

# Electric Field Suppression Of Epileptiform Activity In Hippocampal Slices

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## SUMMARY AND CONCLUSIONS

1. The effects of relatively small external DC electric fields on synchronous activity in CA1 and CA3 from transverse and longitudinal type hippocampal slices were studied.

2. To record neuronal activity during significant field changes, differential DC amplification was employed with a reference electrode aligned along an isopotential with the recording electrode.

3. Suppression of epileptiform activity was observed in 31 of 33 slices independent of region studied and type of slice but was highly dependent on field orientation with respect to the apical dendritic-somatic axis.

4. Modulation of neuronal activity in these experiments was readily observed at field strengths  $\leq 5$ –10 mV/mm. Suppression was seen with the field oriented (positive to negative potential) from the soma to the apical dendrites.

5. In vivo application of these results may be feasible.

decapitated. Four-hundred-micrometer-thick slices were cut transversely or longitudinally with a tissue chopper and placed in an interface type perfusion chamber at 34–36°C. Slices were perfused with artificial cerebrospinal fluid (ACSF) composed of (in mM) 155 Na<sup>+</sup>, 136 Cl<sup>-</sup>, 3.5 K<sup>+</sup>, 1.2 Ca<sup>2+</sup>, 1.2 Mg<sup>2+</sup>, 1.25 PO<sub>4</sub><sup>3-</sup>, 24 HCO<sub>3</sub><sup>-</sup>, 1.2 SO<sub>4</sub><sup>2-</sup>, and 10 dextrose, flowing at 2 ml/min. After 90 min, the ACSF was switched to one containing 8.5 mM KCl, the other ionic constituents remaining the same.

In the presence of elevated potassium, these neuronal networks demonstrate two features that share similarities with spontaneous seizure activity: intermittent synchronized burst discharges similar to interictal epileptic spikes arise in the CA3 region (Pedley and Traub 1990; Rutecki et al. 1985), and more prolonged electrographic seizurelike events are observed in the CA1 region (Traynelis and Dingledine 1988).

For the present study, hippocampal tissue slices exhibiting synchronous activity were placed in the center of a field produced with two parallel-plate electrodes (Ag-AgCl) submerged in the perfusate as illustrated in Fig. 1A. An electric field was imposed on the slice by applying a potential difference to the electrode plates through an isolation amplifier as in Fig. 1B. The electric field in the chamber without a slice present was measured and calibrated to the potential difference applied to the plates. Detailed mappings of the electric field within the chamber with and without a slice present are shown in Fig. 1, C and D, and are found to be quite uniform near and within the slice. The neural layers in the slice can be identified visually and thereby oriented in the chamber with respect to the field.

Differential recordings were made with paired saline-filled micropipettes (1–4 M $\Omega$ ) and a differential DC-coupled amplifier (Grass Model P16). After the recording electrode was placed into the pyramidal cell layer of the CA3 or CA1 region of the slice, the reference electrode position was adjusted to minimize the recording artifact from an external sinusoidal test field (10 Hz). This arrangement placed both electrodes near the same isopotential within the chamber and allowed for continuous recording of neuronal activity despite relatively substantial electric field changes.

## INTRODUCTION

It has long been known that electric fields affect neuronal excitability (Katz and Schmitt 1940; Rushton 1927; Terzoulo and Bullock 1956) and that externally applied electric fields could suppress or enhance stimulus evoked neuronal population spikes (Bawin et al. 1986a,b; Jefferys 1981). Further experiments have shown that direct current injection into tissue could suppress evoked (Kayyali and Durand 1991) or spontaneous (Nakagawa and Durand 1991) epileptiform activity in brain slices. Although there is a long history of attempting epileptic seizure control in humans with stimulation of the nervous system at sites remote from seizure foci (Cooper et al. 1976; Murphy et al. 1995; Van Buren et al. 1978), there has been no attempt to suppress seizures using external electric fields.

The electric field magnitude required to modify the activity of a neuron is much smaller than the magnitude required to provoke neuronal firing from rest (Jefferys 1981). The physics of this interaction has been worked out in detail in recent years (Chan and Nicholson 1986; Chan et al. 1988; Tranchina and Nicholson 1986). An electric field parallel to the dendritic-somatic axis hyper- or depolarizes neurons and thereby changes the threshold for action potential initiation. We postulated that an externally applied electric field would suppress spontaneous epileptiform activity.

## METHODS

Hippocampal slices were prepared from 125 to 150 g Sprague-Dawley rats, which were deeply anesthetized in diethyl-ether and

## RESULTS

The effect of electric field changes on spontaneous activity is illustrated in Fig. 2 for slices cut transversely (Fig. 2, A and B) and longitudinally (Fig. 2, C and D). The electric field change is indicated by the trace above the neuronal activity recording in each panel. More detail of the neuronal activity is shown in the insets at an expanded time base (solid arrows  $\uparrow$  in the panels point to the inset origin). The orientation of the slices with respect to the direction

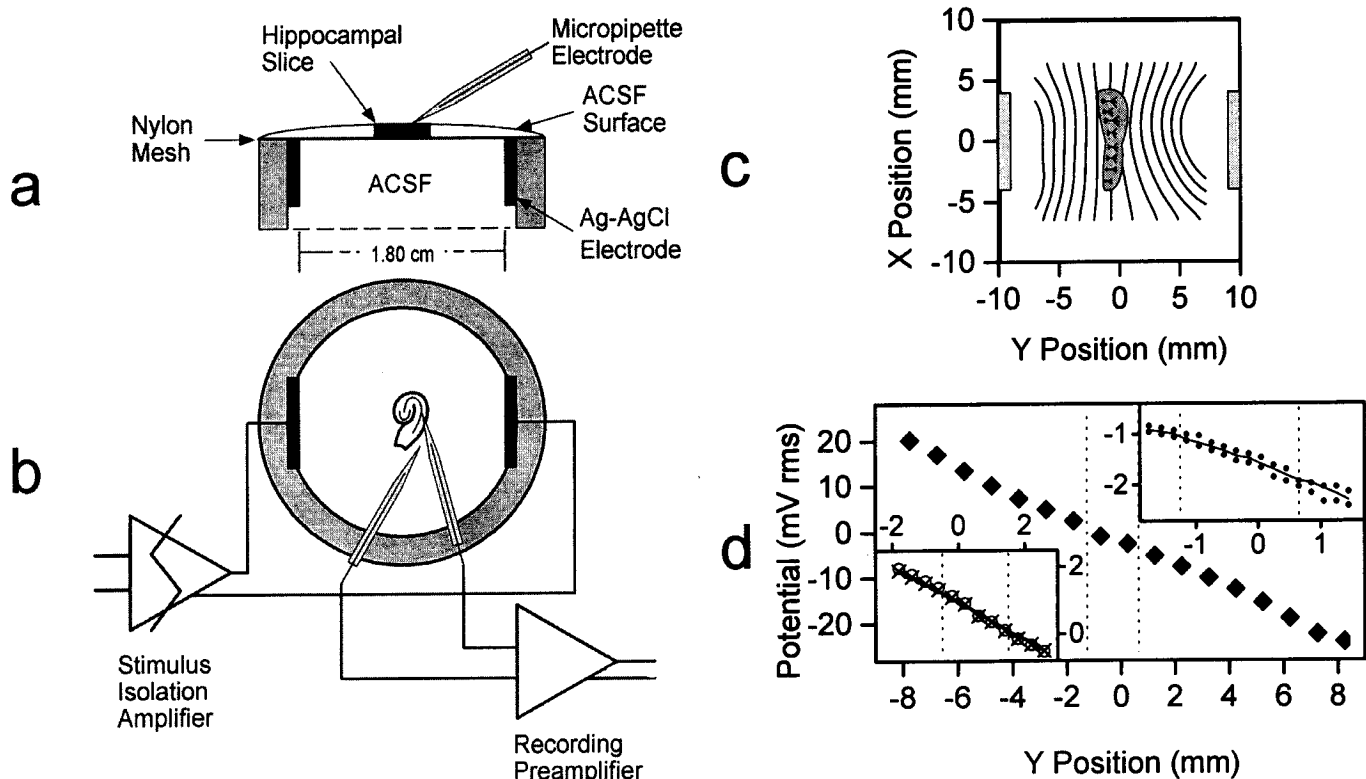


FIG. 1. *A*: schematic of interface type perfusion chamber from side. Parallel Ag-AgCl electrodes are submerged within artificial cerebrospinal fluid (ACSF) with interelectrode spacing of 1.8 cm. Slices rest on a mesh just under the ACSF surface. Slice thickness (350–450  $\mu\text{m}$ ) is not drawn to scale. *B*: perfusion chamber from top. Electric potential is applied through a filtered isolation amplifier (Analog Devices AD210AN) driven by a digital to analog converter (National Instruments ATMIO16X). Filtering (30 kHz) removes high-frequency artifacts from digital to analog conversion, and isolation amplifier allows electrode potentials to “float” independent of ground. *C*: measured isopotentials within chamber. Potential difference applied to plates was 100 mV root mean square (RMS) at 60 Hz, and isopotential lines are spaced 2.5 mV apart. These isopotential lines were measured with a longitudinal slice in the chamber, as indicated by sketch. *D*: electrical potential (RMS) within chamber with and without a slice present. Main graph ( $\blacklozenge$ ) shows potential as a function of position between the electrode plates ( $Y$ ), locally averaged (from 7 measurements) over  $-3 < X < +3$  mm (see *C* for  $X$  and  $Y$  orientation), with a 60 Hz sinusoidal potential applied to plates. Dotted vertical lines indicate boundary of a longitudinal hippocampal slice as in *C*. In *top right inset* is shown potential measured near and within slice at an expanded position scale, showing the potential with a slice present at 2 different positions along  $X$  separated by 0.4 mm ( $\bullet$ ), and the potential without the slice present (solid line —), with a 60-Hz sinusoid applied to the plates. *Bottom left inset* shows a comparison between potentials measured in and near a slice at both 65 Hz ( $\circ$ ) and 5 Hz (crosses X), and at same position without a slice at 35 Hz (solid line —). Field within this chamber is therefore uniform and independent of presence of a slice, throughout range of frequencies tested. Measurements within slices were made at a depth from the slice surface of 50  $\mu\text{m}$ , but similar results were seen at a depth of 100  $\mu\text{m}$  (not shown).

of positive electric field are indicated in the accompanying schematics as well as the orientation of the soma and apical dendrites of the neurons being monitored.

As illustrated in Fig. 2*A*, the CA3 pyramidal cells in a transversely cut slice generate compact bursts of activity whose synchrony shares similarities with interictal spikes (Pedley and Traub 1990). The initial bursts shown here were recorded with an imposed field of  $-10$  mV/mm. This activity was suppressed immediately upon switching the polarity of the field to  $+10$  mV/mm. The CA3 pyramidal cells in this slice were active spontaneously at a slower burst frequency with zero imposed field; a more complete description of the effects of changes in field strength and polarity on this slice will be discussed below (Fig. 3).

As depicted in Fig. 2*B*, the activity observed in the CA1 region of a transverse slice is more complex. Population events in CA3 are propagated through the Schaffer collateral

fibers to the CA1 pyramidal cells, where similar bursts are evoked, interspersed with more prolonged seizurelike events (Traynelis and Dingledine 1988). Note that with the field aligned with the CA1 pyramidal cells (see schematic), seizurelike events are eliminated selectively; however, spike-like events, propagated from CA3 neurons perpendicular to the field, persist.

If the hippocampus is cut longitudinally, the pyramidal cells in CA3 and CA1 are aligned more uniformly than in the more curved configuration in the transverse slice. The synchronous activity in the CA3 (Fig. 2*C*) and CA1 (Fig. 2*D*) regions of longitudinal slices are readily suppressed by electric fields with strengths  $< 13$  mV/mm and aligned parallel to the dendritic-somatic axis of the pyramidal cells.

The relationship between both electric field polarity and magnitude on the CA3 burst frequency can be seen in Fig. 3. With zero external field applied, the burst frequency was

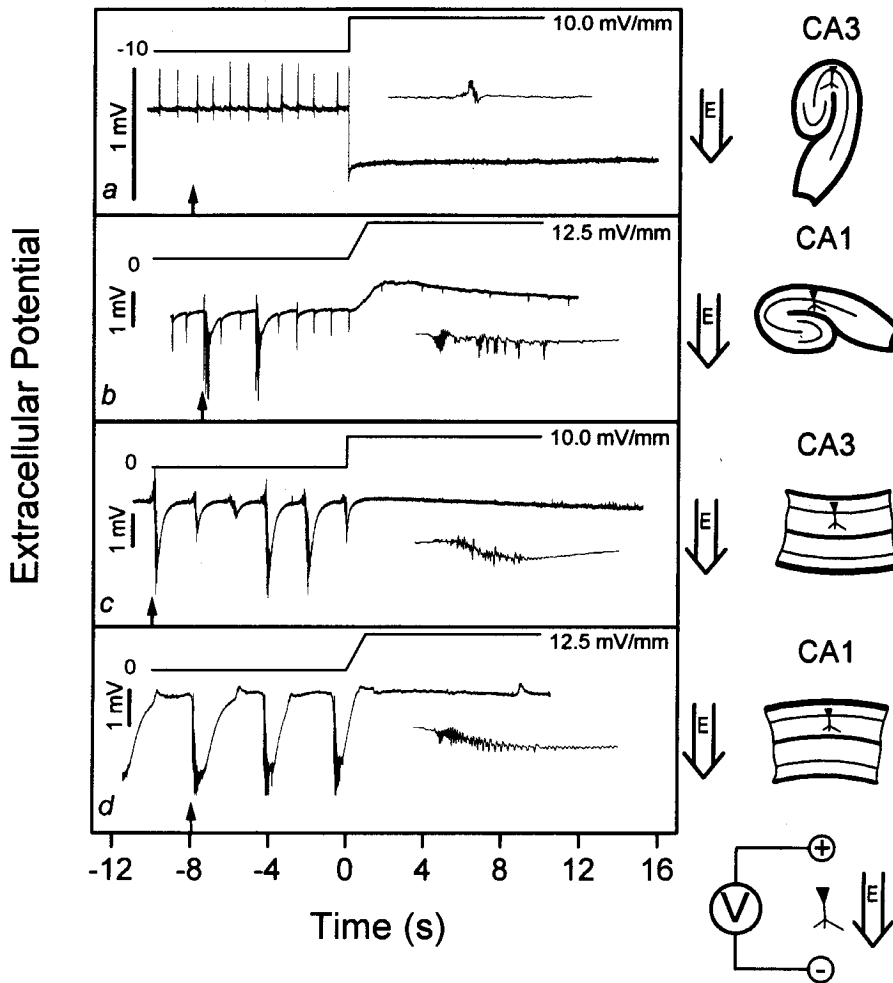


FIG. 2. Examples of suppression of spontaneous neuronal population activity (*bottom traces each panel*) as a function of DC field (*top traces*). Results are shown in transverse slices for (A) CA3 and (B) CA1, and in longitudinal slices for (C) CA3 and (D) CA1, with time base indicated along abscissa and individual 1-mV vertical calibration bars to left of each trace. *Insets* show activity at expanded time base (0.5 s long, arbitrary vertical scale). Origins of *insets* are indicated by the solid arrows ( $\uparrow$ ). Slice anatomy and positive field direction (open arrows) are illustrated, along with orientation of soma and apical dendrites of pyramidal cells monitored. In all cases of suppression, electric field was directed along axis defined from soma to apical dendrite, as shown in schematic at *bottom right*. This orientation and polarity of field would hyperpolarize soma in each example.

approximately one per second. The burst frequency alternately increased and decreased as a direct result of changes in field polarity from negative to positive, respectively. Increased field magnitude resulted in enhanced excitation or

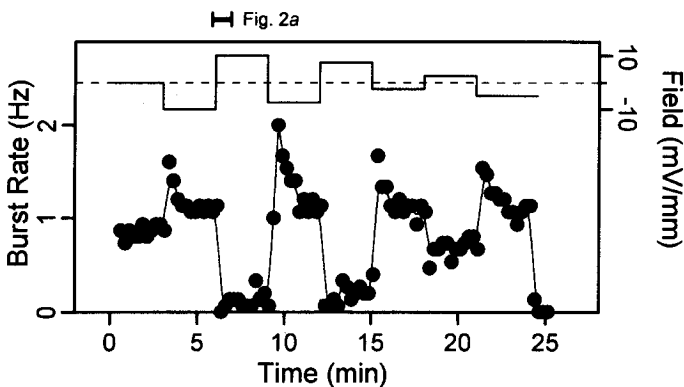


FIG. 3. Burst rate (connected dots ●—●) from transverse CA3 under the influence of external electric field (solid line —) for experiment detailed in Fig. 2A. Overbar indicates time corresponding to Fig. 2A. Burst rate is determined from number of bursts occurring within nonoverlapping 15-s time windows. At baseline, without applied field, pyramidal cells synchronously discharge at  $\sim 1$  Hz. Negative field amplitudes accelerate whereas positive amplitudes suppress burst rate.

suppression. Modulation of neuronal activity in these experiments was readily observed at field strengths comparable or even below the 5–10 mV/mm threshold for modulation reported in previous work (Jefferys 1981).

In 31 of 33 slices, relatively small electric fields suppressed seizurelike activity independent of region studied and type of slice cut. Suppression was observed in CA3 from transverse (9/9) and longitudinal (2/3) slices, and in CA1 from transverse (12/13) and longitudinal (8/8) slices. Neural activity was enhanced from baseline by reversing the polarity for each successful case of suppression, as illustrated in Fig. 3.

#### DISCUSSION

In these experiments, an electric field aligned from the soma to the major apical dendrites of the primary excitatory neurons of a network consistently suppressed spontaneous epileptiform activity; enhancement of such activity was observed with the field polarity reversed. The electric field polarity we observed to be associated with suppression is known (Chan et al. 1986, 1988; Tranchina and Nicholson 1986) to polarize individual neurons along their dendritic-somatic axes in a way that hyperpolarizes the somata and effectively increases their threshold for action potential initi-

ation. It is of interest that although these seizurelike events are emergent properties of a network of neurons, directing our electric fields to suppress a subpopulation of neurons was effective in switching off this activity.

Might external electric fields be clinically applied to controlling human focal epilepsy? The barriers to the application of electric fields to suppress well-defined epileptic foci seem more technical than theoretical. Although the sulcal geometry of the human brain is convoluted, the surface of the gyri contain pyramidal cells in a roughly perpendicular arrangement to the brain surface. Therefore, electrodes external to the tissue could be used to apply appropriately aligned fields. Further work will determine whether suppression of activity in regions of cortex parallel to an applied field will be sufficient to interfere with seizure generation and propagation.

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NOTE ADDED IN PROOF

Using a time-dependent electric field on similar hippocampal slices, we recently demonstrated Stochastic Resonance, a phenomenon in which random noise can optimize the response of a nonlinear system to an otherwise subthreshold signal. This work will appear as Gluckman, B. J., Netoff, T. I., Neel, E. J., Ditto, W. L., Spano, M. L., and Schiff, S. J., Stochastic resonance in a neuronal network from mammalian brain. *Physical Rev. Lett.* 77: 4098–4101, 1996.

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