

Effects of Applied Electric Fields on Low-Calcium Epileptiform Activity in the CA1 Region of Rat Hippocampal Slices

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Effects of applied electric fields on low-calcium epileptiform activity in the CA1 region of rat hippocampal slices. *J Neurophysiol* 84: 274–280, 2000. It is well established that exogenous electric fields can suppress activity obtained in different models of epileptiform discharge such as penicillin and high potassium. In the low-calcium model of epilepsy, spontaneous epileptiform bursting is generated in the absence of synaptic transmission. It has been suggested that ephaptic interactions play a critical role in neuronal synchronization and burst propagation in this nonsynaptic model. We, therefore, tested the hypothesis that low-calcium bursting induced in the CA1 region of transverse and longitudinal hippocampal slices should be highly sensitive to exogenous electric fields. Uniform, low amplitude DC electric fields were applied during spontaneous low-calcium epileptiform activity. Modulation and full suppression of epileptiform activity was observed at field strengths between 1 and 5 mV/mm, a value significantly lower than in other in vitro models of epilepsy. We further investigated the hypothesis that the efficacy of electrical fields was related to changes in the extracellular space. Our results suggest that the osmolality of the perfusate can modulate the efficacy of electric fields. It was also observed that the ability of a field to suppress or modulate low-calcium activity was highly dependent on its orientation, polarity, as well as magnitude. Finally, it was observed that the extracellular potassium “waves” that normally accompany individual epileptiform events was abolished when the individual events were suppressed. These results suggest that DC fields modulate and suppress low-calcium activity by directly polarizing CA1 pyramidal cells.

INTRODUCTION

Direct current injections into hippocampal tissue can modulate evoked (Bawin et al. 1986; Chan and Nicholson 1986; Jefferys 1981; Kayyali and Durand 1991) as well as spontaneous (Gluckman et al. 1996; Nakagawa and Durand 1991; Warren and Durand 1998) epileptiform activity in the hippocampal brain slice preparation. It has been suggested that currents passing from the extracellular space into the cell bodies polarize the cells, thus modulating neuronal activity. Transmembrane voltage recordings have supported this hypothesis by showing that the mechanism of modulation involves a net polarization of the affected somatic membranes (Kayyali and Durand 1991; Nakagawa and Durand 1991).

Perfusion of hippocampal slices with low-Ca²⁺ artificial cerebrospinal fluid (ACSF; ≤ 0.2 mM Ca²⁺) effectively blocks

synaptic transmission (Jones and Heinemann 1987; Konnerth et al. 1986; Taylor and Dudek 1982) and induces paroxysmal events that closely approximate epileptic activity (Haas and Jefferys 1984; Schweitzer et al. 1992; Yaari et al. 1983). The “low-calcium” model of epilepsy retains many characteristics of focal hippocampal seizures in vivo, including a focal origin and local spread, a gradual increase in synchronicity, and a postevent refractoriness (Konnerth et al. 1986). Moreover, in vitro experiments (Benninger et al. 1980; Krnjevic et al. 1982; Somjen and Giacchino 1985) as well as in situ studies (Heinemann et al. 1977; Pumain et al. 1985) have shown that increased neuronal activity, associated with seizures, results in lowered calcium levels in brain tissue.

Each low-Ca²⁺ event is characterized by a negative shift in the extracellular field potential that propagates slowly across the CA1 region and is always accompanied by a transient increase in extracellular potassium. It has been suggested that neurons contribute in a feed-forward manner to the potassium transient (Bikson et al. 1999; Yaari et al. 1986); however, it has not been possible to determine conclusively whether the potassium wave was the cause or a product of the epileptiform activity. In this study, extracellular potassium transients were monitored during the suppression of the spontaneous field bursts with electric fields.

It is known that low-level, endogenous electric fields in the CNS play a significant role in modulating neuronal activity (Faber and Korn 1989; Kayyali and Durand 1991; Snow and Dudek 1984, 1986), particularly under conditions that promote cell swelling, such as during epileptic seizures. Moreover, it has been suggested that ephaptic interactions play a critical role in nonsynaptic epileptogenesis (Haas and Jefferys 1984; Konnerth et al. 1986; Richardson and O’Reilly 1995). We, therefore tested the hypothesis that applied electric fields should be highly effective in controlling and modulating low-calcium epileptiform activity. The efficacy of applied fields as a function of osmolality was similarly evaluated.

METHODS

Electrical recording and data analysis

All experiments were performed in the CA1 region of hippocampal brain slices prepared from adult Sprague-Dawley rats (125–175 g).

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The rats were anesthetized using ethyl ether and rapidly decapitated. The brain was then removed and immediately placed in ice cold "normal" ACSF with the following composition (in mM): 124 NaCl, 3.75 KCl, 1.25 KH_2PO_4 , 2.0 CaCl_2 , 2.0 MgSO_4 , 26 NaHCO_3 , 10.0 dextrose. The hippocampus was dissected out from the brain, and 400- μm -thick transverse and longitudinal slices were prepared on a McIlwain tissue chopper. The slices were then transferred to a holding chamber filled with ACSF at room temperature and bubbled with 95% O_2 -5% CO_2 , where they could be maintained for up to 8 h and used as required.

After >1 h of recovery, a slice was transferred to a standard interface recording chamber with normal ACSF (nACSF) at $35^\circ \pm 0.5^\circ$, and a warmed, humidified 95% O_2 -5% CO_2 vapor maintained over the exposed surface of the slice. After checking for viability (i.e., ≥ 4 mV population spike in CA1, produced by the orthodromic stimulation of the Schaffer collaterals), the slices were perfused with low-calcium ACSF (low- Ca^{2+}) with the following composition (in mM): 124 NaCl, 4075 KCl, 1.25 KH_2PO_4 , 0.2 CaCl_2 , 1.5 MgSO_4 , 26 NaHCO_3 , 10.0 dextrose. Prolonged incubation (60–90 min) in this low-calcium ACSF resulted in spontaneous epileptiform activity in the CA1 region of the hippocampus.

Extracellular recordings of field potentials were obtained using glass micropipettes (2–5 M Ω) filled with 150 mM NaCl. Spontaneous epileptiform activity was monitored with a recording electrode positioned in the somatic layer of the CA1 region (Fig. 1). A second electrode was placed in the bath in an isopotential to allow for differential recording such that neuronal activity could be observed even during significant voltage changes produced by the exogenous fields. The signals were amplified and low-pass filtered (1 kHz) with an Axoprobe-1A amplifier (Axon Instruments), an FLA-01 amplifier (Cygnus Technology, Inc.), and stored on videotape via an A/D converter (Sony PCM-501ES).

The osmolality of the low- Ca^{2+} ACSF was decreased by dilution with 10–20% H_2O , which caused cell swelling and a reduction of the extracellular space (ECS). It was increased by the addition of 10–20

mM sucrose, a membrane-impermeant solute that causes cell shrinkage, and an increase in the extracellular space (Dudek et al. 1990).

Generation and application of electric fields

Uniform, electric fields were generated across individual slices (Fig. 1) by passing current between two parallel AgCl-coated silver wires placed on the surface of the ACSF in the interface chamber. The pulses applied to the wires were generated by a voltage generator (Master-8 Programmable Pulse Generator, AMPI) and converted to a current pulse by a stimulus isolation unit (Grass Instrument Co.).

The electric field (mV/mm) in the chamber was measured by two recording electrodes separated by 1 mm and calibrated to the current passed through the electrodes using the sign convention shown in Fig. 1. "Anodic" and "cathodic" stimulation refer to positive and negative field, respectively. The calibration procedure was repeated before and after each experiment.

Unless otherwise stated, all slices were aligned such that the dendritic-somatic axis was parallel to the direction of the field (0 deg). Experiments were performed where the angle of the external field relative to the apical dendritic-somatic axis was varied to investigate the effect of field orientation on its efficiency in suppressing the activity. The slices were first aligned at 0 deg. Once the minimum field required for full suppression was determined in this configuration, the slice would then be rotated 45, 90, 135, and 180 deg, and the minimum field again determined.

Measurement of extracellular potassium concentration

Potassium-selective microelectrodes (ISM) were constructed using established methods described elsewhere (Amman 1986; Lux 1974; Lux and Neher 1973). We utilized *N,N*-dimethyltrimethylsilylamine (Fluka Chemicals) to silanize the electrode tips, and the Fluka 60398 potassium-selective membrane solution, which contains the potassium ionophore Valinomycin. The ISM was filled with 150 mM KCl. The ISMs were tested and calibrated (Fig. 3, A and B) before and after each experiment and were used only if they yielded a minimum response of 50–55 mV for a 10-fold change in potassium, both for pre- and postexperimental calibrations. For experiments in which extracellular potassium was to be monitored, the ISM was positioned in the CA1 pyramidal cell layer, and a recording electrode would be positioned as close to the ISM tip as possible.

RESULTS

Spontaneous, low- Ca^{2+} epileptiform activity in the CA1 region of transverse and longitudinal hippocampal slices

Following the application of the low- Ca^{2+} ACSF (≤ 0.2 mM Ca^{2+}), synaptic transmission was blocked as demonstrated by a gradual decrease and eventual disappearance of the orthodromic responses in the CA1 region. Spontaneous bursting slowly developed, first appearing as small, short-duration, irregular negative shifts, then developing into larger, more prolonged and regular negative shifts, occasionally superimposed by high-frequency population bursts (Fig. 2A). Both "single peak" and "multiple peak" events were observed (Fig. 2B). Once fully established, the activity remained stable for up to 4–5 h. "Ictal" activity was observed in a small number of slices ($n = 3$). This type of activity was characterized by continuous, vigorous bursting (Fig. 2C).

Individual epileptiform "events" were remarkably similar in duration and amplitude within a given slice, but there was a marked variability among different slices. There were no ap-

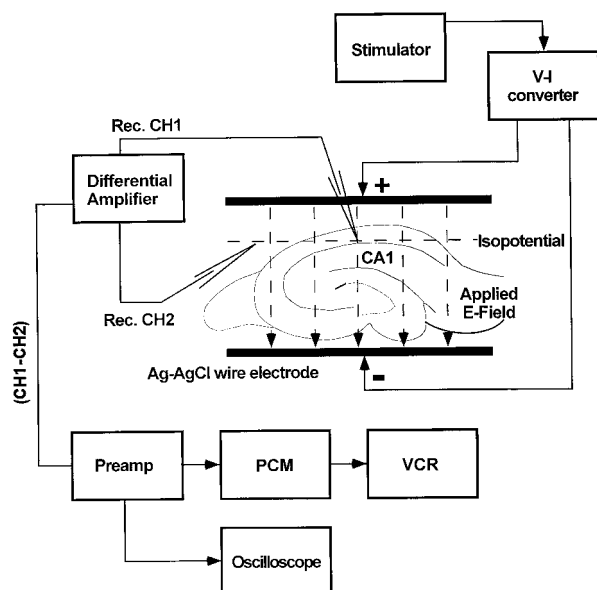


FIG. 1. Schematic block diagram of the experimental set-up. Spontaneous, synchronized activity was induced in the CA1 region of transverse (shown) and longitudinal hippocampal brain slices, using the low-calcium in vitro model of epilepsy. A stimulator applied a preprogrammed pulse to the Ag-AgCl wire electrodes, thus generating the desired electric field across the slice. Differential recordings were obtained to minimize the stimulus artifact. Data was amplified, filtered, digitized (PCM), and recorded to videotape (VCR) for later analysis.

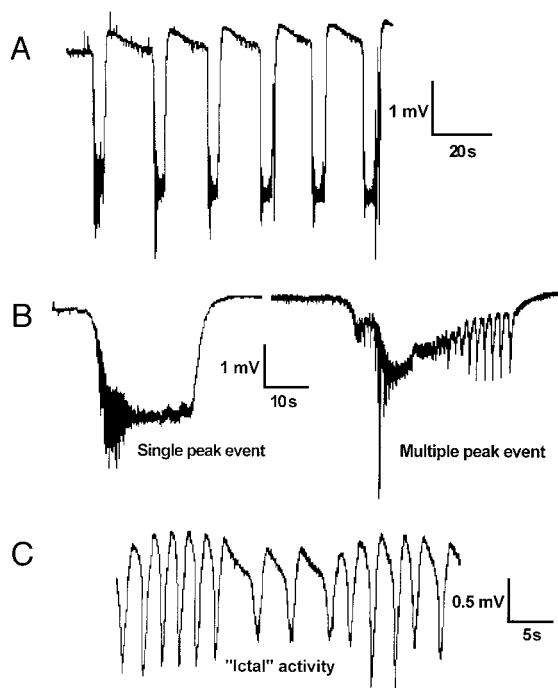


FIG. 2. Spontaneous, epileptiform activity induced in the CA1 region of hippocampal slices by prolonged incubation in low- Ca^{2+} ACSF. *A*: extracellular recordings of typical spontaneous bursting from a transverse slice. *B*: single peak and multiple peak events (with population bursts) from the same slice. *C*: sample of ictal activity from a different slice. Ictal activity was characterized by continuous, vigorous bursting.

parent differences between the activity induced in transverse ($n = 30$) and longitudinal ($n = 12$) slices.

As previously reported (Yaari et al. 1986), spontaneous epileptiform activity in the CA1 pyramidal cell layer was associated with simultaneous transient increases in extracellular potassium concentrations (Fig. 3). This "potassium wave" ranged from 1–3 mM above the baseline potassium level. In most slices ($n = 10$), the potassium wave was closely correlated with the electrical activity waveform (Fig. 3*A*). In a few slices ($n = 2$), the potassium transient appeared to precede the voltage event (Fig. 3*B*).

Effect of electric fields on low-calcium activity

In all slices ($n = 30$), applied anodic fields suppressed the activity while cathodic fields enhanced the activity. Application of exogenous fields resulted in a step increase in the extracellular field potential. The amplitude of the artifact increased linearly with field amplitude. The sign of the artifact was a function of the relative position of the two field electrodes. Larger anodic fields caused greater attenuation of the individual events until complete suppression of the activity was achieved (Fig. 4*A*). Activity was suppressed for the duration of the stimulus. In a majority of the slices, the trailing edge of the field pulses large enough to completely suppress activity caused excitation of the tissue through an anodic break effect (Fig. 4*B*). The generation of an event by anodic break reset the pattern of oscillation, shifting the phase of the activity by an interval equal to the pulse width of the applied blocking field.

The mean minimum field required to suppress spontaneous activity was 3.7 ± 1.8 mV/mm ($n = 30$). Figure 5*A* shows the

frequency distribution of the minimum fields required for full suppression while Fig. 5*B* shows the cumulative distribution of the fields, indicating that a field of approximately 5 mV/mm could suppress 100% of the activity in 90% of the slices. Fields as small as 1–1.5 mV/mm were capable of fully suppressing the low Ca^{2+} activity in approximately 15% of the slices tested.

The minimum field amplitude required for full suppression of activity using transverse (3.8 ± 2.1 mV/mm) or longitudinal (3.5 ± 1.2 mV/mm) slices was not significantly different. There was no correlation between the minimum electrical field required for full inhibition and the mean event amplitude, duration, or frequency.

Effect of field orientation on its efficiency to suppress activity

In several slices ($n = 6$), we imposed fields at different angles relative to the CA1 pyramidal cell layer. As the angle between the imposed field and the apical dendritic-somatic axis was increased from 0 to 45°, the minimum field required for full suppression of the activity increased (not shown). The mean minimum field required for full suppression at the 45° position (13.7 ± 2.9 mV/mm, $n = 6$) was significantly higher

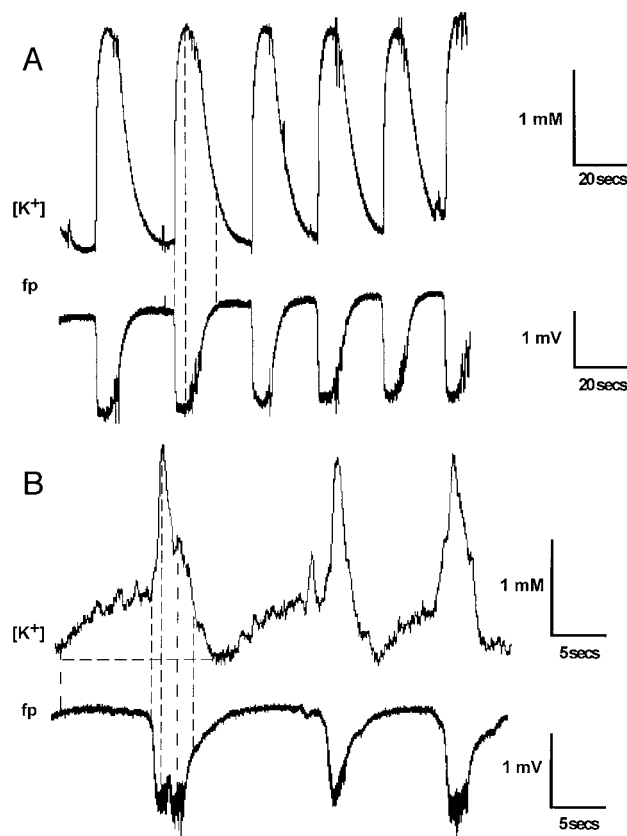


FIG. 3. Extracellular potassium concentrations during spontaneous activity. Simultaneous recordings of extracellular potassium [K^+] made using a potassium sensitive microelectrode, and field potentials (fp) made using a standard glass microelectrode. *A*: spontaneous activity accompanied by transient increases in extracellular potassium. In this case, both waveforms appeared to be closely synchronized, with the sharpest features of both the voltage event and the [K^+] wave appearing to be simultaneous. *B*: in a few slices, the potassium transient appeared to precede the voltage event as a more gradual rise in baseline [K^+].

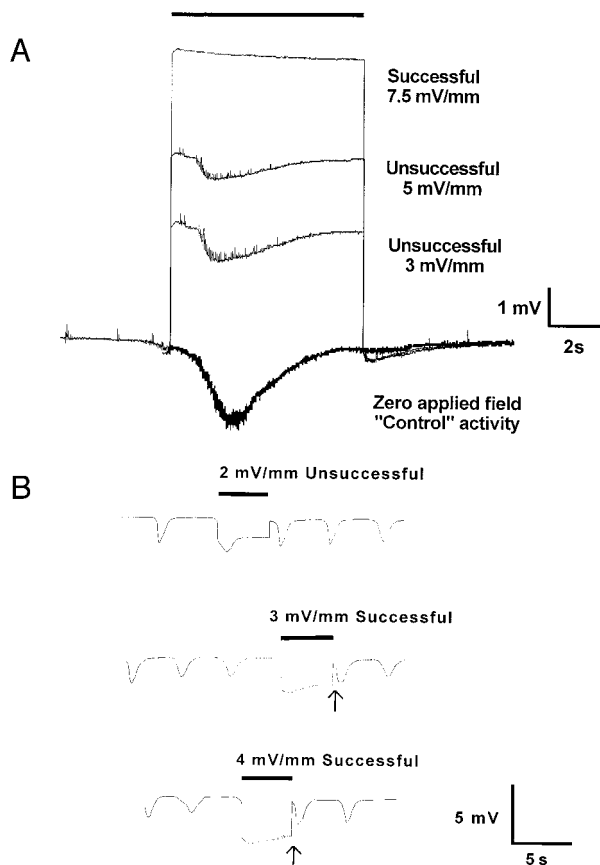


FIG. 4. Electric field suppression of low- Ca^{2+} epileptiform activity. Field application is indicated by the solid bars. *A*: as the amplitude of the applied field is increased, the stimulus artifact increases and the activity is increasingly suppressed until full suppression is achieved at 7.5 mV/mm. *B*: effect of anodic fields on burst phase. A 2 mV/mm field failed to completely suppress activity and did not effect event phase. Larger fields (3–4 mV/mm) suppressed the activity and generated anodic break events at the termination of the field pulse (arrows), thereby resetting the phase of the activity.

than that required at the 0° position (3.7 ± 1.8 mV/mm, $n = 30$). When the angle further increased to 90° , suppression could not be achieved, even at very high fields.

Once the angle increased beyond 90° to 135° , the minimum field magnitudes required for full suppression were very similar to those at 45° , but of opposite polarity. At 180° , the field efficiency in suppressing the activity was maximum, resulting in minimal fields required for full suppression but of opposite polarity to those in the 0° case.

Modulation of ictal activity by changes in field polarity and magnitude

The relationship between both electric field magnitude and polarity and slice excitability was studied in slices which developed ictal activity ($n = 3$). Fields of various amplitudes and polarities were successively applied to modulate the ictal activity. Figure 6A shows that the burst frequency increased during the cathodic phase and decreased during the anodic phase of the applied fields. Burst amplitude and width were not consistently effected. Interestingly, the relationship between the amplitude of the applied field and burst frequency was linear, as shown by the regression analysis in Fig. 6B ($R^2 = 0.96$, slope = 1.55, intercept = 9.45).

Effect of applied electric fields on extracellular potassium concentrations

Extracellular potassium concentrations were measured during application of electric fields. When the voltage events were suppressed by an applied hyperpolarizing field, it was observed that the potassium wave was also completely abolished (Fig. 7B, $n = 6$). This observation could not be attributed to an artifact resulting from interference due to the imposed field on the ISM, since with field amplitudes too low to cause suppression, the ISM responded immediately to changes in extracellular potassium (Fig. 7A). In cases where the trailing edge of a blocking field pulse caused “anodic break” excitation, the ISM again responded immediately to record the expected increase in extracellular potassium.

Effect of osmolality

In several slices ($n = 4$), we tested the effect of changes in perfusate osmolality on spontaneous low- Ca^{2+} activity, as well as on the efficiency of the imposed field. It was relatively difficult to induce robust activity in slices using higher osmolality solutions. Conversely, activity was much more easily

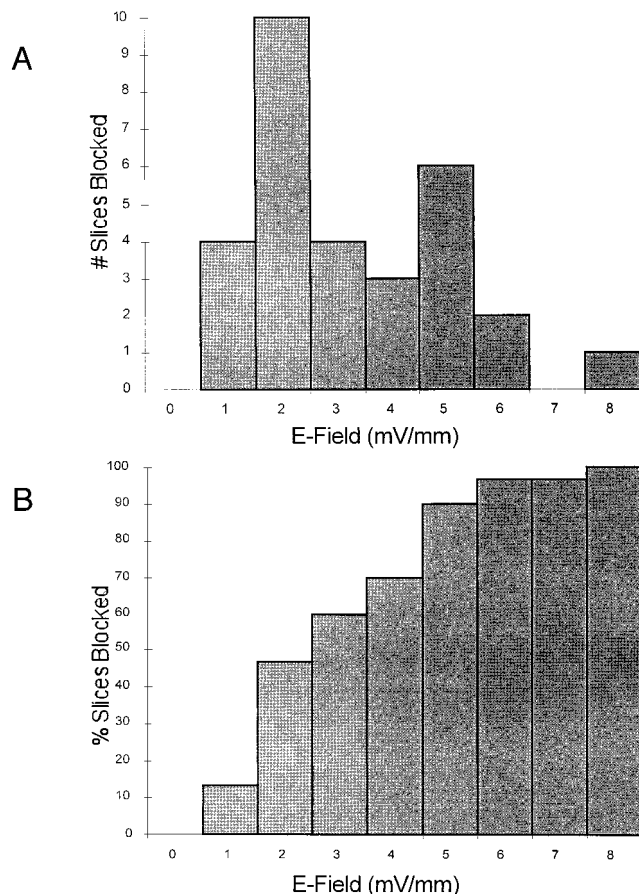


FIG. 5. Distribution of field amplitude required for full suppression. *A*: frequency distribution of the minimum field required for suppression shows that the mean minimum field required to completely block spontaneous events was 3.68 ± 1.82 mV/mm ($n = 30$). *B*: cumulative distribution of minimum field required for full suppression. These results indicate that a field of approximately 5 mV/mm could completely suppress the activity in 90% of the slices, and a field as low as 2 mV/mm could completely suppress the activity in almost 50% of the slices.

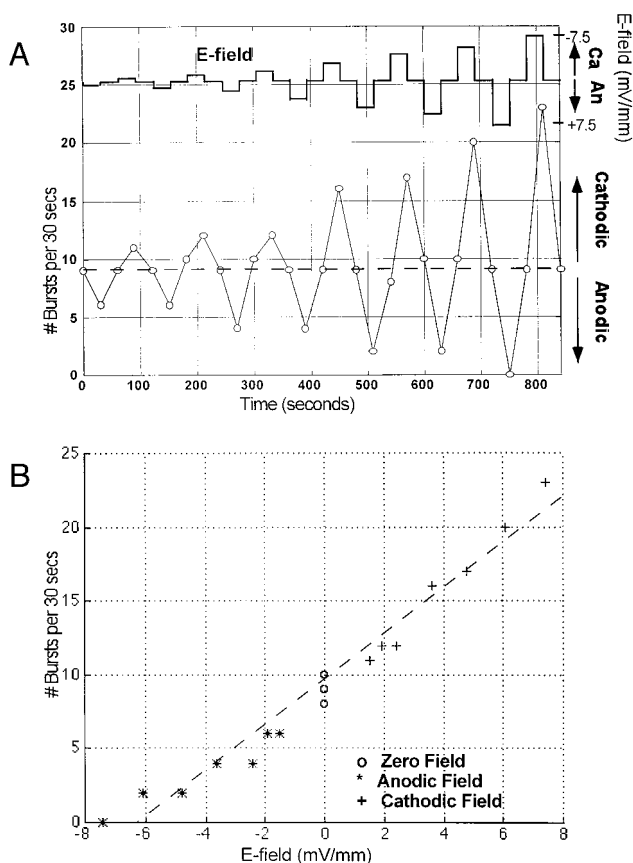


FIG. 6. Modulation of activity by changes in field polarity and magnitude. *A*: modulation of activity. The upper trace represents the amplitude and polarity of the field imposed on the slice at different times. With no field imposed, baseline burst rate was approximately 8–10 bursts per 30 s. Cathodic fields increased the burst rate while anodic fields decreased the burst rate. *B*: relationship between slice activity and the imposed E-field. Event frequency could also be linearly modulated by increasing or decreasing the field magnitude used.

induced using lower osmolarity solutions. A 10% decrease in osmolarity resulted in an average 56% decrease ($n = 4$) in the minimum field required for full suppression, while a 14% increase in osmolarity resulted in an average 81% ($n = 4$) increase in the minimum field required for full suppression.

DISCUSSION

The results of this study show that small externally applied DC electric fields are capable of suppressing or enhancing low-calcium epileptiform activity induced in the pyramidal cell layer of the CA1 region of transverse and longitudinal hippocampal brain slices.

Electric field suppression of low- Ca^{2+} activity, role of osmolarity

One major finding of this study was that the minimum field magnitudes required for full suppression of low- Ca^{2+} activity (3.7 ± 1.8 mV/mm) were considerably lower than those required to suppress high potassium induced epileptiform activity (10–15 mV/mm; Gluckman et al. 1996). We hypothesize that the effects of the imposed fields are enhanced in the low-calcium environment by a decrease in the extracellular

volume caused by cellular swelling. This decrease in the extracellular volume increases the resistance of the tissue, which in turn would increase the efficacy of the applied fields. Consistent with this hypothesis, we found that lowering osmolarity increased the efficiency of the applied fields. Conversely, increasing osmolarity decreased the efficiency of the imposed fields.

Previous studies have shown that decreases in osmolarity can lead to shrinkage of the extracellular volume, resulting in enhanced activity. Increases in osmolarity lead to attenuation or suppression of spontaneous activity (Dudek et al. 1990; Hochman et al. 1995; Traynelis and Dingledine 1989). Our results also confirm these observations and suggest that osmotic modulation of spontaneous activity could be due to changes in the efficacy of ephaptic interaction between neurons.

Alternately, the low threshold for suppression of low- Ca^{2+} activity could be a result of changes in intrinsic cell properties associated with a reduction in extracellular calcium activity (Haas and Jefferys 1984). Changes in membrane input resistance or block of Ca^{2+} dependent K^+ channels could increase cell sensitivity to exogenous fields. Similarly, reducing extracellular calcium increases gap junction coupling (Perez-Velazquez et al. 1994), which might in turn increase the efficacy of a field applied to a network of cells.

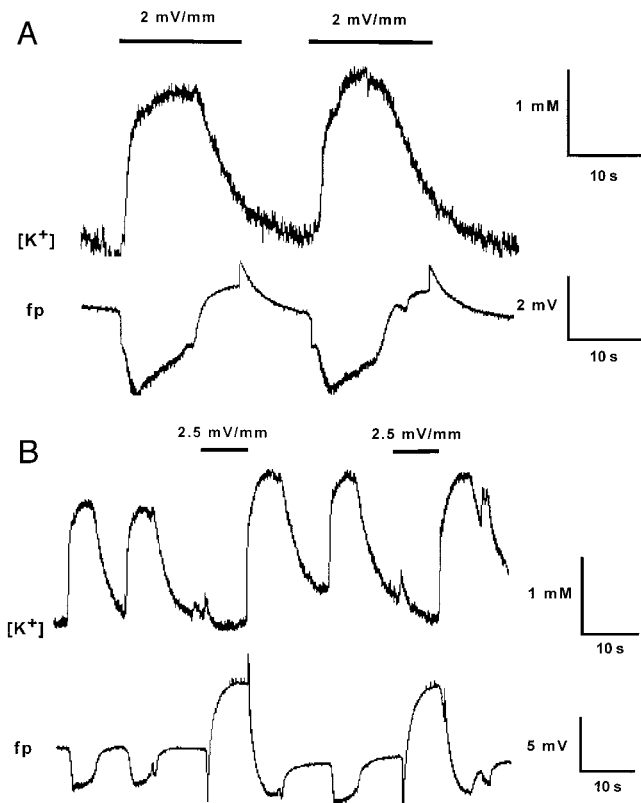


FIG. 7. Extracellular potassium concentrations during attempts to suppress activity. Simultaneous recordings of extracellular potassium [K^+] made using a potassium-sensitive microelectrode, and field potentials (fp) made using a standard glass microelectrode. *A*: 2 mV/mm field pulses failed to suppress activity and did not affect measurement of extracellular potassium transient. *B*: extracellular potassium wave is completely abolished during successful attempts to suppress the activity. Anodic break events, triggered by the falling edge of the anodic field pulse, are also accompanied by a transient increase in extracellular potassium.

Extracellular potassium concentration during suppression of activity

Consistent with earlier studies (Haas and Jefferys 1984; Yaari et al. 1986), transient increases in extracellular potassium were recorded accompanying spontaneous, synchronized epileptiform events in the CA1 pyramidal cell layer of transverse and longitudinal hippocampal brain slices, using potassium-sensitive microelectrodes. It has been hypothesized that these increases in extracellular potassium could be a primary factor in low- Ca^{2+} burst initiation and propagation, but that neuronal firing is required, contributing in a feed-forward manner to the potassium wave (Bikson et al. 1999; Yaari et al. 1986). Our finding of coincident abolition with hyperpolarizing electric fields of the voltage event and potassium wave is consistent with this hypothesis. However, because extracellular potassium concentration never dropped below baseline levels during field application, this result indicates that DC fields do not suppress spontaneous activity by modulation potassium activity.

Mechanism of electric field suppression of activity

Two possible mechanisms that could explain the inhibitory effect of a hyperpolarizing field imposed across the slice are neuronal desynchronization and membrane polarization (Durand and Warman 1994). Desynchronization of neuronal activity by the application of precisely timed, short-duration current pulses that force the neurons to fire out of phase with other cells has been shown to significantly reduce the amplitude of extracellularly recorded population spikes (Durand 1986; Durand and Warman 1994; Warman and Durand 1989). However, this effect requires very small pulses to be applied with precise timing, while in this study we utilized much longer field pulses applied over the entire duration of individual events.

Previous studies, in which current was injected over the entire duration of an event, have shown that activity is suppressed by the hyperpolarization of the somatic membrane (Kayyali and Durand 1991; Nakagawa and Durand 1991). Intracellular recordings from the above studies indicated that there was a large reduction in the number of action potentials during the period of applied current, suggesting that neurons were being hyperpolarized. Similarly, in this study, anodic fields that result in a higher potential at the soma than at the dendrites would force a positive current to flow inward at the soma and out through the dendrites (Jefferys 1981). This would result in the depolarization of the dendrites and the hyperpolarization of the soma. Similarly, cathodic fields which result in a higher potential at the dendrites than at the soma would force a positive current to flow inward at the dendrites and outward at the soma, thus hyperpolarizing the dendrites and depolarizing the soma.

This mechanism is consistent with the results from field orientation experiments which clearly show that a field is maximally efficient in suppressing or enhancing activity when it is aligned parallel to the dendritic-somatic axis. When the field is applied parallel to the dendritic-somatic axis, a larger proportion of the induced current is forced to flow passively through the CA1 pyramidal cells (Chan and Nicholson 1986; Jefferys 1981; Rushton 1927; Tranchina and Nicholson 1986). As the angle increases, less and less current flows through the

cell bodies, hence decreasing the efficacy of the exogenous fields. When the field is applied perpendicular to the axis, no current flows through the CA1 neurons; hence, the activity cannot be affected even at very high field values. Beyond 90 deg, the direction of current flow through the cells reverses and the polarizing effect also reverses.

Modulation of activity by changes in field polarity and magnitude

We have previously shown that low- Ca^{2+} burst frequency is linearly modulated by changes in neuronal polarization (Bikson et al. 1999). Anodic fields, generating a depolarization of the somatic membrane, clearly caused an increase in the excitability of the tissue, as evidenced by an increased burst frequency. Cathodic fields reduced the burst frequency, thus suggesting that they reduced tissue excitability by hyperpolarizing the somatic membrane. Furthermore, by changing the magnitude of the fields, the low- Ca^{2+} burst frequency could be modulated linearly. Taken together, these results suggest that DC exogenous electric fields modulate low-calcium spontaneous bursting by direct polarization of cell bodies.

SUMMARY

The major finding of this study is that spontaneous, synchronized low-calcium epileptiform activity in the CA1 region of rat hippocampal brain slices can be completely suppressed with significantly lower electric field values than those required to block similar activity in the high potassium model. The increased efficiency of the fields in the low- Ca^{2+} environment may be caused by an increase in the tissue resistance due to cell swelling caused by prolonged exposure to low- Ca^{2+} ACSF (Warren and Durand 1998). Our results suggest that activity modulation is mediated by somatic polarization and is thus sensitive to the field orientation. Finally, our results clearly show that the extracellular potassium waves associated with spontaneous, synchronized epileptiform activity are completely abolished when the corresponding voltage events are suppressed.

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