Title: Relationship between individual neuron and network spontaneous activity in developing mouse cortex

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Abstract

Spontaneous synchronous activity (SSA) that propagates as electrical waves is found in numerous CNS structures and is critical for normal development, but the mechanisms of generation of such activity are not clear. In previous work, we showed that the ventrolateral piriform cortex is uniquely able to initiate SSA in contrast to the dorsal neocortex which participates in but does not initiate SSA. In this study we used Ca$^{2+}$ imaging of cultured E18-P2 coronal slices (E17 + 1-4 days in culture) of the mouse cortex to investigate the different activity patterns of individual neurons in these regions. In the piriform cortex where SSA is initiated, a higher proportion of neurons were active asynchronously between waves and a larger number of groups of co-active cells were present in comparison to the dorsal cortex. When we applied GABA and glutamate synaptic antagonists, asynchronous activity and cellular clusters remained while synchronous activity was eliminated, indicating that asynchronous activity is a result of cell-intrinsic properties that differ between these regions. To test the hypothesis that higher levels of cell-autonomous activity in the piriform cortex underlie its ability to initiate waves, we constructed a conductance-based network model in which three layers differed only in the proportion of neurons able to intrinsically generate bursting behavior. Simulations using this model demonstrated that a gradient of intrinsic excitability was sufficient to produce directionally propagating waves that replicated key experimental features, indicating that the higher level of cell-intrinsic activity in the piriform cortex may provide a substrate for SSA generation.

Keywords: spontaneous activity, activity-dependent development, piriform cortex, cortex
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**Introduction**

In many regions of the developing mammalian central nervous system, spontaneous electrical activity that synchronizes large groups of neurons independent of sensory stimulation plays a critical role in development (Moody and Bosma 2005, for review). Such activity has been observed in the retina (Meister et al, 1991), spinal cord (O'Donovan et al. 1998), hindbrain (Gust et al. 2003), hippocampus (Ben-Ari et al. 1989), and cerebral cortex (Corlew et al. 2004). Intrinsic cellular properties and network architecture unique to early development allow this phenomenon to occur during a restricted period. Certain features of immature cells, including a high resistance of the resting cell membrane and prominent low-threshold calcium currents, may enhance excitability in neurons which receive little afferent sensory input. Network properties, including an excitatory effect of GABA and the presence of gap junctions, may permit the propagation of waves of electrical activity. The mechanisms by which such properties interact to produce spontaneous synchronous activity (SSA) remain unclear.

In the cerebral cortex, waves of SSA originate within a well-defined spatial region in the ventrolateral piriform cortex (Lischalk et al. 2009), in contrast to other systems such as the retina in which activity initiates with equal probability throughout the structure (Meister et al. 1991). In imaging experiments, 99% of observed synchronous activity in the cerebral cortex is initiated within this region. Moreover, when the region is surgically isolated from cortical slices, SSA continues to be generated within the isolated region, but SSA is eliminated in other parts of the cortex, suggesting that the ventrolateral piriform cortex is uniquely able to initiate SSA and can do so without input from other cortical regions (Lischalk et al. 2009). In contrast, the dorsal neocortex does not initiate SSA. The existence of a specific region with the ability to initiate SSA allows us to determine how its properties differ from the surrounding tissue and how such properties relate to the generation of SSA.
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In this study, we used calcium imaging to observe the behavior of neurons in the piriform cortex and dorsal neocortex during the developmental period during which SSA reaches its peak frequency. We asked whether asynchronous spontaneous activity generated in individual neurons between synchronous waves differed between piriform pacemaker and neocortical follower regions, and whether such differences were due to synaptic circuitry or cell-autonomous firing properties. We found that individual neurons in the piriform cortex generated a higher level of asynchronous activity than neurons in dorsal neocortex and that this activity remained in the presence of synaptic antagonists, suggesting that it results from intrinsic cellular properties that differ between the two regions. In addition, we observed clusters of ten to two hundred cells synchronously activated in the absence of global SSA. These clusters were more densely distributed in the piriform cortex compared to the neocortex and were reduced by the application of octanol, an antagonist of gap junctions. To investigate the interactions between activity patterns in the different cortical regions and the postulated contributions of gap junctional connectivity, we constructed a conductance-based network model recapitulating our major experimental findings. This model was able to generate propagating waves of SSA initiated in the ventral region, using the ventrally-weighted occurrence of spontaneous asynchronous activity as the only spatial asymmetry.
Methods

Tissue Preparation and Culture

All procedures were performed in accordance with the guidelines of the NIH and were approved by the University of Washington IACUC. Swiss-Webster mice purchased from Harlan (Indianapolis, IN) and Cre-Dlx5a/6a-TdTomato+ CD1 mice provided by R. Hevner (Seattle Children's Research Institute Center for Integrative Brain Research) were time-mated, and pregnant females were euthanized on embryonic day E17 by CO₂ narcosis followed by cervical dislocation. No differences were found between data obtained from the two mouse genotypes; therefore, all data was pooled for analysis. Fetuses were removed, decapitated, and the brains dissected in ice-cold artificial cerebral spinal fluid (ACSF; see Solutions and Drugs) equilibrated with carbogen gas (95% O₂–5% CO₂). The brains were sectioned into 300 µm thick coronal slices in ice-cold ACSF using a Leica VT1200S vibrating microtome. Slices were cultured in an H₂O-jacketed incubator at 36-37°C in 5% CO₂ on sterilized Millicell culture plate inserts (Millipore Corporation, Billerica, MA) in 1 mL cell culture medium (see Solutions and Drugs). Culture media was changed every other day. Experiments were performed on slices from E18 to P2 (E17 + 1-4 days in culture).

We used cultured coronal slices because this was the preparation we used in previous experiments to demonstrate that spontaneous waves were driven by a pacemaker in the piriform cortex, and our goal in this study was to ask whether intrinsic asynchronous spontaneous activity in neurons of that pacemaker region differed from that in dorsal, non-pacemaker regions. We have recorded spontaneous waves in both horizontal (Corlew et al., 2004) and sagittal slices (unpublished) and in sagittal slices have observed initiation of waves in the piriform cortex with propagation dorsally into the neocortex. We used cultured slices also to avoid problems caused by damaged surface-layer cells, which can, for example, show altered chloride gradients and...
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GABA function (Dzhala et al., 2012). In our experience, surface cells in cultured slices are much healthier than in acute slices, as judged both by appearance and by whole-cell recordings (see e.g., McCabe et al., 2007). We have shown previously that many parameters of electrophysiological development (capacitance, voltage-gated sodium and potassium currents, spontaneous waves of activity; McCabe et al., 2006) as well as migration of interneurons (unpublished) occur similarly in acute and cultured slices.

Solutions and Drugs

Cell culture medium was comprised of 75% sterile Neurobasal-ATM Medium (13) (Invitrogen, Carlsbad, CA), 25% horse serum (Sigma), penicillin (100 IU/mL), streptomycin (0.1 mg/mL), and 2 mM L-glutamine (HyClone Laboratories, Logan, UT). Artificial cerebrospinal fluid contained 140 mM NaCl, 3 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 1.25 mM NaHPO₄, 26.5 mM NaHCO₃, and 20mM D-glucose in millipore water. The cocktail of synaptic antagonists contained 10μM picrotoxin, 10μm D-AP5, and 25μm CNQX in ACSF. Tetrodoxin was prepared at a concentration of 1 μM. Drugs were obtained from Tocris Bioscience (Ellisville MO, USA), and salts were obtained from Sigma (St. Louis, MO).

Ca²⁺ Imaging

Slices were removed from the incubator and allowed to rest for 1 hour in warmed, oxygenated ACSF and then immersed in oxygenated ACSF containing 1.5 μm fluo-4AM, a calcium indicator dye, and 0.07% Pluronic Acid (Molecular Probes, Eugene, OR) for 40 minutes. Slices were then placed in the recording chamber and allowed to rest for 30 minutes. High-resolution videos were obtained with an Olympus BX51WI microscope using a 20x water immersion objective. Images were captured with a CoolSnap ES camera (Photometrics, Tucson, AZ) using an exposure time of 300 ms with an interval of 1.0 s. Wide-field imaging was done...
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with a Nikon AZ100 microscope with a 2x objective and a Photometrics QuantEM 512SC or an
Orca Flash 2.8 camera. All data collection was done with NIS Elements software (Nikon
Instruments, Melville, NY). During imaging slices were continuously perfused with oxygenated
ACSF at 30-32 °C. After control traces were obtained, drugs were washed in for 30 minutes
before reimaging under the treatment condition.

Electrophysiology

Electrophysiological experiments were conducted in neurons from coronal cortical slices
cut at E17 and cultured for 2-5 days. Patch pipettes were pulled to a resistance of 8-12 MΩ from
50 μl hematocrit glass capillary tubes using a two-stage puller (Narishige), and filled with
internal solution which contained the following (in mM): 105 potassium gluconate, 28 KCl, 2
MgATP, 3 Na2ATP, 0.2 NaGTP, 10 HEPEs, 1 EGTA, pH to 7.25. During recording, tissue was
maintained in ACSF at 30-32°C bubbled with carbogen gas (95% O2-5% CO2). All salts were
obtained from Sigma. Recordings were made using a List EPC-7 (Heka Elektronik) amplifier.
The number of excitatory postsynaptic potentials were counted by hand in 6 ventral cells (4
slices) and 6 dorsal cells (5 slices).

Data Analysis

High resolution imaging. Data were exported from NIS Elements as a series of tiff
images and analyzed in Matlab. Individual neuron borders were detected using Caltracer 2
(available through: http://www.columbia.edu/cu/biology/faculty/yuste/methods.html) with the
standard parameters. Raw fluorescence traces from individual neurons were exported for further
analysis. Background regions were selected where cells were not present in the image, and their
scaled fluorescence average was subtracted from identified cells to correct for baseline
fluctuations in illumination. After baseline subtraction, fluorescence traces were detrended to
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account for photobleaching over the course of the experiment. Detrending was accomplished using the preprogrammed Matlab detrend function which removes linear trends using fast Fourier transform analysis. Calcium transients in individual cells were detected based on an analysis of the time derivative of the change in fluorescence vs. mean fluorescence ($\Delta F/F_0$), calculated according to:

$$\frac{\Delta F}{F_0} = \frac{F(t) - F(t - 1)}{\bar{F}}$$

where $F(t)$ represents the fluorescence intensity at each time point and $\bar{F}$ represents the mean fluorescence over the course of the imaging trial. For a cell to be considered active during a given imaging trial, it was required to have at least one calcium transient that satisfied the following two criteria: (1) The amplitude of the transient had to exceed a threshold of between 0.02 and 0.1 $\Delta F/F_0$, set individually for each imaging trial based on the level of noise and intensity of the fluorescence signal present in the images; and (2) The rate of rise of the transient ($d(\Delta F/F_0)/dt$) had to exceed the mean plus twice the standard deviation of $d(\Delta F/F_0)/dt$ for that cell. Neurons with noise levels indistinguishable from calcium transients were not considered active. Within the group of active cells defined by these criteria, the initiation points of all calcium transients of activity were defined as time points at which $d(\Delta F/F_0)/dt$ reached a value equal to or exceeding the mean plus twice the standard deviation of $d(\Delta F/F_0)/dt$ for the cell over the course of the experiment. Transients whose amplitude were less than a threshold percentage (20-40%) of the maximum transient amplitude were excluded. This threshold percentage was set independently for each slice based on the fluorescence decay and the level of noise. This last criteria rejected small transients that often occurred late in recordings after dye bleaching reduced transient amplitudes to the point where they were difficult to accurately distinguish from noise.
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After detection of events in all identified cells in the field of view, the average number of cells active during each frame (1.3 seconds) was determined, and the occurrence of global synchrony was defined as those time points during which the number of cells active in a single frame exceeded the mean plus twice the standard deviation. If the mean plus twice the standard deviation of the activity per frame was less than five, we used five as an alternative minimum; this was necessary for imaging trials in which particular drug treatments resulted in very low levels of cellular activity.

**Wide-field imaging.** Images in the form of tiff stacks were imported into MatLab, corrected for bleaching, and converted into $\Delta F/F_0$ images. Activity was thresholded at twice the standard deviation of baseline F noise and then superimposed back onto a camera image of the slice. Superposition was done destructively, by zeroing all camera image pixels underneath the supra-threshold activity to maintain accuracy of the $\Delta F/F_0$ numbers. To detect the spatial distribution of asynchronous activity of cells and clusters, thresholded activity was accumulated for a given stack, and the summed activity superimposed onto a camera image of the slice (see Fig. 1A, e.g.). Cells and clusters were separated by total pixel area (2-20 pixels for cells, and >20 pixels for clusters for a 512 x 512 image). Temporal plots of the activity of individual cells and clusters was done in MatLab by detecting active objects, using the pixel lists of those objects to create masks, and then plotting mean pixel intensity vs. time within each mask over the time course of the image stack.

**Statistical analyses.** Statistical significance was calculated using the Student's t-test. A paired test was used to compare control and experimental conditions when a single slice was exposed to a pharmacological treatment and in the analysis of wide-field imaging data for comparisons between the dorsal and ventral when imaging of both regions was simultaneous. An
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unpaired heteroscedastic test was used for unpaired data comparing ventral and dorsal data that
was obtained sequentially or from different slices. All data are presented as mean ± S.E.M. The
number of samples, $n$, for each experiment is the number of slices, unless otherwise indicated.
Slices were not used for more than one experiment (a single unpaired trial or a control and an
experimental trial) due to bleaching of the calcium indicator dye.

**Modeling**

*Single cell dynamics*

The cell-autonomous property of single neurons was based on intrinsically bursting
model neurons used by Baltz et al. 2011. These neurons can fire bursts of action potentials
without any external input. We used this model for two reasons. First, Baltz et al. 2011 have
shown that these neurons best capture initiation of spontaneous activity patterns as observed in
cultured developing networks, which correspond to the same developmental periods studied in
our slices. Second, simultaneous calcium imaging and patch-clamp experiments in developing
cortex have shown that calcium transients are generated by barrages of activity in the network,
corresponding to bursts of action potentials riding on top of a long-lasting depolarization
recorded in single cells (Alléne et al. 2006).

The model neurons consisted of a single compartment (Smith et al. 2000; Baltz et al.
2011):

$$ C_m \frac{dV_m}{dt} = g_L(E_L - V_m) + g_{sra}(E_K - V_m) + I_T + I_{syn} $$

where $C_m=1 \mu F/cm^2$ is the specific membrane capacitance, $g_L=50 \mu S/cm^2$ is the leak
conductance, $E_L=-70$ mV is the reversal potential of the leak current, and $I_{syn}$ is the synaptic
current (see below). The model also includes a transient calcium current $I_T$.
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\[ I_T = g_T m h(V - V_T) \]

where \( V = 120 \, \text{mV} \) and \( g_T = 40 \, \mu\text{S/cm}^2 \). The variable \( h \) represents the inactivation of the low-threshold conductance. It relaxes to zero with a time constant \( \tau_h = 90 \, \text{ms} \) and relaxes back to unity with \( \tau_{h+} = 150 \, \text{ms} \):

\[
\frac{dh}{dt} = \begin{cases} 
-h/\tau_h^- & \text{if } V_m > V_h \\
(1-h)/\tau_h^+ & \text{if } V_m < V_h 
\end{cases}
\]

This means that when \( V_m \) is below \( V_h = -70.5 \, \text{mV} \), the current \( I_T \) is deinactivated, and when \( V_m \) is above \( V_h \), \( I_T \) is inactivated. The activation function is denoted by \( m = \Theta(V_m - V_h) \), where \( \Theta \) is the Heaviside function. Finally, a negative feedback conductance \( g_{sra} \) was added to enable spontaneous bursting (i.e. weak spike rate adaptation), with potassium reversal potential \( E_K = -75 \, \text{mV} \). The frequency of spontaneous bursts mainly depends on the time constant \( \tau_{sra} \). We chose this number so that the frequency of spontaneous events was similar to that observed experimentally. In fact, to introduce heterogeneity, \( \tau_{sra} \) for each neuron was drawn from a Gaussian distribution with mean 8 seconds and standard deviation of 800 ms.

After reaching threshold, \( V_{th} = -50 \, \text{mV} \), the membrane potential was reset to -60 mV and \( g_{sra} \) was increased by an amount \( \Delta g_{sra} = 15 \, \mu\text{S/cm}^2 \). In addition, a small amount of uniformly distribution random noise was added for slight membrane fluctuations of approximately 0.5 mV that did not affect the firing of the neurons.

**Network architecture**

A three-layered network was constructed wherein each layer consisted of a 50 x 50 rectangular grid of neurons (Figure 11A). The total synaptic conductance received by a neuron was the sum of gap junction and chemical contributions:
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\[ I_{\text{syn}} = I_{gj} + I_{\text{chem}} \]

All neurons in the network were connected to their neighbors by local gap junctional connections. Each neuron was electrically connected to a fraction of the neurons in a rectangular neighborhood of a given size around it. We used a neighborhood of size two, so that each neuron had a total of eight neighbors in its immediate neighborhood, and another 15 in the neighborhood around it. The neuron was randomly connected to half of those neurons via linear gap junctional connections so that the total contribution to the synaptic current for that neuron was taken to be

\[ I_{gj}^i = \sum_j g_{\text{gap}} (V_i - V_j) \]

We explored several values for the gap junctional connection strength (see Results) and settled on the value of \( g_{\text{gap}} = 1 \ \text{S/cm}^2 \).

In addition to gap junctional connections, the three network layers were also connected by long-range excitatory chemical connections. These connections were bidirectional and between neighboring layers only, so that a neuron from layer 1 received input only from neurons in layer 2, a neuron in layer 3 received input only from neurons in layer 2, while neurons in layer 2 received input from neurons in both layers 1 and 3. The connection probability from one layer to the next was taken to be 5%. The connections were random, so that a neuron in a given layer had an equal probability of being connected to neurons in a neighboring layer. The total contribution to the synaptic current for that neuron was taken to be

\[ I_{\text{chem}}^i = \sum_j g_{\text{chem}} (E_{\text{exc}} - V_i) \]
where $g_{chem}$ was the maximal excitatory conductance which obeyed first order linear kinetics with a decay time constant of 5 ms. We varied this number and examined the resulting wave properties, using $0.05 \mu S/cm^2$ for the simulations in Figure 11 and 12. To visualize spiking activity in the two-dimensional grid of neurons in a similar way to calcium imaging in the slice, we convolved the spiking activity for a given cell in space and time. In space, we used a two-dimensional Gaussian filter using a diagonal covariance matrix with standard deviation of 3 cells horizontally and vertically from the chosen cell. In time, we used an exponential filter with a decay time constant of 10 time units. The exact details of this convolution were not important: the spatial convolution helped in visualizing spiking activity of our otherwise point neurons, while the temporal convolution was used to produce longer-lasting activity transients similar to that observed with calcium imaging.
Films of spontaneous calcium transients occurring between spontaneous synchronous waves of activity in coronal slices (E18 – P2; E17 + 1-4 DIV) revealed two types of spontaneous activity. As shown in Supplemental Movie 1, individual neurons show spontaneous calcium transients and small clusters of neurons are also synchronously active. Figure 1A shows a summation of all frames in this 10 minute recording, so that each cell that was active at least once is marked. During this time, 8 clusters (an example is marked by the white arrow) and more than 50 individual cells (red arrow) were active.

Asynchronous activity in individual cells between waves of synchronous global activity

Individual neurons showed spontaneous calcium transients in the period between waves of activity. Figures 1B,C show a fluorescence image of a field of cells in the ventral piriform cortex, along with a plot of $\Delta F/F_0$ signals from three of these cells. A film of this field of view is shown in Supplemental Movie 2. On average, $37 \pm 2.8 \%$ of detected cells in the piriform cortex showed at least one spontaneous calcium transient within a 390 second imaging period ($n = 19$). Within that subset of cells that showed spontaneous activity, the average frequency was $0.543 \pm 0.010$ events/min (19 slices, 1951 cells).

In order to determine if asynchronous activity differs regionally, we used calcium imaging with single-cell resolution in 19 ventral and 12 dorsal regions in cultured cortical slices between P0 and P2 (E17 + 1-3 DIV). We found that in both the ventral and the dorsal cortex, some neurons were active between spontaneous waves. The number of neurons displaying asynchronous activity in the ventral cortex was significantly greater than in the dorsal cortex. Figure 2A displays the number of active cells in 1 second time bins during a typical recording in
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dorsal neocortex and ventral piriform cortex. Figure 2B quantifies the proportion of active cells relative to all cells detected for all recordings in dorsal and ventral cortex, and shows that the ventral regions contained a larger proportion of active cells than dorsal regions. In 19 slices, 37 ± 2.8 % of cells were asynchronously active in the ventral piriform cortex, compared to 9.6 ± 1.4 % (12 slices) in the dorsal neocortex ($p < 10^{-8}$). Table 1 lists the properties of synchronous and asynchronous events measured in the dorsal and ventral cortex in these experiments.

To ensure that these differences were not due to the time of recording or other factors present when dorsal and ventral recordings were done sequentially, we used low-power wide-field imaging (see Methods) to detect spontaneously active cells simultaneously in dorsal and ventral regions of 28 slices between E18 and P2 (E17 + 1-4 DIV). To ensure that our thresholding to separate synchronous and asynchronous activity in the high-power narrow-field imaging above accurately detected spontaneous waves, we compared wave frequencies measured in those experiments to those measured directly by detection of propagating waves in whole-slice experiments. Both ventral and dorsal wave frequencies were the same in both experiment types: ventral ($1.27 \pm 0.14 \text{ min}^{-1}$, narrow-field, vs. $1.70 \pm 0.62 \text{ min}^{-1}$, wide-field, $p=0.54$); dorsal ($0.23 \pm 0.12 \text{ min}^{-1}$, narrow-field, vs. $0.23 \pm 0.10 \text{ min}^{-1}$, wide-field, $p = 1.0$; $n = 340$ events in 28 slices).

As expected from the fact that fewer than 20% of waves propagate out of the piriform cortex into the neocortex at these stages (Conhaim et al., 2011), the frequency of synchronous events detected dorsally is much lower than ventrally.

Figure 2C shows a slice image with each cell that showed at least one spontaneous calcium transient during the 3-min recording period marked. The ventral cortex bilaterally had substantially more active cells than dorsal cortex. This difference as detected in wide-field imaging experiments is quantified in Figure 2D, a histogram of the ratio of the number of
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asynchronously active ventral cells to the number of asynchronously active dorsal cells per unit area in each experiment. This quantification shows that the number of cells with spontaneous activity between synchronous waves in ventral cortex was 3 times higher (36.9 ± 5.6 cells/min/mm²) than in dorsal cortex (12.0 ± 2.0 cells/min/mm²; p < 10⁻⁵). The mean frequency of asynchronous activity did not differ between the ventral (0.543 ± 0.010 events/min, 1951 cells) and dorsal (0.564 ± 0.024 events/min, 316 cells) cortex (p = 0.43; high resolution imaging).

Together these data show that in the pacemaker region of the piriform cortex, which initiates more than 99% of all spontaneous waves (Lischalk et al. 2009), a larger fraction of cells show spontaneous calcium transients between waves than in the non-pacemaker dorsal cortex.

*Synaptic isolation via synaptic antagonists does not eliminate asynchronous activity in individual neurons.*

During the E18 – P2 period (E17 + 1-4 DIV), neurons rely on developing GABAergic and glutamatergic synapses for chemical transmission. In order to determine if the differences in single cell asynchronous activity between ventral pacemaker and dorsal follower regions reflects differences in synaptic circuitry or in intrinsic cellular properties, we determined the effect of synaptic isolation with a combination of CNQX, an AMPA receptor blocker, AP5, an NMDA receptor blocker, and picrotoxin, a GABA_A receptor blocker on asynchronous activity in both ventral (n=9 slices) and dorsal (n = 10 slices) cortex. In agreement with previous data (Conhaim et al. 2011), synaptic isolation eliminated synchronous global activity in both regions (Fig. 3A,B,D; p = 0.001 ventral, p = 0.04 dorsal). Despite the elimination of synchronous activity, a significant proportion of cells in both ventral and dorsal cortex remained asynchronously active in the presence of the combination of synaptic blockers (Fig. 3A,B,C). In the ventral cortex,
synaptic isolation reduced the percentage of asynchronously active cells from 33 ± 2.8 to 25 ± 3.9 % (n = 9; p = 0.01). In the dorsal cortex, the reduction was from 9.0 ± 1.6 to 6.0 ± 0.86 % (n = 10; p = 0.04). In both cases, however, the proportion of asynchronously active cells was significantly greater than zero (p < 10⁻⁵, ventral; p < 10⁻³, dorsal), and the ventral/dorsal ratio of asynchronously active cells was similar under synaptic isolation (4.2) and under control conditions (3.7), (p = 0.001, ventral vs. dorsal proportions of asynchronous cells under synaptic isolation). These results indicate that developing neurons have a cell-autonomous mechanism for producing calcium transients in the absence of synaptic input, and that although a fraction of asynchronous activity depends on synaptic inputs, the higher proportion of spontaneous active cells ventrally primarily reflects a difference in intrinsic cellular properties between these two regions.

Spontaneous activity in clusters of cells between synchronous waves

As shown in Figure 1A (and Supplemental Movie 1), coactive clusters of cells were seen between synchronous waves as well as the individual active cells described above. In the experiment shown in Figure 1A, 8 such clusters were detected during the 10 minute imaging time. Single clusters rarely were active more than once during a single experiment, but the overall occurrence of clusters in the ventral cortex was 4.46 ± 0.54 clusters/min/mm² (n = 29), about nine times lower than the average activity of individual cells measured in the same way (36.9 ± 5.6 cells/min/mm²).

Figure 4 shows activation of a typical cluster. Activity starts with a single centrally-located cell and spreads radially outward over the course of 3-10 seconds to encompass a roughly spherical group of cells. We calculated the number of cells per cluster by extrapolating cell counts in a single plane of focus to an assumed spherical geometry, which gave a mean value
of 77.0 ± 9.7 cells/cluster (n = 77 clusters). The distribution of cluster sizes was not symmetrical (Fig. 5), showing a median of 43 cells and a small number of very large clusters which were sometimes difficult to distinguish from local synchronous waves.

Most clusters were initiated by activity in a single central cell, as shown in Figure 4. Other clusters, however, appeared to be triggered by a central cell whose baseline fluorescence level was high, and decreased abruptly as surrounding cells of the cluster became active. An example of this kind of “off cell” cluster is shown in Figure 6. In a sample of 56 clusters in which we could distinguish either center “on” or “off” cells clearly, 24 (43%) showed central “off” cells. This is likely to be an underestimate because if initial activation of clusters is rapid compared to image capture frequency, it is easier to detect a cell whose calcium signal decreases and stays low than a cell whose signal increases before surrounding cells.

The patterns of activity in clusters did not appear to correspond to the presence of anatomically discrete groups of connected neurons, because individual cells often participated in the activity of more than one cluster. Figure 7A shows an example of sequential activity in two spatially contiguous clusters, with several cells at the border between the two clusters participating in both. Figure 7B shows another example of three nearby clusters active with no overlap in participating cells in two of the clusters, but approximately 50% overlap in participating cells in the other two clusters. Further analysis showed that individual cells could participate sequentially in single cell asynchronous activity, cluster activity, and in synchronous waves within a single 10-min imaging period.
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Active clusters occur more often in the ventral pacemaker region

We next asked whether clusters showed a higher probability of occurrence in the ventral piriform region than in the dorsal cortex, using the same wide-field imaging analysis that showed the preferential ventral distribution of single cell activity (Fig. 2C). Figure 8A shows an image of all active clusters detected in a single slice during a 3-min imaging period. Active clusters are preferentially clustered in the ventral cortex bilaterally. Mean cluster occurrence derived from 29 such slices was $4.46 \pm 0.54$ clusters/min/mm$^2$ in ventral cortex vs. $0.49 \pm 0.10$ clusters/min/mm$^2$ in dorsal cortex ($p < 0.001$) (Fig. 8B).

Asynchronous activity does not depend on voltage-gated sodium channels.

In order to investigate the mechanism behind the generation of autonomous activity in developing neurons, we perfused cortical slices between P0 and P2 with tetrodotoxin (TTX), which blocks voltage-gated sodium channels. As seen in previous studies, TTX eliminated synchronous global events ($p = 0.02$ control vs. TTX, $n = 8$), confirming that sodium-spike dependent synaptic transmission is required for spontaneous waves; however, TTX did not eliminate asynchronous activity between waves ($p = 0.14$ control vs. TTX, $n = 4$).

If TTX-resistant electrical activity in a subset of neurons does in fact underlie the different abilities of dorsal neocortex and ventral piriform cortex to act as pacemakers for spontaneous waves, then piriform neurons should show higher frequencies of synaptic activity between waves than neocortical neurons, as a result of their having inputs from a greater proportion of asynchronously active neighbors. To test this, we made whole-cell current clamp recordings from 6 ventral neurons in 4 slices and 6 dorsal cells in 5 slices. In these recordings, ventral neurons showed a significantly higher frequency of EPSPs than dorsal neurons (Fig. 9;
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0.57 ± 0.14 min<sup>-1</sup> in the ventral cortex, 0.122 ± 0.023 in the dorsal cortex, \( p = 0.006 \). These data indicate that increased asynchronous activity in the ventral cortex is reflected in increased synaptic drive onto those cells which, in turn, is likely to increase the probability of periodic synchronization of their activity. The average frequency of EPSPs in the ventral cortex is 4.7 times the frequency in the dorsal cortex, somewhat larger than the factors of 3.8 and 3.0 comparing the proportion of ventral to dorsal asynchronously active cells in high-resolution and wide-field imaging, respectively. This difference may represent differences in functional connectivity between the two regions.

Clusters do not require voltage-gated sodium channels but do depend on gap junctions

Much like single cell asynchronous activity, clusters persisted in the presence of tetrodotoxin (0.3 ± 0.09/min, control vs. 0.25 ± 0.15/min TTX; \( n = 3; p = 0.8 \)), the combination of CNQX + picrotoxin (1.39 ± 0.67/min, control, vs. 0.74 ± 0.25/min, CNQX+Picrotoxin; \( n = 4; p = 0.29 \)), and thapsigargin (0.77 ± 0.21/min, control vs. 1.06 ± 0.27/min; \( n = 3; p = 0.46 \)). To test the role of gap junctions in the spread of activity within clusters, we applied 3mM octanol. This greatly reduced cluster activity (to 0.048 of control; \( n = 4 \) slices; \( p = 0.029 \)). The fact that single cell activity persisted in the presence of octanol argues against the hypothesis that the action of octanol on electrical activity itself was responsible for the block of cluster activity.

Higher levels of asynchronous activity in the piriform cortex can explain its role as the initiator of spontaneous waves

To determine if the observed differences in cell-autonomous properties between the ventral and dorsal cortices could explain the ventral wave initiation and the directionality of wave propagation towards dorsal areas, we built a network of conductance-based neurons with
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different cell-autonomous properties. The network consisted of three layers, the first corresponding to the ventral region and the last corresponding to the dorsal region (Fig. 10A). To capture our basic experimental findings, the layer designated as ‘ventral’ was given a high proportion of autonomous, asynchronous bursting activity. In this way, we tested the hypothesis that the decreasing gradient of intrinsic excitability is sufficient to generate the directionality of the waves from ventral to dorsal areas.

The network was equipped with two kinds of connectivity. To incorporate the experimental findings of the existence of gap junctionally mediated clusters of activity, within each layer, neurons were connected by gap junctions to their nearest neighbors. In the presence of only local electrical connections, the strength of these connections determined the type of activity generated in the network. Robust waves propagate in the presence of strong local connections (data not shown); however, these waves resemble the waves in the retina and not the cortex. Decreasing the strength of electrical connections creates local clusters of active neurons that propagate in space over a short range and disappear before they reach the edges of the network (data not shown). These local clusters do not propagate across long distances in the network. Because it has been shown experimentally that waves depend on synaptic transmission, these results were not unexpected.

Next, sparse chemical synapses between layers were implemented to establish long-range bidirectional connectivity and generate long-range propagation of activity in the network. Figure 10B shows a network simulation with spontaneously generated wave events. We tuned the adaptation time constant (see Methods) so that waves were generated at frequencies approximating those observed in the experimental system. The two arrows in Figure 10B point to two instances of propagating waves. The raster shown in Figure 11A(left) corresponds to the
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second arrow. At this time resolution, we observed propagating waves that have clear spatial organization: the waves initiate spontaneously in the first network layer, corresponding to ventral cortical areas, and propagate towards the third layer, corresponding to the dorsal cortex. Since the spike rasters do not capture the spatial organization of wave activity in each layer, we also show a snapshot of the temporal wave dynamics (Fig. 11A, right; Supplemental Movie 3). The spiking activity was convolved over space and time (see Methods) to produce activity similar to that observed with calcium imaging. Here, the black areas denote inactive regions in the network, while white areas denote highly active areas. These time points clearly demonstrate that the initiation of wave activity begins with local clusters in the first network layer, amplified by local electrical connections, before the activity spreads to layers two and three propagated by the long-range chemical connections. Why does this unidirectional propagation occur despite the presence of bidirectional chemical long-range connections?

We postulated that the reason for this symmetry in the direction of propagation was the imposed gradient of intrinsically bursting cells in the different network layers. Indeed, eliminating this gradient, by giving all layers the same fraction of intrinsically bursting neurons, eliminated the robust unidirectional wave propagation from layer one to three. Figure 11B,C show two example waves generated in a network with a uniform distribution of intrinsically bursting cells across layers. These cells initiate local activity clusters in each layer, hence large-scale activity can initiate anywhere. Figure 11B (Supplemental Movie 4) shows a large-scale activity that begins in all layers simultaneously, thus there is no directionality of the activity event. In contrast, Figure 11C (Supplemental Movie 5) shows a scenario where a true backward wave is initiated in the third layer, which is then propagated toward the first layer mediated by the long-range synaptic connections.
Model predicts persistence of wave activity near the pacemaker region

One notable feature of wave propagation in the model is that activity not only initiates in the ventral layer, but also persists there at the termination of the wave (see Fig. 11A). That is, the pacemaker region is the first to be active and the last to cease activity. To determine whether this was also a feature of the actual waves, we examined 19 waves that propagated into the dorsal neocortex in 8 P0 slices (E17 + 2DIV). The duration of these waves in the ventral pacemaker, measured from the onset of the macroscopic calcium transient to its 90% decay time was $5.52 \pm 0.5$ sec compared to a dorsal wave duration of $2.95 \pm 0.34$ sec ($p < 0.001$). An example of a propagating wave in which the initiation region shows activity that outlasts the dorsal follower regions is shown in Figure 12A. In some, but not all (5/19), of the waves, the increased duration of activity in the pacemaker region took the form of multiple secondary calcium transients on the falling phase of the primary wave transient. Figure 12B shows plots of calcium transients during wave activity in a slice in which both types of pacemaker activity were observed. The first wave occurs in the left hemisphere and the pacemaker, but not dorsal, wave shows multiple local secondary events. This is followed by a wave in the right hemisphere which shows simple waveforms of activity both ventrally and dorsally. Finally, a second wave occurs in the right hemisphere in which the pacemaker region shows a single secondary transient. These secondary transients were never seen dorsally.
In this study, we investigated the activity of neurons in the ventral (piriform) and dorsal (neocortex) neonatal mouse cortex in the periods between large-scale spontaneous waves in order to gain further insight into the properties that differentiate the two regions. Such differences may explain the spatially asymmetric generation and propagation of SSA, initiating in the ventral (piriform) cortex and propagating dorsally into the neocortex. Two types of activity were observed in the inter-wave period: (1) individual cells produced asynchronous activity and (2) clusters of cells were active in synchronous groups. Individual cells were seen to participate in single-cell asynchronous activity, cluster activity, and synchronous waves, all within a single 5-10 minute imaging period. Wide-field imaging revealed that both types of inter-wave activity were much more common in the ventral compared to the dorsal cortex. We then applied a combination of synaptic antagonists and found that asynchronous cell and cluster activity remained in the absence of glutamatergic and GABAergic synaptic communication. This result indicates that neurons during this period are able to generate activity autonomously and suggests a difference in intrinsic firing properties between ventral and dorsal regions of the cortex. Treatment with octanol eliminated synchronous clusters while preserving single cell activity, suggesting that the propagation of activity within clusters relies on gap junctions.

The higher level of activity during the inter-wave interval in the ventral cortex could provide a mechanism by which the ventral cortex is uniquely able to generate waves. Asynchronous activity generated autonomously by individual neurons and then amplified by gap junctional connectivity may achieve sufficient recruitment of ventral neurons to periodically initiate synchronous waves. To investigate this possibility we constructed a conductance-based network model with a gradient of cell-autonomous activity that decreased over three layers. We
found that this gradient was sufficient to produce directionally propagating waves that replicated key features of experimentally observed SSA. Despite bidirectional synaptic and gap junctional connectivity, waves propagated only from the layer of high autonomous activity towards the layers of lower autonomous activity. When all layers contained an equal proportion of autonomously active neurons, propagation was not unidirectional and synchrony did not simulate experimental findings. Thus, a gradient of autonomously active cells is necessary and sufficient to produce the type of synchronous activity observed experimentally and is likely to represent a critical feature of the biological network architecture. A second prediction of this model is that during wave propagation, the ventral initiator region will not only be the first to activate, but will be the last region to cease activity at the end of the wave. That is, waves will propagate out of and then recede back into the ventral cortex. Analysis of propagating waves in slices bore out this prediction of the model.

The underlying reason for the increased level of autonomous activity in the piriform is not clear, but the piriform cortex does have several unique features that might explain this phenomenon. The piriform cortex, which in the adult is involved in olfactory processing, is phylogenetically older than the neocortex. The neocortex, which is present only in mammalian species, is characterized by a six-layer organization; in contrast, the piriform cortex is organized in a three-layer morphology and lacks columnar organization. Mature neurons in the piriform cortex have widespread axonal arbors that cover much of the piriform cortex and extend into other cortical areas. Superficial pyramidal (SP) neurons in piriform cortex make a small number of synapses onto a large number of other SP neurons (Johnson et al. 2000). Thus, the piriform cortex may be particularly well-structured to coordinate activity both within the region itself and across other regions. Indeed, synchronization orchestrated by the piriform cortex has been
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observed in both physiological and pathological contexts. The region is particularly susceptible
to generating epileptiform activity and is believed to play a crucial role in kindling (Loscher and
Ebert 1996). Spontaneous rhythmic activity comprised of up- and down-states has also been
observed in vitro under physiological conditions (Seamari et al. 2007). Developmentally, the
paleocortex including the piriform cortex matures slightly earlier than the neocortex. The
initiation of SSA may rely on the slightly more mature properties of piriform neurons in contrast
to the developmentally younger neurons in the dorsal neocortex. This prediction is in agreement
with experiments regarding SSA in the hippocampus which have demonstrated that relatively
mature neurons are critical in initiating waves (Picardo et al. 2011).

The clusters of coactive cells we observed appear identical to the domains originally
reported by Yuste et al. (1995), in early postnatal rat cortex. They also reported that domain
activation was not prevented by TTX and was sensitive to gap junction blockers. The only
differences we observed between our clusters and their domains were that we observed almost
half of the clusters apparently triggered by ‘off’ cells, and that in our experiments, we were not
able to trigger clusters by temperature drop. We did not study the effects of temperature on
clusters in general. In addition to the function of domains in the development of the columnar
organization of the cortex, as suggested by Yuste et al., (1995), the present data suggest that they
may also play a role in the propagation of spontaneous waves of activity, and thus more
generally in activity-dependent development of the cortex.

This study identified significant activity differences between the ventral piriform cortex
and the dorsal neocortex during the period between waves. Asynchronously active cells and
active cellular clusters were more prominent in the ventral compared to the dorsal region. Model
Individual neuron and network spontaneous activity to produce directionally specific waves of synchrony that closely mimic our experimental results. The experimental and theoretical data thus indicate that the high level of autonomous activity in the ventral cortex provides a mechanism for location-specific SSA generation.
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Disclosures
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References


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**Figure Legends**

**Figure 1.** Asynchronous activity of individual cells and clusters in the ventral piriform cortex. (A) Image of a cortical slice during calcium imaging. All activity recorded during a 10 minute interval is shown in yellow superimposed on the grayscale slice image. Red arrow indicates a single active cell; white arrow indicates a cluster of synchronously active cells. The image stack from which this figure was made can be viewed as Supplemental Movie 1. (B) Fluo-4 fluorescence image of neurons in the ventral region, 20x magnification. Colored arrows point to the cells whose fluorescence traces are shown in C. (C) $\Delta F/F_0$ traces for three cells indicated in B. The image stack from which these data were taken can be viewed as Supplemental Movie 2.

**Figure 2.** Comparison of single-cell asynchronous activity in the ventral and dorsal cortex. (A) Examples of single-cell activity in dorsal (top) and ventral (bottom) regions. Black bars represent the number of cells active in each 1 sec time bin. Synchronous events (see Methods) were removed and the number of cells at those time points set to zero. (B) Proportion of cells with asynchronous activity in ventral (left, $n = 19$) and dorsal (right, $n = 12$) regions in the 390 s imaging period. Each circles represents one experiment. Black crosses represent the mean, with the height of the vertical bar showing the s.e.m. (C) Image of a coronal slice with all cells showing $\geq$ spontaneous Ca transients during the 3-min recording period marked in yellow. Note that the number of active cells is higher in the ventral (piriform) cortex bilaterally. (D) Histogram of the ratio of ventral:dorsal active cells in 28 whole-slice imaging experiments such as that shown in C. Black cross marks the mean and the vertical bar the s.e.m. The dashed line shows a ratio of 1.0.

**Figure 3.** Effects of synaptic isolation on synchronous and asynchronous activity in the ventral and dorsal cortex. (A) Example of activity in ventral region before (top) and after (bottom) synaptic isolation. Black bars represent the number of cells active at each time point. Synaptic isolation blocks synchronous events but leaves asynchronous activity intact. (B) Same as in A for dorsal region. Note that for both plots, the vertical scale is different for control and drug data. (C) Proportion of cells asynchronously active before (left) and after (right) synaptic isolation in paired experiments. Ventral experiments are shown with grey circles and solid black lines ($n = 9$). Dorsal experiments are shown with white circles and dashed lines ($n = 10$). Black squares represent the mean. Black bars show the s.e.m. Not significant = n.s.; p-values are given. Asynchronous activity in both ventral and dorsal regions is not blocked by synaptic isolation. (D) Frequency ($\text{min}^{-1}$) of synchronous events before (left) and after (right) synaptic isolation in paired experiments, confirming block of synchronous waves by synaptic isolation.

**Figure 4.** Activation of a cellular cluster. Individual frames from a calcium imaging experiment. Times are given in the upper right corner of each image. Cluster activation starts with a single active cell and then spreads radially over the course of 10-11 sec.

**Figure 5.** Distribution of cluster size. Histogram of the number of cells in each detected cluster. Total number of clusters was 77. Average number of cells/cluster was 43.

**Figure 6.** Activation of a cellular cluster triggered by an ‘off’ cell. Individual frames from a calcium imaging experiment. Times are given in the upper right corner of each image. The
central cell is indicated by a white arrow. The decrease in fluorescence of the central cell is seen in the third panel simultaneous with the activation of the cluster.

**Figure 7.** Overlap of cell participation in clusters. (A) Two clusters active at different times in the same region are shown in the first two panels. A merge of the two clusters is shown in the third panel. Red indicates cells that participated in both cluster activations. Thresholding to create the merged image results in some cells in each cluster not displayed. (B) Three cluster activations are shown in the first three panels. In the merged fourth panel, red indicates cells active in both the second and third clusters. Cell activity from panel 1 is show in yellow and did not overlap with either of the other two clusters.

**Figure 8.** Spatial distribution of cellular clusters in the ventral and dorsal cortex. (A) Image of a cortical slice during calcium imaging. All clusters that were active during the 10 minute imaging time are shown superimposed on the image in yellow. White outlines show the division between dorsal and ventral cortex used for quantification. uc = uncut edge of slice, not included in quantified regions; bg = basal ganglia; cc = corpus callosum; sn = septal nucleus. (B) Frequency (min⁻¹ mm⁻²) of clusters in ventral (left) and dorsal (right) regions during each experiment. Black squares represent the mean. Black bars show the s.e.m.

**Figure 9.** Frequency of excitatory postsynaptic potentials in the ventral and dorsal cortex. (A) Sample traces from 4 cells in the ventral(top) and dorsal(bottom) cortex showing the higher frequency of EPSPs in the ventral cortex. Larger events (>20 mV), which presumably represent spontaneous waves, were excluded from the analysis and are truncated in these records. Scale bars in the upper left corner of each set of traces represent 10 mV. (B) Frequencies (min⁻¹) of EPSPs in the ventral (n = 6 cells) and dorsal (n = 6 cells) cortex. Black crosses represent the mean. The height of the vertical bar represents the s.e.m.

**Figure 10.** Network model design and simulation. (A) Model schematic of three-layered network. Percentages of intrinsically active neurons are displayed at the top of each layer. Colored circles represent neurons whose connections are depicted. Colored boxes represent the gap junctional neighborhood to which each of these cells are connected. Arrows represent synaptic connections between layers. V = ventral; D = dorsal. (B) Raster plot of neuronal activity vs. time. Neurons are arranged in vertical layers for each point in time. Black indicates neuronal activity. Waves of synchrony are noted by superimposed vertical black lines.

**Figure 11.** Examples of waves in different network implementations. (A) Example wave in a model simulation in which the proportion of intrinsically active cells increased from the dorsal to the ventral layer. Left panel shows a raster of the wave; Right panels show frames from the simulation (see Movie 3). The waves starts in the ventral layer and propagates dorsally. Note that the activity in the ventral layer decays the most slowly. (B,C) Example waves in a model simulation with a uniform proportion of intrinsically active cells across the three layers. (B) Shows a wave that initiates uniformly across all three layers (Movie 4). (C) Shows a wave that initiates in the dorsal layer and propagates ventrally (Movie 5).

**Figure 12.** Persistence of activity in the ventral piriform cortex at the termination of a propagating wave, as predicted by the model (Fig. 12 A). (A) Montage of images taken during a
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propagating spontaneous wave in a P0 coronal slice. Activity initiates in the right piriform
cortex and propagates throughout the right hemisphere during 1.5 sec. As the wave terminates,
activity persists longer in the piriform initiating region than in the dorsal follower regions. (B)
Macroscopic calcium transients in ventral and dorsal regions taken from another slice that
showed three propagating waves. The first wave, in the right hemisphere (bottom traces) showed
repeated after-discharges in the ventral region that were absent in the dorsal region. The second
wave occurred in the left hemisphere (top traces), and was associated with a simple ventral
calcium transients that was longer in duration than the dorsal transient. The third wave occurred
again in the left hemisphere, and the ventral calcium transient was longer than the dorsal
transient and showed a single after-discharge on the falling phase.

Supplemental Movie 1. Video of wide-field calcium imaging showing asynchronous activity and
cellular clusters occurring between spontaneous waves (not shown). 20 frames per second, 1
frame = 1 second of imaging time.

Supplemental Movie 2. Video of high-resolution calcium imaging showing asynchronous
activity in individual cells. (A) Fluo-4 fluorescence image of neurons in the ventral region, 20x
magnification. (B) ΔF/F₀ traces for three individual cells from the image in A showing
spontaneous calcium transients. Each colored line represents the ΔF/F₀ signal (y-axis) vs. time
(x-axis) for a cell. (10 frames per second; 1 frame = 300 ms exposure time with 1 second
between exposures).

Supplemental Movie 3. Example wave in a model simulation in which the proportion of
intrinsically active cells decreased from the ventral to the dorsal layer. The wave starts in the
ventral layer and propagates dorsally. Note that the activity in the ventral layer decays the most
slowly. (30 frames per second; 1 frame = 0.1 ms)

Supplemental Movie 4. Example wave in a model simulation with a uniform proportion of
intrinsically active cells across the three layers. The wave initiates simultaneously across all
three layers. (30 frames per second; 1 frame = 0.1 ms)

Supplemental Movie 5. Example wave in a model simulation with a uniform proportion of
intrinsically active cells across the three layers. The wave initiates in the dorsal layer and
propagates ventrally. (30 frames per second; 1 frame = 0.1 ms)
A) Graph showing the number of active cells over time.  

B) Scatter plot comparing the proportion of asynchronous cells in Ventral vs. Dorsal regions.  

C) Image of a brain section highlighting active cells.  

D) Bar graph showing the proportion of asynchronous cells across different Ventral:Dorsal ratios.
Clusters (min⁻¹ mm⁻²)

p < 10⁻⁷
A

B

p = 0.02
<table>
<thead>
<tr>
<th></th>
<th>Ventral</th>
<th>Dorsal</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synchronous event frequency</td>
<td>1.3 ± 0.14 min⁻¹</td>
<td>0.23 ± 0.12 min⁻¹</td>
<td>&lt; 10⁻⁵</td>
</tr>
<tr>
<td>Synchronous event fractional participation</td>
<td>0.35 ± 0.16</td>
<td>0.58 ± 0.064</td>
<td>0.002</td>
</tr>
<tr>
<td>Asynchronous event frequency (per active cell)</td>
<td>0.54 ± 0.01 min⁻¹</td>
<td>0.56 ± 0.024 min⁻¹</td>
<td>0.4</td>
</tr>
<tr>
<td>Fraction of cells showing asynchronous activity</td>
<td>0.37 ± 0.028</td>
<td>0.096 ± 0.014</td>
<td>&lt; 10⁻⁸</td>
</tr>
<tr>
<td>Calcium transient amplitude (ΔF/F₀)</td>
<td>0.299 ± 0.0039 (σ = 0.4690)</td>
<td>0.138 ± 0.0035 (σ = 0.1526)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table I. Data collected from: 19 ventral slices (157 synchronous events, 1951 cells showing asynchronous activity, and 14478 calcium transients) and 12 dorsal slices (18 synchronous events, 316 cells with asynchronous activity) and 1914 calcium transients. Values represent mean ± s.e.m.