-up studies. Age bins in increments of 3 years (8–10, 11–13, 14–16, 17–19, 20–22 years) were matched for race, IQ, and gender (these groups are henceforth referred to as age bins 1 through 5, respectively). Potential participants were excluded using the MINI (Sheehan et al. 1998) for having a history of DSM IV Axis I or mental

### Table 1

Demographic and behavioral summary for current sample (N = 188)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Count</th>
<th>Age bin (in years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 (8–10)</td>
</tr>
<tr>
<td>n</td>
<td>44</td>
<td>32</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Left-handed</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Caucasian</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Other/not reported</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WASI IQ</td>
<td>107.5</td>
<td>105.1</td>
</tr>
<tr>
<td>Average SES</td>
<td>41.6</td>
<td>44.4</td>
</tr>
<tr>
<td>Standard Trial False Alarm Rate</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>Standard Trial Reaction Time</td>
<td>245</td>
<td>198</td>
</tr>
<tr>
<td>Trial Segment Count (Hz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>67.0</td>
<td>87.9</td>
</tr>
<tr>
<td>30</td>
<td>66.3</td>
<td>81.4</td>
</tr>
<tr>
<td>20</td>
<td>67.7</td>
<td>85.8</td>
</tr>
</tbody>
</table>

Note: Average SES represents a composite SES score using the Hollingshead Scale. WASI IQ represents an age normalized IQ score derived from the Wechsler Abbreviated Scale for Intelligence test. Participant demographics do not reflect withdrawn participants.
retardation diagnosis, or a first-degree relative with a history of psychosis. Additional demographic information can be found in Table 1.

Task
Participants were seated ∼80 cm from an LCD computer monitor used to present visual stimuli and wore ER-3A insert earphones (Etymotic Research, Elks Grove, IL, USA) for auditory stimuli. All stimuli were presented using E-Prime software (Psychological Software Tools, Pittsburgh, PA, USA). Click trains of 500-ms duration were presented binaurally at 65 ± 5 dB. The click train repetition frequencies were 20, 30, or 40 Hz and presented in the context of an auditory oddball paradigm to ensure participant attention to the stimuli. Standard stimuli were click trains with individual clicks being 1-kHz carrier frequency whereas Oddball stimuli were click trains with clicks of 2-kHz carrier frequency. During click train presentation and for 200 ms after click train cessation, the screen remained blank (black). Participants were then prompted by the appearance of a central index finger for Standard stimuli (110 trials per block) or their right index finger for Oddball stimuli (10 trials per block). There was one block for each click repetition frequency (20, 30, and 40 Hz) for a total of 3 blocks, with block orderings counterbalanced across subjects. Behavioral analyses were conducted to confirm attention to task but only correct Standard trials were submitted for EEG analyses. Neither behavioral nor EEG analyses were carried out for oddball trials due to insufficient trial counts.

EEG Acquisition and Preprocessing
EEG sessions were conducted in an electrically shielded, sound-attenuated room lit with a low-level ambient light. EEG data were collected using a 128-channel Geodesic Sensor Net and Netstation software (EGI, Eugene, OR, USA) sampling at 250 Hz and referenced to a common reference (Cz). Online filtering was applied, setting a 0.01- to 100-Hz elliptical bandpass hardware filter, and electrode impedances were maintained at or below 50 kΩ. Epochs were defined as −500 to 1000 ms relative to stimulus onset adjusted to a −350 to −150-ms prestimulus baseline. Only correct Standard trials were included and epochs containing artifacts were excluded (greater than ±100 µV amplitude within epoch or a consecutive sample difference of 60 µV). Segments identified by these criteria were visually inspected prior to rejection. Ocular and ECG artifacts were removed with ICA-based rejection. Ocular and ECG artifacts were removed with ICA-based rejection. Ocular and ECG artifacts were removed with ICA-based rejection. Ocular and ECG artifacts were removed with ICA-based rejection.

Spectral Analyses
Data were transformed by a complex Morlet wavelet transform as basis for all spectral analytic measures. Morlet wavelets are a Gaussian-shaped sinusoidal function, thus yielding frequency-specific information in a time-specific manner: 

\[ w(t, f_0) = A \times \exp\left(-t^2/2\xi^2\right) \times \exp(2i\pi f_0 t) \]

where \( A \) is a normalizing constant, \( \sigma_i \) gives the spread of the Gaussian, and \( f_0 \) is the frequency of the sinusoid, with \( \xi = 1/2\sigma_i \). The transform of the EEG data, then, was a convolution of the wavelet with the EEG data \( d(t) \) where

\[ W_j = \int w(t, f_0) \times d(t) \]

The modulus and arguments of the resulting complex value in polar form are estimates of the amplitude and phase values for the particular time point and frequency band. The ratio \( f_0/\sigma_i \) is constant and defines the frequency versus time resolution (higher values favoring higher frequency resolution). A value of 10 was used for phase-sensitive measures (evoked activity and PLF) at the entrainment frequencies. This value emphasized frequency resolution with minor loss of time resolution at such relatively high frequencies. For measures involving lower frequencies (induced activity and cross-frequency coupling), time resolution was emphasized by using a value of 5.

The evoked amplitudes were calculated on wavelet transforms of the averaged EEG data (see Supplementary Fig. 1 for ERP averages), whereas induced amplitudes were calculated as averages of wavelet transformed data on a per trial basis. PLF, the phase consistency with respect to the click trains, was calculated as the modulus of the vector average of the normalized transformed data:

\[ 1/p \sum_{n=1}^{p} (a + bi) \cdot (a' + b'i)^{-1} \]

where the summation is over the number of data points \( p_i \) and \( w+bi \) is each wavelet transformed data point. PLF values are bounded from 0 to 1, and being equivalent to 1 minus the circular variance of phases, a value of 1 represents no variance, that is, identical phase across trials. The unit normalization of magnitude ensures that PLF yields an independent, complementary measure to evoked amplitude.

CFC analysis addressed the degree of dependence of amplitude on the phase of lower frequencies, that is, phase-amplitude coupling. This can be quantified by measuring the distance between the phase and amplitude distributions (Tort et al., 2010). By binning amplitude values at a frequency on the basis of the concurrent phase of a lower frequency, the latter can be said to modulate the former if its distribution is not uniform. This approach was refined in order to capture coupling even in fast dynamics during short time windows that could otherwise be compromised by uneven sampling of lower frequency phase values. To this end, both phase and amplitude were binned and coupling measured in terms of their mutual information.

Statistical Analysis
All statistical differences across age bins (i.e., 8–10, 11–13, 14–16, 17–19, 20–22 years) were assessed through one-way analyses of covariance (ANCOVA) controlling for gender, handedness, IQ, ethnicity, and socioeconomic status (SES) as assessed on the Hollingshead scale (Hollingshead 1975). SES data were incomplete for 5 participants; data for these participants were imputed, derived from the mean SES for their respective age bins. ANCOVAs were conducted for Standard trial reaction times and error rates. For each age group, separate ANOVA models were run for evoked amplitudes, induced amplitudes, PLF and CFC. As CFC analyses identified delta–gamma coupling, the total delta and gamma amplitudes were also evaluated separately. Owing to heteroscedasticity of delta amplitudes, a weighted least squares approach was taken where group weights were calculated as the inverse of the variance of residuals from the unweighted model. Note that 3 participants had no viable trials in 1 of the 3 conditions (20 Hz: one from 22–24 year age bin; 50 Hz: one each from age bins 11–13 and 17–19 years); therefore, analyses of those conditions reflect the remaining participants' data. Standard trial error rate was arcsine transformed to correct for significant differences in variance across age bins. For evoked and induced amplitudes and PLF, means were extracted from 225- to 525-ms poststimulus onsets to avoid differential influence from the initial mid-latency response (50- to 150-ms postonset). Age-related trajectories were assessed using polynomial trend analyses with post hoc t-tests carried out to assess group differences. Lastly, we characterized relationships between measures with partial correlations controlling for age. Post hoc t-tests and partial correlations were evaluated at a Bonferroni corrected \( \alpha = 0.05 \) to assess possible inflation of type I error.

Results

Behavioral Performance
Error rates were significantly higher for the youngest age bin, \( F_{1,469} = 2.55, \ P = 0.041, \eta_p^2 = 0.06 \), and followed a negative quadratic trend with age, \( F_{1,469} = 6.41, \ P = 0.012, \eta_p^2 = 0.04 \). However, pairwise contrasts only found a trend-level difference between the first and third age bins, \( t_{(79)} = 2.72, \ P = 0.058 \). Reaction times showed no clearly discernable pattern by age,
and no significant differences were observed across age bins. See Table 1 for performance summaries.

**Evoked Amplitude**

Analyses of evoked amplitude revealed significant main effects of age bin for 20- and 40-Hz steady-state responses, $F_{1,169} = 3.32, P = 0.012, \eta^2_p = 0.07$; $F_{1,169} = 5.83, P < 0.0005, \eta^2_p = 0.12$, respectively (Fig. 3 and Supplementary Fig. 5; see Supplementary Fig. 2 for 20- and 30-Hz time–frequency plots). Polynomial trend analyses of 20-Hz responses across age bin revealed a significant negative linear trend reflecting decreases in evoked response with age, $F_{1,169} = 10.71$, $b_{\text{linear}} = -5.21$, $SE_{\text{Linear}} = 1.59$, $P = 0.001$, $\eta^2_p = 0.06$. Consistent with this trend, mean amplitude significantly decreased from the first to the last age bin, $t_{(73)} = 3.57, P = 0.005$.

In contrast, evoked amplitude for the 40-Hz response increased from age bins 1–3 before decreasing again from age bins 3–5 (Figs 1A and 3 and Supplementary Fig. 5). Polynomial contrasts confirmed this pattern to be statistically significant with positive linear and strongly significant negative quadratic trends, linear: $F_{1,169} = 5.39$, $b_{\text{linear}} = 2.61$, $SE_{\text{Linear}} = 1.12$, $P = 0.021$, $\eta^2_p = 0.03$; quadratic: $F_{1,169} = 15.35$, $b_{\text{Quadratic}} = -4.51$, $SE_{\text{Quadratic}} = 1.15$, $P < 0.0005$, $\eta^2_p = 0.08$. These patterns reflect significant increases between the first age bin and bins 2, 3, and 4: $t_{(74)} = -3.01, P = 0.030$; $t_{(79)} = -4.47, P < 0.0005$; and $t_{(78)} = -3.63, P = 0.004$, respectively. The 40-Hz trajectory is especially noteworthy given that its pattern is distinct from the 20-Hz trajectory.

To rule out any developmental trends due to differential build-up or attenuation of the ASSR during the course of the experiment, we compared responses from the first and second halves of the experiment. However, there were no significant differences between the first and second halves (Supplementary Fig. 7).

**Induced Amplitude**

Only induced activity at 40-Hz differed significantly across age bins, $F_{4,169} = 4.27, P = 0.003, \eta^2_p = 0.09$ (see Fig. 3, Supplementary Figs 4 and 5). Polynomial trends showed the trajectory of the 40-Hz response to be the inverse of the evoked responses. With increases in age, induced 40-Hz amplitudes demonstrated significant negative linear and positive quadratic trends indicating that across development induced 40-Hz activity declined decreased at a decreasing rate with a slight increase for the final age bin, linear: $F_{1,169} = 5.50$, $b_{\text{linear}} = -1.38$, $SE_{\text{Linear}} = 0.59$, $P = 0.020$, $\eta^2_p = 0.03$; quadratic: $F_{1,169} = 8.95$, $b_{\text{Quadratic}} = 1.80$, $SE_{\text{Quadratic}} = 0.60$, $P = 0.003$, $\eta^2_p = 0.05$. Consistent with an inverse pattern to the evoked response, this trend was driven by decreases from age bin 1 to bins 2, 3, and 4: $t_{(74)} = 2.97, P = 0.035$; $t_{(79)} = 3.26, P = 0.013$; and $t_{(78)} = 3.59, P = 0.004$, respectively. Note that the 40-Hz induced amplitudes were negative indicating a significant reduction from baseline levels of nonphase-locked activity; however, since the absolute magnitudes of the reductions did not quantitatively mirror the increases in evoked amplitudes, the evoked activity could not be entirely explained by a phase realignment of ongoing cortical gamma-band activity.

**Phase-Locking Factor**

Measures of phase-locking mirrored the results for the evoked 40-Hz response, $F_{4,169} = 10.15, P < 0.0005, \eta^2_p = 0.19$ (see Figs 1B and 3 and Supplementary Fig. 5); however, neither the 20- nor 30-Hz phase locking significantly differed across age bins (Supplementary Fig. 2B, D). Similarly, trend analyses of 40-Hz
Correlation Analyses

As evoked oscillations may be due, in part, to phase resetting of ongoing oscillations, we evaluated for negative correlations between evoked and induced amplitudes. Partialling out the linear and quadratic trend effects of age, evoked 40-Hz amplitude correlated negatively with induced 40-Hz amplitude (partial correlation $r_{p} = -0.83$, $df = 170$, $P < 0.0005$). We also evaluated the correlation between 40-Hz evoked amplitude and PLF, finding evidence for a close relationship ($r_{p} = 0.87$, $df = 170$, $P < 0.0005$). Finally, as CFC measures of coupling may be influenced by the amplitudes of the coupled oscillations, total sum of induced and evoked activity delta and gamma amplitudes were correlated with CFC during 40-Hz stimulation. There were significant positive correlations between CFC and delta ($r_{p} = 0.29$, $df = 177$, $P < 0.0005$) and gamma ($r_{p} = 0.70$, $df = 177$, $P < 0.0005$) amplitudes.

Discussion

This study investigated age-related changes in multiple aspects of gamma oscillatory activity as indexed by ASSR in healthy subjects 8–22 years old. The common pattern across the measures, including evoked power, PLF and CFC, was an inverted-U trajectory, with monotonic increases from age childhood (8–10 years) to mid-adolescence (14–16 years), with subsequent decreases towards adulthood (20–22 years) (Fig. 3). This trajectory stood in stark contrast to the entrained responses to the 20-Hz and 30-Hz stimuli which were either uniformly monotonic decreasing or showed no appreciable changes across the same age span. While the initial increases in gamma activity are consistent with the findings of prior studies that show similar trends, this is the first study with sufficiently dense sampling of this age range to capture the decreases in gamma activity later in development. The inverted-U trajectory is suggestive of 2 underlying developmental processes with opposing effects, such as increases in alpha-1 GABA receptors promoting increases in gamma activity with age that are later offset by synaptic pruning that decreases pyramidal cell excitation and, as a consequence, gamma activity.

Prior studies of ASSR gamma oscillations have characterized the general first-order developmental trend towards increased gamma, starting with poor ASSR gamma in infants and children (3 months to 6 years) (Stapells et al. 1988) and increases in childhood (Maurizi et al. 1990; Poulsen et al. 2009) that appear to continue monotonically, plateauing in early adulthood (Rojas et al. 2006). In general agreement with these prior reports, we found an initial frequency-specific pattern of increases in evoked gamma power. This increase is consistent with parallel changes in the subunit composition of cortical PV fast-spiking interneuron GABA-A receptors from alpha-2 to alpha-1 subtype that results in a shift from slower to the faster inhibitory decay kinetics (Hashimoto et al. 2009) that are important to the higher frequency oscillations in the gamma-band range (Bartos et al. 2007). This shift follows a protracted developmental course, beginning postnatally and continuing through to adulthood (Hashimoto et al. 2009). Similar observations have been made in hippocampus where PV neurons...
undergo developmental shifts toward producing stronger, more precise inhibitory currents with faster kinetics that characterize the more mature state and support high-frequency gamma oscillations (Doischer et al. 2008). Interestingly, an integrated MEG/computational modeling study of the ASSR in schizophrenia (Vierling-Claassen et al. 2008) showed that the decreases in 40-Hz power and increases in 20-Hz power for patients could be explained precisely by slower kinetics in GABA neurotransmission through its shifting of the peak frequency to the lower portion of the spectrum. Future developmental studies could use finer sampling of frequencies (cf., Krishnan et al. 2009) to more explicitly demonstrate such age-related shifts.

If GABA-A-receptor kinetics were the only rate-limiting factor, one would expect gamma oscillatory activity to follow a similar monotonic increasing trajectory, perhaps plateauing by early adulthood. Rojas et al. (2006) reported just such a trend but while the study may have been adequately powered \( (n = 69, \text{ages } 5–52 \text{ years}) \) to detect this first-order pattern, the sampling of younger subjects was relatively sparse \( (n = 30 \text{ subjects } < 20 \text{ years old}) \) precluding a more detailed characterization. Our much denser sampling of the same age range permitted a more refined characterization, revealing the decreases in gamma activity beyond mid-adolescence toward early adulthood. (While the Rojas et al. (2006) study demonstrated a first-order monotonic increase toward adulthood, a re-examination of their data (provided courtesy of Dr Rojas) revealed a trend toward an inverted-U trajectory resembling the findings of the current study. See Supplementary Data.) This nonmonotonic pattern suggests that in addition to factors such as GABA-A-receptor kinetics, other important developmental processes help to shape the trajectory of gamma activity.

Given that oscillatory activity measurable at the scalp results largely from postsynaptic potentials in the apical dendrites of pyramidal cells, synaptic pruning is one such developmental process that could act counter to that of evolving GABA-A-receptor kinetics, perhaps eventually enough to cause decreases in measured gamma activity. Interestingly, synaptic pruning occurs more in supragranular than infragranular layers (Rakic et al. 1986; Bourgeois et al. 1994; Anderson et al. 1995), precisely the layers where gamma oscillations are more prominent (Chrobak and Buzsáki 1998; Quilichini et al. 2010; Buffalo et al. 2011; Spaak et al. 2012). Gamma oscillations are also thought to primarily reflect local circuitry dynamics in the superficial layers and so it is notable that the extensive pruning in these layers during adolescence occurs in local intrinsic circuitry as opposed to synapses associated with long-range associational axons (Woo et al. 1997). Interestingly, this pruning during adolescence does not appear to depend on the functional immaturity of synapses (Gonzalez-Burgos et al. 2008), as is the case earlier in development (Mirmics et al. 2001). Further, synaptic elimination occurs beyond adolescence into the third decade of life (Jacobs et al. 1997; Petanjek et al. 2011). Consistent with pruning processes that continue into adulthood, the temporal cortex peaks relatively late in gray-matter thickness \( (\sim 17 \text{ years}) \) (Giedd et al. 1999) and the posterior superior temporal gyrus undergoes linear decreases in gray-matter thickness that continue through adolescence into early adulthood (Gogtay et al. 2004). Thus, the temporal course of functional refinements due to GABA-A-receptor kinetics and structural refinements mediated by synaptic pruning may together shape the resultant developmental trajectory of gamma oscillatory activity. Of note, both these factors could also explain the monotonic decreasing pattern for the 20-Hz ASSR, since the faster receptor kinetics would be progressively less optimal for responses at this lower frequency (Doischer et al. 2008; Vierling-Claassen et al. 2008) and pruning processes could generally decrease the magnitude of any type of measurable EEG signal, including the 20-Hz ASSR.
In addition to changes in GABA-A-receptor kinetics and synaptic pruning processes, other developmental maturation of excitatory inputs onto pyramidal cells (Gonzalez-Burgos et al. 2008), there are substantial changes in glutamatergic receptors on fast-spiking interneurons during adolescence (Wang and Gao 2009, 2010). The presence of NMDA receptors on the majority of fast-spiking interneurons prior to adolescence reduces to a minority by adulthood (Bitanhirwe et al. 2009; Wang and Gao 2009) while calcium-permeable AMPA receptors increases over the same period (Wang and Gao 2010). The decreases in NMDA-mediated excitation on fast-spiking interneurons could result in a relative disinhibition of pyramidal cells thereby contributing to increases in gamma activity, as would be suggested by increases in ASSR gamma after acute administration of ketamine, an NMDA receptor antagonist (Plourde et al. 1997) and findings that excessive NMDA conductance in fast-spiking cells can decrease gamma power (Rotaru et al. 2011). The sustaining of AMPA-mediated excitation on the other hand could provide the necessary much faster kinetics to support high-frequency oscillations (Johnston and Wu 1997; Compte et al. 2000; Rotaru et al. 2011; Buzsáki and Wang 2012).

Gamma oscillatory development could also be affected by another aspect of GABA neurotransmission, namely, the cannabinoid system, which undergoes significant changes during adolescence. Stimulation of type 1 cannabinoid receptors (CB1R) on cholecystokinin (CCK)-containing interneurons suppress their output (Katona and Freund 2008) and modulation of these receptors can substantially decrease gamma oscillations (Holderith et al. 2011), although the particular effect can depend on the specific circuit (Morgan et al. 2008). CB1R density significantly increases in layers deep 3 and 4 during adolescence (Eggan et al. 2010), possibly contributing to development of gamma oscillations (which predominantly arise from layer 3 (Maier et al. 2010; Buffal et al. 2011; Spaak et al. 2012) as well as to the particular vulnerability to cannabis use during this period (Ehrenreich et al. 1999; Pope et al. 2003; Moore et al. 2007). Interestingly, a recent rodent study showed that adolescent but not adult exposure to cannabis decreased gamma activity in association with working memory impairments (Raver et al. 2013).

The maturation of cognitive processes such as response inhibition and cognitive control (which is associated with gamma activity (Cho et al. 2006; Kieffaber and Cho 2010; Minzenberg et al. 2010), reaches adult levels during mid-late-adolescence (Luna et al. 2004), paralleling our findings of increases in evoked gamma until mid-adolescence. The later decreases in gamma activity toward adulthood may be consistent with structural refinements via synaptic pruning that serve to enhance cortical efficiency, as activity in the gamma band is more metabolically demanding than lower frequency activity (Mukamel et al. 2005; Niessing et al. 2005). In fact, fMRI studies using BOLD imaging, which indirectly indexes metabolic demand and correlates strongly with gamma activity (Niessing et al. 2005; Magri et al. 2012), have shown an inverted-U pattern over development in association with working memory and cognitive control tasks, with higher activation levels in executive control regions during adolescence compared with childhood and adulthood (Luna et al. 2001; Giesielski et al. 2006; Scherf et al. 2006).

Induced gamma activity followed a mirror opposite trend to that of evoked gamma, with values decreasing below baseline, consistent with ongoing prestimulus gamma oscillations becoming phase-aligned to the stimulus upon its presentation. Also supportive of this idea are the high negative correlations between induced and evoked activity, even after partiaillling out the effects of age. However, the absolute values of evoked gamma were higher than induced gamma suggesting that over and above any such phase realignment, a portion of evoked activity was also due to de novo additional excitation of pyramidal cells due to the auditory stimulus.

The decreases in induced gamma activity in the current study during steady-state evoked responses can be contrasted with the increases typically found in association with tasks that engage gamma oscillations in association with processes that are not generally phase-locked to stimulus onset, such as perceptual decision-making. In a developmental study of gamma oscillations using a Gestalt perception task, Uhlhaas et al. (2009) found that induced gamma in parietal scalp regions had a generally monotonic increasing trend from childhood to adulthood that was interrupted by a relative dip during late-adolescence. These age-related changes more closely resemble that observed for evoked gamma responses in the current study. This suggests that from childhood to mid-adolescence, there may a generic increased ability of cortical circuits to engage in gamma oscillatory activity that can be indexed through different means, including evoked activity as elicited by periodic stimuli or induced activity as elicited by perceptual decision-making. During late-adolescence through to adulthood, the specific pattern of age-related changes may depend on the specific task or region investigated. Future studies could further elucidate the dependencies of age-related changes on the specific paradigm or anatomic region examined to index gamma oscillations.

In contrast with induced and evoked averages which both depend on the amplitude of oscillations, PLF is an index of phase consistency across trials that analytically do not have any dependency on amplitude. PLF showed a similar profile to that of evoked activity, displaying an inverted-U trajectory with a peak at mid-adolescence (14–16 years). The earlier increases in PLF could be understood in terms of enhanced temporal precision of action potentials in more mature PV interneurons, with 4-fold reductions in the coefficient of variation of basket cell conduction velocities observed for mature versus young rodents (Doischer et al. 2008). This enhanced precision, however, is inconsistent with the decreases in PLF observed beyond mid-adolescence. Rather, these later decreases may be attributable to the PLF estimates being affected by signal-to-noise issues. While the analytic determination of PLF depends solely on the phase of the oscillation and not the amplitude, the very estimate of phase may be affected by the relative amplitude of the oscillation compared with the background noise. Thus, assuming constant noise levels, higher amplitudes will tend to yield higher PLF estimates even if the actual PLF remains constant (as opposed to ideal situation of a complete absence of noise in which case amplitude would not affect PLF estimates). Consistent with this idea, we found strong correlations between PLF and evoked power. In contrast to the 40-Hz condition, the evoked amplitude for 20 Hz showed a significant downward trend with age while 20-Hz PLF showed no effect of age, demonstrating that, while correlated, amplitude and phase estimates are not simply redundant indices. Thus, the observed
age-related changes in PLF are likely a function of both under-
lying enhancements in the temporal precision of neuronal 
activity as well as measurement properties of PLF estimates 
in non-noise-free time series.

CFC analyses showed significant delta phase modulation of 
gamma amplitudes in the 40-Hz condition which followed a 
similar age-related inverted-U trajectory to 40-Hz evoked 
power and PLF, increasing from childhood, peaking in mid-
adolescence, and decreasing toward adulthood. The initial 
CFC increases may also be attributable to developmental 
changes in GABA neurotransmission. Wulff et al. (2009) 
reported on the critical role of fast synaptic inhibition of PV 
fast-spiking interneurons in hippocampal theta-gamma CFC 
using experimental studies in rodents and a computational 
model, showing that ablations of inhibition of PV cells reduced 
both theta and its coupling to gamma. Delta–gamma coupling 
has also been observed in the primary visual cortex 
(Whittingstall and Logothetis 2009; Ito et al. 2013) and modeled 
computationally as slow shifts (delta frequency) in cortical excit-
ability that modulate the engagement of excitatory–inhibitory 
loops that produce gamma oscillations (Mazzoni et al. 2010). 
However, it is not clear how this putative mechanism may 
evolve over development. Changes in the magnitude of delta 
band activity could affect the degree of engagement of excit-
atory–inhibitory loops that produce gamma, as well as affect-
ing the precision of phase estimates in the calculation of CFC 
due to SNR issues (see preceding discussion of similar issues 
with PLF measure). Delta amplitude progressively decreased 
with age, however, indicating that the increases in CFC from 
childhood to mid-adolescence did not simply result from in-
creases in delta band activity (for further discussion relating to 
measurement issues, see Supplementary Data).

This study had a number of limitations, including the age 
range not extending beyond early adulthood (sampled in this 
study as 20–22-year olds). As such, while the dense sampling 
of the current study allowed a detailed characterization of 
gamma activity over a broad, developmentally critical age 
range (8–22 years), the precise trajectory by which higher 
levels of gamma activity reached again later in adulthood 
(Rojas et al. 2006) needs to be investigated. Also, although 
longitudinal designs offer the strongest inferences regarding 
developmental trajectory, the current data were collected cross-
sectionally. Accordingly, while the age subgroups were 
matched demographically, cohort effects cannot be completely 
rules out. Another limitation is that while the ASSR paradigm 
has the advantages of excellent SNR and a robust ability to 
detect gamma-band-specific disturbances in clinical popu-
lations, in its standard implementation (as in the current study), 
it does not directly link the oscillatory activity to a cog-
nitive process. Future investigations could vary task par-
eters to produce meaningful behavioral responses that 
enhance the direct cognitive relevance of the EEG findings. 
Finally, while much finer sampling of frequencies was possible 
(Krishnan et al. 2009), just 3 frequency bands were selected from 
those commonly examined in auditory steady-state para-
digms in the interest of making the experiment time tolerable 
(especially to the youngest of subjects). Refinements to the 
ASSR paradigm that allow more refined sampling of the fre-
quency spectrum while maintaining reasonable experimental 
duration are currently under development.

In summary, the current study investigated the development 
of gamma oscillations in 8- to 22-year-old individuals 
performing the ASSR paradigm, finding that evoked power, PLF 
and CFC all followed an inverted-U trajectory, with increases 
from childhood to mid-adolescence with subsequent decreases 
toward early adulthood. This detailed characterization of mul-
tiple aspects of gamma oscillatory dynamics could inform our 
understanding of the neurodevelopmental basis of sensory and 
cognitive processing abilities during this developmental period 
of dynamic changes, as well pathophysiologic mechanisms of 
disturbances that arise in this neurodevelopmental context, such as 
schizophrenia. Disturbances in gamma oscillations, thought 
to be a core pathophysiologic mechanism in this disorder, 
have been extensively characterized using the ASSR paradigm 
(Kwon et al. 1999; Light et al. 2006; Spencer et al. 2008; 
Krishnan et al. 2009; Kömek et al. 2012). Schizophrenia has also 
been hypothesized to arise from dysregulation of synaptic 
pruning and has well characterized pathologic changes in 
layer III pyramidal cells and PV fast-spiking interneurons across 
cortical regions including the auditory cortex and PFC (Lewis 
and Sweet 2009). The findings of the current study could 
provide important insights regarding the timing and physiologic 
nature of pathologic changes that interact to give rise to dis-
turbances in gamma oscillations in the illness. A particularly 
clinically useful outcome would be the ability to detect physio-
logic disturbances prior to psychosis onset. Prior studies have 
examined gamma-band responses to auditory stimuli in genetic 
high-risk subjects, finding that evoked power and PLF were 
reduced (Hong et al. 2004; Leicht et al. 2010). However, given 
that the subjects were older than the typical age of psychosis 
onset and were not followed longitudinally, future studies using 
longitudinal designs in younger clinical or genetic high-risk sub-
jects could better evaluate the potential for detection and prog-
nostic value of identifying disturbances in gamma activity 
earlier in the developmental trajectory.

Supplementary Material
Supplementary can be found at: http://www.cercor.oxfordjournals.org/.

Funding
This work was supported by the National Institute of Mental 
Health at the National Institutes of Health (K08 MH080329 to 

Notes
The authors acknowledge Debra Montrose and Alicia Thomas for their 
contributions to subject recruitment and RyAnna Verbiest, Megan Carl, 
Polina Radchenkova, Tanisha Hill-Jarrett, Annette Richard for their 
contributions to data collection. They also thank Dr Don Rojas for pro-
viding the Rojas et al. (2006) data to permit re-analysis. This work was 
supported by the National Institute of Mental Health at the National 
Institutes of Health (K08 MH080329 to R.Y.C. and P50 MH084053 
to D.A.L.). These data were presented, in part, at the 2011 Annual 
Meeting of the Society of Biological Psychiatry, San Francisco, CA, 
USA. Conflict of Interest: None declared.

References
Anderson SA, Classey JD, Condé F, Lund JS, Lewis DA. 1995. Synchro-
nous development of pyramidal neuron dendritic spines and 
parvalbumin-immunoreactive chandelier neuron axon terminals in 


Delenno A, Malenck H, Wang T-T. 2009. GABAergic inputs to pyramidal neurons in layer 3 are reduced in schizophrenia. Schizophr Res. 70:293–301.


control independent of medication status in first-episode schizophrenia. Neuropsychopharmacol. 35:2590–2599.


