SUPPLEMENTARY FILES

Supplementary Discussion:

The role of experience in the desynchronization of cortical network activity:

The neocortex is capable of generating its own patterns of activity, presumably as a result of spontaneous activity in the primary sensory organs. For example, in the developing visual system, before eye opening, waves of correlated spontaneous activity periodically sweep across the retina and are propagated to the thalamus and then onto the neocortex (Meister et al., 1991; Wong et al., 1993; Feller et al., 1996; Feller et al., 1997; Penn et al., 1998; Hanganu et al., 2006). Similarly, in the developing somatosensory system, periodic myoclonic jerks in the limbs trigger localized bursts of activity in the neocortex, referred to as spindle bursts (Khazipov et al., 2004). Importantly, this spontaneous activity in the neocortex persists even when it is deprived of inputs from the periphery (Weliky and Katz, 1999; Chiu and Weliky, 2001; Hanganu et al., 2006). This suggests that the developing cortex plays the dominant role in generating the correlated activity within its recurrently connected circuits. Interestingly, blockade of action potential driven activity prevents the clustering of horizontal collaterals in the developing cat visual cortex but enucleation does not (Ruthazer and Stryker, 1996), implying that internally generated activity in the cortex may be sufficient to mediate some aspects of activity-dependent refinement of circuits. Our data extend these findings by demonstrating that even the state transition from highly synchronous to largely sparse and decorrelated firing of neurons is not dependent on sensory inputs, even though it coincides with eye opening and more vigorous sensory exploration of the environment.
Changes in the Balance of Excitation and Inhibition Underlying Developmental Decorrelation

We find an increase in the contribution of inhibitory input after the second postnatal week that could mediate the decorrelation in network activity around P12. However, the technique we used to look at the balance of excitation and inhibition has limitations: because inhibitory postsynaptic currents will not occur at the same precise time within each UP state, it is difficult to determine a precise reversal potential for the composite conductance. Still, the effect of this variability in timing of postsynaptic currents is probably low because we averaged multiple UP states. Furthermore, others have shown a similar time course for the change in the balance of excitation and inhibition in brain slices (Agmon and O'Dowd, 1992).

Given that the chloride equilibrium potential is near its mature hyperpolarized level by P11 in neocortex (Daw et al., 2007) and that bumetanide, which causes a shift in the reversal potential of GABA\textsubscript{A} mediated responses, does not block spindle bursts (Minlebaev et al., 2007), we favor a scenario whereby increased activation and recruitment of GABA\textsubscript{A} receptors, rather than a shift in the chloride equilibrium potential, leads to decreased neuronal excitability.

Brain states and correlated neuronal firing:

We considered the possibility that different brain states could influence the extent of correlated activity at different ages, as has recently been shown in the adult brain (Poulet and Petersen, 2008). We carefully monitored the behavioral state of most unanesthetized animals used for calcium imaging experiments by closed video circuit. After P12, mice appeared to be in a state of quiet wakefulness and were most likely not asleep. We infer this, because in most videos, brief periods of movement were associated with grooming movements or voluntary changes in posture (not with startle arousals or myoclonus). Furthermore, patch clamp recordings from animals older than P15 showed the presence of
2-5 Hz oscillations, and not the < 1 Hz slow oscillations associated with slow-wave sleep. For animals younger than P12, it was difficult to judge the exact brain state we were observing. The exact delineation of different brain states in neonatal rodents is controversial (Blumberg et al., 2005). Many investigators believe that before the emergence of slow-wave sleep in neonatal mice, brain states may be undifferentiated, and cannot be further characterized (Frank and Heller, 2003, 2005).

To address this concern (i.e., different brain states at different ages could explain the developmental desynchronization of activity), we subjected mice at different postnatal ages to the same brain state by anesthetizing the animals with two anesthetics, isoflurane or urethane. As reported in Fig. 2, we found that while these anesthetics have small effects on the magnitude of correlation coefficients (Suppl. Fig. 4), the time course of developmental decorrelation was identical to that seen in unanesthetized mice. Given that these two anesthetics have different mechanisms of action, the data indicate that this decorrelation is a very robust phenomenon and is present regardless of the brain state of the animal.

**Open questions regarding cell-type specific and laminar differences in correlational structure of neocortical networks:**

Our study did not differentiate between excitatory cortical neurons and the various subtypes of inhibitory neurons. It is possible that the distribution of correlation coefficients of inhibitory-inhibitory, and inhibitory-excitative pairs will be different from excitatory-excitative pairs. This is likely given that excitatory → inhibitory connections are usually stronger than excitatory → excitatory connections, and spikes in one or few excitatory neurons can drive inhibitory neurons to fire (Bartho et al., 2004). In addition, we focused on the correlational structure within L2/3. It is possible that inter-and intra-laminar correlations between and within other layers could be different. This is likely true for L5, where studies
show that L5 neurons spike on almost every cycle of the slow cortical oscillation (Luczak et al., 2007). Further studies are needed to address these important questions.
References:

Supplemental Data Figure Legends

Supplemental Figure S1:

A-B Somatic ΔF/F calcium traces and simultaneous cell-attached recordings for 2 individual neurons stained with Fluo-4 and imaged at 15.6 Hz in a P13 mouse anesthetized with isoflurane. The actual firing rate as determined from electrophysiological recordings (black) and the calculated firing rate from deconvolved somatic calcium trace (red) are also shown for each neuron. These conditions are identical to those used to generate data shown in Supplemental Fig. S6.

C-E: Somatic ΔF/F calcium traces and simultaneous cell-attached recordings for 3 individual neurons stained with OGB and imaged at 3.9 Hz from P15 and P19 mice anesthetized with isoflurane (C and D) and from a P15 mouse anesthetized with urethane (E). These conditions (indicator, objective, acquisition speed, anesthesia) are identical to those used to generate data shown in Figs. 4 and 6. They are also identical to conditions in experiments shown in Figs. 1 and 2, except that those data were generated in unanesthetized mice.

F: Somatic ΔF/F calcium traces and simultaneous cell-attached recordings for one neuron stained with OGB and imaged at 15.6 Hz and simultaneous cell-attached recording in an unanesthetized P12 mouse. These traces should be compared with panels A and B, obtained with Fluo-4 (see also Supplemental Table 1).

G. Graph of simulated data demonstrating a stable ratio of the mean correlation coefficients of early vs. late developmental stages after exclusion of 10-65 % of single spikes. Arrows represent the percentage
of single spikes missed with Fluo-4 and OGB using 20X and 40X objectives at 3.9 and 15.6 Hz imaging frequency in experiments with simultaneous cell-attached recordings and in vivo calcium imaging. Single spikes were defined as spikes separated in time from other spikes by more than 300 ms.

**Supplemental Figure S2:**

Somatic $\Delta F/F$ calcium traces from 5 representative L2/3 neurons at four postnatal ages, expanded from Fig. 1C.

**Supplemental Figure S3:**

A. Each graph demonstrates the first singular value squared (the first eigenvalue) from the singular value decomposition of the extrapolated firing rate matrix (red arrow) and the distribution of the first null eigenvalues from 100 circularly shuffled firing rate matrices (blue). Note that the first eigenvalues at P5 and P9 (arrows) are far outliers compared to the tightly distributed null values. Data from 4 different representative experiments in unanesthetized mice at a range of developmental stages are shown.

B. Graph demonstrating the ratio of the first eigenvalues to first ten median eigenvalues values obtained from 100 shuffled firing rate matrices at P4-P7, P8-P11, P12-P15, and > P16 stages.
Supplemental Figure S4:

A: Average neuropil ΔF/F calcium traces in L2/3 at P5, P8, P11, P13, P18, >P30.

B. Left: Four consecutive images of OGB loaded L2/3 neurons at P15 with each separated from the next by 6 µm in the z dimension. Arrowheads demonstrate a layer 2/3 neuron sectioned through the equator (filled arrowhead). Right: contours of cells were automatically detected in the image using custom written routines in ImageJ and MATLAB, at settings designed to detect the maximal number of cells (top), and at settings designed to detect only optimally sectioned cells. The cut off boundaries for the cross-section area of optimally sectioned cells were measured from image stacks where individual slices were collected at 2-µm intervals through L2/3 at P10 and P15.

C: Graph demonstrating the relationship of correlation coefficient vs distance separating cell pairs at P8-11 and P14-P16, solely from optimally sectioned cells.

Supplemental Figure S5:

A. Mean correlation coefficient vs. normalized distance between pairs of cells at P4-P7, P8-P11, P12-P15 and >P16 (unanesthetized mice; same data as Fig. 2B). Normalization was performed to correct for the increase in distance between neighboring cells during development.

B. Mean distance separating neighboring neurons at P4-P7, P8-P11, P12-P15 and >P16 stages.
Supplemental Figure S6:

A. Somatic ΔF/F calcium traces from 5 representative L2/3 neurons stained with Fluo-4 AM at P9 and P14, and imaged under isoflurane with the 40 X 0.8NA objective at 15.6 Hz.

B. Mean correlation coefficients of all pairs of Fluo4-stained neurons located within 100 µm of each other at P6-P9 and P13-15 stages (n= 2 and n= 3 mice, respectively; Student’s t-test: p < 0.05).

Supplementary Figure S7: Post-hoc brain reconstruction and registration of two-photon images with barrel architecture.

A. Cartoon of a coronal section of a mouse brain showing the approximate location of the cranial window and the plane of sectioning for reconstructions. Fixed brains were sectioned on a vibratome. The spacing between slices was 60 µm. The slices corresponding to Layer 3 and deeper were processed for cytochrome oxidase (COX) histochemistry.

B. The first step is to create a montage of photographs of individual brain slices, by aligning them with respect to one another (using the contour of the slice, the blood vessel imprints on the edges, and penetrating vessels in brain parenchyma). The slices are ordered from the most superficial (top) to the deepest (bottom). This is done in Adobe Photoshop and is the step that that introduces the largest error in alignment of the imaging field of view with respect to barrel architecture. We estimate this error is <30 µm in the x-y direction for any 2 slices, but is not
cumulative, because it should cancel out when all the slices are registered. The boxed region is shown in higher magnification in panels C-F.

C. Higher magnification of the top 2 slices and a slice through Layer 4 stained with COX, showing individual barrels. The scale bar is the same for panels C-F.

D. Higher magnification view of the aligned brain slices showing the detail of the imprints of the surface vasculature on the cortical surface. Arrows in D-F point to the same vessel bifurcations.

E: The next step is to draw the cortical vessels.

F. These drawings are then used to align the reconstructed brain to the photograph of the same blood vessels as seen through the cranial window (through the two-photon microscope). The edge of the glass coverslip can be seen as a brighter curved streak.

G. Higher magnification view of the blood vessels through the window. The scale bar is the same for panels G-J.

H. A two-photon image stack through Layer 1 from the red channel (taken soon after injection of the OGB-1 AM and Alexa-594 dye mixture) corresponding to the imaged region has been matched to the photograph of blood vessels. This is quite easy because blood vessels can be seen on the two-photon stack as dark shadows. The 512x512 image has a lateral dimension of ~ 720 µm.

I. Same as H, except the two-photon image is from the green channel showing the OGB-stained neurons in Layer 2/3.
J. Final reconstructed image of the outline (green) of 4 barrels in Layer 4 with the actual imaging field of view for fast calcium imaging.

**Supplemental Figure S8:**

A. Top: representative in vivo current clamp recordings demonstrating passive responses of L2/3 neurons to 25 pA hyperpolarizing pulses in P10 and P16 mice under light isoflurane anesthesia. Bottom: input resistance of L2/3 neurons at P8-P10 (n= 7) and P14-P16 (n= 8) (* p = 0.0003, Mann-Whitney test).

B. Top: representative in vivo voltage clamp recordings of synaptic currents underlying network events at a range of membrane potentials at P9 and P17. Bottom: extrapolated reversal potential of network events (peak amplitude of the events and net charge transfer) at P9-P11 and P13-P17 (* p < 0.05, Student’s t-test; n= 3 for each group). These experiments were performed under light isoflurane anesthesia.

**Supplemental Movies:**

Movie S1: In vivo calcium imaging with OGB-1AM in a P5 mouse. 3:00 min movie acquired at 3.9 Hz, played here 10 times faster than real time.
Movie S2: In vivo calcium imaging with OGB-1AM in a P9 mouse. 3:00 min movie acquired at 3.9 Hz, played here 10 times faster than real time.

Movie S3: In vivo calcium imaging with OGB-1AM in a P13 mouse. 2:15 min movie acquired at 3.9 Hz, played here 10 times faster than real time.

Movie S4: In vivo calcium imaging with OGB-1AM in a P26 mouse. 3:00 min movie acquired at 3.9 Hz, played here 10 times faster than real time.

Movie S5: In vivo calcium imaging with OGB-1AM in a P6 mouse. 18:00 min movie acquired at 1.95 Hz, played here 10 times faster than real time.
**Supplemental Table 1**: Correlation of spikes detected by cell-attached electrophysiological recordings to deconvolved calcium traces using OGB and Fluo4, under different acquisition frequencies and with different microscope objectives.

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient between e-phys and deconvolved trace</th>
<th>Percent of single spikes detected</th>
<th>Percent of spike doublets detected</th>
<th>Percent of bursts of 3 or more action potentials detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OGB</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>20X 0.95 NA 3.9 Hz</td>
<td>0.69 ± 0.03</td>
<td>38 ± 13%</td>
<td>80 ± 12%</td>
<td>98 ± 2 %</td>
</tr>
<tr>
<td>n = 5 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40X 0.8 NA 15.6 Hz</td>
<td>0.83 ± 0.30</td>
<td>63 ± 13%</td>
<td>98 ± 1%</td>
<td>100 ± 0 %</td>
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<tr>
<td>n = 4 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Fluo-4</strong></td>
<td></td>
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<tr>
<td>20X 0.95 NA 15.6 Hz</td>
<td>0.85 ± 0.05</td>
<td>76 ± 20%</td>
<td>97 ± 3%</td>
<td>100 ± 0 %</td>
</tr>
<tr>
<td>n = 3 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40X 0.8 NA 15.6 Hz</td>
<td>0.90 ± 0.02</td>
<td>87 ± 14%</td>
<td>100 ± 0 %</td>
<td>100 ± 0 %</td>
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<tr>
<td>n = 2 cells</td>
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**Supplementary Table 2:** Developmental changes in network activity as measured by two-photon calcium imaging in unanesthetized mice.

<table>
<thead>
<tr>
<th>Age</th>
<th>Event Duration (s)</th>
<th>Inter-Event Interval (s)</th>
<th>Mean Correlation Coefficient (All Distances)</th>
<th>Mean Correlation Coefficient (10-100 μm)</th>
<th>Mean Correlation Coefficient (200-500 μm)</th>
<th>Proportion of Cell Pairs Significantly Correlated (10-100 μm)</th>
<th>Proportion of Cell Pairs Significantly Correlated (200-500 μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P4-P7</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>N= 4 mice</td>
<td>1.96 ± 0.10</td>
<td>10.28 ± 1.60</td>
<td>0.36 ± 0.05</td>
<td>0.58 ± 0.09</td>
<td>0.12 ± 0.07</td>
<td>0.95 ± 0.02</td>
<td>0.27 ± 0.17</td>
</tr>
<tr>
<td>n=139 events</td>
<td>N= 65,106 cell pairs</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><strong>P8-P11</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N= 5 mice</td>
<td>2.82 ± 0.49</td>
<td>23.42 ± 0.42</td>
<td>0.32 ± 0.05</td>
<td>0.39 ± 0.06</td>
<td>0.21 ± 0.02</td>
<td>0.81 ± 0.08</td>
<td>0.49 ± 0.05</td>
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<tr>
<td>n=121 events</td>
<td>N=38,714 cell pairs</td>
<td></td>
<td></td>
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<tr>
<td><strong>P12-P15</strong></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>N= 6 mice</td>
<td>1.97 ± 0.38</td>
<td>13.44 ± 2.60</td>
<td>0.20 ± 0.02</td>
<td>0.22 ± 0.03</td>
<td>0.15 ± 0.01</td>
<td>0.54 ± 0.08</td>
<td>0.31 ± 0.06</td>
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<tr>
<td>n=172 events</td>
<td>N=44,458 cell pairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>&gt;P16</strong></td>
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<tr>
<td>N= 4 mice</td>
<td>1.28 ± 0.15</td>
<td>14.34 ± 6.85</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.31 ± 0.03</td>
<td>0.24 ± 0.01</td>
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<tr>
<td>n=65 events</td>
<td>N=9,798 cell pairs</td>
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**Supplemental Table 3:** Average firing rate of cells recorded in whole-cell or cell-attached mode in unanesthetized mice.

<table>
<thead>
<tr>
<th>Cell ID</th>
<th>Age</th>
<th>Recording Configuration</th>
<th>Firing Rate (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>032108</td>
<td>P10</td>
<td>Whole-Cell</td>
<td>0.63</td>
</tr>
<tr>
<td>021208</td>
<td>P12</td>
<td>Whole-Cell</td>
<td>0.41</td>
</tr>
<tr>
<td>021308c1</td>
<td>P15</td>
<td>Whole-Cell</td>
<td>0.20</td>
</tr>
<tr>
<td>031108c1</td>
<td>P20</td>
<td>Whole-Cell</td>
<td>0.03</td>
</tr>
<tr>
<td>031108c2</td>
<td>P20</td>
<td>Whole-Cell</td>
<td>0.34</td>
</tr>
<tr>
<td>021208</td>
<td>P12</td>
<td>Cell-Attached</td>
<td>1.50</td>
</tr>
<tr>
<td>013008</td>
<td>P12</td>
<td>Cell-Attached</td>
<td>0.97</td>
</tr>
<tr>
<td>020408</td>
<td>P13</td>
<td>Cell-Attached</td>
<td>1.20</td>
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<td>P15</td>
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<td>021308c1</td>
<td>P15</td>
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<td>020808</td>
<td>P17</td>
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SUPPLEMENTAL FIGURE S2

P5
P9
P13
P26
**SUPPLEMENTAL FIGURE S8**

**A**

- Input resistance
  - MegaOhms
  - Graph showing resistance values for P8-P10 and P14-P16.

**B**

- Reversal potential (peak amplitude)
  - Bars showing reversal potential values for P9-11 and P13-P17.
  - Graph showing a significant difference (*).

- Reversal potential (charge)
  - Bars showing reversal potential values for P9-11 and P13-P17.
  - Graph showing a significant difference (*).