Sensory-driven and spontaneous gamma oscillations engage distinct cortical circuitry

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Abstract

Gamma oscillations are a robust component of sensory responses but are also part of the background spontaneous activity of the brain. To determine whether the properties of gamma oscillations in cortex are specific to their mechanism of generation, we compared in mouse visual cortex in vivo, the laminar geometry and single neuron rhythmicity of oscillations produced during sensory representation with those occurring spontaneously in the absence of stimulation. In mouse visual cortex under anesthesia (isoflurane and xylazine), visual stimulation triggered oscillations mainly between 20-50 Hz, which, because of their similar functional significance to gamma oscillations in higher mammals, we define here as gamma range. Sensory representation in visual cortex specifically increased gamma oscillation amplitude in the supragranular (L2/3) and granular (L4) layers, and strongly entrained putative excitatory and inhibitory neurons in infragranular layers, while spontaneous gamma oscillations were distributed evenly through the cortical depth, and primarily entrained putative inhibitory neurons in the infragranular (L5/6) cortical layers. The difference in laminar distribution of gamma oscillations during the two different conditions may result from differences in the source of excitatory input to the cortex. In addition, modulation of superficial gamma oscillation amplitude did not result in a corresponding change in deep layer oscillations, suggesting that superficial and deep layer of cortex may utilize independent, but related networks for gamma generation. These results demonstrate that stimulus-driven gamma oscillations engage cortical circuitry in a manner distinct from spontaneous oscillations and suggest multiple networks for the generation of gamma oscillations in cortex.

Keywords: gamma oscillation; visual cortex; cortical column; spontaneous activity; GABAA
Oscillatory activity in the gamma frequency band is a ubiquitous rhythm throughout the brain that has been implicated in multiple aspects of information processing. Gamma oscillations have been associated with sensory representation (Gray et al., 1989; Ribary et al., 1991; Eckhorn et al., 1993), sensorimotor integration (Sanes and Donoghue, 1993; Schoffelen et al., 2011) and cognitive processes including memory (Sederberg et al., 2003; Gruber et al., 2004; Herrmann et al., 2004), attention (Fries et al., 2001; Debener et al., 2003) and perception (Keil et al., 1999; Ribary, 2005). The association of gamma-band rhythmic activity with information processing is found across a wide range of species from insects (Laurent, 1996) and rodents (Sukov and Barth, 1998; Nase et al., 2003) to non-human primates (Eckhorn et al., 1993) and humans (Ribary et al., 1991).

In addition to its association with information processing, gamma-frequency activity is a robust component of the ongoing background activity of the brain. Spontaneous gamma oscillations occur during REM and slow wave sleep, under various anesthetics, and in the awake, restful state (Contreras et al., 1996; MacDonald et al., 1996; Steriade and Amzica, 1996; Steriade et al., 1996a; Destexhe et al., 1999; Steriade, 2006). Given that cortical activity during the spontaneous and sensory-representation brain states clearly generates different functional and perceptual outcomes, it seems likely that the networks participating in these states may show different spatial and temporal properties. However, a number of studies have reported that spontaneous and sensory-driven cortical activities have a similar geometry, and may share core networks of neurons (Arieli et al., 1996; Tsodyks et al., 1999; Kenet et al., 2003; MacLean et al., 2005; Luczak and Maclean, 2012). These results have been interpreted to suggest that spontaneous activity consists of samples from all of the different possible patterns of sensory-driven cortical activity (Luczak et al., 2009). However, recent work looking at cortical network
activity during different brain states has identified key state-dependent differences related to laminar structure that are consistent with the functional anatomy of cortex (Maier et al., 2011; Sakata and Harris, 2012). In the auditory cortex, stimulus-driven population activity originates in the thalamoreceptive layers 4 and 6 and spreads quickly across columns, while spontaneous activity initiates in deep layers and propagates upwards slowly. Results such as these suggest that the functional spread of activity through the cortical laminae may differ with brain state.

Like the similarities observed in the broadband LFP, the gamma frequency component of cortical activity has been reported to have a similar magnitude and frequency between spontaneous and sensory-driven cortical activity (Lakatos et al., 2005). Yet, the modulation of the laminar properties of gamma frequency activity with respect to the brain state has been incompletely described. Characterizing the modulation of the spatiotemporal distribution of gamma oscillations in the vertical axis of the cortex would help identify the components of gamma frequency spatiotemporal geometry that are specific to the representation of sensory information.

This work investigates the columnar circuitry and excitatory and inhibitory network contribution during stimulus-driven and spontaneous gamma activity in mice in vivo. The well-documented modulation of gamma activity by visual stimuli, the robust gamma oscillations in the mouse V1 (Nase et al., 2003), and the characterization of its single unit response properties (Niell and Stryker, 2008) make the mouse primary visual cortex ideally suited for our study of the characteristics of gamma frequency oscillations. In higher mammals, visually-driven oscillatory activity occurs in the frequency range from 30 – 80 Hz, ascribed to the gamma frequency. In mouse visual cortex, visual stimulation triggered oscillations mainly between 20-
50 Hz, which, because of their similar functional significance to gamma oscillations in higher mammals, we define here as gamma range.

We show that in the primary visual cortex of the anesthetized mouse in vivo, visually-driven and ongoing spontaneous gamma oscillations have similar magnitudes and peak frequencies but differ in their distribution through the depth of the cerebral cortex. Sensory representation in visual cortex specifically increases gamma oscillation amplitude in the superficial layers, and primarily entrains putative excitatory neurons. In contrast, spontaneous gamma oscillations are distributed evenly through the cortical depth, and strongly entrain putative inhibitory neurons in the deep layers. The laminar distribution of gamma oscillations during different functional states may result from differences in the source of excitatory input to the cortex. In addition, modulation of superficial gamma oscillation amplitude without a corresponding change in deep layer oscillations suggest that superficial and deep layer of cortex may utilize independent, but related networks for gamma generation. These results imply that the laminar geometry of gamma frequency activity in V1 depends on the functional context in which they are generated, and suggest that the laminar spatiotemporal structure of gamma activity may be a specific component of the sensory response.
Materials and Methods

Surgery

All animal experiments were performed in accordance with the guidelines of the National Institutes of Health and the University of Pennsylvania Institutional Animal Care and Use Committee. Adult C57/B6 mice (12 – 24 weeks) were sedated with an initial dose of xylazine (13 mg/kg) and anesthetized with brief exposure to a high concentration of isoflurane (5%). Anesthesia was maintained with light isoflurane (0.1 – 0.7 %) and the administration of booster doses of xylazine as needed, typically every 2 hours. Anesthetic level was monitored by toe pinch, respiration and pupil dilation. This anesthesia protocol was chosen because it allowed for the stable, long-term maintenance of the animal at a relatively light anesthesia state. Under very deep anesthesia, such as the state achieved using 1% - 1.5% isoflurane without xylazine, the visually-evoked responses were eliminated. Body temperature was maintained at 36° – 37° C throughout the experiment with a heating pad (FHC). Mice were positioned in a stereotaxic apparatus (David Kopf Instruments) and rotated 60° so that one eye was directed towards a LCD monitor. Skin incisions were infused with lidocaine and the eye was covered with ointment (Lacri-Lube) to prevent drying. During the recording session, ointment was removed, and eye condition was carefully monitored. A craniotomy (~1.5 x 1.5 mm) was made above the primary visual cortex contralateral to the eye facing the monitor. The dura was removed to allow the insertion of either a silicon multi-electrode probe (NeuroNexus Technologies) or multiple tetrodes (Thomas Recording Technologies).

Electrophysiology

Local field potential (LFP) recordings were obtained from multi-electrode probes (Neuronexus Technologies) with 16 channels arranged in a vertical configuration, with either 50 or 100 μm
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spacing between probes (model a1x16-3mm50-177 or a1x16-3mm100-177), inserted normal to
the surface of the cortex. In some experiments, two probes were inserted with ~1mm space
between the probes. LFP signals were filtered 0.1 to 3000 Hz online (n= 18 animals, 21 probes).
For single-unit recording, simultaneous extracellular and LFP recordings were obtained from
multiple tetrodes inserted via a microcontroller (Thomas Recording). The extracellular signal
was filtered from 600 to 9000 Hz and spiking events were detected on-line by voltage threshold
crossing (N=14 animals, 47 tetrodes, 215 cells). A 1.5 ms waveform sample was acquired around
the time of threshold crossing and was analyzed offline. All signals were recorded with the
Cheetah 32-channel acquisition system at 30 kHz (Neuralynx).

Pharmacology

To specifically manipulate the gamma oscillations in superficial layers, in a subset of
experiments, superficial gamma activity was disrupted through pharmacological manipulation of
the GABAergic system using picrotoxin. Because gamma oscillation production depends on
inhibitory function (Cardin et al., 2009; Sohal et al., 2009; Takada et al., 2014), the application of
the GABA antagonist, picrotoxin, increases overall depolarization, but decreases the magnitude
of gamma oscillations. In these experiments, a small dose (75 μL) of picrotoxin (0.75 μM,
Sigma) was applied to the surface of the cortex, and the excess was immediately removed.
Recordings were started 5 minutes after the application of picrotoxin and lasted for ~20 minutes.
This dose was selected as the lowest possible dilution that still produced an increase in the
magnitude of the evoked response in the LFP. The very dilute dose of picrotoxin in combination
with the short duration of recordings allowed us to isolate the effects of picrotoxin in the
superficial layers of cortex. The penetration of picrotoxin into the cortex was monitored
indirectly by changes in the size of the evoked response in the LFP. Under these conditions, only the evoked responses in the superficial layers of cortex were affected.

**Visual stimuli**

Visual stimuli were generated using a ViSaGe stimulation generation system (Cambridge Research Systems, Cambridge, UK) and the accompanying MATLAB toolbox. Stimuli were displayed on a gamma-corrected 19-inch LCD monitor configured at 75Hz refresh rate, and positioned 30cm away from the mouse's eye to occupy ~70° of visual space. Full screen drifting gratings were presented for 1s with long (2-5 s) inter-stimulus intervals to allow for a sufficient return to baseline between trials. Stimulus parameters (95% contrast, 0.08- 0.1 cycles/degree, 3 Hz) were chosen to maximally drive the visual response in both single units(Drager, 1975; Niell and Stryker, 2008) and LFPs.

**Analysis**

Analysis was carried out using custom routines in Igor (Wavemetrics) except where specified. Neural signals were filtered 0.1 – 300 Hz for LFP recordings and 20 – 50 Hz to isolate gamma frequency oscillations. LFPs from the 16-channel probes were used to calculate the current source density (CSD) of the cortical stimulus-evoked responses according to the methods of Swadlow and colleagues (Swadlow et al., 2002). Briefly, the one-dimensional CSD was derived from the second spatial derivative of the LFP data as described by Freeman and Nicholson (Freeman and Nicholson, 1975):

\[
\frac{(\partial^2 \Phi/\partial z^2)}{} = \frac{[\Phi(z+2\Delta z)-2\Phi(z)+\Phi(z-2\Delta z)]}{(2\Delta z)^2}
\]

where \(\Phi\) is the LFP, \(z\) is the vertical coordinate depth of the probe, and \(\Delta z\) is the inter-recording site distance (50 or 100 μm in the present study). Upper and lower boundaries for CSD calculation were obtained by extrapolating recordings from the first and last recording sites.
Cortical layers were identified by the appearance of current sinks and sources, relating to extracellular current flow following sensory input. The earliest current sink appears in L4, followed by sinks in L2/3 and L5/6. The latency and amplitude of the LFP evoked response was quantified by finding the local peak (which is negative since the excitatory input causes a negative wave in the LFP) following stimulus onset. Values reported in text correspond to those found in L4, based on CSD calculations, unless otherwise specified. Power spectra were generated using Fast Fourier Transform at a single trial level for 1s epochs either immediately before (baseline condition) or after (stimulus condition) the onset of the drifting grating. Baseline and stimulus spectra were averaged across trials for each electrode and then divided (stimulus/baseline) to give a ratio measurement of the fold-increase above baseline. Peak frequency, amplitude and width measurements were calculated from the ratio spectra (see Fig. 2A for an example). Latency to onset of gamma oscillations was calculated from LFP traces filtered for the gamma range (20-50 Hz) and was measured as the time from stimulus onset to the first positive peak of the gamma filtered trace that crossed a significance threshold (2.5 SD above the mean). Coherence measurements were computed using multitapered methods and the Chronux package (http://chronux.org/) (Mitra and Bokil, 2007) in Matlab (Mathworks). Wave triggered averages were calculated by averaging LFPs on the peaks of gamma oscillations that occurred during the presentation of the drifting grating and were above the significance threshold (2.5 SD above the mean). Representative LFPs from L2/3, L4 and L5/6 were averaged on the peaks of gamma from the L4 LFP. Phase shift was quantified by comparing the time of the center peak in the wave triggered average of LFPs from L2/3, L4 and L5/6. Single units were first identified using automated clustering algorithm based on the mixture-of-Gaussians model (Harris et al., 2000). Clusters were refined manually on the basis of
waveform shape and interspike interval (SpikeSort3D, Neuralynx). Quality of separation was determined based on the Mahalanobis distance and L-ratio (Schmitzer-Torbert et al., 2005) using the MClust package (Matlab). Units were identified as visually responsive if they maintained an average firing rate of 0.05 Hz during at least 20 presentations of the stimulus.

All 215 units were then classified as regular or fast spiking based on properties of their average waveforms, at the electrode site with largest amplitude. Three parameters were used for discrimination: the height of the positive peak relative to the subsequent negative trough, the time from peak to trough, and the slope of the waveform 0.5 ms after the initial peak. These parameters have been found optimal for separation of neurons in all layers of the mouse V1 (Niell and Stryker, 2008).

To determine if single units were entrained to the gamma oscillations in the LFP recorded on the same tetrode, we created peri-event histograms (PEHs) using the peaks of the gamma oscillations that crossed a significance threshold as timestamps. To ascertain if the modulation of these events was significant, we computed a rhythmicity index (RI) for each cell. The RI was calculated by averaging the spike counts of the difference between the three center peaks and troughs, and dividing this by the average of the entire histogram, which normalizes the measurement for spike rate (Popescu et al., 2009). This calculation was repeated 1,000 times with shuffled spike times, and the RI of the cell was normalized with respect to the resulting histogram of spike-shuffled rhythmicity values. A normalized rhythmicity value of 0.95 or greater, corresponding to a RI greater than 95% of the shuffled values, was considered significant. All rhythmicity values reported in the text and figures are normalized. Another measure of spike to LFP relationship, the spike field coherence, was computed using
multitapered methods and the Chronux package (http://chronux.org/)(Mitra and Bokil, 2007) in
Matlab (Mathworks).

To confirm that the spike waveforms of rhythmic cells were not contributing to the
gamma power measured on the corresponding LFP, we removed 4 ms of the LFP waveform
centered on each spike timestamp. This 4 ms snippet was replaced with the average value of the
point on the LFP waveform immediately on either side of the missing snippet. This segment was
median filtered to remove any abrupt transitions in LFP values and then the gamma oscillation
power was recalculated for the LFP.

Statistical analysis was performed using the Mann-Whitney U test or the Wilcoxon
signed-rank test where appropriate. Variability is reported as the standard deviation (SD) unless
specified to be the standard error of the mean (s.e.m.). All error bars in the figures represent the
standard error of the mean.
Results

Spontaneous and visually-driven gamma frequency oscillations occur robustly through the depth of V1.

Our goal was to characterize the spatiotemporal distribution of gamma oscillations through the depth of the mouse primary visual cortex (V1) and compare visually-driven oscillations with those occurring during spontaneous background activity, i.e., in the absence of visual stimulus. The response in visual cortex was dominated by oscillatory activity between 20 and 50 Hz, which we defined as our gamma range because of their functional identity with gamma oscillations in higher mammals (30-80 Hz). We recorded local field potentials (LFPs) using a multisite probe (Neuronexus, Ann Arbor, MI) with 16 evenly spaced recording sites (50 or 100 µm interelectrode distance) inserted normal to the cortical surface and spanning the cortical depth. In a separate cohort, we recorded single cells from layers 2-6 using 5-7 independently movable tetrodes (Thomas Recordings, Giessen, Germany). Mice were anesthetized with a mix of isoflurane and xylazine in order to obtain a stable low-amplitude baseline pattern recorded in the LFPs (Fig. 1A). The visual responses described here were readily abolished or became highly variable under deep anesthesia, as indicated by large-amplitude slow oscillations in the background activity, and all recording sessions of this nature were discarded. We obtained recordings from 21 probes from 18 animals, from which we selected 17 probes from 13 animals for analysis based on the stability and amplitude of visual responses and the absence of slow oscillations in the baseline. Among these, we further selected 9 probes from 7 animals for the analysis of baseline activity because of the presence of long periods of stable non-stimulated activity.
The presentation of high-contrast, full screen drifting gratings evoked a large response in the LFP signal, which was evident at the single trial level (Fig. 1, green boxes) both in the broadband (0.1 to 300 Hz, Fig. 1A) and gamma frequency (20 – 50 Hz, Fig. 1D) filtered data. The stimulus-locked average of the visual response in the broadband LFP (Fig. 1B) consisted of a negative deflection with a latency to peak of 90 ms after stimulus onset, as quantified by the local minimum of the LFP trace within layer 4. The amplitude and latency of the stimulus-locked response varied systematically with cortical depth, which we quantified using current source density (CSD) analysis (Freeman and Nicholson, 1975; Swadlow et al., 2002). Because the sinks generated by sensory inputs have the shortest latency in L4, we were able to use the CSD analysis to reliably identify channels located in L4. We included the channels that were 300 μm superficial to the upper boundary of L4 in our analysis of L2/3 and those that were 400 μm deeper than the lower boundary of L4 in our analysis of L5/6. In this example, the CSD revealed an initial large sink in L4 with a latency to peak of 90 ms followed 92 and 111 ms later by sinks in L2/3 and L5/6, respectively (Fig. 1C). For the population, the latency to peak of the evoked response in L4 was 95.7 ± 15.5 ms (population measures are mean ± SD, unless otherwise noted), the latency to peak of the CSD response for L4 was 103±16 ms followed by the peaks in L2/3 and L5/6 at additional latencies of 69±66 ms and 78±73 ms respectively. These latencies are longer than others have previously reported for rodent primary visual (Heynen and Bear, 2001; Niell and Stryker, 2008) and auditory cortex (Szymanski et al., 2009). However, this may be a function of the drifting grating stimuli, which produces a more gradual onset than a flashed or contrast reversing grating. Additionally, differences in peak latency may be anesthesia dependent.
Visual stimulation also triggered a robust increase in gamma frequency activity (Fig. 1D). Unlike the LFP evoked response, which is time-locked to the stimulus, sensory driven gamma showed a variable trial-to-trial latency from stimulus onset, and, therefore, was greatly reduced in magnitude by the stimulus-locked averaging (Fig. 1E, notice the change in vertical scale). Indeed, the CSD analysis of the averaged gamma revealed multiple sinks and sources, whose amplitudes were not above background noise and therefore showed no laminar arrangement (Fig. 1F, note change in z-scale). For this reason, our quantification of visually-driven gamma frequency activity was performed at the single trial level and not on the stimulus locked average.

In addition to visually-driven gamma oscillations, the spontaneous background activity (not related to the visual stimulus) showed bouts of gamma activity with similar amplitude and duration to those triggered by the drifting gratings. Spontaneous gamma bouts occurred robustly and regularly throughout the depth of V1 in all recording sessions (Fig. 1D, spontaneous). The presence of spontaneous gamma oscillations agrees with previous observations indicating that gamma oscillations are a robust component of the background activity of the brain (Steriade et al., 1996b).

**Sensory-driven and spontaneous gamma frequency oscillations have different spatiotemporal geometries.**

We compared the characteristics of visually-driven and spontaneous gamma oscillations through the vertical depth of the cortex. Because visually-driven gamma oscillations are not time-locked to stimulus onset, our quantification is based on single trial data. Single trial analysis is immune to the reduction in amplitude of gamma oscillations that inevitably results from averaging responses with varying latency. We calculated, from the raw unfiltered LFP in each trial, the power spectrum from a 1 second window after stimulus onset (response) and from a 1 second
window immediately preceding stimulus onset (baseline). We then averaged the single trial broadband spectra for the response and baseline (Fig. 2A, left panel) and calculated the ratio between the two for all 16 channels (Fig. 2A, right panel). Thus, the ratio spectra quantify the fold-increase in power, *across all frequencies*, triggered by the visual response with respect to the spontaneous activity immediately preceding stimulus onset. The ratio spectra for the example illustrated in Figure 2A showed an increase in power over baseline limited to a relatively narrow band of frequencies (width = 11.3±1.8 Hz; Fig. 2A, right plot, “w”) surrounding a sharp peak at 26 Hz ± 1 Hz (Fig. 2A, right plot, “f”), at which the maximum increase of 6.4 fold was observed for the electrode at the top of L4 (Fig. 2A, right plot, “a”). This specific increase in power, centered in the gamma band, was not uniform through cortical depth but was much stronger in granular (L4) and supragranular (L2/3) layers. This was illustrated by representing the ratio spectra as a 2D plot in which magnitude was color coded (Fig. 2B). The asymmetry between layers was clear when plotting the fold-increase of the peak frequency (26 Hz) versus cortical depth (Fig. 2B, right column), which shows a 2.8 fold difference between L4 and L5/6 and a maximum increase of 6.4 fold at top of L4 (Fig. 2B, right).

Spontaneous gamma oscillations had distinct spectral and spatiotemporal properties compared to visually-driven gamma. We detected spontaneous gamma bouts by the crossing of a significance threshold of 2.5 SD above the mean applied to the gamma band filtered data (see Methods). We then used those time stamps to calculate the power spectrum from the raw, unfiltered data of 1 second after each detection time and their corresponding 1 second preceding baselines. We averaged these single trial spectra (Fig. 2C, left panel) and calculated the ratio spectra (spontaneous bout/baseline) for all 16 channels (Fig. 2C, right panel). As illustrated by the example in Fig 2C, spontaneous bouts were similar to sensory-triggered in their amplitude.
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and peak frequency (maximum 6.2 fold increase at the peak frequency of 25.2 ± 1 Hz). In contrast, spontaneous oscillatory bouts included a much wider spectral increase, spanning beyond the gamma range (between 10-60 Hz, example of Fig. 2C, right panel: width = 18.5 ± 1.4 Hz) and were relatively uniform in magnitude over the cortical layers. The more uniform distribution of spontaneous gamma bouts through cortical depth is illustrated by the pseudocolor plot of the ratio spectra and by the laminar profile of the peak frequency of 26 Hz (Fig. 2D). Thus, in contrast with those driven by visual stimuli, spontaneous gamma oscillations were much wider spectrally and lacked a distinct laminar structure.

To generate population measures, we averaged the responses of the channels located in the supragranular (L2/3), granular (L4) and infragranular (L5/6) laminae, according to the L4 location indicated by the CSD analysis. Because of the variability in spectral power magnitude across experiments, we normalized the ratio spectra of L2/3 and L4 to that of L5/6 for each experiment (Fig. 2E). The population data showed that the increase in visually-driven gamma was significantly higher in L2/3 (1.83 fold greater, p= 0.022) and L4 (1.86 fold greater, p= 0.012) than L5/6. In contrast, the increase in gamma power during spontaneous bouts did not show significant differences between layers, despite a tendency to a larger increase in L2/3 (Fig. 2E).

The width (in Hz) of the increased power in the ratio spectra was measured as the distance between points rising 1 SD above spectral noise (as in Fig. 2A, right panel, “w”). The width did not change as a function of depth in either the spontaneous or stimulus-driven condition, and thus was averaged across all channels for each experiment. The width was significantly larger for spontaneous (23.2 ± 1.9 Hz) than for visually-driven bouts (13.4 ± 2.7 Hz) (Fig. 2F), and while the visually-driven increase generally fell between 20 – 50 Hz,
spontaneous oscillations increased over a broader range from 10 – 60 Hz. Consistent with the example in Figure 2, the population peak frequency in the ratio spectra did not change over cortical depth and was averaged across all channels. The average population peak frequency was not different between spontaneous (29.5 ± 3.4 Hz) and visually-driven (29.1 ± 1.8 Hz) activity (Fig. 2G). We quantified the latency to onset of visually-driven gamma oscillations as the time from onset of the drifting grating presentation to the first threshold-detected peak on the gamma-band filtered LFP. The population average showed a small but not significant difference in onset latency between L2/3 (524 ± 246 ms) and L4 (495 ± 252 ms), but the latency in L5/6 was significantly longer than in L4 (652 ± 279 ms, p = 0.04). In summary, sensory-driven and spontaneous gamma activity showed peaks with similar frequency and magnitude but different bandwidth and distribution through cortical depth.

To further characterize the differences between spontaneous and visually-driven gamma oscillations and their distribution in the cortical depth, we investigated the correlation of gamma phase and magnitude between the cortical layers. We performed coherence measurements (see Methods) between representative channels in L4, L2/3 and L5/6, in which the two non-granular channels were equidistant to L4. In the example of Figure 3A (left panel) the coherence of the response to drifting gratings was strong between L4 and the other two layers along most of the frequency spectrum, but was consistently larger between L4 and L2/3. The visually-driven coherence between supra and infragranular layers was considerably smaller throughout the spectrum. In all three cases, coherence increased in the gamma band (20-50 Hz) but was highest between L4 and L2/3 (Fig. 3A, left panel). We quantified coherence at the gamma frequency band for the population by averaging the coherence values for frequencies from 20 - 50 Hz across the population. The visually-driven coherence between L4 and L2/3 (0.83±0.1) was
greater than the coherence between L4 and L5/6 (0.64±0.2, p< 0.0001). Coherence between L2/3
and L5/6 (0.46±0.2) was lower than for either of the other pairs. The coherence analysis for the
spontaneous gamma oscillations produced the same results as the stimulus-driven (L4 to L2/3 =
0.82±0.1; L4 to L5/6 = 0.64±0.2) Thus, our results show a large degree of broadband spectral
coherence within the cortical column with a strong functional coupling between L4 and L2/3.
More importantly, our results show that coherence is enhanced in the gamma frequency band
both for spontaneous and visually-driven gamma. The similar coherence values between
spontaneous and visually-driven oscillations suggest that, despite the differences in laminar
distribution and frequency band, the phase relations across the cortical depth are equally stable
over a wide spectral band.

Coherence analysis quantifies the stability of the phase relationships between waveforms
but does not provide a measure of the phase value. To measure the phase delay (in ms) of gamma
oscillations between layers, we averaged the gamma oscillations in all channels around the
threshold-detected positive gamma cycles from one chosen electrode in L4. As illustrated by the
example of Figure 3B, these wave triggered averages (WTA) centered on L4 showed a positive
phase shift in supragranular layers progressively increasing towards the pia, and a negative phase
shift that progressively decreased towards the white matter. These peaks can be seen clearly in
the corresponding CSD (Fig. 3B, pseudocolor plots). To obtain a population measure of phase
shift we measured the delay of the central peak with respect to L4 from the same two
representative channels (L2/3 and L5/6) as for the coherence analysis (Fig. 3C). We measured
the phase shift both for stimulus-driven and spontaneous gamma. The example in Figure 3C of
stimulus-driven gamma showed a L4-L5/6 negative delay of -2.6 ms and a L4-L2/3 positive
delay of 2.8 ms. Population averages revealed a positive delay in L2/3 during visually-driven
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gamma (2.7 ± 1.7 ms) that was larger than the positive delay during spontaneous gamma (1.9 ± 1.6 ms; p = 0.029), as shown in Figure 3D. The average phase between L4 and L5/6, in contrast, showed a negative delay (visually-driven = -2.2 ± 1.5 ms; spontaneous = -1.9 ± 1.8 ms). In addition, the WTA analysis showed a much larger central peak for L2/3 than L5/6, emphasizing the functional coupling of the superficial layers with L4. Thus, the large coherence values and the small phase shifts of the gamma positive peaks between layers assures effective interactions in the gamma band along the vertical axis of the cortical column, with particular interaction between granular and supragranular layers. In addition, sensory-driven gamma oscillations have a better-defined spatial and temporal structure than spontaneous oscillations.

**Single units entrain to gamma differently through the cortical layers.**

In order to determine if the laminar structure of the LFP oscillations was reflected in the spike output of V1, we recorded single units and LFPs throughout V1 using independently movable tetrodes (n=215 units). Single units in the mouse primary visual cortex responded robustly to full screen drifting gratings (mean firing rate to visual stimulation = 2.2 ± 3.2 Hz; baseline firing rate = 0.5 ± 1.1; n= 176 cells). However, since response firing rates rarely reached firing rates in the gamma range, responsive cells typically fired only once every several gamma cycles. An example of a cell responding to various orientations of a full screen drifting grating is illustrated by the rastergram in Fig. 4A. The spike response of this example cell was robust as shown by the peristimulus histogram (PSTH) at the bottom, and it faithfully followed the evoked potential recorded in the LFP from the same electrode (superimposed trace on Fig. 4A, polarity inverted for clarity). However, despite the strong responses and the presence of gamma oscillations in the LFP no gamma frequency activity was visible in the PSTH. Therefore, to quantify the rhythmicity of cells given their low firing rates, we generated perievent histograms (PEH) which
are spike time histograms around the threshold-detected positive gamma peaks in the LFP (Fig. 4B). From the PEHs we calculated a rhythmicity index (RI, see methods) as the average distance between trough and peak of the center 3 peaks of the PEH (Popescu et al., 2009). We set a criteria for significance at 95% over the level of the shuffled PEH (Fig. 4C, significance threshold) and found that 38% of single units showed a significant entrainment to stimulus driven gamma-frequency fluctuations in the LFP (mean RI 0.99±0.6) but only 15% to the spontaneous gamma oscillations (mean RI 0.74±0.4) (Fig. 4C). For example, the PEH of the cell in Fig. 4B (green histogram, RI = 2.59) showed a strong entrainment to the visually-driven gamma oscillations, which were very robust as shown by its WTA (Fig. 4B, green line, vertical dotted line indicates the zero time of the central gamma peak). The same cell showed a much smaller entrainment to the spontaneous gamma oscillations (blue histogram, RI = 1.03) which in this example also produced a less robust WTA (Fig. 4B, blue line). Variations in the degree of entrainment of the spikes to the LFP could be a trivial result of variations in the magnitude of the gamma oscillations. However, the entrainment of a given cell could not be predicted by the overall magnitude of gamma activity in the LFP rhythm (Fig. 4D) or the rhythmicity index of other cells in very close spatial proximity (data not shown). In contrast, cell rhythmicity was dependent on the laminar position of the cell during visually driven gamma (Fig. 4E). The mean RIs were highest in L2/3 (1±0.6, p=0.025) and L5/6 (1.18±0.6, p=0.0039) compared to L4 (0.78 ± 0.3). During spontaneous gamma, there were no significant differences in RI (L2/3 = 0.76±0.5; L4 = 0.67±0.3; L5/6 = 0.77±0.4) throughout the cortical depth (Fig. 4E, left panel). Furthermore, compared to spontaneous gamma, visual stimulation only triggered significant increases in gamma rhythmicity index in L2/3 and L5/6 but not in L4 (evoked vs. spontaneous for L2/3 p=0.003872; L4 p=0.12634; L5/6 p=0.000413).
Similar results were obtained when examining the percentage of rhythmic cells by layer. During stimulus-driven gamma L2/3 and L5/6 had a greater percentage of rhythmic cells (38% and 58.5%, respectively) than L4 (20.9%). During spontaneous gamma, the differences were much smaller, although L4 still had the smallest percentage of rhythmic cells (9.3% compared to 17.1% in L2/3 and 26.8% in L5/6; Fig 4E, right panel). To provide additional insight, we performed an alternative quantification to measure the correlation between spikes and the local field signal, spike field coherence. The spike field coherence for L2/3 (0.28 ± 0.1) was significantly greater than that of L4 (0.18 ± 0.06, p=0.0001), while the spike field coherence for L5/6 (0.22±0.1) trended towards an increase over L4, but did not reach significance (p=0.07). In sum, cells in L2/3 and L5/6 showed higher entrainment to visually-driven gamma oscillations than those in L4, and as compared to spontaneous oscillations.

**RS and FS cells entrain differently during spontaneous and stimulus-driven gamma.**

In cortex, gamma oscillations rely strongly on excitatory and inhibitory network interactions. To determine the potential influence of excitatory and inhibitory cells on rhythmogenesis throughout the cortex, we classified all 215 units as putative excitatory (regular spiking, RS) or putative inhibitory (fast spiking, FS) cells on the basis of the shape of their waveform (Fig. 5A) (n= 195 RS, 20 FS)(Niell and Stryker, 2008). Unlike what has been observed in other species(Bruno and Simons, 2002), FS and RS both showed high variability in firing rates and very similar mean rates during visual stimulation (FS = 2.65±2.8 Hz ; RS=2.13±3.3 Hz). However, the average rhythmicity of FS cells was much more pronounced than that of RS cells (RI: FS = 1.42±0.7; RS = 0.95±0.5, p=0.002; Fig. 5B, top panel) and a higher percentage of FS cells were rhythmic compared to RS cells (FS = 75 %, RS = 35%; Fig. 5B, bottom panel). Similarly, spike field coherence was greater for FS cells (0.32±0.1 vs. RS 0.22±0.1, p=0.009). Both FS and RS cells
Sensory-driven and spontaneous gamma oscillations in V1 showed laminar structure in their rhythmicity during stimulus-driven gamma oscillations. FS cells showed no differences in average rhythmicity between L2/3 (1.12±0.6) and L4 (1.16±0.6), but rhythmicity in L5/6 was significantly greater than in either of the superficial layers (2.03±0.67, p=0.02, p=0.022; Fig 5C, dark brown). Similarly, the percentage of rhythmic FS cells in L5/6 (100%) was higher than in L2/3 (66.7%) or L4 (60%; Fig 5E, dark brown). The laminar profile of the rhythmicity of RS cells during stimulus-driven gamma was distinct from that of FS cells (Fig. 5D, dark blue). RS cells had significantly higher rhythmicity in L2/3 (1±0.6, p = 0.006) and L5/6 (1.06±0.46, p=0.0006) than L4 (0.73±0.25), and a similar pattern in the percentage of rhythmic cells across layers (L2/3 = 36%; L5/6 = 52.8%; L4 = 15.8%; Fig 5F, dark blue).

Spike field coherence measurements revealed similar trends, although differences between infragranular and granular layers failed to reach significance. For FS cells, there is a trend toward a larger coherence in L5/6 (0.37±0.1) as compared to L4 (0.29±0.1) and L2/3 (0.31±0.2). RS cells were more coherent in L2/3 (0.27±0.1) than L4 (0.17±0.04, p=0.00002) and show a greater coherence in L5/6 cells (0.2±0.1, n.s.) as compared to L4.

During spontaneous gamma, the mean RI decreased in both cell classes and across all layers. However, the RI of FS cells maintained its laminar structure during spontaneous gamma, with L5/6 cells showing significantly greater rhythmicity than cells in more superficial layers (L5/6 = 1.24±0.47; L4 = 0.75±0.7, p=0.038; L2/3 = 0.82±0.042, p= 0.033) (Fig. 5C, light brown). In contrast, despite the significant entrainment of cells throughout the cortex, RS cells did not show laminar specificity during spontaneous gamma (L2/3 = 0.76±0.5; L4 = 0.66±0.27; L5/6 = 0.71±0.34) (Fig 5D, light blue). The percentage of rhythmic cells followed the same trends as the mean RI for both FS and RS cells during spontaneous gamma (Fig 5E,F, light colors). Taken
together, these results show that the RS cells in L2/3 and L5/6 are specifically engaged during stimulus-driven gamma activity and that both types of gamma activity engage more powerfully FS cells in the deep layers.

Strong entrainment of deep layer cells is in apparent contradiction to the relatively lower amplitude of gamma oscillation in deep layers, as compared to superficial layers (see Fig 2E and Fig 5C-F). To ensure that this contradiction was not due to contamination of the LFP by spike waveforms, we removed spike waveforms of rhythmic from the LFP and recalculated gamma spectral measurements (see methods). The gamma power ratio measurements changed less than 1.5% from those reported in Fig 2E. As an additional confirmation, we calculated the correlation between the LFP gamma power ratio and the number of cells entrained to gamma and found no relationship, allowing us to conclude that the spike waveforms are not contributing significantly to the measured gamma power of the LFP.

**Stimulus-driven, but not spontaneous, gamma frequency activity depends on GABAergic transmission between RS cells.**

Anatomical and electrophysiological studies of cortical circuits have found a strong efferent drive from L2/3 to L5/6 (Thomson et al., 2002; Binzegger et al., 2009). We hypothesized that the increase in gamma frequency activity in the LFP and single units of L2/3 during stimulus-associated gamma might be driving the increased rhythmicity seen in FS and RS cells in L5/6. To test this hypothesis, we specifically reduced gamma frequency activity in the superficial layers through topical application of a weak concentration of picrotoxin (0.75 µM, 75 µL) to the surface of the cortex and presented drifting gratings over the course of 20-30 minutes. Within 5 – 10 minutes, the picrotoxin had noticeably increased the size of the evoked response in the LFP throughout all cortical layers (L2/3 increased from 0.004 mV to 0.01 mV, p = 0.02; L4 increased...
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from 0.004 mV to 0.005 mV, n.s.; L5/6 increased from 0.003 mV to 0.007 mV, n.s.) (Fig 6A, for example voltage traces, Fig 6B). Correspondingly, the magnitude of stimulus-driven gamma activity in L2/3 was selectively reduced (L2/3 gamma ratio value dropped from 21.1±7.9 to 11.4±2.4, p=0.02; L4 raised from 2.1 to 2.5; L5/6 dropped from 6.5±2.2 to 6.3±2, ns) (Fig 6C).

The reduction of the GABAA dependent transmission also reduced the percentage of rhythmic cells in L2/3 (50% reduction) and L5/6 (15%) (Fig. 6D). FS and RS cells were identified on the basis of their waveform shape, as described earlier (n= 30 RS, 7 FS). In L2/3, the number of rhythmic cells was reduced for both FS cells (33% reduction, n = 3) and RS cells (100% reduction, n = 9). In L5/6, the percentage of RS cells with significant rhythmicity was reduced (88% reduction, n=18), but the number of rhythmic FS cells in L5/6 was unchanged (n=3).

These results suggest that networks of FS and RS cells in L2/3 generated gamma oscillations driven by sensory stimulation through a mechanism that was dependent on GABAergic transmission. This network activity underlying gamma generation in L2/3 during visual stimulation contributed to the rhythmicity of RS, but not FS cells in L5/6. This result suggests a mechanism for L5/6 RS cell entrainment based either on the location of their apical dendrites in L2/3 or on excitatory vertical connectivity, and suggests that networks of FS cells in L5/6 are capable of generating gamma oscillations independently of their RS neighbors and of afferent drive from L2/3. Furthermore, the magnitude of stimulus-driven gamma in L5/6 was unchanged following the application of picrotoxin (Fig. 6C), suggesting that L5/6 stimulus driven gamma at a population level does not depend on activity in L2/3 or RS cell entrainment in L5/6. This suggestion is consistent with the hypothesis that FS cells are the primary drivers of gamma frequency activity in L5/6.
Sensory-driven and spontaneous gamma oscillations in V1
Discussion

Our goal was to compare the laminar distribution of gamma frequency activity driven by visual stimuli with that occurring spontaneously in the primary visual cortex of the mouse. We use the term spontaneous simply to indicate oscillations that occur in the absence of a visual stimulus. We do not imply that gamma band activity which is not directly triggered by a sensory stimulus is devoid of function or information, as abundant evidence indicates that ongoing gamma oscillations may underlie a multiplicity of information processing operations (Llinas et al., 1998; Llinas and Ribary, 1998; Llinas et al., 1999; Llinas and Steriade, 2006; Llinas et al., 2007; Roy and Llinas, 2008; Sekar et al., 2013; Llinas et al., 2015). Our results show that visual stimuli drive a 4-20 fold increase of a narrow spectral band of gamma oscillations (peak =29 Hz, width =13 Hz) in the LFP of granular and supragranular layers, which is 1.5 to 2 times larger than the fold increase in gamma in infragranular layers. In contrast, spontaneous gamma is spectrally wider (23 Hz), albeit with the same peak frequency (29Hz), with a non-significant tendency for an increase in gamma power from deep to superficial layers.

Surprisingly, the study of single units revealed discrepancies between the distribution of LFP gamma and rhythmic units. These differences were much more pronounced for evoked than spontaneous gamma. During spontaneous gamma, the percentage of rhythmic units was highest in infragranular layers (27%), followed by supragranular (17%) and granular (9%), while rhythmicity index was not different among layers, consistent with the evenly distributed LFP gamma power. Visual stimulation drove the highest percentage of rhythmic cells and mean rhythmicity index in infragranular (59%, 1.2) layers, followed by supragranular (38%, 1.0) and granular (20%, 0.8), which is exactly the inverse of the arrangement of LFP gamma power (granular>supragranular>>infragranular). Thus, compared with spontaneous gamma, visual
stimuli drove many more units into gamma oscillations and tightened their phase relations with the local field (i.e., increased their rhythmicity index). However, both types of gamma led to an uneven distribution of cellular rhythmicity with higher values in infragranular and supragranular as compared with granular layers.

Breaking the number of rhythmic cells into RS (putative excitatory) and FS (putative inhibitory) cells showed a higher percentage and a larger rhythmicity index of FS cells in infragranular layers compared to the other layers for both spontaneous and evoked gamma. In contrast, rhythmic RS cells were equally distributed across depth during spontaneous gamma, but were more rhythmic in supra- and infragranular than granular layers during visual stimulation.

Thus, overall a higher percentage of cells demonstrating higher rhythmicity index values were present in infragranular and supragranular layers than granular layer.

This apparent discrepancy between units and fields may originate from their different levels of spatial resolution. Single unit recordings report, by definition, from a single cell, but LFPs represent the average synaptic activity from a surrounding volume of tissue much larger than a single cell. Even when recorded by the same electrode as a single unit, the LFP represents an unknown number of activity sources which introduce noise and blur the correlation between the cell and the LFP activity. As the LFP captures a more diverse set of voltage fluctuations, synchrony between these sources is likely to decrease, resulting in increased ‘spectral noise’, which represents an increase in the SD of the power spectrum. Increased spectral noise appears to have an inverse relationship with cellular rhythmicity. For example, in Fig 2B. L4 has the largest spectral noise, and shows proportionately the least neuronal rhythmicity, despite having the largest fold increase in gamma power. Infragranular layers, in contrast, have the least amount
of noise and the largest cellular rhythmicity, despite demonstrating the smallest fold increase in gamma. Supragranular layers have intermediate levels of noise and neuronal rhythmicity. Thus, the discrepancy between laminar distribution of rhythmic cells and LFP gamma power may result from the existence of overlapping ensembles of oscillating cells in granular and supragranular layers with different and shifting phase relations with the average gamma oscillations recorded in the LFP. By representing the average of synaptic potentials in the local vicinity, the LFP may mask the fine grain synaptic structure underlying the entrainment of specific population of cells into synchronized ensembles. Moreover, individual neurons may rapidly switch between ensembles and participate in synchronized gamma networks having different phase relations to our “average” gamma LFP, thus leading to lower rhythmicity. In contrast, in infragranular layers, the almost absent spectral noise suggests quieter networks in response to visual stimulation and, consequently, a cleaner LFP average. Less noisy networks would lead to an average LFP which is more representative of the cell recorded by the same electrode, thus leading to higher rhythmicity index and larger percentage of rhythmic cells. This interpretation implies that gamma activity would be tighter, or less flexible, in terms of participating neurons in infragranular layers compared to granular and supragranular. If our interpretation is correct, the signal to noise ratio is a critical factor in understanding and interpreting measurement of gamma power and neuronal rhythmicity.

The different population dynamics between supra and infragranular layers is compatible with their diverse role in cortical function, which in turn is in agreement with the evolutionary view of the thalamus (Llinas et al., 1998; Llinas and Ribary, 1998; Llinas et al., 1999; Llinas and Steriade, 2006; Llinas et al., 2007; Roy and Llinas, 2008; Sekar et al., 2013; Llinas et al., 2015).
Supragranular layers integrate cortico-cortical information with rapidly changing thalamocortical input, while infragranular layers are responsible for generating subcortical outputs. This work demonstrates that the spatiotemporal characteristics of gamma oscillations may undergo state-specific modulation, and suggests that aspects of gamma oscillation laminar geometry are a component of the sensory response in primary cortical areas.

**Gamma oscillations are ubiquitous during sensory processing and spontaneous, background activity**

Over the last several decades, many studies have produced evidence that gamma oscillations in neocortex are relevant to information representation. Initial work demonstrated increased synchrony in gamma frequency activity in single units in the primary visual cortex (Gray and Singer, 1989; Gray et al., 1989). Later studies revealed that the gamma component of local field potentials in V1 is directly modulated by stimulus characteristics (Friens et al., 2000; Henrie and Shapley, 2005). Work in humans showed that gamma oscillations are modulated by perceptual processes, such as the interpretation of Gestalt stimuli (Keil et al., 1999; Tallon-Baudry and Bertrand, 1999), and are structured differently in patients with psychiatric disorders such as schizophrenia (Kwon et al., 1999; Spencer et al., 2004). Furthermore, results from magnetoencephalography (MEG) have shown that gamma oscillations display a specific spatiotemporal organization over the entire human cortex which are reset by sensory stimuli (Ribary et al., 1999).

However, gamma frequency activity is present not only during information processing related activity, but also occurs spontaneously as part of the background activity of the brain. Spontaneous gamma activity is found during the depolarization phases of anesthesia and slow
wave oscillations, REM sleep, brain activation by stimulation of neuromodulatory systems of the brainstem and in the waking state not in association with motor or sensory activity (Steriade et al., 1996b); reviewed in (Steriade, 2006). The ubiquity of gamma oscillations during a wide variety of brain states brings into question whether gamma frequency activity is stereotypic or state-specific, both regarding its spatiotemporal properties and the cellular networks it entrains. 

**Spatiotemporal structure of gamma oscillations during background activity and sensory processing**

While there are few studies that directly compare the structure of gamma oscillations in different functional or behavioral contexts, a number of studies have described the structure of spontaneous gamma activity. It was shown that spontaneous gamma oscillations have limited spatial synchrony in the cortex of awake cats (Steriade et al., 1996a) and rats (Sirota et al., 2008), and there are multiple distributed current sinks and sources through the depth of the cortex (Steriade and Amzica, 1996). The study by Steriade and Amzica (1996) reported for the first time that gamma oscillations occur in tight phase relations across the depth of the cortex and that single cell firing was tightly locked to the oscillations, indicative of local generation rather than volume conduction. That LFPs are indeed local in nature has been quantified in cat primary visual cortex (Katzner et al., 2009; Xing et al., 2009). We have found a similar tight relation between cell firing and LFPs as quantified by peri-event histograms centered on the peaks of gamma oscillations in the LFP. Furthermore, in our study we quantified the phase shift of visually driven gamma across layers and found a systematic positive shift from L4 to L2/3 of ~ 3 ms and a systematic negative phase shift from L4 to L5/6 of ~ -2 ms. Thus, gamma oscillations occur with very small (<6 ms) shifts of their positive peaks across the cortical depth which may allow effective communication between layers of gamma frequency spikes.
Gamma oscillations produced by stimulation of brainstem cholinergic systems has a homogenous amplitude distribution through the layers of the cortex (Amzica and Steriade 1996), similar to our results for spontaneous gamma activity. Brainstem cholinergic systems depolarize thalamic non-specific nuclei, which in turn globally activate the neocortex through glutamatergic transmission (Steriade, 2006). A study comparing spontaneous gamma oscillations with those evoked by stimulation of an intralaminar, non-specific thalamic nucleus showed a similar and homogenous distribution through the depth of the cortex (Sukov and Barth, 1998). The similarity between gamma oscillations due to activation of diffusely projecting thalamic nuclei and those occurring spontaneously is consistent with the hypothesis that non-specific thalamic inputs may be an important component of the background gamma activity that characterizes functional brain states (Llinás and Pare, 1997; Steriade, 2000; Jones, 2002) and which is altered in several pathological conditions (Llinás et al., 1999). Furthermore, it clearly distinguishes spontaneous gamma oscillations with a non-specific thalamic contribution from those generated by specific sensory inputs as reported in this study, and suggests that the origin and structure of excitatory inputs to cortex may produce the state-specific modification of gamma oscillations. Finally, despite the differences in structure, our study showed that spontaneous and sensory driven gamma share a common mean frequency and spectral amplitude which is in agreement with a similar comparison made in the primary auditory cortex of the rat (Lakatos et al., 2005).

Our results show an increase in gamma oscillation magnitude in the superficial layers that occurs only during stimulus-driven gamma. Similar findings of enhanced gamma activity in superficial layers during visual stimulation have been reported previously in visual cortex of awake macaque (Xing et al., 2012; van Kerkoerle et al., 2014). Specific activation of L4 and L2/3 gamma oscillations with high interlayer coherence during the processing of sensory stimuli
is consistent with the known functional architecture of the cortex. Pyramidal cells in L2/3 have widespread horizontal connections that are known to be critical for cortico-cortical information processing (Felleman and Van Essen, 1991; Barbas et al., 2005; Binzegger et al., 2009), and strong excitatory projections from thalamorecipient L4 neurons drive excitatory activity in L2/3 during the representation of sensory stimuli. Furthermore, the coherence at gamma frequency along the vertical axis of the cortex is highly increased during visual responses but mostly between L4 and L2/3.

In apparent contrast to our findings, (Maier et al., 2011) report higher gamma oscillation magnitude in superficial than deep cortical layers during spontaneous activity in awake monkeys. However, their study did not compare the magnitude of stimulus- and spontaneously-driven gamma oscillations, making these results difficult to directly compare to ours. In addition, this work may be consistent with the slight trend towards larger gamma oscillation magnitude in superficial layers during spontaneous activity (see Fig 2 E). Finally, a portion of this difference in our findings and those from Maier et al. may be attributable to the reduction in top-down activity in anesthetized animals, which may decrease excitatory input to the superficial layers of cortex (Barbas et al., 2005).

It has been suggested that gamma oscillations may ‘bind’ together assemblies of neurons that are representing a single percept, but recent studies have shown that gamma activity depends instead on stimulus intensity, not perceptual grouping (Henrie and Shapley, 2005; Chalk et al., 2010; Ray and Maunsell, 2011). We find that gamma oscillations that are produced during sensory representation in the primary sensory areas are distinct from those occurring in the absence of sensory stimulation. Our work does not reflect a role for ‘binding’ of a percept, but does suggest that gamma activity is a distinct component of the sensory response in V1.
In vitro cortical slice studies have suggested that there may be two independent gamma generating circuits in the cortex, one in the superficial layers and one in deep layers, because a cut in L4 does not reduce the power of pharmacologically induced gamma in either L2/3 or L5/6 (Roopun et al., 2006; van Aerde et al., 2009). Similarly, in vivo experiments demonstrate coherence in the gamma band between either superficial to superficial or deep to deep layer LFPs, with a distinct transition between the two zones in the thalamorecipient layers (Maier et al., 2010). Supra- and infragranular layer gamma oscillations were differentially modulated by ongoing alpha oscillations in the awake, behaving macaque, providing evidence for laminar specificity in gamma activity (Spaak et al., 2012). Additionally, in both in vitro studies, superficial layer gamma was dependent on chloride GABAergic transmission. The laminar functional architecture of sensory-driven gamma reported here depends on GABAergic transmission as well, since superficial application of picrotoxin abolished gamma activity in L2/3. Furthermore, superficial reduction of gamma oscillation amplitude by picrotoxin did not reduce gamma activity in the LFP of deep layers but did reduce the RI of RS cells, suggesting that in vivo as well deep and superficial layers of cortex may have distinct, but related, networks for generating oscillations.

Rhythmicity in supragranular layers may be related to infragranular layers either by direct projections to L5 or by contacting L5 apical dendrites in L2/3, this second possibility is supported by the much stronger LFP gamma in supragranular layers. In addition, PTX in superficial layers reduced the rhythmicity index of deep RS cells (which presumably would have apical dendrites in superficial layers), further supporting the notion that such cells receive entraining input in their apical dendrites. From the preceding arguments it follows that not all L5 cells would be entrained by supragranular input but only those with apical dendrites in L2/3.
Excitatory and inhibitory network contribution to gamma oscillations in cortex

A strong case has been made for the importance of the FS cell network, mediated by gap junctions, in the generation of cortical gamma oscillations. Inhibitory transmission is thought to carry a large portion of the gamma rhythm (Hasenstaub et al., 2005) and rhythmic depolarization of FS cells produces gamma activity in the LFP and increases spike precision (Cardin et al., 2005). Inhibitory interneurons are necessary for the robust generation of gamma oscillations in cortex (Cardin et al., 2009; Sohal et al., 2009; Takada et al., 2014). In addition, in vitro studies have shown the generation of cortical gamma to be abolished by the blockade of GABA A transmission (Traub et al., 2005; Roopun et al., 2006). However, excitatory neurons do contribute to the generation of gamma oscillations through increased excitatory drive, as demonstrated by the depolarization of superficial RS cells which produced gamma activity in the LFP and drove the firing of RS cells in L5/6 (Adesnik and Scanziani, 2010), consistent with the known excitatory drive from L2/3 to L5/6 in sensory cortex (Thomson et al., 2002; Binzegger et al., 2009).

Our work highlights the involvement of both putative excitatory and inhibitory networks in gamma rhythmogenesis, but suggests that the entrainment of excitatory and inhibitory cells varies with the context in which gamma oscillations are generated. RS cells in L2/3 and L5/6 show increased rhythmicity during stimulus-driven activity compared to ongoing gamma oscillations. Furthermore, disruption of rhythmic activity in the superficial layers by the GABA A antagonist, picrotoxin, produces a corresponding decrease in the entrainment of all superficial neurons, along with a specific decrease in the entrainment of only RS cells in the deep layers. This is consistent with the hypothesis put forward by Adesnik and Scanziani, that gamma
activity in L2/3 contributes to the entrainment of RS cells in L5/6. Thus, the excitatory cell network seems to be specifically engaged by stimulus-driven gamma oscillations in superficial and deep cortex.

In contrast, putative inhibitory cells are more rhythmic than putative excitatory cells throughout the cortex, but only display context-specific modulation of rhythmicity in the deep layers. Superficial FS cells display similar rhythmicity regardless of context, and always are less rhythmic than their deep layer FS counterparts. In contrast, FS cells in deep layers show increased overall rhythmicity during stimulus-driven gamma, but also maintain high rhythmicity during spontaneous activity. The specific reduction of superficial gamma oscillations with picrotoxin does not produce a corresponding reduction in deep layer gamma oscillation amplitude or FS cell entrainment to gamma activity, even as the rhythmicity of deep layer RS neurons declines. The relative independence of deep layer FS neurons from superficial excitatory drive is consistent with in vitro studies showing that FS-RS connected pairs of neurons in L5/6 are less likely to receive strong excitatory input than non-FS-RS connected pairs (Otsuka and Kawaguchi, 2009). These results suggest an independent gamma generation mechanism in deep layers that may depend primarily on FS interneuron contribution.

Taken together, these findings show that the dynamic geometry of gamma oscillations through the cortical laminae is influenced by the state in which they occur. The spatiotemporal distribution observed may reflect the divergent sources of excitatory drive to the cortex inherent to different brain states. In addition, excitatory and inhibitory cell networks contribute differently depending on context of the oscillations, with putative excitatory neurons generally showing greater context specificity than putative inhibitory neurons. Our results also are consistent with other reports suggesting that superficial and deep layers of cortex have related
but independent networks for generating gamma oscillations. In addition, they suggest that the
GABAergic-dependent mechanism for the generation of gamma oscillations in the superficial
cortex is a component of the representation of sensory information in the cortex.

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Spontaneous and stimulus-driven gamma oscillations in V1
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Figure 1. Local field potential activity during the presentation of a drifting grating.

(A) Sixteen LFPs, filtered 0.1 – 300 Hz, showing the evoked response to a single presentation of a full screen vertical drifting grating (95% contrast, 3 Hz, 0.08 cycles/degree) for 1 second, as indicated by the green rectangle. An evoked response and corresponding fast frequency activity following stimulus onset are clearly visible during this single trial.

(B) Average evoked response for 50 presentations of the drifting grating. The maximum amplitude of the evoked response occurs 90 ms following stimulus onset within layer 4.

(C) Current source density analysis of the average response in (B) shows a large initial sink in L4 at 90 ms, followed by sinks in L2/3 (182 ms) and L5/6 (201 ms).

(D) Same data as presented in (A) but filtered for the gamma frequency range, 20 to 50 Hz. A stimulus-driven increase in gamma activity occurs during the presentation of the drifting grating, and a spontaneous bout of gamma activity occurs several seconds later.

(E) Average evoked response as in B filtered for gamma frequency range. Because gamma activity is not time-locked to stimulus onset, these averages only represent a small portion of the total gamma activity, as reflected by the smaller amplitude of the averages compared to the single trial activity in (D).

(F) Current source density analysis of the average evoked gamma. Note difference in ordinate scale. Unlike the CSD analysis of the broadband LFP (panel C), there is not one initial sink in L4 followed by distinct sinks in the other layers, but instead non-significant, multiple sinks and sources distributed over space and time.

Spontaneous and stimulus-driven gamma oscillations in V1
Figure 2. Laminar characteristics of stimulus-driven and spontaneous gamma activity.

(A) Left. Power spectra from each of the 16 channels for one experiment. Power spectra are calculated for the 1s window during the presentation of the stimulus (green) and for the baseline period 1 second immediately before the stimulus onset (black). Spectra are calculated for each single trial and then averaged across all trials. Right. For each channel, the stimulus spectrum (green) is divided by the baseline spectrum (black) to reveal the frequencies with the greatest increase above baseline during the presentation of the stimulus. The largest difference is within the gamma frequency band (20-30 Hz).

(B) The values of the stimulus/baseline ratio (A, right hand graph) are plotted with respect to cortical depth. The fold increase over baseline is represented by pseudocolor, with red indicating the largest increase (~6 fold increase). Values for the frequency with the largest increase (26 ± 1 Hz, designated by arrow in panel A, right), are plotted with respect to depth to the right of the color plot. The width of the gamma peak is 11.3±1.8 Hz.

(C) Left. Spontaneous bouts of gamma are identified manually and confirmed by threshold crossing (2.5 SD + mean). Power spectra are calculated in the same manner as in (A), for the 1 second window during the spontaneous bout (blue) and the 1 second baseline period immediately preceding each bout (black). Right. The spectra during the spontaneous bout are then divided by the corresponding baseline spectra for each channel to reveal a ratio measurement.

(D) The ratio measurements calculated in (C) were plotted with respect to depth. The pseudocolor scale indicated the increase over baseline and is the same as in (B). The maximum increase (~6 fold) occurs at frequency 25.2 ± 1 Hz, and the width of the gamma peak is 18.5 ± 1.4 Hz.

Spontaneous and stimulus-driven gamma oscillations in V1
(E) The ratio measurements shown in (A) and (C) for stimulus and spontaneous gamma, respectively were quantified (stimulus n=18 probes, 13 animals; spontaneous n=9 probes, 7 animals). The increase over baseline was quantified as the maximum amplitude for each channel, demonstrated by “a” in panel (A, right). To compare between stimulus and spontaneous, these values were normalized to the value in L5/6. The increase in visually driven gamma was significantly higher in L2/3 (1.83 fold greater, p= 0.022) and L4 (1.86 fold greater, p= 0.012) than L5/6, only during stimulus driven gamma.

(F) The width of the gamma peak includes those frequencies in which the ratio measurement is greater than baseline spectral noise, demonstrated by “w” in panel (A). The width of the gamma peak was similar throughout the layers of cortex, but encompassed a wider range of frequencies during spontaneous gamma (23.2 ± 1.9 Hz) than stimulus (13.4 ± 2.7 Hz, p=0.02).

(G) Peak frequency was designated as the frequency with the largest increase over baseline, demonstrated by “f” in panel (A, right). Peak frequency was constant through the layers of cortex and was the same during stimulus-driven (29.5 ± 3.4 Hz) and spontaneous (29.1 ± 1.8 Hz) bouts of gamma.

(H) The latency to the first bout of gamma was only calculated for stimulus-driven gamma, since this measurement is made from stimulus onset it cannot be calculated for spontaneous gamma. Latency was determined by finding the first gamma peak to cross the significance threshold following the onset of the stimulus. The population average showed no difference in onset latency between L2/3 (524 ± 246 ms) and L4 (495 ± 252 ms), but the latency in L5/6 was significantly longer than in L4 (652 ± 279 ms, p = 0.04).

Spontaneous and stimulus-driven gamma oscillations in V1
Figure 3. Coherence and phase measurements between cortical laminae.

(A) Coherence ± jackknife error measurements for 50 trials of drifting grating presentation. The coherence of L4 and L2/3 between 20-50 Hz (0.83±0.1) was greater than the coherence of L4 and L5/6 (0.64±0.17, p<0.0001). Coherence between L2/3 and L5/6 was lower than both of the other pairs (0.46±0.23). These differences were significant over the population (right panel, N=5 animals), and were similar between stimulus-driven and spontaneous gamma (not shown).

(B) Wave-triggered averages (WTAs) using the peaks of gamma oscillations in L4 as timestamps shows a distinct phase relationship through the cortical depth. The phase relationship is seen even more clearly in the CSD plot to the right of the WTAs. Similar phase shifts are seen in both stimulus-driven (left panel) and spontaneous (right panel) gamma activity.

(C) Detailed view of the WTA averages seen in (B) for one channel from each lamina. L2/3 and L5/6 are equidistant to L4.

(D) Quantification of the phase relationships shown in (B) and (C) for the population shows a larger phase precession through the layers during stimulus-driven gamma than spontaneous (N = 5 animals). The positive delay between L2/3 and L4 is larger for visually-driven (2.7 ± 1.7 ms) than for spontaneous (1.9 ± 1.6 ms; p = 0.029) gamma. The average phase between L4 and L5/6, in contrast, showed a negative delay (visually-driven= -2.2 ± 1.5 ms; spontaneous = -1.95 ± 1.8 ms).

Spontaneous and stimulus-driven gamma oscillations in V1
Figure 4. Single units are modulated by gamma oscillations differently through the cortex.

(A) Raster and PSTH from an RS cell in L2/3 during the presentation of a drifting grating. The evoked response in the LFP (black trace, inverted polarity) follows a similar time course to the PSTH (gray) of the unit.

(B) Peri-event histograms for stimulus-driven (green) and spontaneous (blue) spiking events, calculated with respect to the positive peaks of gamma oscillations that crossed the significance threshold (2.5 SD above mean).

(C) Distribution of rhythmicity indices (RIs) for 176 cells during stimulus-driven (green) and spontaneous (blue) gamma. RI for one third (38%) of the neurons were greater than the significance threshold during stimulus-driven gamma (mean RI 0.99±0.6), and RI for 15% of the neurons crossed threshold during spontaneous gamma (mean RI 0.742±0.4). Box and whisker plots demonstrate the median and interquartile values for each condition (stimulus median = 0.79, lower quartile = 0.62, upper quartile = 1.16; spontaneous median = 0.66, lower quartile = 0.51, upper quartile = 0.84).

(D) The RI did not correlate with the magnitude of gamma oscillations in the LFP from the same tetrode for either spontaneous or stimulus-driven gamma.

(E) Distinct laminar differences in rhythmicity were observed during stimulus-driven, but not spontaneous gamma. Neurons in both L2/3 (1±0.6, p=0.025) and L5/6 (1.18±0.6, p=0.0039) had significantly higher mean RI than L4 neurons (0.78 ± 0.3) (left panel). During spontaneous gamma there were no differences between the mean RIs for each layer (L2/3 = 0.76±0.45; L4 = 0.67±0.33; L5/6 = 0.77± 0.4). Similarly, a greater percentage of neurons in L2/3(38%) and L5/6 (58.5%) were rhythmic compared to L4 (20.9%) during stimulus-driven gamma (right panel).
There was a similar trend during spontaneous gamma, but the differences between layers were smaller than during stimulus-driven gamma (L2/3 = 17.1%; L4 = 9.3%; L5/6 = 26.8%).

Spontaneous and stimulus-driven gamma oscillations in V1
Figure 5. FS and RS classification and modulation through the cortex.

(A) FS and RS cells were well separated on the basis of their peak/trough amplitude ratio, peak-to-trough time and ending slope of the trough.

(B) FS cells showed significantly greater rhythmicity than RS cells both in terms of the mean RI (FS 1.42±0.7; RS 0.95±0.5, p=0.002), and the percentage of rhythmic cells (75% FS, 35% RS).

(C) FS cells displayed a laminar structure to their rhythmicity during both stimulus-driven and spontaneous gamma. During stimulus-driven gamma (dark lines) FS cells in L5/6 were more rhythmic (2.03±0.7) than those in L4 (1.16±0.6, p=0.022) or L2/3 (1.12±0.6; p= 0.02). FS cells had a similar laminar profile during spontaneous gamma (light lines) (L5/6 1.24±0.7; L4 0.75±0.7, p=0.038; L2/3 0.82±.042, p= 0.033). The rhythmicity in L5/6 was greater during stimulus-driven than spontaneous gamma activity (p=0.0217).

(D) RS cells in L2/3 (0.998±0.6) and L5/6 (1.06±0.46) showed greater rhythmicity than cells in L4 (0.734±0.25, p<0.0001). During spontaneous gamma, RS cells showed no distinct changes in rhythmicity through the depth of the cortex (L2/3 0.76±0.5, L4 0.66±0.27, L5/6 0.71±0.34). RS cell rhythmicity in both L2/3 and L5/6 was greater during stimulus-driven than spontaneous gamma activity (p<0.0001).

(E) Similar to the mean RI shown in (C), more FS cells were rhythmic in L5/6 (100%) than in L2/3 (66.7%) or L4 (60%) during stimulus-driven gamma. This same relationship held true for spontaneous gamma (L5/6 = 80%; L2/3 = 33.3%; L4 = 20%).

(F) Like the data presented in (D), more RS cells were rhythmic in L2/3 (36%) and L5/6 (52.8%) than in L4 (15.8%), during stimulus-driven gamma. During spontaneous gamma, there was a trend in the same direction, but the difference between layers was much smaller (L2/3 = 17%; L4 = 7.9%; L5/6 = 19.4%).

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Figure 6. Gamma activity is reduced following the application of picrotoxin.

(A) The GABAA antagonist, picrotoxin, increases the amplitude of the evoked response in the LFP during the presentation of the drifting grating through all layers of cortex, with the largest increase in L2/3 (n = 3 animals, p = 0.0215).

(B) Single traces of evoked responses per layer showing the effect of picrotoxin is to increase the amplitude of the visually evoked response only in supragranular layers.

(C) Picrotoxin reduces the gamma power (20-50 Hz) during the presentation of the visual stimulus in L2/3 (p = 0.0313).

(C) The percent of rhythmic RS cells (n=30) in both L2/3 and L5/6 decreases following the application of picrotoxin (100% and 88% decrease). The percent of rhythmic FS cells (n=7) falls in L2/3 (33% decrease) but not in L5/6 (0% decrease).


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