Long-range intralaminar noise correlations in the barrel cortex

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Recently, a novel study (Olsen et al. 2012) established that layer (L) 6 neurons in the visual cortex modulate the gain of sensory responses and proposed that the different layers have distinct roles within cortical circuitry. Accepting this notion, it becomes crucial to establish the spatial dimensions of the laminar effect relative to the vertical column. Intracortical connectivity data highlight extensive vertical axons linking the layers within local domains, while the probability of monosynaptic connections between pairs of neurons drops sharply with horizontal distance (Boucsein et al. 2011). Indeed, Olsen et al. (2012) reported that the effect of L6 is restricted to the cortical domain directly above the activated region. Accordingly, it is expected that the laminar influence would be highly constrained by the vertical columnar organization.

Challenging this notion, we assessed the spatial dimensions of the laminar effect in cortical networks by analyzing spike-count noise correlations between multiple neurons with identified laminar and columnar position during spontaneous activity (Averbeck et al. 2006). Numerous studies have been devoted in recent years to the study of spike-count correlations in complex central nervous system structures such as the neocortex. It has been argued that these correlations have important implications for cortical processing and information encoding (Zohary et al. 1994; Reich et al. 2001; Averbeck et al. 2006). Functional connectivity data highlight extensive vertical axons linking the layers within local domains, while the probability of monosynaptic connections between pairs of neurons drops sharply with horizontal distance (Boucsein et al. 2011). Indeed, Olsen et al. (2012) reported that the effect of L6 is restricted to the cortical domain directly above the activated region. Accordingly, it is expected that the laminar influence would be highly constrained by the vertical columnar organization.

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Here we show that the laminar position of a cell is a central determinant of the firing rate fluctuations over horizontal distances of several functional columns. We also provide a spatial measure and quantitative estimate to the relative weight of the spike-count correlations in the two dimensions of the cortical network.

MATERIALS AND METHODS

In vivo recordings. A more detailed description of our methods can be found in Reyes-Puerta et al. (2014), a study reporting complementary analyses of sensory-evoked activity from the same animals. All procedures were approved by the local ethics committee (No. 23177-07/G110-1-010) and followed the European and German national regulations (European Communities Council Directive, 86/609/ECC). Male Wistar rats (postnatal days 28–42, 80–200 g) were anesthetized using urethane (1.5 g/kg) and fixed into a modified stereotaxic device. A 3 × 3 mm craniotomy was performed over the barrel cortex of the left hemisphere. Body temperature was held at ~37°C. The depth of anesthesia was maintained at stage III/IV (Friedberg et al. 1999) during the whole experiment.

We identified the barrel-related columns of interest by performing voltage-sensitive dye (VSD) imaging (VSD RH1691: Optical Imaging, Rehovot, Israel). VSD signals were evoked by single whisker deflections to determine the insertion points of the 128-channel “silicon probes” (NeuroNexus Technologies, Ann Arbor, MI), which contained eight shanks separated by 200 µm, presenting a vertical spacing of 75 µm between the recording sites. The position of the electrode insertion was determined by the VSD imaging responses and the pattern of the blood vessels on the cortical surface (Fig. 1B1).

In five of the experiments, the electrode shanks were oriented along barrel rows, and in two other experiments the recordings were done along the barrel arc. For postmortem histological verification of the electrode tracks, the probes were labeled with DiI (Molecular Probes, Eugene, OR) before insertion. For whisker stimulation, individual whiskers were briefly deflected in the vertical direction by inserting them into a capillary tube glued to a piezoelectric bimorph actuator (Physik Instrumente, Karlsruhe, Germany), controlled by a voltage pulse generator (Master-8: A.M.P.I., Jerusalem, Israel). The voltage pulse was a 2-ms square step. The stimuli were applied in blocks of typically 100 or 200 trials, and the frequency of stimulation changed between blocks, from 0.2 Hz and up to 10 Hz. Stimulation-free, spontaneous activity was recorded for a period of between 8.3 and 33.3 min.

Histology. Animals were deeply anesthetized after the experiment with ketamine (120 mg/kg, ketamine, 50 mg/ml; Hameln Pharma, Hameln, Germany) and xylazine (5 mg/kg, Rompun 2%; Bayer, Leverkusen, Germany), being afterwards perfused through the aorta with 10 IU/ml heparin in phosphate-buffered saline and 4% paraformaldehyde. The brain was carefully dissected out of the skull, and the barrel cortex was tangentially cut and flattened from 2 to 1 mm. After 24 h fixation in 4% paraformaldehyde, the cortex was rinsed in 0.1 M phosphate buffer and incubated in 30% sucrose (in phosphate buffer) overnight. Tangential 80-µm-thick sections were then processed for cytochrome oxidase histochemistry (Staiger et al. 2004). Finally, sections were intensiﬁed with 0.5% copper (II) sulfate (Sigma) for 2–3 min, air-dried, and mounted (Fig. 1B2).

Data analysis. All analyses were performed offline using custom programs written in Matlab (Mathworks, Natick, MA) and C. Raw data were accessed using functions included in the FIND Matlab toolbox (Meier et al. 2008) and processed at full sampling rate (20 kHz) for spike sorting (see below). For local field potential (LFP) analyses they were downsampling to 1 kHz.

Multichannel spike sorting was performed as described previously (Reyes-Puerta et al. 2014). In brief, the continuously recorded raw signals were high-pass ﬁltered (0.8–5 kHz). Nonoverlapping groups of two or four contiguous channels were selected as “virtual tetrodes.” Amplitude thresholding was used to perform spike detection in each group of channels independently. Extracted spikes included the amplitude values from all channels in the group in the time range from −0.5 to +0.5 ms (relative to the waveform negative peak). From the spike waveforms, we then computed feature vectors containing for each channel three values (the negative peak amplitude plus the two first principal components derived from the whole waveforms).

Fig. 1. Multiple silicon-probe electrode recordings in the barrel cortex. A: schematic illustration of the experimental setup for selective mechanoelectric stimulation of single whiskers and voltage-sensitive dye (VSD)/multielectrode recordings. B1: mean VSD responses averaged from 6 deflections of whiskers D2 and D3. White stars mark the insertion position of the eight probe shanks. B2: photomicrograph of a cytochrome oxidase-stained tangential section through L4 of the barrel field. Yellow points indicate the location of the 8 shanks. C: illustration of the 8 × 16 channels probe, showing the position of the barrels D2 to D5 and the location and orientation of the different layers. Channels selected for further illustration in Fig. 2 are marked with an orange box. D: spontaneous local field potentials (LFPs) recorded in L4 in each of the barrels D2 to D5 (see C) during 10 s of spontaneous activity. E: spike times of sorted neurons. The numbers marked at right (1–50) represent the ordering of the recorded neurons according to their position across the shanks, i.e., belonging to the barrel-related columns D2-D5. Symbol color represents the laminar position of the recorded cells (see legend at right). Orange box marks the selected neurons and time period (~6.6 s) selected for display in Fig. 2. F: trace representing multisorted-unit (MSU) activity computed using all neurons in the ensemble and smoothed with a 10-ms Gaussian kernel. Periods marked in gray belong to detected up states and black to down states.
vectors of \((n^3)\)-dimensions (where \(n\) represents the number of channels within a group) were sorted using KlustaKwik (http://klustakwik.sourceforge.net) and Klusters (http://klusters.sourceforge.net) (Harris et al. 2000; Hazan et al. 2006). Further details are described in Fig. 2. All neurons included in the present study passed all validated criteria for proper isolation quality: 1) a clear refractory period, 2) a stable spontaneous firing rate during the whole duration of the recordings, and 3) a valid “isolation distance” during the spike sorting procedure (Reyes-Puerta et al. 2014).

The cortical depth of the recording electrodes was assessed from the current-source density (CSD) maps computed from the LFPs and the stereotaxically estimated depth of the probe tip. Early CSD sinks present around 7–10 ms after sensory stimulation at the thalamorecipient layers (L4 and LSB/6) (Bernardo and Woolsey 1987; Cruikshank et al. 2010) allowed us to assign the individual channels to specific cortical layers (Reyes-Puerta et al. 2014). For the purpose of the current analysis, all neurons at the level of the low early sink and below were marked as LSB/6. The somatic location of the spike-sorted neuron was further assigned to the recording site containing the mean waveform with maximum negative peak amplitude. The recorded neurons were assigned numbers according to their location on the shank (from superficial to deep), and the order of the shanks and, hence, their numerical order largely reflects their horizontal position.

Up and down states were detected using the activity of all sorted single units (Luczak et al. 2009). First, we computed the multisorted-unit (MSU) trace from the spontaneous activity smoothed with a 5 ms Gaussian kernel, which represents the average spike rate of the population activity. The MSU trace was then used to establish the thresholds defining the transitions to up and down states, respectively. The onset of an up state was detected when 1) the MSU trace crossed the up-state threshold, and 2) the mean of the MSU trace in the following 60 ms was higher than the up-state threshold. The subsequent end of the up state was defined as the time point in which the mean MSU trace was below the down-state threshold in the following 30 ms. Down states were similarly detected. The time values used for the detection of state onsets and offsets (60 and 30 ms, respectively) matched those used in previous studies (Luczak et al. 2009; Sakata and Harris 2009; Luczak and Barthó 2012), yielding the best separation between periods of population activity and periods of network silence when the raw data (raster plots and MSU traces) were visually inspected.

We counted the number of spikes per time bin for each neuron at several bin sizes. For control, these spike-count bins were shuffled randomly on time 100 times. For the poststimulus data, we analyzed time intervals of 80 ms following whisker deflection, in blocks where the stimulus frequency was between 0.067 and 10 Hz. Typically, 1 block of 200 trials (low stimulation frequency, 0.067 to 0.2 Hz) and 10 blocks of 100 trials each (higher frequencies, 1 to 10 Hz) were included in this analysis. To prevent the contribution of signal-driven correlations on the poststimulus activity, the mean poststimulus time histogram was calculated and subtracted from the neuronal spiking responses for each block separately.

The Pearson correlation coefficients \((r_{SC})\) of the spike counts between pairs of neurons were calculated as follows:

\[
    r_{SC} = \frac{E(N_1N_2) - EN_1EN_2}{\sigma_1\sigma_2},
\]

where \(E\) is the expected value approximated by the average firing rate, \(\sigma\) is the standard deviation of the responses, and \(N_1\) and \(N_2\) are the spike counts of cells 1 and 2, respectively.

Data are expressed as means ± SD, unless otherwise stated. Mann-Whitney U-tests, ANOVA followed by Scheffé’s post hoc analysis, and Kolgomorov-Smirnov tests were used to determine differences between groups at 5% significance level.

RESULTS

In the present study we used data only from experiments in which more than 30 neurons were recorded simultaneously (average number 51.9 ± 11.4), and all 8 shanks were verified to reside within the barrel field, yielding 7 data sets. Overall, the data are biased towards the infragranular layers, as the total number of neurons in each layer in all experiments together is as follows: L2/3: 28; L4: 54; L5A: 170; LSB/6: 111. The limited sample from L2/3 can be attributed either to a technical difficulty of sampling supragranular layers with the silicon probes (Reyes-Puerta et al. 2014) or to the sparse firing of neurons in this layer (Barth and Poulet 2012). In five of the seven analyzed experiments, spontaneous activity was recorded for periods between 8.3 and 33.3
min (average 19.6 ± 9.01 min). In the two additional experiments, we used 200 time segments of 0.4 to 4.9 s following whiskers’ stimulation at 0.2 Hz.

To study the possible dependence of firing correlations on the laminar position of the neuronal pairs, we examined the noise correlations between spontaneously firing neurons recorded over prolonged periods in anesthetized rats. Figure 1 presents the experimental setup: silicon probes containing 8 shanks, each 1 hosting 16 electrodes, were used for simultaneous recording of single neurons in the barrel field. Barrel-related columns were located by VSD imaging and verified post hoc by visualizing the DiI traces of the electrode shanks in cytochrome-oxidase stained slices (Fig. 1B). In each experiment, the cortical layers were identified by CSD depth profiles (Reyes-Puerta et al. 2014). Using this approach, we were able to allocate the recorded neurons to specific cortical columns and layers (Fig. 1C). The network activity was generally organized into periods of simultaneous spiking and periods of silence, which have been termed up and down states in previous studies (Steriade et al. 1993; Destexhe et al. 2007). Negative LFP deflections were correlated with increased synaptic activity (Luczak et al. 2009; Sakata and Harris 2009). A mean of 0.47 ± 0.4 spikes was elicited per recorded neuron during up states, while only 0.03 ± 0.05 spikes were elicited during down states (Mann-Whitney U-test, P < 0.001), implying that most spikes (~93.5%) occurred during up states, and only a minority of them (~6.5%) between these synchronized firing periods. Examining the laminar contribution to each of these up states, there were no obvious differences between them, i.e., neurons located in different layers took part similarly in the firing periods (Fig. 1E) (Sakata and Harris 2009).

Calculating the correlations of firing rate fluctuations. We next examined the cross correlations between the firing rate fluctuations of neurons. Matrices of correlation coefficients were calculated from the periods of spontaneous activity using time bins of 5 (Fig. 3A), 10 (Fig. 3B), 50, and 100 ms (not shown). Increasing the time bin resulted in a general increase of the correlation coefficients (Fig. 3E) (Reich et al. 2001). Isolating the spikes that occurred only during up states resulted in almost identical coefficients’ matrices (the average similarity index calculated by cross correlating the matrices for all experiments was 0.99), ruling out the possibility that the correlations between neuronal pairs were driven mostly by state transitions.

To examine the exact position of correlated pairs, we plotted diagrams of all neurons according to their columnar and laminar position (Fig. 3, C and D, corresponding to the matrices in Fig. 3, A and B, respectively). These diagrams reveal that pairs of highly correlated neurons are located in a variety of relative spatial locations and over large horizontal distances: within the same layer, within same barrel-related column, and in various oblique relationships. Also apparent is the great similarity between the diagrams of the two time bins.

Figure 3E shows an example of the cumulative distribution of the correlation coefficients between all neurons for each
time bin (shown by the different colors). As a control, the spike counts were shuffled randomly on time. This procedure practically eliminated all significant correlations in all experiments, producing coefficient distributions symmetrically centered on zero (Fig. 3F).

To further explore the structure of the neuronal correlations we applied eigen decomposition of the correlation coefficient matrices. Essentially, this analysis extracts from the matrices several “principal components,” representing groups of cells that fluctuate together and thus form “collective modes” of network behavior (Zohar et al. 2013; Shamir 2014). The eigenvalues represent the variance of the specific mode and are ordered such that successive components account for decreasing magnitudes of variance. The corresponding eigenvector represents the spatial structure of the mode. For example, an eigenvector in which all components are either positive (or negative) represents a collective mode in which all the population fluctuates together above or below its mean firing rate. There are several noteworthy advantages to this approach: (1) the dimensionality of the problem is reduced from \( N(N-1)/2 \) pairs to only \( N \) collective modes (where \( N \) is the number of neurons); (2) the collective modes’ are uncorrelated with each other (i.e., orthogonal) so are simpler to examine than the mutually correlated neural responses; and (3) the eigenvalues provide natural ranking of the different modes according to the magnitude of their fluctuations. Note that the collective modes with the largest eigenvalues are also termed the principal components.

Figure 4 illustrates an example of this analysis for 5 (black)- and 10 (red)-ms time bins (results for 50 and 100 ms time bins are qualitatively the same). Figure 4A shows the eigenvalue spectrum of the correlation coefficient matrices used in the analysis. As can be seen, the vectors associated with the real data largely overlap. Using the cosine as a measure of the similarity between the vectors, we calculate that for all three principal components, the cosine between the eigenvectors of the 5 and 10 ms is 0.99. On the other hand, the eigenvectors associated with the shuffled data are randomly scattered (cosine between the eigenvectors of the shuffled 5- and 10-ms bins is 0.09 for the first component, 0.11 for the second, and 0.02 for the third). Moreover, whereas the eigenvectors of the shuffled data do not show any specific structure, a clear structure could be seen emerging from the principal components of the real data. The coefficients of the eigenvectors associated with the first principal component are all positive (Fig. 4B); this first vector reflects a uniform collective mode of fluctuations, wherein all neurons increase or decrease their firing rate concurrently. This collective mode may represent, for example, fluctuations between the up and down states described above (Harris et al. 2011).

The vectors associated with the second and third components display both positive and negative values. The second principal component (Fig. 4C) does not show a structure that varies along the x-axis; its components are almost equally distributed between positive and negative values in all barrels (but see below). The eigenvector corresponding to the third principal component (Fig. 4D) represents a collective mode of fluctuation, which can be interpreted as follows: when neurons in D2 fire above their mean, neurons in D3 and D4 fire below and vice versa. Such behavior was noted in most of our
analyzed experiments (6 out of 7). It is tempting to attribute this type of collective mode of fluctuation to a propagation of the “up state” along the barrel row, such that has been observed in our experiments (see also Ruiz-Mejias et al. 2011; Civillico and Contreras 2012). In summary, the eigen decomposition analysis yielded three uncorrelated collective modes of fluctuation: one uniform mode and two modes with a spatial structure (i.e., the second and the third principal components), which coexist and are uncorrelated. The resultant variability in the firing of individual neurons is the sum of all these modes. Consequently, the mean correlations between different neurons will be positive, as sum of all collective modes that are weighted by their eigenvalue.

Noise correlations are stable. A crucial point related to the significance of noise correlations is their stability over long periods of time. To assess this issue, we analyzed the three experiments in which the period of spontaneous activity was longer than 20 min. This period was divided into two halves, and the rate covariance was calculated for each half separately. Subsequently, the resulting matrices were subtracted. The subtraction matrix (Fig. 5A) reveals very low coefficient values, mostly distributed around zero, thus being different from the almost identical distribution of the coefficients calculated for the two half-periods (Fig. 5B). We also used eigen decomposition to examine the vectors associated with the first three principal components (Fig. 5C). It can be noted that these vectors are almost identical (cosines for the first 0.99 ± 0.001; second 0.95 ± 0.028; and third 0.93 ± 0.024, Fig. 5D). From the fourth vector the similarity index drops sharply and reaches an asymptotic low value by the sixth. This is because of the following reason: the eigenvalues of rank higher than three are practically degenerate, i.e., very close to each other (see example in Fig. 4A). Consequently, their corresponding eigenvectors are not determined uniquely and can be rotated within the degenerate subspace, i.e., can be mixed with eigenvectors of corresponding degenerate eigenvalues. Thus the eigenvectors of ranks higher than three are governed, to a large extent, by small noise fluctuations that result from finite data.

An additional step was taken to examine whether new correlations emerge if we introduce a time shift between the neuronal spikes. The diagram in Fig. 5E shows an example of the correlations between neuron number 20 and all the other simultaneously recorded neurons, over 10 time shifts of 5 ms each (5 to 50 ms). It can be noted that all correlations decline with time and approach 0 at ~50 ms. The correlations remained low for longer time shifts (up to 12 s, not shown). The same declining correlations were found for all cells examined, in this experiment and others, such that the SD of the mean correlation coefficient for all pairs in each experiment approached its asymptotic value at 50- to 60-ms time shift (Fig. 5F). Overall, this result implies that there were no other significant correlations among pairs of neurons with any time shift between their spikes.

**Significant role of the cortical layers in determining correlations.** As the elements of the eigenvectors are related to the sampled neurons, we examined whether the structure of the

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**Fig. 5. Noise correlations are stable.** A: example of a matrix calculated by subtracting 2 correlation coefficient matrices from half-periods of spontaneous activity (each one 10-min long). B: cumulative distributions of coefficients from the 2 half-periods (red: initial 10 min; black: final 10 min) are almost identical; therefore, the distribution of the subtraction matrix (gray) is centered around 0. Data are from the same experiment as in A. C: eigenvalues’ spectrum calculated for the initial and final halves of the data, same experiment as in A, color coding as in B. D: cosine similarity index between the first 20 eigenvectors calculated for the 2 half-periods (means ± SD; n = 3 experiments). Note that values are close to 1 for the 3 principal components. E: from another experiment, a diagram of the correlation coefficients between neuron number 20 and all other neurons recorded simultaneously, computed over 10 time shifts of 5 ms bins each. The colors represent the time-shift bins. The bar representing the correlation of neuron 20 with itself at the time shift of 0 ms (R = 1) is truncated. F: for the same experiment as in E, the mean correlation coefficient for all pairs (solid line) and the standard deviation of the mean (broken line) are plotted against the time shifts between spikes, demonstrating the lack of firing correlations at time intervals longer than ~50 ms.

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eigenvectors corresponding to the principal components is influenced by the laminar position of the neurons. Figure 6A displays the first eigenvector from the same illustrative experiment as in Figs. 1, 2, and 3. The coefficients of the 50 recorded neurons were color coded for their laminar position. It can be noted that all coefficients were positive (Fig. 6A, see also Fig. 4B) and color coding did not disclose any specific structure. On the other hand, color coding the neuronal coefficients for layers in the second eigenvector revealed a clear structure, where coefficients of most neurons from L2/3, L4, and L5A were positive, while those of almost all neurons located in L5B/6 were negative (Fig. 6B, same eigenvector as in Fig. 4C). A summary of seven experiments is displayed in Fig. 6C: L5B/6 coefficients presented more negative values than all other layers, a result that was consistent when performing the analyses using time bins of 5, 10, 50, and 100 ms. A two-way ANOVA test confirmed significant differences between L5B/6 and each of the other layers for time bins of 5 and 10 ms (P < 0.001). The difference between L5B/6 and other layers was somewhat less remarkable for longer time bins.

Analyzing the spiking fluctuations following whisker stimulation revealed essentially the same structure; even decomposition of the correlation coefficient matrices disclosed a small number (2 or 3) of significant principal components (Fig. 6D). The coefficients of the eigenvectors associated with the first principal component were almost all positive, or close to zero (Fig. 6E), while those associated with the second (Fig. 6F) and third (not shown) components displayed both positive and negative values. Moreover, color coding the coefficients of the second eigenvector for the laminar position of the neurons revealed comparable layer-dependent structure to the structure revealed from spiking data acquired during spontaneous activity (Fig. 6F). However, it should be noted that the eigen decomposition results of the poststimulus data are based on a much smaller number of spikes, due to the much shorter time interval used (~1–2 min after concatenating the stimulation trials from all blocks, see MATERIALS AND METHODS). This fact is most likely responsible for the “noisier” structure compared with that of the spontaneous activity and precludes detailed quantitative comparison between the two conditions. Together, these findings suggest that the second most significant factor influencing noise correlations is the neuronal laminar position, such that the firing rates of L5B/6 neurons fluctuate in an opposite direction to that of neurons in the other more superficial layers.

It has been well accepted that the density of axonal connectivity in the vertical domain is much higher than that of horizontal connections, contributing to the columnar similarity of sensory-evoked responses and highly correlated firing (e.g., Feldmeyer 2012). To further assess the relative weight of the layers’ impact, we compared it to the column pairwise noise correlations. Since the extension of the barrel-related column is

Fig. 6. The second factor underlying spike-count correlations is the cortical layers. A: example of an eigenvector corresponding to the first eigenvalue, color coded for the laminar position of each neuron (legend in B). The histologically confirmed barrel columns are marked at top. B: for the same experiment as in A, the eigenvector corresponding to the second eigenvalue is color coded for the laminar position of the neurons. Note that the 0 line largely separates L5B/6 neurons from the rest. C: summary of 7 experiments, presenting the second eigenvector values across neurons grouped by their laminar location and averaged using 4 different time bins (presented as means ± SE). Significant differences are found between L5B/6 and all the other layers for 5- and 10-ms time bins (P < 0.001). For 50 ms, there is a significant difference between L5B/6 and L2-3/L4 (P < 0.01), but no significant difference between L5B/6 and L5A. For 100 ms, there is a significant difference between L5B/6 and L5A (P < 0.001) but no significant differences between L5B/6 and L2-3/L4. Significant differences are marked only for the 5 ms bin. D: for the same experiment as in A and B, eigenvalues spectrum of the spike count correlation coefficient matrices computed using 5 ms time bins from spontaneous (gray circles) and poststimulus activity data (80 ms following whisker deflections, red circles). E: eigenvector corresponding to the first eigenvalue, color coded for the laminar position of each neuron, calculated from poststimulus data. F: eigenvector corresponding to the second eigenvalue, color coded for the laminar position of the neurons. For E and F, data are from the same experiment as in A and B (legend in B).
large and these megacolumns contained up to three electrode shanks in our experiments, we chose to analyze a narrower domain of vertically aligned neurons, recorded on the same shank. Thus we reanalyzed the correlation matrices and compared the distribution of the correlations between same-shank neurons to that of the correlations between neurons in the same layer. Cumulative probability plots (Fig. 7A) disclosed highly similar distributions for most experiments. In fact, for the distance examined (1.4 mm), the average intralaminar correlation coefficient was 0.015 ± 0.017, and the average intracolumnar was 0.015 ± 0.019 (means ± SE). For comparison, the distribution of the correlation coefficients between pairs with various oblique relationships was significantly shifted to the left (Fig. 7B, Kolmogorov-Smirnov test, \( P < 0.001 \)), and their average was 0.010 ± 0.013 (means ± SE). The relative strength of the intralaminar correlations was further emphasized by comparing the correlation coefficients between neuronal pairs according to their laminar position (Fig. 7C).

It should be noted that the average of the correlation coefficients decayed almost linearly with the horizontal distance (Golshani et al. 2009; Boucsein et al. 2011) for all time bins examined (not shown) for both intralaminar and various oblique pairs (Fig. 7D). This decay was especially steep for L4 and comparable across the infragranular layers (Fig. 7E). Interestingly, the strength of these deep horizontal correlations was not affected by the barrel borders; when we compared the strength of the correlations between pairs located 200 \( \mu m \) apart, there was no difference in the correlation coefficients between pairs within the same barrel, and pairs located in neighboring barrels (Fig. 7F). Yet, and although the influence of the different layers on noise correlations is spatially restricted, its potential effect exceeds considerably the columnar functional domain.

**DISCUSSION**

Our study makes use of the unique spatial information obtained from neurons in the barrel cortex (both columnar and laminar) to provide a complete description of the spatiotemporal structure of noise correlations. Mainly, it reveals the importance of the cortical layers in determining the network’s spiking behavior. We find that noise correlations between pairs of neurons located in the same layer and up to 1.4 mm apart present a similar distribution to those of vertically aligned neurons and are significantly higher than noise correlations between neuronal pairs located in the same horizontal distance.
but belonging to different layers. The substantial dependence of spike-count correlations on the cortical layers is further emphasized by the resulting principal components of the covariance coefficient matrices. The purpose of this analysis was to identify the factors underlying the correlations. We identified three principal components, namely coexisting, uncorrelated, collective modes of fluctuation: one uniform mode and two modes with a spatial structure. We can only conjecture using indirect evidence about the factors underlying the first and third principal components (see above). However, the vector associated with the second eigenvalue (i.e., the second principal component) displays a clear laminar structure, demonstrating that the factor second in rank is the cortical layers. Especially remarkable is the finding of a collective mode of fluctuations, in which the firing of L5B/6 neurons fluctuates in antiphase to those of neurons in the laminae above it. This finding can be explained in light of a recent studies demonstrating that L6 inhibits the more superficial layers (Olsen et al. 2012; Bortone et al. 2014). Therefore, when L5B/6 neurons “accelerate,” neurons in other layers “decelerate,” and vice versa.

The exact laminar definition in our study differs somewhat from that of Olsen et al. (2012), mostly due to technical differences. In the present study we used 1,200-μm long probes such that L5B/6 neurons were recorded at depth greater than 1,150 μm from the pia, corresponding to the lower sink in the sensory-evoked CSD and below it. Using this method, we cannot separate L5 from L6, yet, we deduce that there is considerable overlap between the neurons referred to as L6 in Olsen et al. (2012) and those labeled as L5B/6 in the present study.

The relative strength of the intralaminar correlations in our study may be affected by the higher sampling rate of neurons from the infragranular layers in our data, as these deep neurons are known to have stronger noise correlations compared with granular neurons (Sakata and Harris 2009; Hansen et al. 2012; Smith et al. 2013). Yet, in agreement with our findings, significant firing correlations \( R \sim 0.02 \) between infragranular neurons residing in neighboring columns have been noted before, during both sensory-evoked and spontaneous activity (Zhang and Alloway 2004). Moreover, there is evidence in the literature for pairwise noise correlations between neurons located at horizontal distances of 1.5 mm and even more (Smith and Kohn 2008; Smith and Sommer 2013). However, note that those studies were performed in monkeys, thereby making direct distance comparisons difficult, yet they underline how remarkable these distances are in the rat.

What mechanisms could account for such long-range correlations? It has been assumed that they derive from the architecture of the local circuitry, either from shared inputs between the correlated neurons or from direct intracortical axonal connections between them. A direct thalamic input to neighboring barrels exists, but is rather limited (Petersen and Diamond 2000; Arnold et al. 2001; Oberlaender et al. 2012), and probably cannot explain the extent of the correlations. The alternative, long intracortical horizontal axonal connections in infragranular layers have been described (Gilbert and Wiesel 1979; Luhmann et al. 1986; Chagnac-Amitai et al. 1990; Zhang and Deschenes 1997; Bannister 2005). Yet, the probability of detecting horizontal excitatory connections between pairs of neurons falls off dramatically with distance and approaches 0 at \( \sim 500 \mu m \) (reviewed by Boucsein et al. 2011). Such partial synaptic component is not likely to drive alone frequency alterations coordinated over three to four barrel columns, and additional factors probably serve to facilitate this behavior. Several G-coupled modulatory receptors have been demonstrated to have strong laminar pattern of expression (Lein et al. 2007). The neuromodulatory systems are well positioned to modify the strength and extent of firing rate fluctuations, yet direct evidence for this is insufficient. In this regard, only muscarinic receptors have been shown so far to suppress noise correlations in the visual cortex (Goard and Dan 2009). The nonspecific thalamocortical inputs also display broad and laminar specific axonal trajectories (Ohno et al. 2012) and have a modulatory effect (Viaene et al. 2011). A modulatory innervation with laminar specificity may participate in enabling the long-range correlations revealed here.

Our data are derived from “spontaneous activity” recorded under anesthesia, when sensory drive of the cortical column is minimal. We also demonstrated here that the basic pattern of correlations is preserved when a robust sensory stimulus is delivered. The principal uniform mode, reflecting fluctuations in a global parameter (such as levels of attention, awareness etc.), may be more pronounced during anesthesia. Indeed, it has been recently shown that pairwise noise correlations measured over horizontal distances are enhanced under network states of sleep or anesthesia (Ecker et al. 2014). However, the following principal components show distinct structure. Consequently, we hypothesize that these modes reflect an underlying structure of connectivity and, hence, should also appear during wakefulness. Therefore, one may assume that the relative effect of the columnar vs. laminar connectivity on shaping firing patterns varies with the different brain states. Nevertheless, this study highlights the role of laminar processing on shaping neuronal responses. We further suggest that specific laminar effect over cortical processing is modulated under various brain states, to shape the network’s behavior.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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