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Stimulus-dependant augmented gamma oscillatory activity between the functionally connected cortical neurons in V1.

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Abstract.

Neuronal assemblies typically synchronize within the gamma band (30-80 Hz) and are fundamental to information processing. Despite numerous investigations, the exact mechanisms and origins of gamma oscillations are yet to be known. Here, through multiunit recordings in the primary visual cortex of cats we show that the strength of gamma power (20-40 Hz and 60-80 Hz) is significantly stronger between the functionally connected units than the unconnected units within an assembly. Furthermore, there is increased frequency coherence in the gamma range between the connected units than the unconnected units. Lastly, the higher gamma rhythms (60-80 Hz) are mostly linked to the fast-spiking neurons. These results led us to postulate that gamma activity is intrinsically generated between the connected units within cell-assemblies (microcircuits) in relation to the stimulus within an emergent ‘50 ms-temporal window-of-opportunity’.

Introduction

Micro-circuitries in the brain are fundamental in encoding information about the lived experience. Despite numerous investigations, a great deal has to be revealed about the mechanisms by which these networks process information. Coactive neurons with tightly correlated neural activities have been shown to be implicated in encoding processes, wherein neurons fire in close time relationships to each other (Singer, 1999; Buzsaki, 2010, Barthó *et al.*, 2004, Fujisawa *et al.*, 2008, Bharmauria *et al.*, 2014, Bachatene *et al.*, 2015, Molotchnikoff & Rouat, 2011).

Oscillatory rhythms are ubiquitous in cortical networks across different species (Buzsáki *et al.*, 2013; Fries *et al.*, 2007; Bosman *et al.*, 2014) and are believed to be an additional factor to synchronized activity in encoding sensory stimuli. Neuronal assemblies typically synchronize in gamma-frequency spectrum (30-80 Hz) (Gray & Singer, 1989; Engel *et al.*, 2001; Gray & McCormick, 1996; Womelsdorf *et al.*, 2006; Fries *et al.*, 2007) and these rhythms are widely postulated as the fundamental precursor of computational mechanisms within brain networks. Recently it has been suggested that neuronal coherence assigns oscillatory excitation-rhythms in assemblies to eventually create windows of temporal communication (Siegle *et al.*, 2014 and Bharmauria *et al.*, 2014). These oscillations sub-serve various cognitive aspects (perceptual binding, attention, short- and long-term memory). Despite plethora of literature, the specific functions of gamma remain debatable (Fries *et al.*, 2007; Bosman *et al.*, 2014; Ray & Maunsell, 2014;), and a great deal is yet to be known about how these rhythms are generated and maintained.

A widely accepted hypothesis is that inhibitory interneurons embedded in networks are implicated in generation of gamma band synchrony (Wang & Buzsaki, 1996; Bartos *et al.*, 2007; Bartos *et al.*, 2002; Fries *et al.*, 2007; Cardin *et al.*, 2009; Tiesinga & Sejnowski 2009), however, recurrent excitatory connections are a characteristic feature of neural circuits (Shu *et al.*, 2003; Douglas & Martin, 2004) and have also been linked to these chattering reverberations (Gray & McCormick, 1996; Tiesinga & Sejnowski 2009).

Previously, we have reported that a specific functional network is activated within a cell-assembly (simultaneously recorded neurons) that is contingent upon a particular orientation (Bharmauria *et al.*, 2012). The functionally connected neurons within these circuits synergistically cross-interact within a 50-ms emergent ‘window-of-opportunity’ to encode the

presence of stimulus (Bharmauria *et al.*, 2014). During this ‘window-of-opportunity’ the firing rate of the target neuron significantly increases following the spike-occurrence of the reference neuron. Holding this latter investigation as a premise, we sought to investigate how one neuron impinges upon the oscillatory rhythm of its simultaneously recorded companion neuron. Firstly, we report that the connected neuron-pairs in an assembly exhibit higher power at the gamma oscillatory range (**20-40 Hz and 60-80 Hz**) in comparison to the unconnected neuron-pairs; secondly, there is an increased coherence between them. Lastly, the higher gamma mode (**60-80 Hz**) is exclusively related to the fast-spiking neurons. This finding leads us to postulate that gamma routing is specific within cortical circuitry and is an intrinsically generated substrate within an assembly for feature processing.

Materials and methods

Ethical approval

Four animals (Cats) were prepared for electrophysiological recordings in the primary visual cortex, as per the guidelines of Canadian Council on Animal Care and approved by the Institutional Animal Care and Use committee of Université de Montreal. The full description of the protocol is below:

Animals, anaesthesia and surgical procedures

Animals premedicated with acepromazine maleate (Atravet, Wyeth-Ayerst, Guelph, ON, Canada; 1 mg.kg⁻¹, intramuscular) and atropine sulphate (ATRO-SA, Rafter, Calgary, AB,

Canada; 0.04 mg.kg⁻¹, intramuscular), were anesthetized with ketamine hydrochloride (Rogarsetic, Pfizer, Kirkland, QC, Canada; 25 mg.kg⁻¹, intramuscular). The cats were then paralyzed with 40 mg and maintained with 10 mg.kg⁻¹.h⁻¹ gallamine triethiodide (Flaxedil, Sigma Chemical, St. Louis, MO, USA; intravenous) administered in 5% dextrose lactated Ringer's nutritive solution. General anesthesia was maintained by artificial ventilation with a mixture of N₂O/O₂ (70:30) supplemented with 0.5% isoflurane (AErrane, Baxter, Toronto, ON, Canada). Electroencephalogram, electrocardiogram, rectal temperature and end-tidal CO₂ partial pressure were monitored throughout the experiment, and kept in physiological ranges. Pupils were dilated with atropine and Plano lenses with artificial pupils (5 mm diameter) were fixed. The loci of the area centrales were inferred from the position of the blind spots.

Visual stimulation

Monocular stimulation was done. The multi-unit receptive fields (RF) were mapped as the minimum response fields (Barlow *et al.*, 1967) by using a hand-held ophthalmoscope after clearly detectable activity were obtained. These preliminary tests revealed qualitative properties such as dimensions, velocity preference, orientation and directional selectivity. Visual stimuli were generated with a VSG 2/5 graphic board (Cambridge Research Systems, Rochester, England) and displayed on a 21-inch monitor (Sony GDM-F520 Trinitron, Tokyo, Japan) placed 57 cm from the cat's eyes, with 1024 × 768 pixels, running at 100-Hz frame refresh. Stimuli were sine-wave drifting gratings covering the excitatory RF of neurons at both electrodes. Stimuli were presented randomly in 8 different orientations. Contrast was set at 80%. Mean luminance was 40 Cd.m⁻². Optimal spatial and temporal frequencies were set at 0.24 cycles.deg⁻¹ and 1.0-

2.0 Hz range respectively, where V1 neurons are driven maximally by sine-wave drifting gratings (Bardy *et al.*, 2006). Each drifting grating was presented 25 times (each trial lasted 4.1 s) with varying interstimulus (1-3 s) intervals. Thus the presentation of a stimulus lasted 180 s (with all trials and inter-stimulus intervals).

Electrophysiological recording, single-unit selection and OSI (Orientation Selectivity Index)

Multi-unit activity in the primary visual cortex was recorded at 410-820 μ (laterally) apart by a tungsten multi-electrode (Frederick Haer & Co, Matrix Electrode. The multi-electrode had four columns, and each column had one row). For each recording, only two contacts were used (spaced by 410-820 μ). Fourteen recordings were done across all cats either in the left or the right hemisphere. In total, 28 sites were obtained. Each site corresponds to a tip (contact). Recordings were performed in the supragranular layers (cortical depth < 1000 μ m; mean = 650 μ m). The signal from the microelectrodes was amplified, band-pass filtered (300 Hz - 3 kHz), digitized and recorded with a 0.05 ms temporal resolution (Spike2, CED, Cambridge, England). Spike sorting from the multi-unit signals was done, and each electrode yielded isolated single units. All cells were discriminated on the basis of three criteria: 1) the spike-waveforms' difference 2) principal component analysis showing well dissociated clusters 3) and auto-correlograms showing no events at the central point (Fujisawa *et al.*, 2008; Barthó *et al.*, 2004; Csicsvari *et al.*, 1998). The stability of each cell's activity across conditions was verified qualitatively by the visual control of the disposition of clusters and the shapes of waveforms. The details are present in Bharmauria *et al.* (2014).

Cluster analysis was done using Spike2, CED, Cambridge, England in a 3-dimensional plot. The isolation distance was calculated as the Mahalanobis distance. Mahalanobis distance is the

distance from the cluster center within which as many events belong to the other clusters as many belong to the specified cluster (Harris *et al.*, 2001). In other words, given multivariate data values for which the values in each variable are normally distributed around a mean, this measure allows us to define boundaries of constant probability around the multi-dimensional centre of the distribution. This estimation allows the separation of a cluster from the nearest cluster. Units within Mahalanobis distance of 2.5 were considered for the further analysis of the spike trains of neurons to reveal the functional connections between them. Further, the J3 values were calculated for every neuron pair (clusters) that indicates the separation of the clusters. Clusters with J3 value of 1.5 were set as the benchmark for cluster separation. The insert in the figure shows an example of a neuron pair (clusters) that has a J3 value of 1.56. The respective waveforms are shown adjacent to the clusters. Across all recordings, 276 pairs were analysed. Figure 1A shows this metanalysis.

Once single cells were sorted out off-line from multi-unit spike trains accumulated during data acquisition, orientation tuning curves of cells were obtained from raw data and fitted with the von Mises function (Swindale, 1998).

$$M(\theta) = A \cdot e^{b(\cos(\theta-c))} + d \quad (i)$$

where, A is the value of the function at the preferred orientation c, and b is the width parameter.

An additional parameter, d, represents the spontaneous firing rate of the cell. $M(\theta)$ is the firing rate of the neuron at orientation, θ . This allowed us to determine with precision, the preferred orientation of every cell.

An orientation selectivity index (OSI) was calculated to ensure the tuning of neurons. It was measured using the fitted tuning curves by dividing the firing rate at baseline (orthogonal

orientations) by the firing rate for the preferred orientation, and subtracting the result from one (Ramoia *et al.*, 2001, Liao *et al.*, 2004; Bachatene *et al.*, 2013). The closer the OSI is to one, the stronger the orientation selectivity. Figure 1B presents the OSI for all the analysed neurons ($n = 141$). The mean OSI with standard deviation was found to be 0.81 ± 0.14 .

Cross-correlograms and network-formation

Cross-correlograms were constructed between the trains of action potentials of every recorded neuron at all the sites, and all the applied orientations to reveal the functional connections. The raw cross-correlograms were shift-corrected to eliminate the putative significant peaks due to the simultaneous stimulation of both cells during each trial (to remove the stimulus evoked and locked components) (Perkel *et al.*, 1967).

A significant peak of 2 ms (two adjacent 1 ms bins) or at least one significant bin (Alloway & Roy, 2002) was searched within a window of ± 5 ms around zero (excluding the ± 1 ms bins around zero) in the shift-corrected cross-correlograms to reveal a functional connection between two neurons. The statistical threshold for the significant peak was set at 95% (red curved line in cross-correlogram), and the probability (P) of the neuron firing in a bin is calculated as follows (Abeles, 1982):

Considering the spike train is a Poisson train, the probability (P) of the neuron to fire in the small bin of the size b is: $P = F \cdot b$ and $F = N/T$ (ii)

where, F is the neuron frequency, T is the total time interval, and N is the number of spikes in that interval.

The confidence limit was calculated as per (Abeles, 1982):

The expected bin count (C) for the histogram is then:

$C = P \cdot N_{\text{Ref}}$, where N_{Ref} is the number of the reference events.

The confidence limits for C are calculated using the assumption that C has a Poisson distribution.

Assume that a random variable S has a Poisson distribution with parameter C. Then the 95% confidence limits are calculated as follows:

Low Conf. = x such that $\text{Prob}(S < x) = 0.005$

High Conf. = y such that $\text{Prob}(S > y) = 0.005$

Based on the shift corrected-cross-correlograms, functional networks between all neurons at all orientations were wired. In case when two cells recorded from the same tip fired in synchrony, their respective waveforms are summed and consequently the shape of the resultant waveform is rejected as it falls outside the range of the template. In the present investigation ‘synchrony’ of spikes from the same tip is excluded from the analysis. We considered only spikes that fell beyond ± 1 ms after the central point in the cross-correlogram to make significant projections. In addition, cumulative sums based on all histograms of the firing rates indicated that the reference spike modulates the discharge of the target neuron. For every cross-correlogram the cumulative distribution of spikes was computed (it is displayed above every cross-correlogram). This computation indicates the modulation of the firing rate of the target cell after the reference cell fired an action potential. The reference spike occurred at point zero, that is, the central point in the cross-correlogram and the corresponding cumulative distribution is shown above. This further ascertains the functional connectivity between both cells since the activity of the target cell depends upon the activity of the companion unit (reference neuron).

The cumulative sum (cs) graphs above histograms are calculated as:

If we have a histogram with bin counts $bc[i]$, $i = 1, \dots, N$.

then the cumulative sum $cs[i]$ for a bin is:

for bin 1: $cs[1] = bc[1] - A$

for bin 2: $cs[2] = bc[1] + bc[2] - A * 2$

for bin 3: $cs[3] = bc[1] + bc[2] + bc[3] - A * 3$, etc.

Peri-event spectrogram and coherence analysis

The analysis was performed using NeuroExplorer 4, Nex Technologies. For each selected variable, this analysis calculates multiple spectrograms that start at the specified time after the occurrence of each reference event (spike). These spectrograms are then averaged over all the reference events. The power spectra were calculated using 512 frequencies between 1 and 120 Hz, smoothed with a Gaussian Kernel with bin width of 3. The analysis window started from -0.75s (relative to the reference event) until 0.75s. The sliding window (shift) was set at 20 ms (that is, 100 shifts for the window of analysis).

The power spectrum is calculated for the specified number (Number of Shifts) of windows as follows:

For a timestamped variable, the rate histogram is calculated and copied into a Signal array. The following parameters are used:

$$\text{Histogram Start} = \text{Start} + \text{Shift} * (\text{Window Number} - 1)$$

$$\text{Bin} = 1 / (2 * \text{Maximum Frequency})$$

$$\text{Number of Bins} = 2 * \text{Number of Frequency Values}$$

In other words, each timestamp of the reference neuron (t) was taken, then a bunch of histograms of another neuron in time intervals ($t + \text{Histogram Start}$, $t + \text{HistogramStart} + \text{Bin} * \text{Number of bins}$) was calculated.

where, 'Histogram Start' formulism as described above.

For each interval, rate histograms are calculated and detrended, Hanning window is applied and FFTs (Fast Fourier transform) were calculated. Then, individual and cross-densities were calculated. Finally, the FFT analysis on these rate histograms produced a single spectrogram for timestamp t . The same was repeated for another timestamp of the reference neuron and so on. In a similar fashion the peri-event spectrograms were calculated for all the analysed neurons ($n = 141$) in response to the stimulus (that is, when the stimulus was set as trigger instead of the spike of the neuron).

Thereafter the coherence was calculated. Coherence measures the correlation between two time series as a function of frequency (Sakkalis *et al.*, 2009; Pereda *et al.*, 2005). In our study, we computed coherence between the time-series (spike trains) of two neurons using 512 frequencies between 1 and 120 Hz. The overlap-window was set at 5% and it was smoothed with a Gaussian kernel with binwidth of 3. For each time stamped variable coherence was calculated in a similar fashion as above.

Results

Anaesthetized cats were used for study in these experiments. Sine-wave drifting gratings were presented within the receptive fields of neurons in V1 to record their activities simultaneously with multielectrodes. Thereafter, neurons were spike sorted from the multiunit activity and traced

at all the orientations. As described in the methods section, the functional connections were revealed at the tested orientations between the recorded neurons (Bharmauria *et al.*, 2012). Such simultaneously recorded neurons from a single tip of a multi-electrode were termed as a cell-assembly. Following this, the goal of the current investigation was to observe the changes in firing frequency of a neuron with reference to the firing of another simultaneously recorded neuron in the assembly. Some neurons in the assembly connect to each other while others do not exhibit connections (Bharmauria *et al.*, 2012; Bharmauria *et al.*, 2014) depending upon the applied orientation. In total, 198 pairs were analysed from 28 recording sites. The connected (n = 98) and unconnected (n = 100) neuron-pairs were investigated across the same assemblies. Table 1 summarizes the recordings. It is to be mentioned that in the current analysis, a single orientation where an assembly exhibited most number of connections was chosen for the peri-event spectrogram analysis, although the networks were revealed at all the tested orientations.

Augmented gamma activity between functionally connected neurons

We began with the peri-event spectrogram analysis for the same pairs for which cross-correlations were computed. Figure 2 describes a typical example of cross-correlogram and peri-event spectrogram analysis of a neuron-pair. The functional connection between the pair was revealed by computing a cross-correlogram (shift-corrected, see methods) between the spike trains of both neurons. In Figure 2A, the blue raster corresponds to the reference neuron and the cyan raster represents the target neuron. The cross-correlogram analysis (Fig. 2B) reveals a significant peak (95% statistical threshold highlighted by the cyan background, see methods) within 5 ms after the zero (black broken line), thereby indicating that the blue neuron projects

onto the cyan neuron (that is, it excites the blue neuron in the circuit). This is highlighted by the black horizontal arrow linking the two neurons (blue and cyan circles above the cross-correlogram). The probability of the significant peak was found to be 0.13 and depicts the strength of the connection. The black curved line above the cross-correlogram represents the cumulative sum based on histogram and further ascertains the upsurge in spike-activity of the target neuron after the reference neuron's firing as described previously (Bharmauria et al., 2014). This upsurge suggests the augmented excitability. Thereafter, the peri-event spectrogram analysis (see methods) was performed for the same neuron-pair (Fig. 2C). It was found that once the reference neuron (blue) spikes at zero (indicated by the red broken line), firstly, there is an increase in frequency-power (indicated by the colored scale) of the target neuron (cyan) that straddles zero. Secondly, there is a distinct zone of increased frequency that ranges approximately from 20-40 Hz (low-gamma oscillatory band) as depicted by the pink arrow in Figure 2C.

Some neuron-pairs did not exhibit connections between them. In a similar fashion, the peri-event spectrogram analysis was performed for the unconnected neuron-pairs. Figure 2D shows the respective spike-trains for the unconnected neuron-pair (red neuron is the reference). The absence of a significant peak in the cross-correlogram (Fig. 2E) indicates that no functional link exists between neurons. The spectrogram analysis (Fig. 2F) for the pair shows that activity of target neuron is unmodulated and this indicates the absence of a distinct gamma activity band between neurons (it is to be noticed that all frequencies are almost equally represented).

Figure 2G depicts that there is an augmented gamma power in the 20-40 Hz range (highlighted by the shaded gray area) for the connected neuron-pair (blue curve) than the unconnected

connected pair (pink curve). It is to be mentioned that these four neurons have been isolated from a single tip, thus constitute a cell-assembly.

Significant difference between the connected and unconnected units

Figure 3 shows the averaged analysis for the connected ($n = 98$) and unconnected ($n = 100$) neuron-pairs. Figure 3A illustrates that there is a significant difference between the time course of evolution (0.75 s to $+ 0.75$ s) of the oscillatory activities between the connected (green curve, $n = 98$) and unconnected (red curve, $n = 100$) neuron-pairs (unpaired t-test $p < 0.0001$). The shaded area depicts the SEM. The curves are aligned with the responses of the reference neuron at the zero mark (indicated by the red broken line). To better compare the gain in activity the raw curves were normalized. The normalization was done with the lowest value set to zero. Figure 3B shows the normalized curves for connected and unconnected pairs that are significantly different (paired t-test, $p < 0.0001$). The power straddling zero for connected pairs is approximately twice (indicated by the black arrow) the activity for the unconnected neuron-pairs. The shaded purple area represents the characteristic “temporal widow-of-opportunity” and coincides with the 50 ms-epoch (the peak of the green curve is offset from the peak of the red curve approximately by 50 ms, and the increase in the rhythmic activity before zero is due to the application of stimulus as it is presented at the beginning of the trace) in our previous finding (Bharmauria et al., 2014).

In one of the most significant results of the current investigation (Fig. 3C), we found that there is an augmented power of gamma activity (~ 20 -40 Hz and 60-80 Hz) between the connected (blue curve) pairs than the unconnected (pink curve) pairs (unpaired t-test, $p < 0.0001$). The

normalized curves (Fig. 3D) further show that the power is substantially different (paired t-test, $p < 0.0001$) in the low and high gamma bands (indicated by the gray shaded areas) for the connected and unconnected pairs. To further confirm that indeed the revealed connections and the consequent higher power between the connected neurons are an outcome of stimulus presentation (each spike of the reference neuron is aligned at zero); we investigated the same connected pairs for the frequency-power analysis at spontaneous (black curve) oscillations (Fig. 3E). The curves at both conditions were significantly different (unpaired t-test, $p < 0.0001$). Figure 3F depicts the normalized curves and reflect the significant difference in gamma activity (shaded gray area) between the spike-triggered activity of pairs at stimulus presence and absence (spontaneous oscillations) fluctuations (paired t-test, $p < 0.0001$). These distinct gamma bands have been extensively reported in literature to the presentation of stimulus (Brunet *et al.*, 2014; Fries, 2005; Fries *et al.*, 2007; Miller *et al.*, 2014), thus it was important to examine whether this spike-triggered gamma activity was also present when the stimulus was set as trigger. To this goal, we calculated the peri-event spectrograms of all neurons ($n = 141$) with reference to the stimulus. Figure 3G (dark cyan curve) represents the mean power of spectrogram analysis and shows that these two zones of gamma activity (shaded gray area) were also present when the stimulus was set as trigger. Figure 3I (normalized curves) depicts that the spike-triggered power (blue) was significantly higher and different (paired t-test, $p < 0.0001$) than the stimulus-triggered power (dark cyan). Collectively, this reference-neuron-triggered difference in spectral power between the two classes points to the fact that gamma activity is specifically routed between the functionally connected neurons in a stimulus-activated-assembly. Moreover, it seems from the latter analysis that gamma is masked within microcircuits, thus a contentious debate (Fries *et al.*, 2007; Bosman *et al.*, 2014; Ray & Maunsell, 2014) prevails over their

occurrence (as all previous studies are related to LFP that capture the activity of all the neuronal population within in a volume).

Augmented coherence between the connected neuron-pairs

We then calculated the coherence for all the analysed pairs as it has been suggested as an inevitable mechanism for the generation of oscillatory rhythms within an assembly (Fries, 2005). Coherence measures the relationship (correlation) between frequencies of two time-series (Sakkalis *et al.*, 2009). If either the power or phase of the signals changes, it leads to a change in the coherence value of the analysed signals. That is, if two signals have a phase or power-relationship they will have a higher value of coherence.

Figure 4 illustrates this analysis. Figure 4A shows the difference in coherence between a connected (purple curve) and an unconnected (orange curve) neuron-pair. There is an increased coherence (0.08) for the connected pair around 20-40 Hz, although the pair also exhibits coherence at higher frequencies. It is to be noticed that these are the same pairs as in Figure 2. Figure 4B shows the global trend for the connected (purple curve, $n = 98$) and unconnected (orange curve, $n = 100$) pairs, with SEM (shaded area). The global coherence analysis between the two classes shows that there is an added coherence for the connected pairs in the gamma spectrum especially in the lower gamma zone (the shaded gray area) than the unconnected pairs and it is significantly different (unpaired t-test, $p < 0.0001$). This suggests that inter-neuronal connections facilitate multiple rhythmic activities, however, it is more pronounced at the lower gamma-band. On the other hand, unconnected neurons appear to be lacking in these rhythmic

oscillations. Figure 4C depicts the normalized curves for connected (purple curve) and unconnected pairs (orange curve) and it further shows that the increase for connected pairs is significantly different than unconnected pairs in the gamma range (20-40 Hz and 70 Hz, shaded gray areas), (paired t-test, $p < 0.0001$). This distinct augmented coherence in low and high frequency domains further points to the fact that connected neurons share an oscillatory phase between them.

Interaction between the regular spikes (RS) and fast spikes (FS)

Inhibitory interneurons embedded in excitatory-neuron networks have been widely postulated to be generators of gamma oscillations (Wang & Buzsaki, 1996; Bartos *et al.*, 2007; Bartos *et al.*, 2002; Fries *et al.*, 2007). To investigate this, we dissociated the spike waveforms based on their ascending slope into regular and fast spikes (Niell & Stryker 2008; Vinck *et al.* 2013) that are putatively linked to pyramidal neurons and inhibitory interneurons respectively (Niell & Stryker 2008; Bachatene *et al.* 2012; Vinck *et al.* 2013). The histogram in Figure 5A shows the dissociation of spike-waveforms into regular (red) and fast spikes (gray) – waveforms with ascending slope of ≤ 0.3 ms were categorized as fast spikes and > 0.3 ms were defined as regular spikes. Out of a total of 141 waveforms (neurons), 40 were separated as fast spikes and 101 were dissociated as regular spikes. A typical example of a fast spike (putative inhibitory interneuron) and a regular spike (putative pyramidal cell) is shown as an insert in Figure 5A.

Thereafter, all the connected pairs were dissociated as regular-spike-regular-spike (RS-RS) connections, fast-spike-regular spike (FS-RS) connections and fast-spike-fast-spike (FS-FS) connections. The distribution is illustrated in Figure 5B — 73 connections were between regular spikes (RS-RS); 21 were FS-RS and only four were between fast spikes (FS-FS). Further, we

analysed the respective peri-event spectrograms and coherence values for connections between only regular spiking neurons (RS-RS) and fast-spiking neurons' interactions with any other neuron (FS-RS and FS-FS). Figure 5C depicts the spectrogram analysis and it was noticed that RS-RS connections (red curves) only exhibited the low-gamma oscillation, whereas, the high-gamma rhythm was absent. On the contrary, interestingly, when the FS was involved in a connection (FS-RS or FS-FS) the higher gamma band around 60-80 Hz stood pronounced, although the low-gamma hump was still present. The raw curves were significantly different from each other (unpaired t-test, $p < 0.0001$). The results are further accentuated in the normalized curves (Fig. 5D) that were significantly different from each other (paired t-test, $p < 0.0001$). The shaded cyan areas underline two distinct epochs of gamma activity. Furthermore, the coherence analysis on the same pairs yielded results that further corroborated the above findings. The RS-RS coherence (red curve) was present only at low gamma band, whereas, fast spiking neurons exhibited higher coherence (black curve) with other neurons (FS-RS and FS-FS) at low as well as high gamma rhythms. The curves were significantly different from each other (unpaired t-test, $p < 0.0001$). The significant difference (paired t-test, $p < 0.0001$) between normalized curves further shows the difference in low and high gamma rhythms between two classes as highlighted by the shaded cyan areas. In summary, these latter analyses reflect that higher gamma rhythms are mostly linked to the fast spiking neurons or putative inhibitory interneurons.

Discussion

In the present investigation, using peri-event spectrogram analysis we showed that once a neuron spikes in a connected neuron pair in an assembly, it switches the target neuron firing into the

gamma-oscillatory mode. The power of gamma oscillations in the connected pairs is significantly higher than unconnected neuron-pairs, although all neurons were activated with the stimulus. Since there is an added coherence for the connected pairs, this suggests that the oscillatory rhythms originate from this systematic phase-relationship between the firing of connected neurons.

Anaesthetized animals were used in current experiments. A stimulating orientation grating was presented within the receptive field of the neurons. It has already been shown by other reports that different oscillatory rhythms might be present in anaesthetized animals either spontaneously or in relation to the stimulus (Brunet *et al.*, 2014a; Fries, 2005; Fries *et al.*, 2007; Miller *et al.*, 2014; Brunet *et al.*, 2014). Moreover, we have already reported that the revealed connections between neurons are strongly related to the stimulus rather than the spontaneous fluctuations in the brain (Bharmauria *et al.* 2012; Bharmauria *et al.*, 2014).

Functional Consequences

Previously, it has been shown by many authors that there is rhythmic gamma oscillatory activity that develops within the population in relation to the stimulus (Brunet *et al.*, 2014; Fries, 2005; Fries *et al.*, 2007); however, all these studies have been done on LFP's (local field potentials) that provide the activity of all the neurons captured in a local population. Interestingly, the major result that we found in current investigation shows that the connected neurons within an encoding population of neurons (a cell-assembly) exhibit heightened gamma activity than the unconnected neurons within the same assembly. Moreover, there is an increased frequency coherence related to these connected neurons. Recently, we have reported that after the reference neuron spikes in a connected pair, it leads to the upsurge in the excitation of the target neuron for a short time-period of 50 ms (window-of-opportunity) and, thereafter, the activity gradually

subsidies (Bharmauria *et al.*, 2014;). Furthermore, the comparison of the spectrograms of the same connected neurons in relation to the spike-triggered activity at stimulus presence and absence (spontaneous oscillations) shows that the reference neurons switch the target neurons into the gamma mode.

Few decades ago an emerging hypothesis (binding-by-synchrony) emerged explaining the encoding of sensory signals. This hypothesis rests on the principle that selected neurons within an assembly synchronize their spikes within gamma oscillations to frame a specific network (Salinas & Sejnowski, 2001; Singer, 1999; Engel *et al.*, 1991; Milner, 1974). In general, a precise synchrony is attributed to the common afferent input; however, quasi-synchronous delays (~ 5ms) between neuronal spike trains are indicative of putative functional links between cells (Barthó *et al.* 2004; Fujisawa *et al.* 2011; Bharmauria *et al.* 2014). Thus, within the framework of a distributive system (Bernardet & Verschure, 2002; Behrmann & Plaut, 2013), wherein circuits are specifically programmed to encode a stimulus, we suggest the following process for information retrieval within circuits: To the presentation of a stimulus, firstly a group of neurons (typically defined as a cell-assembly, but is synonymous to synfire chains, reservoir, ensemble) become simultaneously active, wherein they share frequency-coherence eventually switching the neurons within the assembly into a recurrent gamma-oscillatory mode (Fries, 2005; Miller *et al.*, 2014). Functional connections are then engineered between neurons in an assembly that have an added coherence as suggested by Fries, (2005) because the input and output gates are open for these neurons at the same time. This ultimately cascades into the temporal “window-of-opportunity” (Bharmauria *et al.*, 2014; Fries, 2005; Siegle *et al.*, 2014) for neurons to encode the relevant information downstream and wire a specific network contingent upon the input (synonymous to a classifier, as postulated by Singer, 2013). In other words, an intrinsically

generated functional network is constructed by a cell-assembly depending upon the input as has been recently postulated by numerous authors (Miller *et al.*, 2014; Singer, 2013; Harris & Mrsic-Flogel, 2013). Sparse activity of neurons has emerged as a major phenomenon for neural coding within cortical circuits. Authors have postulated that the temporal codes and sparseness may well arise from each other (Molotchnikoff & Rouat, 2011; Guyonneau *et al.*, 2004; Ratté *et al.*, 2013). Sparseness implies the low firing rate of neurons in response to a stimulus and it very well explains the redundancy reduction and multiplexing (Molotchnikoff & Rouat, 2011; Ratté *et al.*, 2013) of neural codes (temporal and rate codes) within circuits, however, it has a few problems associated to it — that rest on the fragmented organization of the sparse activity and seems contrary to the coherent perception of everyday events (Molotchnikoff & Rouat, 2011; Barth & Poulet, 2012; Jeyakumar *et al.*, 2013). This so called ‘window-of-opportunity’ (Bharmauria *et al.*, 2014; Siegle *et al.*, 2014) organised within oscillatory phases seems to overcome these hindrances that sparseness poses, that is, the increase in firing during this period. Thus such ‘window-of-opportunity’ creates a burst of cooperative neuronal discharges that potentiate coding within sparse circuits. Indeed, this study differs from previous studies (Brunet *et al.*, 2014; Fries, 2005; Fries *et al.*, 2007; Salinas & Sejnowski, 2001; Singer, 1999) in that it rests upon the increased neuronal coherence between ‘functionally connected’ neurons within an assembly; however, it also integrates synchrony (quasi- synchronous functional links within 5ms) and coherence hypothesis that are at the heart of dynamic communication structure in cortical circuits. Lately, Womelsdorf *et al.* 2012 have shown that high OSI of neurons was linked to high gamma (although the experiments were performed in awake monkeys, thus the gamma bands differed across monkeys), we may infer that most of neurons’ firing in the current investigation was in sync with gamma as the mean OSI was high and gamma was present in

relation to the stimulus.

PING (pyramidal-interneuron gamma) or ING (interneuron gamma)?

Excitatory pyramidal neurons and inhibitory interneurons are two major classes of neurons that fill the cortex, and synchrony within a limited space volume (e.g. an orientation column) can be achieved by their specific interactions (Tiesinga & Sejnowski 2009) – ING (interneuron gamma related to mutually connected FS cells) or PING (pyramidal-interneuron gamma, related to mutually connected FS-RS cells). Since in our investigation, we found that low and high gamma oscillations were simultaneously generated by the interaction of fast spikes with other spikes (since RS-RS interactions were at low gamma only), we suggest that PING gamma may be implicated in generation of these synchronous outputs (Tiesinga & Sejnowski 2009; Cardin *et al.*, 2009); whereas, the RS-RS gamma synchrony might be an outcome of feedforward flow from the upstream areas (Tiesinga *et al.*, 2009).

Recently the role of gamma oscillations has been questioned (Ray & Maunsell, 2014) — mainly for the following reasons: first, they have low power, particularly during the first 200 ms after stimulus onset (Ray & Maunsell, 2014). Secondly, the irregularity of their occurrences which appears to depend on several systemic (brain state) and stimuli factors. Contrary to these investigations, here we associated gamma oscillations to the neuronal activity of a companion cell instead of the stimulus onset. In our investigation, although the stimulus-triggered gamma was present (nevertheless weak) but our strategy (spike-triggered spectrograms) led us to uncurtain the heightened power of these oscillations between connected units in this range. Thus, this study might help reconcile the debate on the occurrence of gamma oscillations.

We conclude that co-active neurons in response to a stimulus feature a cell-assembly; however, most importantly, it is the specific network and gamma routing between neurons within a characteristic “temporal window-of-opportunity” that eventually lead to the feature encoding. The intrinsically generated gamma oscillations specifically spread between connected neurons within V1 circuits in relation to the stimulus that consequently lead to preliminary processing of information, thereafter, passing this information downstream in cortical areas through feed-forward flow.

Conflict of interest

The authors declare no conflict of interest.

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Abbreviations

FS, fast spikes; FFT, fast fourier transform; ING, interneuron gamma; OSI, orientation selectivity index; PING, pyramidal-interneuron gamma; RF, receptive field; RS, regular spikes.

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Table 1. Statistics for analysed neuron-pairs

Total number of sites (Cell assemblies)	Average number of cells isolated	Connected cell-pairs analysed	Unconnected cell-pairs analysed
28	5.1	98	100