Low-intensity electric fields induce two distinct response components in neocortical neuronal populations

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Xu W, Wolff BS, Wu JY. Low-intensity electric fields induce two distinct response components in neocortical neuronal populations. J Neurophysiol 112: 2446–2456, 2014. First published August 13, 2014; doi:10.1152/jn.00740.2013.—Low-intensity alternating electric fields applied to the scalp are capable of modulating cortical activity and brain functions, but the underlying mechanisms remain largely unknown. Here, we report two distinct components of voltage-sensitive dye signals induced by low-intensity, alternating electric fields in rodent cortical slices: a “passive component,” which corresponds to membrane potential changes directly induced by the electric field; and an “active component,” which is a widespread depolarization that is dependent on excitatory synaptic transmission. The passive component is stationary, with amplitude and phase accurately reflecting the cortical cytoarchitecture. In contrast, the active component is initiated from a local “hot spot” of activity and spreads to a large population as a propagating wave with rich local dynamics. The propagation of the active component may play a role in modulating large-scale cortical activity by spreading a low level of excitation from a small initiation point to a vast neuronal population.

Transcranial alternating current stimulation (tACS) can have significant modulatory effects on cortical activities and brain functions (Kirov et al. 2009; Marshall et al. 2006; Polania et al. 2012; Reato et al. 2013). Unlike suprathreshold stimulation therapies such as repetitive transcranial magnetic stimulation (rTMS), tACS involves stimulation intensities that are far below the threshold to induce action potentials directly in cortical neurons. An in vitro study showed that action potentials in cortical neurons are only evoked by electric fields greater than ~28 V/m (Radman et al. 2009). However, under a slow alternating field, effective modulation of cortical activity can occur at much lower field intensities (Fröhlich and McCormick 2010), ~1/10th of that necessary to induce action potentials directly. This raises a question: how can such small membrane potential changes induced by an alternating electric field integrate within neuronal populations to bring about large-scale changes in brain activity?

One possible answer is that cortical local circuits serve as an amplifier to convert small membrane potential fluctuations into large-scale population (network) activity. Cortical local circuits have a highly divergent and convergent connectivity pattern; each principal neuron receives thousands of excitatory inputs and in turn sends excitatory outputs to thousands of postsynaptic neurons (Douglas and Martin 2004). Under a slow alternating field, the probability of spontaneous firing in each neuron fluctuates with the field (Radman et al. 2007; Reato et al. 2010). Since the alternating field simultaneously affects a large population of neurons, small fluctuations in firing probability may lead to a large change in the total number of spikes in the population. This increase in spiking can in turn lead to more excitatory postsynaptic potentials (EPSPs) throughout the divergent network and may ultimately have a much larger effect on membrane potential than the field itself.

This possibility may be verified experimentally by examining how the membrane potentials of neuronal populations respond to application of a slow, alternating field. The response can be separated into two components by a pharmacological blockade of excitatory synaptic transmission. Any changes in membrane potential sensitive to the blockade are likely caused by excitatory synaptic activity and will be referred to as the “active component.” Changes resistant to this blockade are likely caused by direct, field-induced neuronal polarization and will be referred to as the “passive component.”

The active and passive components should also be separated by their spatial patterns in the cortical tissue. Under low-intensity electric fields, neurons passively polarize in a compartment-specific fashion with depolarization in compartments closer to the cathode and hyperpolarization in compartments closer to the anode (Radman et al. 2009). Principal neurons have a uniformly vertical arrangement in the cortex, so the degree and direction of compartmental polarization will to a large degree depend on depth within the cortex. In contrast, the active component consists of EPSPs, which should be depolarizing at all locations in the cortex.

In this report, we use voltage-sensitive dye (VSD) imaging to examine the spatial and temporal patterns of population membrane potential fluctuations in mouse cortical slices under applied, low-frequency (1- to 4-Hz) sinusoidal electric fields. Optical recording with VSD is not affected by the volume conductance artifact of the applied field, allowing for high-sensitivity measurements of subthreshold changes in membrane potential.

We indeed found that the two components can be clearly distinguished in neocortical tissue. The properties of the passive component are highly dependent on the underlying cortical cytoarchitecture as seen with a phase-reversal zone and a number of low-amplitude zones arranged parallel to the cortical laminae. VSD signals show that the amplitude of the active component can be ~10 times larger than the passive component, suggesting that the direct effect of the applied field on membrane potential can be dwarfed by the resulting synaptic
activity. We also found that the active component is organized as propagating waves, which may play a role in spreading the field-induced activity from a local hot spot to vast regions of cortex.

MATERIALS AND METHODS

C57BL/6 mice (n = 52) of both sexes from postnatal day 17 (P17) to P28 were used in the experiments. The animal protocol (preparation of acute cortical slices) was approved by the Institutional Animal Care and Use Committee of Georgetown University following the guidelines of the National Institutes of Health.

Slice preparation. After being deeply anesthetized by isoflurane or an intraperitoneal injection of ketamine (100–200 mg/kg), the animals were decapitated. The whole brain was then quickly removed and chilled in cold (0°C) sucrose-based artificial cerebrospinal fluid (ACSF) containing (in mM): 252 sucrose, 3 KCl, 2 CaCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 10 dextrose, bubbled by 95% O2-5% CO2. Neocortical slices (400 μm thick) were cut in coronal sections with a vibratome (Leica VT1000S) between bregma 1 and −3 mm. After sectioning, the slices were transferred into an incubation chamber with ACSF containing (in mM): 132 NaCl, 3 KCl, 2 CaCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 10 dextrose, saturated with 95% O2-5% CO2 at 26°C. The slices were incubated for about 90–120 min before staining with VSD.

VSD staining, signals, and optical imaging. An oxonol dye, NK3630 (Nippon Kankoh-Shikiso Kenkyusho), was used as an indicator of transmembrane potential. The slice was stained with 5–10 μg/ml dye dissolved in ACSF for 120 min (26°C). During staining, the ACSF was circulated and bubbled with 95% O2-5% CO2. After staining, the slices were transferred back to the incubation chamber for at least 1 h before each experiment.

NK3630 is in the dye family that binds to the external surface of the membrane of all cells without interrupting their normal function (for review, see Chemla and Chavane 2009). The absorption spectrum of the dye shifts linearly with the changes in the membrane potential (Ross et al. 1977). The VSD signal in this report is the change in absorption of light with a 705-nm wavelength. In all experiments, the molecules do not generate fluorescence, so no noticeable phototoxicity is detected (Jin et al. 2002).

In most of our experiments, we adjusted the slice and microscope plane to make the somatosensory cortex in the center of the imaging field. The VSD signals were recorded by a 464-channel photodiode array (Olympus BX51WI) with a transillumination arrangement. We imaged at 2 spatial resolutions: with a ×5 objective (0.1 numerical aperture (NA); Zeiss) or macroscope (0.40 NA, modified from a Navitar 25 mm F 0.95 video lens), the imaging field was ~4 mm in diameter, and each recording channel (pixel) collected VSD signals from an area of cortical tissue of 150 μm in diameter, with a ×20 objective (0.95 NA; Olympus), the imaging field was ~980 μm in diameter, and each recording channel collected signals from a tissue area of 38 μm in diameter. With a transillumination arrangement, neurons through the whole thickness of the slice (400 μm) contribute relatively equally to the VSD signal. A tungsten filament lamp was used for illumination, and a 705-/10-nm interference filter (Chroma) was placed in the illumination path during optical recording. During imaging experiments, the slice was continuously perfused in a submersion chamber with ACSF (same as the incubation solution) at 28°C and at a rate of >20 ml/min. Intermittent imaging trials were performed with at least 5-min intervals between each trial. The total light exposure for each slice was <600 s, far below what is necessary for detectable dye bleaching or phototoxicity (Jin et al. 2002).

Generation and application of electric fields. A pair of parallel Ag-AgCl coils or powder Ag-AgCl half-cells were used to generate an electric field in the solution. Both kinds of electrodes provide large surface areas, reducing electrolytic effects such as bubbling. The electrodes were placed in a long (65-mm) and narrow (10-mm) chamber so that the field was evenly distributed around the slice at the center of the chamber as indicated by measurements from paired probing electrodes. Unless otherwise indicated, the electrical field was perpendicular to the cortical laminae in the imaging field (Fig. 1A). In most experiments, we used oscillating electric fields of 1 Hz (alternating current) for 3 cycles to induce the active component and fields

Fig. 1. Activation of cortical slices under a 1-Hz alternating current field. A: experiment arrangement. A mouse coronal slice containing the barrel cortex is imaged by a 464-channel diode array with a ×20 objective (the imaging field is marked with a hexagon). A sinusoidal alternating field is applied by a pair of parallel Ag-AgCl electrode coils (E). B, top: the voltage applied to the electrodes. Middle: the waveform measured from the center of the chamber (the measuring electrode probes are 1.5 mm apart). Bottom: the current passing through the chamber, measured by a 10-Ω resistor connected to the chamber in series. C, the calculated electric field from the probes vs. the voltage applied to the electrodes. D: the active component simultaneously recorded by a local field potential (LFP) electrode and voltage-sensitive dye (VSD) imaging (Optical). Note that the LFP signals contain a large artifact from the applied field due to imperfect cancellation. The LFP recording electrode was placed outside of the imaging area (marked in A) with a reference electrode (not shown) adjusted to reduce the artifact.

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of 4 Hz for 40 cycles to examine the properties of the passive component. The sinusoidal waveforms were generated by a programmable signal generator (Wavetek 10-MHz DDS function generator model 29). The field strength at the center of the chamber was measured using two parallel electrode probes 1.5 mm apart and calibrated to the voltage output of the signal generator (Fig. 1B). The current flowing through the chamber was monitored with a 10-Ω resistor in series. The measured field strength was linearly related to the voltage output from the generator (Fig. 1C), and thus the intensity of field is linearly correlated to the sine wave from the output voltage.

In some of the experiments (19 animals), we added 6-cyano-7-nitroquinolinic acid-2,3-dione [CNQX; non-N-methyl-D-aspartate (non-NMDA) glutamate receptor antagonist, 20 μM], 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzof[1]quinoline-2,3-dione (NBQX; non-NMDA glutamate receptor antagonist, 20 μM), or (2R)-amino-5-phosphonopentanoate (AP5; NMDA receptor antagonist, 20 μM). These drugs are used for two purposes: first, to verify that the active component is dependent on excitatory synaptic transmission; and second, to block the active component so that the passive component can be imaged in isolation.

**Local field potential recordings.** Local field potential recordings were performed with a pair of glass micropipettes (~200-kΩ impedance). The recording electrode was inserted to cortical layers II–III, ~1 mm from the edge of the imaging field. The reference electrode was adjusted in the bath to reduce the artifact from the applied field. Local field potential signals were used for verifying the activity of the active component seen in the VSD signals (Fig. 1D).

**Data analysis.** The optical data were analyzed using the program NeuroPlex (RedShirtImaging) and programs written in MATLAB (MathWorks). Raw optical signals were digitally filtered between 0.4 and 200 Hz. The 0.4-Hz high-pass filter was chosen to eliminate baseline drift from the optical recording, which causes a background color shift in the pseudocolor images. Filtered signals were compared with unfiltered signals to verify that the 0.4-Hz high-pass does not affect amplitude measurements of the active component. When working with the amplitude of the passive component, we used a 4-Hz alternating field to avoid reduction by the 0.4-Hz filter. The singular value decomposition (SVD) method (Prechtl et al. 1997) was also used to remove random noise from the signal.

A Student’s t-test was used to evaluate the statistical significance of the pharmacological effects. A Wilcoxon rank-sum test was used to evaluate differences in median propagation velocity for different cortical layers.

**RESULTS**

The VSD signals of the active component closely match local field potential electrode recordings (Fig. 1D). Comparing the optical and electrical signals demonstrates that the applied electrical field does not generate any artifact in the VSD signals (Fig. 1D, Optical). In contrast, the artifact could be seen in the local field potential signals if adjustments to the reference electrode did not completely cancel out the field artifact (Fig. 1D, LFP).

VSD signals are clearly seen in single trials in each optical detector at low field intensities (Fig. 2A, right traces). The VSD signals reflect the membrane potential of neuronal populations in the brain tissue with each optical detector receiving integrated signals from a large number of cortical neurons. The amplitude of the population VSD signal is proportional to the area of stained membranes; thus, if the soma and dendrites are depolarized to the same degree, dendrites should contribute much more to the population signal than the soma. Action potentials directly contribute very little to population VSD signals; most...
of the signal comes instead from postsynaptic potentials (for review, see Chemla and Chavane 2009).

Following the convention of Ghai et al. (2000), we refer to a “positive” field as when the electric current flows from the pial surface into the deeper cortex and a “negative” field as one in which current flows outward the pial surface (positive and negative are labeled “P” and “N”, respectively, in Fig. 2A, right). Using these terms, an “anodal field” in the literature (Nitsche et al. 2008; Paulus 2011) will be the same direction as a positive field here.

The active and passive components in VSD signals. Weak alternating electric fields induced a cortical population response with two distinct components: the active and passive (Fig. 2A, right traces, and Fig. 2B). In VSD signals, the active and passive components can be distinguished in several respects. The active component is a depolarizing VSD signal that emerges during the positive phase of the alternating field. The waveform of the active component does not closely resemble the waveform of the applied field (Fig. 2B, left), and at a low field intensity the active component may appear only on the first few cycles (Fig. 2A, traces). The passive component, in contrast, occurs in both directions of the alternating field and closely follows the sinusoidal waveform (Fig. 2B, right).

The amplitudes of the passive and active components show a striking difference; the passive component is linearly related to the field intensity with no apparent threshold (Fig. 2D). In contrast, the amplitude of the active component has a nonlinear relationship to the field intensity and quickly saturates at field strengths above 15–20 V/m (Fig. 2C). When using low field intensities, the amplitude of the active component can be 5–10 times higher than that of the passive component at the same location (Fig. 2A, right, and Fig. 2B).

Spatially, the active component is a depolarizing signal through all cortical laminae (Fig. 2B, left). In contrast, the passive component has opposite phases in the superficial and deep layers (Fig. 2B, right). With the spatial phase reversal, a positive field will induce a passive component that hyperpolarizes the infragranular regions but depolarizes the subgranular regions. In a band between these superficial and deep polarizations, the passive component is undetectable (Fig. 2B, center trace). With these spatiotemporal characteristics, under a positive field, both response components are depolarizing deep within the cortex. In more superficial regions of cortex, however, the passive component has a hyperpolarizing influence during active component depolarization. This summation of passive hyperpolarization and active depolarization may explain why, in more superficial regions of cortex, the amplitude of the active component appears to reduce slightly at higher field intensities (Fig. 2C).

Manipulations with synaptic transmitter blockers further distinguish the active and passive components. The active component is extremely sensitive to the disruption of local excitatory circuits. It can be completely blocked by 20 μM CNQX or NBQX, antagonists of non-NMDA ionotropic glutamate receptors (representative trials shown in Fig. 3A and aggregated data in Fig. 3B). The active component can also be blocked by the NMDA receptor antagonist AP5 (Fig. 3, C and D). In contrast, the passive component is resistant to excitatory synaptic blockers (CNQX and AP5, both 20 μM) and the action potential blocker TTX (5–10 μM). These results suggest...
that the passive and active components are generated by different mechanisms. Excitatory synaptic interactions are necessary to generate the active component but are not involved in generating the passive component.

Threshold and time lag for inducing the active component.
The active component emerges when the positive field reaches a threshold intensity. When the field intensity is close to this threshold, the active component varies considerably with some cycles failing to induce the active component (Fig. 4A). We use the occurrence rate of the active component to define the threshold. For each field intensity we test, we run three trials, each containing three cycles of a 1-Hz sine wave. This gives nine chances for inducing the active component (Fig. 4A). If the active component occurs during at least two of the nine cycles, then the field intensity is considered above the threshold. Whereas the threshold varies considerably from slice to slice, the occurrence rate generally increases quickly >4 V/m and then reaches 100% at 20 V/m (Fig. 4B). In an attempt to estimate the threshold more accurately, we found it affected by many factors, including perfusion, temperature, slice incubation time, and the interval between imaging trials. After optimizing these conditions, we measured an average threshold of 9.6 ± 2.6 V/m (mean ± SE) across 31 slices from 25 animals with the lowest recorded threshold being 4 V/m. Without controlling for optimal conditions, the threshold across 61 slices increased to 12.9 ± 4.8 V/m.

The emergence of the active component often lags behind the waveform of the field, most obviously seen when the field intensity is just slightly above the threshold (Fig. 4A). Plotting the 50% peak time of the active component against the waveform of the applied field (Fig. 4C) reveals large phase delays. These phase delays can nearly reach \( \pi \) under low field intensities (Fig. 4D), although in all 1,090 cases of active component examined, none emerged during the negative field (phase delay > \( \pi \); Fig. 4D). The phase delay of the active component suggests that a buildup of recurrent excitation may be needed for the active component to emerge.

The passive component is a structure-specific signal. Whereas the passive component has a stationary pattern in space, its amplitude is dependent on the neuronal architecture in the cortex (Figs. 5 and 6). To study the passive component in greater detail, we blocked excitatory synapses with CNQX or NBQX. This prevents the active component from emerging in field strengths as high as \( \sim 50 \) V/m. Higher field strengths improve the signal-to-noise ratio of the passive component (Fig. 5A, traces) and allow us to investigate better its spatial patterns.

In all cortical slices examined (between bregma 0 and \( -2.5 \) mm), there is a nominal amplitude and phase reversal (NA-PR) zone between superficial and deep cortical layers (Fig. 5B, top image, blue band). The NA-PR zone is parallel to cortical lamina and located below layers II-III. In barrel cortex, the NA-PR zone is about 491 ± 27 \( \mu \)m (mean ± SE, \( n = 21 \) slices from 13 animals) deep from the pial surface (Fig. 5, B and C). Phase analysis demonstrates that the phase reversal line coincides with the line of the lowest amplitude in the NA-PR zone (Fig. 5C). In a planar field, the NA-PR line always follows the curvature of the cortex (Figs. 5 and 6), which is not dependent on the direction of the field vector (Fig. 5, B and D). When the field vector is rotated from +30 to \(-30°\) oblique to the depth axis, the direction of the NA-PR does not rotate (Fig. 5D), demonstrating that the NA-PR line is related to the cytoarchitecture of the cortex but not the field vector. At larger oblique angles (e.g., 60–90°), the amplitude of the passive component was too small to measure accurately.

Using mouse whisker barrel fields, we examined how detailed the passive component amplitude can represent the cortical cytoarchitecture (Fig. 6). We used a coronal slice

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**Fig. 4.** Threshold and phase delay of the active component. A: emergence of the active component at fields near the threshold. The threshold for this slice is 5 V/m. Note that at near-threshold intensity, some cycles of the stimulus fail to induce the active component. B: the occurrence rate of active component under different field strengths (5 animals labeled by colors and shapes). The occurrence rate is defined as the fraction of cycles during which the active component occurred. C: a representative trace showing the applied field and a delayed emergence of the active component. The vertical dashed lines represent the time at which the VSD signal reaches half of its maximum with the phase of the sinusoidal field (in radians) listed below the trace. D: aggregated phase delay data from 1,090 occurrences of the active component. The phase delay is defined as the phase of the field when the active component reaches half of its maximum value as shown in C.

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sectioned at bregma −1.5 mm (400 μm thick) where the whisker barrel subfield S1BF was included between two other areas of the somatosensory cortex, S1Tr (trunk region) and S2 (secondary). Under a ×20 water-immersion lens, each of our optical detectors received VSD signals from an area 38 μm in diameter. With this higher resolution, the whisker barrels were clearly visible in the passive component amplitude map (Fig. 6, inset). Using the whisker barrels as a reference for cortical layer IV, we know that the NA-PR zone is located between cortical layers II–III and IV. The barrel structures were contrasted out by intermittent low- and high-amplitude VSD signals. There are two additional low-amplitude zones (Fig. 6, L1 and L2) observed between the white matter and the NA-PR zone. Unlike the NA-PR zone, phase reversal was not seen around these two low-amplitude zones. These two zones were also visible in other sensory regions of cortex, such as visual and auditory, but were not seen outside of sensory areas, suggesting they are related to the cytoarchitecture within the granular layer of sensory cortex.

**Spatiotemporal patterns of the active component.** The active component starts from a small region (a local hot spot) and expands to a larger area as in the example shown in Fig. 7. This point-start pattern was seen in 33 slices from 16 animals when the activity pattern over a larger imaging area is visualized with lower magnification objectives (×5 or ×10). Globally, the active component manifests as propagating waves of variable velocity (Supplemental Video S1, available in the data supplement online at the *Journal of Neurophysiology* Web site). Rich spatiotemporal dynamics were seen in the spreading of the active component, including the emergence of multiple hot spots, variations in the velocity and direction of propagation, and interactions between local waves (Fig. 7). This suggests that the spreading of the active component involves polysynaptic excitations instead of direct, long-range axonal conductance.

Figure 7, A and B, shows three episodes of the active component induced during the positive phase of a 1-Hz oscillating field. In VSD signal traces, the waveform of the active component varies largely across different episodes at the same location and at different locations during the same episode (Fig. 7A, traces). Pseudocolor images selected from one episode (Fig. 7B, a–n) further show that the active component first starts from a small region (hot spot) and quickly spreads to a larger population (arrow in frame b). A second hot spot appears later in the medial region and also expands (arrow in frame c). These two active regions expand, fuse, and then propagate in the medial and lateral directions across several cortical areas. Nonuniform spreading is also seen, for example when the active component moves outward horizontally from the deep layers and then vertically to the superficial layers (Fig. 7B, frames e–f and f–g; Supplemental Video S1). In some instances, the active component oscillates a few times at the same location without propagation (frames h–n).
near the initiation site (depth 1), one above (depth 2), and one below (depth 3). We found no significant difference between cortical areas; as shown in the amplitude maps (Fig. 7). This zone is seen in all cortical areas we have examined, suggesting it is related to the basic laminar cytoarchitecture of the cortex. This result is consistent with the phase reversal found in three-layer hippocampal CA3 tissue (Akiyama et al. 2011; Bikson et al. 2004).

Three aspects of the cellular organization may account for the low-amplitude zones: 1) long cells arranged in the columnar structure form dipoles with proximal and distal dendrites in opposite polarities and a middle section with near zero polarization; 2) an abundance of spherically symmetrical cells are not extended in space and are less affected by the field; and 3) a balanced mix of depolarized and hyperpolarized cell components results in a zero net sum in the population VSD signal. It may be that all three aspects contribute to the amplitude contrast of the passive component map.

The main source of the passive component is likely to be associated with the layer V pyramidal neurons. A subthreshold external field can polarize neurons in a compartment-specific fashion as confirmed in both intracellular recordings (Chan et al. 1988; Delgado-Lezama et al. 1999; Jefferys 1981; Park et al. 2005; Radman et al. 2009) and VSD imaging (Akiyama et al. 2011; Bikson et al. 2004) in the three-layered hippocampus. The pyramidal neurons in layer V are large with their apical dendrites extending all the way into layer I (Peters 1993). As a result, these neurons have a larger vector length under the field and are likely to have larger amplitude of polarization (Radman...
et al. 2009). Thus layer V pyramidal neurons form dipoles; they contribute large and antiphase population signals in layers I–II and in layer V while contributing nominal signal near the border between layer III and layer IV. In addition, the signals from basal dendrites of layer II/III cells and apical dendrites of short layer VI pyramidal neurons should have opposite polarities between layers III and IV, canceling each other in the VSD signals. This may also contribute to the low signal in the short layer VI pyramidal neurons should have opposite polarities from basal dendrites of layer II/III cells and apical dendrites of layer V cells. The low-amplitude signals associated with barrel structures (Fig. 6) are another example of the association between the passive component and the cortical cytoarchitecture. Although these amplitude contrasts may be associated with the barrel hollows or granular cells, we cannot rule out the possibility that these amplitude contrasts may be associated with the barrel field division (16 or 24 V/m, respectively). Amplitude is defined as in Fig. 2C. A white broken line marks the putative boundary between S1BF and S1Tr. Note that the active component only propagates beyond this boundary under the support of a stronger (16 or 24 V/m) positive field.

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field vector. When the electric field was rotated further, resulting in an orientation parallel to the cortical laminae, we were unable to detect any passive component in fields as large as 50 V/m. At first glance, this may appear incompatible with a recent report of field-direction-specific polarization of somatodendritic branches (Rahman et al. 2013). However, our VSD signals show a population summation of membrane polarizations in the area under each detector; due to dendritic branches extending horizontally in both directions, contributions to the VSD signal from branches specifically polarized by a field with a horizontal orientation would likely be canceled out by contributions from dendrites of opposite polarization.

From the passive to the active component. When the applied field is in its positive phase, the passive component in the deep layers of cortex is depolarizing; the active component was never induced outside of this phase of the field (Fig. 4D). Under low-intensity fields, the depolarization of the active component is ~10 times larger than that of the passive component (as seen in Fig. 2B in the VSD signal amplitude), suggesting the active component can have a larger effect on cortical neuronal populations than the passive component. The active component is extremely sensitive to the AMPA/kainate receptor antagonist CNQX (Fig. 3), suggesting that local glutamatergic excitatory synapses play a key role in integrating the small effects of a field on individual neurons into a large population event. The threshold for inducing the active component (Fig. 4B) is far below the reported threshold for directly evoking action potentials in pharmacologically isolated cells (Radman et al. 2009). This suggests network mechanisms are involved such as an elevation of spontaneous firing rate or a modulation of spike timing distributed across many cells (Anastassiou et al. 2010; Radman et al. 2007; Reato et al. 2010).

Alternating fields elevate the firing probability only when the field is in its positive phase, resulting in coherent increases of activity across the network (Deans et al. 2007; Francis et al. 2003; Park et al. 2005). The coherent increase of firing in excitatory cells may be critical for the generation of the active component, as it can lead to temporal summation across the highly divergent and convergent cortical network. The spontaneous firing in a large population of neurons may also engage stochastic resonance (Bezrukov and Vodyanoy 1995; Collins et al. 1995), increasing the probability of hot spots that can generate the active component. To investigate further the emergence of the synchronized depolarization of the active component from asynchronously spontaneous firing, multiunit spike recording and cell-attached patch recording may be needed. Both methods can be high-pass filtered to avoid the artifact from a sinusoidal electric field. Combined with VSD imaging, spike recordings near the origins of the active component could elucidate the dynamic process of forming hot spots in greater detail.

It is somewhat surprising that AP5 can also block the active component. VSD signals of the active component suggest a low level of depolarization, and NMDA receptors should contribute little to subthreshold membrane potentials. However, the mild population depolarization in a large population of neurons may be sustained by a small fraction of spiking neurons. The activation of NMDA receptors in these spiking neurons might contribute to prolonging their depolarization and increasing the total number of spikes. The delayed emergence of the active component (Fig. 4D) suggests that a buildup of recurrent excitation may generate the active component; NMDA conductance might play a critical role in this buildup of excitation. The large phase lag may also explain why the active component only follows low-frequency fields; in our experiments, the active component in most of the slices can follow 1 Hz or lower but not 2 Hz or higher (data not shown).

The field intensity that can induce the active component in our quiescent cortical slices (threshold ~10 V/m) is larger than the intensity for modulating spontaneous rhythms in neuronal networks (~1 V/m or lower; Ali et al. 2013; Deans et al. 2007; Francis et al. 2003; Fröhlich and McCormick 2010; Reato et al. 2010). This may be because spontaneous rhythmic activity facilitates the excitation that generates the active component. The field intensity used in human subthreshold transcranial stimulation protocols was also much lower than ours (estimated to be 1 V/m or lower; Datta et al. 2009; Marshall et al. 2006). This may in part be due to the higher neuronal density and longer axonal length in the human cortex as well as resonance (reviewed by Fröhlich 2014) between rhythmic human cortical activity and the tACS.

In a continuous alternating field, the active component occurs only during the first few cycles (Fig. 2A), suggesting the involvement of complex factors at both cellular and population levels. On the cellular level, voltage-gated calcium currents ($I_{Ca}$ and $I_{K}$) may facilitate the active component during the early cycles of the field but may also trigger calcium-activated potassium currents ($I_{Ca-K}$; McCormick 2004) that reduce its likelihood during the later cycles. On the population level, the resources for the initiation, spreading, and sustaining of the active component may be inversely related to the involvement of neurons in the previous episode of active component. If more neurons are active in an earlier cycle, then more neurons may be refractory in the later cycles, and fewer neurons will be available for generating a subsequent episode of active component. We noticed that the active component may fail to reoccur after a particularly high-amplitude active component, e.g., the last active component in Fig. 2 traces. This large VSD signal indicates that more neurons are involved or that the neurons involved are more depolarized. In the former case, there may be a decreased number of neurons in the population available for subsequent propagating waves (Gao et al. 2012); in the latter case, cellular mechanisms may suppress further episodes of active component.

Active component organized as propagating waves. The active component starts in a small area and spreads as a propagating wave (Fig. 7). It appears there is a two-step process in which the active component first becomes self-sustained in a local area (as a hot spot) and subsequently spreads under the support of the electric field. This is consistent with a recent computational modeling study of spontaneous slow-wave oscillations under tACS where activity emerges in one or more local hot spots and propagates outward (Ali et al. 2013).

Propagation of the active component may serve as a mechanism for spreading electric field-induced activity beyond areas directly affected by the field such as a sulcus where the field vector is not optimally aligned. The propagating waves of the active component may be similar to self-sustained waves seen in vivo; the magnitude and propagating velocity of the activity at a distance from the initiation site may not depend on the...
magnitude at the initiation site. Instead, local excitability and prior wave dynamics may play major roles in determining local activity (Gao et al. 2012). Such self-sustained, locally controlled propagating waves may cause the large variations in propagating velocity and amplitude seen in the active component (Fig. 7B, frames h–n).

Propagating waves of the active component can be distinguished from other waves such as epileptic waves or waves of spreading depolarization (SD; Reiffurth et al. 2012). Epileptiform activity involves recurrent excitation of a much larger magnitude than the active component with a VSD signal amplitude about 3–5 times larger (Jin et al. 2002). In addition, the intrinsic optical signal (light scattering of the cortical tissue) of the active component is about 1/10 that of an epileptic wave (Jin et al. 2002; Tsau et al. 1998) and 1/100th that of an SD (Aitken et al. 1999). The characteristics of propagation are also different; epileptiform waves in cortical slices are fast (100–600 mm/s; Demir et al. 1998; London et al. 1989) and robust, whereas the active component propagation is slower (~13.1 mm/s; Fig. 7D) and shows more varied patterns of propagation. The propagating velocity of the SD waves are even slower (<0.1 mm/s; Reiffurth et al. 2012) than the active component. The SD also has a long refractory period (several minutes), whereas the active component can have a series of episodes occurring at ~1 Hz (Figs. 1 and 2).

Propagating waves of the active component are similar to waves that are widely seen in the cortex accompanying sensory and motor events (reviewed by Sato et al. 2012; Wu et al. 2008). These sensory- or motor-evoked waves bring low levels of membrane depolarization from the cortical representation site to a vast neuronal population via distributed, nonspecific synaptic interconnections. Similar propagating waves also occur spontaneously in the cortex during sleep states (Huang et al. 2010b; Massimini et al. 2004). In human experiments with tACS, interactions have been suggested between a weak external electric field and cortical sleep waves. The effects of an external oscillating field were largest during slow-wave sleep, where a 0.75-Hz field was more effective than a 5-Hz field in modulating sleep oscillations and facilitating declarative memory consolidation (Marshall et al. 2006). In such an experiment, the propagating waves of the active component may be interacting with ongoing cortical waves, leading to modulation of brain activity and ultimately behavior.

The active component in this report is produced by a low-frequency, alternating electric field. A persistent direct current field, like those used in standard transcranial direct current stimulation (tDCS) protocols, may have different effects on cortical activity (Nitsche et al. 2008; Zaghii et al. 2010). For instance, the effects of tDCS can last minutes to hours after exposure to the applied field. This long-term effect is likely due to plastic changes in the network caused by a sustained period of elevated cortical activity (Paulus 2011). Oscillating fields, in contrast, can trigger large but transient increases in cortical activity (the active component). They may also produce long-term changes in network activity (Reato et al. 2013), likely depending on the relationship between endogenous brain activity and the frequency (Marshall et al. 2006) and phase (Polania et al. 2012) of the applied field.

In conclusion, the cortical response to subthreshold alternating electric fields not only consists of passive neuronal polarization, but also can have a network-mediated active component that is many times larger. The active component is organized as propagating waves that spread the activity from a local hot spot to a larger population and may serve as a mechanism for modulating widespread cortical function with a small, subthreshold field.

REFERENCES


DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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