Spiral Waves in Disinhibited Mammalian Neocortex

Xiaoying Huang, William C. Troy, Qian Yang, Hongtao Ma, Carlo R. Laing, Steven J. Schiff, and Jian-Young Wu

1Department of Physiology and Biophysics, Georgetown University Medical Center, Washington, DC 20057, 2Department of Mathematics, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, 3Department of Mathematics, Massey University, 102-904 Auckland, New Zealand, and 4Department of Psychology and Program in Neuroscience, Krasnow Institute, George Mason University, Fairfax, Virginia 22030

Spiral waves are a basic feature of excitable systems. Although such waves have been observed in a variety of biological systems, they have not been observed in the mammalian cortex during neuronal activity. Here, we report stable rotating spiral waves in rat neocortical slices visualized by voltage-sensitive dye imaging. Tissue from the occipital cortex (visual) was sectioned parallel to cortical lamina to preserve horizontal connections in layers III–V (500-/um-thick, ~4 × 6 mm). In such tangential slices, excitation waves propagated in two dimensions during cholinergic oscillations. Spiral waves occurred spontaneously and alternated with plane, ring, and irregular waves.

The rotation rate of the spirals was ~10 turns per second, and the rotation was linked to the oscillations in a one-cycle–one-rotation manner. A small (<128 µm) phase singularity occurred at the center of the spirals, about which were observed oscillations of widely distributed phases. The phase singularity drifted slowly across the tissue (~1 mm/10 turns). We introduced a computational model of a cortical layer that predicted and replicated many of the features of our experimental findings. We speculate that rotating spiral waves may provide a spatial framework to organize cortical oscillations.

Key words: voltage-sensitive dye; tangential slice; optical imaging; oscillation; partial differential equations; spiral waves

Introduction

A spiral wave in the broadest sense is a rotating wave traveling outward from a center. Such spiral waves have been observed in many systems (Winfree, 2001; Murray, 2003), including biological systems, such as heart ventricular fibrillation (Davidenko et al., 1992), retinal spreading depression (Gorelova and Bures, 1983), fertilizing Xenopus oocyte calcium waves (Lechleiter et al., 1991), and glial calcium waves in cortical tissue culture (Verkhovsky et al., 1998). Although neuronal traveling waves have been observed in many systems from invertebrates to mammals (Ermentrout and Kleinfeld, 2001), spiral waves of neuronal activity have not been confirmed in mammalian brain despite considerable effort (Petsche et al., 1974; Fuchs et al., 1987; Friedrich et al., 1991).

Demonstrating a true spiral wave requires that the medium under study be relatively smooth and isotropic. Such demonstrations must exclude spurious rotation of the measured activity as a result of spatial undulations in the excitable medium and its properties (e.g., human EEG cannot detect true spirals if recorded on a scale larger than a single gyrus or from the scalp). If one labels the phases of oscillation at each point in the medium, a "phase singularity" should be observed at the center that distinguishes spiral waves from other kinds of rotating waves (Winfree, 2001).

The most rigorous demonstration of spiral wave formation in cortex that we are aware of is the finding of phase singularities in optical imaging of turtle visual cortex, which demonstrated circular waves persisting for up to four rotations (Prechtl et al., 1997).

Although circular waves were predicted from early models of cortical activity (Beurle, 1956), true spiral wave formation was not observed until the more sophisticated Wilson–Cowan formulation (Wilson and Cowan, 1972, 1973) and modern computing simulation strategies (Milton et al., 1993). Our experimental work was inspired by such theoretical considerations. Nevertheless, a close link between computational models of spiral wave formation in cortex and experiment has not been attempted previously.

In this report, we present evidence for stable spiral waves (up to 30 cycles) in rat neocortical slices with robust phase singularities. We also introduce a computational model of a cortical layer that predicts and replicates many of the features of our experimental findings. Our results suggest the possibility that spiral dynamics participate in the spatial organization of prolonged and periodic activities such as seizures and oscillations in neocortex related to sensory and motor events.

Materials and Methods

Tangential slice. Neocortical slices were obtained from Sprague Dawley rats (postnatal days 21–35). Tangential slices were cut with a vibratome on the rostrocaudal and mediolateral coordinates of bregma –2 to –8 mm and lateral 1–6 mm, respectively (see Fig. 1, left). The first cut was made 300 µm deep from the pial surface, and the tissue was discarded. The second cut was made 500 µm deeper to obtain a 500-µm-thick slice of middle cortical layers. The slice was perfused with artificial CSF (ACSF) containing the following (in mM): 132 NaCl, 3 KCl, 2 CaCl₂, 2 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 dextrose (saturated with 95% O₂ and 5% CO₂ at 28°C for 1 hr before experiments). When the
slices were perfused with 100 μM carbachol and 10 μM bicuculline, oscillations (4–15 Hz) occurred spontaneously, and the activity appeared as spiral and other waves in the voltage-sensitive dye imaging. These activities lasted as long as the preparation was perfused with carbachol and bicuculline, similar to coronal slices (Lukatch and Maclver, 1997; Bao and Wu, 2003).

Voltage-sensitive dye imaging. An oxonol dye, NK3630 (Nippon Kankoh-Shikiso Kenkyusho, Okayama, Japan) was used as an indicator of transmembrane potential. Slices were stained with 5–10 μg/ml of dye dissolved in ACSF for 60–120 min (26°C) and perfused in a submersion chamber during the experiment (28°C). Imaging was performed with a photodiode array on an upright microscope with transillumination (absorption) arrangement (Wu et al., 1999; Jin et al., 2002). Data were analyzed using NeuroPlex software (RedShirt Imaging, Fairfield, CT) and displayed in the form of traces (see Figs. 1, 2B, 3) (digitally filtered 2–50 Hz) and pseudocolor images (see Figs. 2A, 3A) (digitally filtered 3–30 Hz). Supplementary movies S1–S4 (supplemental material, available at www.jneurosci.org) are made with consecutive pseudocolor images.

Phase analysis. The phase map of the spiral (see Fig. 2C) was made using programs written in the MATLAB (Mathworks, Natick, MA) according to methods described by Prechtl et al. (1997). Briefly, raw data (filtered 2–50 Hz) recorded by each photodetector (see Fig. 2C, Raw data) was further filtered by a multistaged projection filter at an optimal frequency (see Fig. 2C, Filtered). Phase was then assigned to the filtered signal (see Fig. 2C, Phase). The phase value at each location at a given time was assigned to a color according to a linear color scale, and the map was composed of colors at all locations.

Computational methods. In our model, we reduce the size of “neurons” to points in a continuum in which the cells have excitation u and recovery a, but, as in our experiments, no inhibition:

\[ \frac{du(x, y, t)}{dt} = -u(x, y, t) - \int \int w(x, y, p, q) f(u(p, q, t) - \theta) dp dq - a(x, y, t) \]

\[ \frac{da(x, y, t)}{dt} = \beta u(x, y, t) - a(x, y, t), \]

where w represents the connectivity between neurons as a function of position (x, y). We have taken w to be a product of the following form:

\[ w(x, y, p, q) = w_r \left( \sqrt{(x-q)^2 + (y-p)^2} \right) g(q, p), \]

where \( w_r \left( \sqrt{(x-q)^2 + (y-p)^2} \right) \) is a symmetric decreasing Gaussian-type function, and \( g(q, p) \) represents a mild anisotropy. In polar coordinates, \( (r, \phi) \), \( g(q, p) \) has the simple form \( g = 0.5 (1 + \exp(0.02 \sin(0.5q))) \). The firing rate is \( f(u - \theta) \), where \( \theta \) represents threshold. In our simulations, \( f(u) \) is represented by a sigmoid or Heaviside function (equal to 0 for \( u < 0 \) and equal to 1 for \( u > 0 \)). The a represents a recovery or adaptation variable whose rate of increase is proportional to \( \beta u \), and \( \gamma \) is the time constant for change in a relative to change in u. For efficiency, we used a Fourier transform method (Laing and Troy, 2003) to transform the system into an equivalent system of partial differential equations. These were solved using a finite difference scheme with Neumann (free) boundary conditions. Spirals and ring waves were initiated with both boundary conditions. Spirals and ring waves were initiated with both waves (Golomb and Amitai, 1997; Wu et al., 1999; Bao and Wu, 2003). In tangential slices (Fig. 1, left) (Fleidervish et al., 1998), such oscillations develop into two-dimensional waves. Four wave patterns were observed: spiral, plane, ring, and irregular (Fig. 2A) (movies 1–4, available at www.jneurosci.org as supplemental material). Spiral waves appeared as a wave front rotating around a center (Fig. 2A, top row). Each cycle of the rotation was associated with one cycle of the oscillation (movie 1, available at www.jneurosci.org as supplemental material). Plane waves had straight traveling paths across the tissue, and each wave was associated with one cycle of oscillation (Fig. 2A, middle row) (movie 2, available at www.jneurosci.org as supplemental material). The plane waves appeared to evolve from ring waves as the wavefront curvature decreased when propagating outward from the center of the ring (movie 4, available at www.jneurosci.org as supplemental material). Irregular waves had multiple simultaneous wavefronts with unstable directions and velocities (Fig. 2A, bottom row) (movie 3, available at www.jneurosci.org as supplemental material). These four patterns alternately occurred within an oscillation epoch. Irregular waves usually occurred at the beginning and the end of the oscillation epochs; the plane, ring, or spiral waves typically occurred in the middle of the epochs (Fig. 2B) and were relatively stable, i.e., similar wave patterns repeated with each cycle of the oscillation. Spiral waves appeared to arise out of the interactions of waves (movie 4, available at www.jneurosci.org as supplemental material).

Spiral waves were observed in 48% of trials (116 of 242 trials from 15 slices of 12 animals). Of these 116 observations of spirals, 66 had at least four rotations (57%) and 10 had >10 rotations (9%). Both clockwise and counterclockwise rotations were seen during different oscillation epochs from the same slice. However, each emergent spiral always rotated in the same direction.
Phase singularity

To distinguish the spirals from other types of rotating waves, we analyzed the spatial phase distribution of the spirals (Fig. 2C). During the entire period of the spiral, the phase distribution within the field of view was mapped between \(-\pi\) and \(\pi\) (Fig. 2C). The highest spatial phase gradient was observed at the pivot of the spiral (Fig. 2C, white dots). The presence within such a phase gradient of a phase singularity would be the hallmark of a true spiral wave (Ermentrout and Kleinfeld, 2001; Winfree, 2001; Jalife, 2003).

We hypothesized that a phase singularity in the slice would be observed as a small region containing oscillating neurons with nearly all phases represented between \(-\pi\) and \(\pi\). Such phase mixing would result in amplitude reduction in the optical signal. In the experiment in Figure 3, we used higher spatial resolution to search for the singularity. Using a 25 \(\times\) 25 hexagonal array with 464 elements, each detector covered a circular area 128 \(\mu\)m in diameter (total field of view, 3.2 mm in diameter). All of the detectors showed high-amplitude oscillations before the formation of spirals (Fig. 3A, traces a\(-\)e, before the first broken vertical line). During spiral waves, the phase singularity drifted slowly across the tissue (~1 mm/10 turns). The four detectors, a\(-\)d, alternately recorded reduced amplitude as the spiral center approached each detector in turn. Such amplitude reduction was localized at the spiral center, and this reduced amplitude propagated with drift of the spiral center (Fig. 3A, traces a\(-\)d). In locations distant from the spiral center (Fig. 3A, location e), the amplitude remained high during all rotations of the spiral. In plane or ring waves, no localized region of oscillatory amplitude reduction was seen.

To further confirm that the amplitude reduction was caused by superposition of anti-phased oscillations, we examined the signals surrounding a spiral center. Figure 3B shows the signals from a group of detectors when a spiral center drifted over the center detector. As the spiral center hovered briefly (~200 msec, two rotations) (Fig. 3B, p) over the center detector, the amplitude was reduced (Fig. 3B, trace C). Simultaneously, the six surrounding detectors (1\(-\)6) did not show amplitude reduction, but the oscillation phases exactly opposed each other symmetrically across the center (Fig. 3B, traces 2, 5). These results indicate that the area of amplitude reduction was less than or equal to the size of our optical detector field of view (128 \(\mu\)m diameter). When we added the signals from the six surrounding detectors, the averaged waveform showed a similar amplitude reduction to the center detector (Fig. 3B, AVG). This combined signal was nearly identical to the central signal, as demonstrated by the small residual when the two signals were subtracted (Fig. 3B, AVG-C). These findings strongly support that the amplitude reduction was not caused by inactivity but rather by the superimposition of multiple widely distributed phases surrounding the singularity and that the spiral center was fully confined within the area of the central detector.

In all of the 63 cases of spiral waves examined by the 25 \(\times\) 25 array, we found that localized amplitude reduction always occurred at the center of the spiral. Such phase singularities were not anchored to a fixed location within the tissue but were observed to drift while rotating.

**Spirals in model**

We modeled the spiral waves in cortical slices with a homogeneous and isotropic two-dimensional excitatory medium. The
most widely used models for such medium are based on the Wilson-Cowan equations (Wilson and Cowan, 1972, 1973). Later, modifications by Pinto and Ermentrout (2001) described one-dimensional wave propagation in excitatory disinhibited neural networks. We extended this approach into two dimensions.

We seek the simplest model possible, reducing the neurons to points in a continuum that has excitation and recovery but, as in our experiments, no inhibition. Such a model represents the qualities of a disinhibited network dominated by fast excitation (perfused by carbachol and bicuculline) and with an intact recovery adaptation that combines the refractory effects of spike inactivation and voltage- and calcium-activated potassium currents. Point stimulation of this model can successfully reproduce ring and plane waves propagating away from the point of stimulation (Fig. 4A, left and center).

The spirals in the model were initiated by breaking a wave-front with an inhibitory stimulus applied where the wave meets the boundary of the medium, creating a free end of sufficiently high curvature to initiate spiral rotation. Such spiral waves emerged macroscopically as a property of the network in the absence of pacemaker or oscillatory microscopic dynamics and were sustained as a prolonged and periodic activity in the network (Fig. 4A, right) (movie 5, available at www.jneurosci.org as supplemental material). The model also showed irregular waves with multiple wave fronts and annihilation after collisions. These behaviors were consistent with the waves observed in the cortical slices. When virtual detectors were placed at different locations in the field of the rotating spiral waves (Fig. 4A, right, a–d), amplitude reductions were observed near the spiral center but not at other locations, consistent with the observations in slices (Fig. 3).

Discussion
Spiral in neocortex
Our results support the existence of true spiral waves of cortical neuronal activities in four respects. First, a phase singularity (Figs. 2, 3) was observed in spiral waves but not in other wave patterns. Second, the oscillation amplitude was reduced at the spiral center (Fig. 3A), and the reduction only occurred when a spiral was formed. Third, the center of the spiral was smaller than the field of view of a single photodetector at our highest spatial resolution (Fig. 3B). Last, spirals were not an artifact of the boundary constraints from the edge of the slices because nonrotating waves (plane, ring, and irregular) were observed to dynamically alternate with rotating spiral waves.

Phase singularity
It would be interesting and challenging to understand the cellular organization within the phase singularity. The observed spiral center was small, ~100 μm in diameter (Fig. 3B). We speculate that, at the center of the spiral, intracellular measurement would show high-amplitude oscillations but that neighboring neurons would have different phases. Future experiments with simultaneous intracellular measurements from neurons near the phase singularity would be required to fully characterize such dynamics.

Waves and oscillations
Neuronal population wave activity in slices cut normal (perpendicular) to the surface of the cortex produce unidirectional traveling waves as if the cortical circuits were functionally one-dimensional (Chagnac-Amittai and Connors, 1989; Wandman and Gutnick, 1993; Golomb and Amitai, 1997; Tsau et al., 1998; Wu et al., 1999, 2001; Bao and Wu, 2003). Population oscillatory wave activity in slices cut tangentially to the surface of the cortex (Feldervish et al., 1998) can generate activities that suggest that the cortical circuits are functionally two-dimensional. Oscillation patterns in two-dimensional networks can be complex, and at least four types were described in this report. These patterns occurred spontaneously and alternatively in the same tissue, suggesting that the patterns are organized dynamically rather than associated with particular anatomical structures within the network.

Golomb and Amitai (1997) have proposed that the speed of the wave front and implicitly the direction of the wave front were related to the spread of horizontal connections. In our results, emerging dynamics of the activity in the system also play a significant role in the propagating velocity, direction, and the curva-
A Model waves

Ring
Plane
Spiral

Figure 4. Model waves. A, Examples of wave patterns from the computational model, including ring waves from central stimulation and plane and spiral waves initiated from stimulation at the edge of the excitable sheet. B, Computational output of amplitude from individual detectors (a–d in A) with differing spatial and temporal relationships to spiral center. Amplitude reduction occurred in detectors a–d at different times, indicating drift of the spiral center. T, Time (computational steps).

B Amplitude reduction

Figure 4.

References


Pinto DJ, Ermentrout GB (2001) Spatially structured activity in synaptical-


