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**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 sperm in a mammalian egg (Muller and

tion 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 (Fitzpatrick et al., 1997).

Although circular waves were predicted from early models of cortical activity (Beurlé, 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 network that predicts and replicates many of the features of our experimental findings. Our results suggest the possibility that such dynamics participate in the spatial organization of prolonged periodical activities such as seizures and oscillations in neocortex related to sensory and motor events.

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**Materials and Methods**

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 CaCl<sub>2</sub>, 2 MgSO<sub>4</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, and 10 dextrose (saturated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 28°C for 1 hr before experiments). When the

slices were perfused with 100  $\mu$ M carbachol and 10  $\mu$ M 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 MacIver, 1997; Bao and Wu, 2003).

An oxonol dye, NK3630 (Nippon Kankoh-Shikiso Kenkyusho, Okayama, Japan) was used as an indicator of transmembrane potential. Slices were stained with 5–10  $\mu$ g/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

In the experiment in Figure 3, we used higher spatial resolution to search for the singularity. Using a 25 × 25 hexagonal array with 464 elements, each detector covered a circular area 128 μm 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).  
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### 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 0 and 2π (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 0 and 2π. Such phase mixing would result in amplitude reduction in the optical signal.

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 recov-

- Chagnac-Amitai Y, Connors BW (1989) Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. *J Neurophysiol* 61:747–758.
- Davidenko JM, Pertsov AV, Salomonsz R, Baxter W, Jalife J (1992) Stationary and drifting spiral waves of excitation in isolated cardiac muscle. *Nature* 355:349–351.
- Eckhorn R, Bauer R, Jordan W, Brosch M, Kruse W, Munk M, Reitboeck HJ (1988) Coherent oscillations: a mechanism of feature linking in the visual cortex? Multiple electrode and correlation analyses in the cat. *Biol Cybern* 60:121–130.
- Ermentrout GB, Kleinfeld D (2001) Traveling electrical waves in cortex: insights from phase dynamics and speculation on a computational role. *Neuron* 29:33–44.
- Fleiderer IA, Binshtok AM, Gutnick MJ (1998) Functionally distinct NMDA receptors mediate horizontal connectivity within layer 4 of mouse barrel cortex. *Neuron* 21:1055–1065.
- Franowicz MN, Barth DS (1995) Comparison of evoked potentials and high-frequency (gamma-band) oscillating potentials in rat auditory cortex. *J Neurophysiol* 74:96–112.
- Friedrich R, Fuchs A, Haken H (1991) Spatio-

ture of the wave front, because, within a given set of anatomical connections, different wave patterns occur (Fig. 2) (movies 1–4, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material). Interestingly, all of the patterns were associated with the oscillation in the same manner: one-cycle–one-wave for nonrotating waves and one-cycle–one-rotation for spirals. This is consistent with previous characterizations of one-dimensional waves in coronal slices (Wu et al., 1999; Bao and Wu, 2003).

Although oscillations are commonly observed in sensory (Gray and Singer, 1989; Franowicz and Barth, 1995) and associational (Pesaran et al., 2002) cortices, little is known about the spatial organization that accompanies such oscillatory activity. It has been shown in visual cortex that sensory-evoked oscillations can demonstrate intercolumnar coherency (Eckhorn et al., 1988; Gray et al., 1989). We speculate that rotation waves of spirals may provide a spatial framework to organize cortical oscillations. Dynamic stability of spirals might extend the duration of evoked activity and interact with incoming input streams, and, in pathological conditions, might contribute to seizure generation. Spiral waves might serve as emergent population pacemakers to generate periodic activity in a nonoscillatory network without individual cellular pacemakers. Spirals might be used for coordinating oscillation phases over a population of neurons, serving functions such as binding sensory information or dynamical temporal storage in working memory.

## References

- Bao W, Wu JY (2003) Propagating wave and irregular dynamics: spatiotemporal patterns of cholinergic theta oscillations in neocortex in vitro. *J Neurophysiol* 90:333–341.
- Beurle RL (1956) Properties of a mass of cells capable of regenerating pulses. *Philos Trans R Soc Lond B Biol Sci* 240:55–94.

- lycoupled neuronal networks. I. Traveling fronts and pulses. *SIAM J Appl Math* 62:206–225.
- Prechtl JC, Cohen LB, Pesaran B, Mitra PP, Kleinfeld D (1997) Visual stimuli induce waves of electrical activity in turtle cortex. *Proc Natl Acad Sci USA* 94:7621–7626.
- Ross WN, Salzberg BM, Cohen LB, Grinvald A, Davila HV, Waggoner AS, Wang CH (1977) Changes in absorption, fluorescence, dichroism, and birefringence in stained giant axons: optical measurement of membrane potential. *J Membr Biol* 33:141–183.
- Tsau Y, Guan L, Wu JY (1998) Initiation of spontaneous epileptiform activity in the neocortical slice. *J Neurophysiol* 80:978–982.
- Verkhatsky A, Orkand RK, Kettenmann H (1998) Glial calcium: homeostasis and signaling function. *Physiol Rev* 78:99–141.
- Wadman WJ, Gutnick MJ (1993) Non-uniform propagation of epileptiform discharge in brain slices of rat neocortex. *Neuroscience* 52:255–262.
- Wilson HR, Cowan JD (1972) Excitatory and inhibitory interactions in localized populations of model neurons. *Biophys J* 12:1–24.
- Wilson HR, Cowan JD (1973) A mathematical theory of the functional dynamics of cortical and thalamic nervous tissue. *Kybernetik* 13:55–80.
- Winfree AT (2001) *The geometry of biological time*. New York: Springer.
- Wu JY, Guan L, Tsau Y (1999) Propagating activation during oscillations and evoked responses in neocortical slices. *J Neurosci* 19:5005–5015.
- Wu JY, Guan L, Bai L, Yang Q (2001) Spatiotemporal properties of an evoked population activity in rat sensory cortical slices. *J Neurophysiol* 86:2461–2474.