Suppression of epileptiform activity by a single short-duration electric field in rat hippocampus in vitro

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Mikkelsen R, Andreasen M, Nedergaard S. Suppression of epileptiform activity by a single short-duration electric field in rat hippocampus in vitro. J Neurophysiol 109: 2720–2731, 2013. First published March 13, 2013; doi:10.1152/jn.00887.2012.—The mechanisms behind the therapeutic effects of electrical stimulation of the brain in epilepsy and other disorders are poorly understood. Previous studies in vitro have shown that uniform electric fields can suppress epileptiform activity through a direct polarizing effect on neuronal membranes. Such an effect depends on continuous DC stimulation with unbalanced charge. Here we describe a suppressive effect of a brief (10 ms) DC field on stimulus-evoked epileptiform activity in rat hippocampal brain slices exposed to Cs⁺ (3.5 mM). This effect was independent of field polarity, was uncorrelated to changes in synchronized population activity, and persisted during blockade of synaptic transmission with Cd²⁺ (500 μM). Antagonists of A₁, P₂X₅, or P₂Y receptors were without effect. The suppressive effect depended on the alignment of the external field with the somato-dendritic axis of CA1 pyramidal cells; however, temporal coincidence with the epileptiform activity was not essential, as suppression was detectable for up to 1 s after the field. Pyramidal cells, recorded during epileptiform activity, showed decreased discharge duration and truncation of depolarizing plateau potentials in response to field application. In the absence of hyperactivity, the applied field was followed by slow membrane potential changes, accompanied by decreased input resistance and attenuation of the depolarizing afterpotential following action potentials. These effects recovered over a 1-s period. The study suggests that a brief electric field induces a prolonged suppression of epileptiform activity, which can be related to changes in neuronal membrane properties, including attenuation of signals depending on the persisting Na⁺ current.

epilepsy; electric field; CA1; membrane properties; persistent sodium current

DEEP BRAIN STIMULATION with implanted electrodes is a promising method for treatment of drug-resistant epilepsy (Li and Mogul 2007; Tellez-Zenteno and Wiebe 2011; Theodore and Fisher 2004). The mechanisms behind the beneficial effects of electrical brain stimulation in epilepsy and other disorders are, however, poorly understood. To enable future efforts in development of rational designs of stimulus paradigms, further knowledge of the dynamic properties of the abnormal network being stimulated and of the basic effects of electrical stimulation in brain tissue is needed (Jiruska et al. 2010; Kringelbach et al. 2010; Stacey and Litt 2008; Sunderam et al. 2010). In most clinical settings used for inhibiting epileptic activity, short-duration pulses (<1 ms) are delivered at high frequency (>100 Hz) (Jobst et al. 2010; Tellez-Zenteno and Wiebe 2011; Theodore and Fisher 2004). In vitro studies have shown that repetitive pulse trains or high-frequency sinusoidal current leads to suppression of epileptiform activity. Such effects have been attributed to either depolarization block of neuronal discharge concomitant with a rise in extracellular K⁺ concentration ([K⁺]) (Bikson et al. 2001; Lian et al. 2003; Su et al. 2008) or depression of excitatory synaptic transmission, which could be combined with decreased membrane excitability at high-frequency stimulation (Schiller and Bankirer 2007). Another stimulation approach, which has proven successful in experimental conditions, is the use of electric fields applied with long durations and low frequency. A uniform electric field generates a voltage gradient in the extracellular tissue, which will cause a differential change in the transmembrane potential (TMP) of cells or cellular elements lying in parallel with the field (Chan and Nicholson 1986; Tranchina and Nicholson 1986). In hippocampal pyramidal cells, which have a preferential orientation of the soma-dendritic axis and asymmetric location of the cell body, the applied field results in somatic hyperpolarization or depolarization of the TMP depending on the polarity of the field (Andreasen and Nedergaard 1996; Bikson et al. 2004; Gluckman et al. 1996). The ability to suppress or enhance epileptiform activity by applied fields was first investigated in hippocampal brain slices (Ghai et al. 2000; Gluckman et al. 1996), but the method has also been developed for seizure modulation in an in vivo setup (Richardson et al. 2003; Sunderam et al. 2009). In general, these previous studies show that a uniform DC field enhances or inhibits epileptiform activity in a polarity-dependent manner and the effect subsides immediately upon termination of the field, consistent with alteration of pyramidal cell excitability through polarization of the TMP at the soma. These original findings from slices were done in models of spontaneous epileptiform discharge induced in high extracellular [K⁺] (Gluckman et al. 1996) or low [Ca²⁺] (Ghai et al. 2000).

In the present study, we have examined the effects of a single brief (10 ms) DC field on epileptiform discharge evoked by orthodromic or antidromic stimulation during perfusion of Cs⁺ in rat hippocampal slices. We show that such field stimulation can suppress epileptiform activity independent of the field polarity and hence is not related to the direction of change in the TMP in a specific part of the pyramidal cell. We hypothesize that this suppressive effect of the DC field, which has not previously been described, involves the initiation of cellular processes persisting after termination of the field. The study provides evidence that such processes may include alteration of pyramidal cell membrane properties.

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MATERIALS AND METHODS

Preparation of brain slices. Animal care and housing facilities as well as the experimental protocol for the euthanasia of animals were in accordance with Danish and European law and approved by the Animal Experimentation Board under the Danish Ministry of Justice. Experiments were performed on hippocampal slices prepared from adult male Wistar rats (4–5 wk). The rats were anesthetized with isoflurane and decapitated. The brain was removed and quickly placed in a dissection medium (see below) at 4°C. The hippocampus was dissected free, and 400-μm slices were cut on a McIlwain tissue chopper. One slice was immediately transferred to the recording chamber, where it was placed on a nylon mesh grid at the interface between warm (31–33°C) standard perfusion medium (see below) and warm humidified carbogen (95% O2, 5% CO2). Perfusion flow rate was 1.2 ml/min. The slice was allowed to rest for at least 45 min before recordings were started. The remaining slices were stored in dissection medium bubbled with carbogen at room temperature.

Electrophysiological recordings. Extracellular recordings were obtained with borosilicate glass microelectrodes (1.2-mm OD; Clark Electromedical, Pangbourne, UK) filled with 1 M NaCl (tip resistance: 15–30 MΩ). The recording electrode was placed in stratum pyramidale in area CA1 unless otherwise noted. Intracellular recordings were done with the same type glass electrode filled with 4 M K+-acetate (tip resistance 40–90 MΩ). Intra- and extracellular electrodes were placed as close to each other as possible under visual guidance. For orthodromic stimulation of the Schaffer collateral-commisural fibers, constant-current pulses (100 μs, 100–800 μA) were applied at 5- to 20-s intervals via a bipolar, Teflon-insulated, platinum electrode placed in stratum radiatum at the border between area CA3 and area CA1. For antidromic stimulation, a part of the alveus was physically isolated from the stratum oriens by microdissection. The stimulus electrode was placed on the isolated part of the alveus, and trains of 10–15 pulses (50 μs, 100–800 μA, 100 Hz) were delivered at 5- to 20-s intervals. Conventional recording techniques were employed with a high-input impedance amplifier (Axon lamp 2A, Axon Instruments) with bridge balance and current injection facilities. Signals were digitized with a CED 1401 A/D converter and analyzed on a PC by Signal software (Cambridge Electronic Design).

Slices were accepted for recording if they displayed a normal orthodromic field potential (FP), consisting of a field excitatory postsynaptic potential (EPSP) and a single population spike of maximal amplitude between 5 and 20 mV. During epileptiform activity, spreading depression occasionally occurred in a few experiments. After a spreading depression the stimulus-evoked response was monitored closely, and recordings were stopped if the baseline potential and FP did not fully recover or if spreading depressions occurred three times.

Electric field induction. An electric field was applied in the CA1 region by passing pulses of constant current between two parallel wire electrodes: a platinum electrode fixed to the edge of the recording chamber (reference electrode) and a tungsten electrode mounted on a micromanipulator and placed horizontally on the slice surface near the hippocampal fissure (Fig. 1A). The electrodes were connected to a constant current source (Isolator-11, Axon Instruments). The induced extracellular voltage gradient was measured in four slices by moving the recording electrode along a track perpendicular to stratum pyramidale in steps of 100 μm (Fig. 1A) and at a depth of 250 μm. The voltage gradients induced by (±)0.1- and 0.5-mA command currents are shown in Fig. 1B. Note the nonlinear profile with increased gradient toward the fissure. This is presumably due to an “edge-effect” of the nearby field electrode, as nonlinearity is not observed with both electrodes placed away from the slice (Andreasen and Nedergaard 1996). To test whether electrode arrangement is important for the effects reported in this study, we performed control experiments using two fixed electrodes on either side of the slice in the same experiment. With this arrangement, the applied field showed similar efficacy in inhibiting epileptiform activity (not shown). The profiles obtained (Fig. 1B) were numerically similar for both polarities, and the average field intensity in the CA1 was found to be 880 mV mm⁻¹ mA⁻¹ (mean value obtained with F⁺ and F⁻). The terms F⁺ and F⁻ are used here to designate field polarities generated by current running in the fissure-to-alveus direction (F⁺) and in the opposite direction (F⁻). In experiments where the slice was rotated 90°, the CA3 was closest to the reference field electrode and the electrode located on the slice was placed in the subicular end of the CA1 and oriented perpendicular to the stratum pyramidale. Recording electrodes were placed at similar distance to the field electrodes as in other experiments. When applied fields were combined with ortho- or antidromic stimuli, the field pulse was triggered at a preset delay from the stimulus in each sweep. After each pulse, one to three records of control stimuli were sampled before the next field application.

During intracellular recordings from CA1 pyramidal cells, an extracellular recording electrode was placed in the stratum pyramidale close to the intracellular recording electrode. The potential measured by the two electrodes was continuously subtracted by a custom-built differential amplifier with a gain balance. Short pulses of current were passed though the slice and the gain balance adjusted so that the signal was correctly compensated before the experiment. In some recordings, the collected signals were stored on videotape. After the experiment, the electrode was retracted from...
the cell and the extracellular voltage changes, in response to ±0.05- to 0.2-mA field pulses, were recorded by both electrodes. The data were once more fed through the differential amplifier to fine-tune the compensation before digitization.

Data analyses. The stimulus-evoked Cs⁺-induced epileptiform potential consists of an initial positive phase followed by a prolonged negative phase (Fig. 2A). The area of the negative phase (used for quantifying the amount of epileptiform activity) was measured with respect to the baseline potential, starting at the point where the decay of the positive phase crossed the baseline potential and ending where the potential returned to baseline. Data with field application in combination with ortho- or antidromic stimulation were normalized to the averaged value of the two control sweeps sampled immediately before and after the field application. The threshold value for the suppressive effect of the applied field (Tₑ) was determined as the lowest applied current within the range of currents that gave consistent reduction of ≈5%. The threshold for the excitatory effect of the field alone (T₀) was determined as the lowest applied current that induced a negative phase. The amplitude of the action potential (AP) and area (mV·ms) of the depolarizing afterpotential (DAP) were measured with respect to the membrane potential prior to the stimulation pulse. The coastline index (CI), a quantitative approximation of synchronized spike activity, was measured as the sum of the distances between individual data points in the digitized signal [unit: (ms² + mV²)¹/₂]. For individual measurements, the CI was corrected for noise and subsequently expressed as a normalized value relative to control.

For a detailed description of the procedure, see Skov et al. (2009).

Drugs and solutions. The composition of the dissection medium was (in mM) 120 NaCl, 2 KCl, 1.25 NaH₂PO₄, 6.6 HEPES acid, 2.6 NaHEPES, 20 NaHCO₃, 10 d-glucose, 2 CaCl₂, and 2 MgSO₄, bubbled with carbogen. The composition of the standard perfusion medium was (in mM) 124 NaCl, 3.25 KCl, 1.25 NaH₂PO₄, 20 NaHCO₃, 2 CaCl₂, 2 MgSO₄, and 10 d-glucose, bubbled with carbogen (pH 7.3). In experiments where Cd²⁺ was applied, phosphate and sulfate were omitted to prevent precipitation. Most pharmacological compounds were made up in aqueous stock solutions of 100–1,000 times the required final concentration and diluted in the perfusion solution as appropriate. Experiments with the light-sensitive compounds suramine and pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) were performed in a dark environment. Suramine, PPADS, CsCl, CdCl₂, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), bicuculline methobromide, 2-amino-5-phosphonopentanoic acid (APV), (5R-10S)-(+) -5-methyl-10,11-dihydro-5H-dibenzo(a,c)cyclohepten-5,10-imine (MK-801), and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were all purchased from Sigma.

RESULTS

Effect of an applied electric field on the epileptiform Cs-FP.

In this study, we examined the effect of a brief electric field...
field intensity in the CA1, the mean values for \( T_s \) and \( T_e \) were significant for both polarities (Fig. 4). In terms of average, \( T_s \) and \( T_e \) differed by a factor of 3.8, which was significant (see also Fig. 3). The overall dose-response relation (Fig. 4B) showed a gradual increase in the suppressive effect of the field in the current range of 0.02–0.30 mA, which suggested that the suppressive effect persists, and may even increase, at intensities larger than \( T_e \). To make a quantitative evaluation of this point, we determined, in each experiment, the maximal effect recorded within the range of field intensities below \( T_e \) and at or above \( T_e \), respectively. With intensities below \( T_e \), we found that the orthodromic field potential was maximally reduced to \( \sim 36\% \) of control [average between \( F^- \) and \( F^+ \), both of which gave significant suppression compared with control (\( P < 0.05, n = 7 \))], whereas higher intensities (\( \geq T_e \)) gave a maximal average reduction to 13% of control (Fig. 4C). The difference between low- and high-intensity fields, however, did not reach statistical significance \( [F^-: P = 0.17 (n = 6), F^+: P = 0.09 (n = 7); \text{paired } t\text{-test}] \). Together, these data suggested that the suppressive effect of the applied field is independent of its excitatory effect and, in terms of field intensity, a window exists in which the suppressive effect is isolated.

Generation of a long-lasting negative phase of the Cs-FP depends on sustained high-frequency discharge in pyramidal neurons, initiated by events taking place during the early phase of the response (Andreasen et al. 2007; Andreasen and Nedergaard 2012). In the above experiments, the field pulse was applied during the early part of the positive phase. To test whether this temporal alignment was significant for the suppressive effect of the applied field, we altered the delay of the field pulse to 500 ms from the orthodromic stimulus. This delay covers the time span from initiation to full development of the negative deflection. At 500-ms delay, we still detected a suppressive effect of the field pulse (10-ms duration) that was intensity dependent and was present with both polarities (Fig. 3, B and D). In the total sample (\( n = 6 \)), the average effects were somewhat less than at 10-ms delay (Fig. 4C), but significant suppression was still present with field intensities below \( T_e \) (\( F^-: 46 \pm 11\% \) of control, \( P < 0.05 \); \( F^+: 52 \pm 14\% \) of control, \( P < 0.05 \), paired \( t\text{-test} \)). These results therefore seemed to suggest that the effect of the field could not merely be ascribed to interference with the induction of the epileptiform activity.

Effect of applied field on synchronized activity. The orthodromic Cs-FP is characterized by a period of high-frequency synchronized population activity, which begins during the positive deflection and extends through the transition phase into the early part of the negative deflection. It has been shown that electrical stimulation can interfere with synchronization (Durand and Warman 1994), and we have found that changes in the degree of synchronization during the transition phase are correlated with changes in the total area of the following negative deflection (Skov et al. 2009). To examine whether the effect of the applied field on the negative deflection was related to changes in synchronization during the preceding transition phase, we determined the CI of individual traces in a 175-ms period after the field, which covers the transition phase (Fig. 5A). Paired measurements of the Cs-FP negative FP area and the CI value (both normalized to control) are plotted in Fig. 5B. There was no significant correlation between the two parameters. Hence, there seemed to be no supporting evidence that the field-induced suppression is related to either enhanced or reduced synchronization of network discharge. We noted, however, that the CI often increased in the presence of field...
Fig. 3. Dependence of the field efficacy on intensity, polarity, and delay. A, top: full Cs-FP evoked by orthodromic stimulation. Bottom: responses obtained with incrementing field intensities (top to bottom, applied current indicated). The field pulse (10 ms, F+) was applied alone (left) or at 10-ms delay from the orthodromic stimulus (right). Insets show expansion of the initial portion of the trace. Note that field alone at the higher intensities is followed by an epileptiform potential with initial population spike activity and a slow negative phase. B: from the same experiment, records of Cs-FPs with the field applied at 500-ms delay (10 ms, F+, incrementing intensity). Asterisk marks time of field application. Calibrations as in A. C: summary data from the same experiment showing the area of the slow negative FP, recorded with applied fields of different intensities, and expressed as % of the control area obtained with orthodromic stimulation alone (= 100%). Field pulse delay: 10 ms. Bars represent applied field alone (either polarity, F− or F+) and applied field combined with stimulation (ortho/F). In this experiment, the thresholds for field-induced suppression of epileptiform activity (T*) and for evoking epileptiform activity by the field alone (T*) were estimated to be 0.02 mA and 0.10 mA, respectively, for both F− and F+. Note that since 0.02 mA was the lowest intensity used the estimated threshold (T*) is likely inaccurate, and the true threshold could be lower.

Application (>100% of control, Fig. 5), indicative of a general synchronizing effect.

Blockade of calcium channels. We examined the effect of unselective blockade of voltage-dependent calcium channels by including Cd2+ (500 μM) in the perfusion medium. Since the orthodromically evoked Cs-FP is abolished by Cd2+ (Skov et al. 2005), we activated CA1 pyramidal cells antidromically by a train of stimuli delivered to the alveus (see MATERIALS AND METHODS). With this protocol, epileptiform potentials could be generated reliably, which were similar in shape to the Cs-FP, including the expression of a slow negative phase (Fig. 6A). Using 10-ms field pulses applied 15 ms after train onset, we observed a reduction of the antidromically evoked epileptiform response, which was present at intensities where the field alone failed to evoke hyperactivity (Fig. 6A). Overall, the effect was statistically significant with either polarity of the field [F−: 28 ± 4% of control (n = 5), P < 0.05, F+: 27 ± 8% of control (n = 5), P < 0.05; paired t-test], and there was no difference between the two polarities (P = 0.90, paired t-test). Antidromic population spikes were not reduced in amplitude or area by the field application, suggesting that the effect was not secondary to propagation failure. We also used the antidromic protocol to test the effect of a field applied prior to stimulation. Pooled data from seven experiments showed significant attenuation of the epileptiform potential with a 10 ms F− field pulse applied 150 ms before the antidromic train (area reduced to 57 ± 13% of control, P < 0.05). In some experiments we observed attenuation upon further increases in delay up to 1 s. These data were, however, not quantitatively compared across the population.

Blockade of adenosine and purinergic receptors. The above results obtained in the presence of Cd2+ indicated that the suppressive effect of the applied field was independent of synaptic release of neurotransmitters. A possible cause of the effect could, however, be adenosine, as the extracellular concentration of this substance (resulting from ATP degradation) has been shown to increase under deep brain stimulation (Bekar et al. 2008). We therefore included the A1 receptor antagonist DPCPX (0.5 μM) together with Cd2+ (500 μM). However, this treatment gave no detectable change in the
the plateau amplitude, as well as duration, was clearly reduced in response to field application (Fig. 7B). The firing rate generally decreased during the initial 100–200 ms after the field pulse. In some neurons (Fig. 7A), the firing rate was unaltered or enhanced in the period beyond 200 ms until termination. Complete abolishment of APs or plateau was rarely observed.

While these results seemed to suggest that the excitability of pyramidal cells is altered by the applied field, they did not indicate whether the effects are direct on the individual neuron. To exclude the possible contribution from synaptic signaling, we included Cd$^{2+}$ (500 μM) in the perfusion medium, in addition to Cs$^+$ (3.5 mM), to block synaptic transmission. Field pulses (10 ms, 0.2 Hz) were adjusted to a strength that gave no epileptiform activity ($<T_\text{p}$) and did not trigger APs in the recorded cell. We observed that after termination of the field pulse the membrane potential deviated from the resting baseline in all eight of the cells recorded. Interestingly, the polarity differed, being depolarizing in five cells (range: 1.0–5.3 mV, measured 30 ms after field pulse) and hyperpolarizing in three cells (0.3–2.2 mV). In individual cells, the polarization had the same direction after F+ and F− fields (Fig. 8A). The average polarization declined from about 1 mV to near zero within 200 ms after the field (Fig. 8A, inset). We also monitored the membrane input resistance ($R_\text{m}$) by injection of current pulses (−0.3 nA, 50 ms). $R_\text{m}$ was markedly decreased 30 ms after the field (70 ± 8% of control, $n = 8$, $P < 0.05$).

The efficacy of the applied field ($n = 6$, not illustrated). Blockade of the purinergic P$_{2X}$ and P$_{2Y}$ receptors by co-application of the antagonists PPADS (5 μM) and suramin (50 μM) was also without effect ($n = 5$, not illustrated), hence suggesting lack of involvement of ambient ATP in the suppressive effect of the applied field.

Orientation of the applied field. With the standard experimental setup (Fig. 1A), the current flow was oriented parallel to the longitudinal axis of the CA1 pyramidal cells, leading to differential polarization of these cells (Andreasen and Neder-gaard 1996). We tested the significance of the orientation by rotating the field by 90° (see MATERIALS AND METHODS), so that the current ran perpendicular to the somato-dendritic axis of the pyramidal cells. With this orientation, we could detect no significant effect of the applied field on antidromically evoked epileptiform field potentials (Fig. 6B; F−: area = 94 ± 10% of control, $P = 0.48$; F+: area = 101 ± 6%, $P = 0.75$, $n = 5$, paired $t$-test).

Effect on pyramidal cell properties. We next monitored the effects of the applied field on the membrane properties of individual CA1 pyramidal cells recorded with sharp electrodes. The somatic TMP, measured with respect to the extracellular signal recorded by a nearby extracellular electrode, was usually altered during a field pulse, being depolarized with F+ and hyperpolarized with F−. Orthodromic stimulation resulted in high-frequency firing during the Cs-FP. Firing always began shortly after the stimulus and was generally maintained during the negative phase. Co-application of a 10-ms field pulse (4- to 10-ms delay from orthodromic stimulus) consistently gave a reduced duration of the discharge in nine of nine cells (Fig. 7).

In two of seven cells, which expressed a depolarizing plateau potential (Andreasen and Nedergaard 2012), repolarization from the plateau was seen to occur earlier despite an unaltered amplitude of the plateau (Fig. 7A). In the remaining five cells,
paired t-test) and recovered gradually within the following 1 s (Fig. 8C). AP, triggered by injection of short (4 ms) depolarizing current pulses, showed unaltered height 30 ms from the field pulse compared with control (94 ± 3 mV vs. 94 ± 3 mV, n = 8). The AP half-width was slightly, but insignificantly, increased (after field 1.5 ± 0.2 ms vs. control 1.4 ± 0.2 ms, n = 8). The DAP following the AP was, however, clearly attenuated over a period of several hundred milliseconds after the field pulse (Fig. 8, B and C). When normalized to control, the average area of the DAP (see MATERIALS AND METHODS) recorded 30 ms after F− was 49 ± 9% of control (n = 9, P < 0.05, paired t-test) and recovery followed a time course equivalent to the Rin (Fig. 8C). Similar results were obtained with F+ (not illustrated). Application of the field with 90° rotation induced little or no change in Rin or DAP (Fig. 8D). On average, Rin was reduced to 94 ± 2% of control (n = 7) and the DAP was reduced to 81 ± 7% of control (n = 7) at 30-ms delay. Neither of the values deviated significantly from the controls. We compared the normalized values obtained with 0° versus 90° field orientation measured at 30-ms and 200-ms delay. This analysis revealed a significant difference in Rin at 30-ms but not 200-ms delay. The DAP differed significantly at both delays (Fig. 8E). As the observed change in the DAP is indicative of an altered activation of a persistent sodium current (INaP) (Bean 2007; Golomb et al. 2006), we used an alternative protocol to induce this current. Previous studies have shown that a somatic INaP is behind amplification of depolarization in a narrow voltage range below AP threshold, with negligible contribution from T- or R-type Ca2+ current (Andreasen and Lambert 1999). The INaP-dependent component, elicited by a brief subthreshold current pulse (+0.15 nA, 15 ms), is revealed as a depolarizing envelope following the pulse (Fig. 8F). We observed that this component, recorded in normal perfusion medium with no blockers, was inhibited when a field (10 ms, 0.05–0.15 mA) was applied prior to the intracellular pulse (Fig. 8F). In these experiments, the membrane potential reached at the end of the current pulse was kept constant by adjustment of DC holding current, when necessary. An inhibitory effect on the evoked depolarizing envelope was found in 12 of 12 cells with F− and in 5 of 7 cells with F+. Of the remaining two cells with F+, one showed an increase and the other showed no effect.

**DISCUSSION**

We show here that a short (10 ms) electric field applied across the CA1 region can suppress the progression of epileptiform activity evoked by orthodromic or antidromic stimulation. The applied field could provoke epileptiform activity by itself, but this required significantly higher intensity (factor of
suggesting that a selective suppressive effect can be achieved within a wide safety margin. The suppressive effect could be evoked in the presence of the nonselective calcium channel blocker Cd$_2^+$ (500 μM), indicating that it was generally independent of synaptic transmission and transmembrane Ca$_2^+$ influx.

The present findings differ in several respects from previous in vitro studies on the suppressive effects of applied fields or focal current stimulation on epileptiform activity. Our stimulation technique involves contact between one of the stimulus electrodes and the brain tissue, which is the case also in deep brain stimulation in vivo or focal stimulation in vitro. Because of the parallel alignment of the stimulus electrode with an external electrode, the current sets up a voltage gradient, which is reminiscent of the uniform field generated with two external electrodes (Andreasen and Nedergaard 1996; Chan and Nicholson 1986; Gluckman et al. 1996). The voltage gradient was, however, not constant through the tissue (Fig. 1B). It appears that tissue-to-electrode contact or nonuniformity of the field is not a prerequisite for the effect, as we could reproduce our results by using two external electrodes. This finding furthermore indicates that the shunting of current through the perfusion medium surrounding the slice, which is much larger with two external electrodes, has negligible influence on the suppressive effect in the tissue apart from the requirement of a higher command current to obtain the same field intensity.

The epileptiform activity was suppressed when the external field was aligned with the pyramidal cell somato-dendritic axis (0°), whereas no significant effect was detected with 90° rotation. Such orientation dependence is in accordance with previous studies (Ghai et al. 2000; Gluckman et al. 1996, 2001), suggesting that polarization of the pyramidal cell membrane, effective with 0° fields but not with 90° fields, is...
essential for inducing the effect. For a 0° field in CA1, it is well documented that current running in the alveus-fissure direction (our F— polarity) is capable of inhibiting epileptiform activity, whereas the opposite field polarity exacerbates the activity (Ghai et al. 2000; Gluckman et al. 1996). Because the proximal end of the pyramidal cell including the soma/initial segment is hyperpolarized by F—, these previous findings are explained by inhibition of AP discharge resulting from somatic membrane hyperpolarization, a notion that is supported by the observation that such suppression depended on the continuous presence of the field (Ghai et al. 2000). This is contrary to the present results, showing that both polarities of the field were suppressive and the effect was detectable in a period of at least 150 ms after termination of the field. In addition, we found that the threshold intensity required for suppression was, on average, 28 mV/mm, which is higher than reported for modulation (5–10 mV/mm; Gluckman et al. 1996) or blockade (3.7 ± 1.8 mV/mm; Ghai et al. 2000) of spontaneous epileptiform activity with a continuous field.

Stimulus protocols using AC current (sinusoidal field) or repetitive application of short-duration DC square pulses have been shown to suppress epileptiform activity through a different mechanism. During such stimulation, the pyramidal cells are reported to undergo a large depolarization, concomitant [K+] by a rise in extracellular [K+] leading to depolarization block (Bikson et al. 2001; Lian et al. 2003). In coherence with our data, this effect is independent of synaptic transmission and persists after stimulation, albeit the recovery time is much longer (minutes) and the threshold intensity (for full blockade) is >100 mV/mm (Bikson et al. 2001). The possibility that the present results, obtained with a single short-duration stimulus, could be reminiscent of such effects (e.g., represent the effects of the first event in a series of repetitive stimulations) is unlikely. First, we never observed depolarization block of pyramidal cell discharge, and second, the effect here was orientation dependent, whereas AC protocol-induced suppressive effect can be achieved with equal efficacy by a field rotated by 90° (Bikson et al. 2001). In
addition, however, evidence suggests that long-term repetitive field stimulation (minutes) gives distinct responses in different phases of the stimulation, indicating that multiple processes are initiated that operate at different timescales (Su et al. 2008). Such events have not yet been clearly defined, preventing direct comparisons with the present data; however, it seems possible that the effect described here may represent a previously unidentified process evoked during repetitive field application in other model systems.

As a third possible mechanism, it has been shown that a single, short (100 μs) current pulse applied with a unitary electrode in stratum pyramidale can desynchronize epileptiform discharge of individual CA1 pyramidal cells (Durand and Warman 1994). Although our procedure may be superficially similar (use of a single, brief stimulus), the present results deviate substantially from those of Durand and Warman (1994). First, their desynchronizing effect depended on the delivery of the stimulus in precise timing with respect to interspike intervals, a constraint that we did not observe. Second, there was no overall desynchronization associated with the field stimulation, nor was there any correlation between the efficacy of the applied field and the amount of synchronization as determined by CI. It should be noted that CI can be an imprecise indicator of synchronization, as it is sensitive to shifts in neuronal baseline potential causing altered driving forces for ionic currents. We found, however, that the applied field induced only modest changes in membrane potential of pyramidal neurons (+1 mV on average), suggesting no large contribution from polarizing shifts to the CI.

In summary, based on available reports in the literature, it seems evident that the effect observed in this study is mediated by a mechanism that has not been described previously. To evaluate possible discrepancies from earlier work, differences in stimulus protocols, quantification methods, and, not least, epilepsy models should be considered. The previous studies, using applied fields to inhibit epileptiform activity, were done in models of spontaneous activity, whereas we have used stimulus-evoked epileptiform potentials. It is possible that the sensitivity to the field could vary depending on parameters such as the type and intensity of the hyperactivity and on the mode of initiation.

**Effect on pyramidal cell properties.** Our intracellular data show that, during orthodromically induced epileptiform activity, the applied field causes a consistent decrease in the duration of firing and a slowing of the firing rate of pyramidal cells, at least within the first 100–200 ms after the field pulse. Recent results suggest that maintenance of prolonged afterdischarge during the slow negative phase of the Cs-FP depends on the expression of plateau potentials in a subset of the pyramidal cells. The plateau potential is voltage dependent and, under standard conditions, generated in an all-or-nothing manner showing no intermediate amplitudes (Andreasen and Nedergaard 2012). In the present study, we found that the applied field reduced the amplitude of the plateau potential in a high proportion of the cells, indicating that the suppressive effect on epileptiform activity might be related to an interference with the plateau property. Evidence suggests that the plateau potential largely depends on $I_{\text{NaP}}$ (Andreasen and Nedergaard 2012), which, in turn, is known to be facilitated during epileptiform events (Somjen and Muller 2000; Yue et al. 2005). Because of this interdependence, the field-induced reduction of the plateau potential could either be secondary to the decrease in epileptiform activity or vice versa. In the absence of population activity, the $I_{\text{NaP}}$ is activated during single APs and contributes, along with $\text{Ca}^{2+}$ current, to inward currents forming the afterdepolarization (ADP) (Golomb et al. 2006; Metz et al. 2005). We observed that the ADP, recorded during nonselective inhibition of $\text{Ca}^{2+}$ channels, was diminished after the applied field, suggesting that the field can attenuate depolarizing signals induced by the $I_{\text{NaP}}$ in individual cells that do not participate in epileptiform activity. Together, these data seem to suggest that field-induced decrease in the $I_{\text{NaP}}$ is the primary event leading to suppression of epileptiform activity. The slow decay of this effect (1 s) is consistent with the extracellular data, showing that suppression of epileptiform activity could be obtained with preceding field application. In support of the involvement of $I_{\text{NaP}}$, a subthreshold depolarization, known to depend on this current (Andreasen and Lambert 1999), was also inhibited by field application. In addition, we observed a decrease in $R_{\text{in}}$, the time course of which paralleled the average change in the ADP. This observation is compatible with the possibility that inhibition of the ADP resulted from shunting of the $I_{\text{NaP}}$, secondary to the increase in membrane conductance. However, the possibility remains that the applied field caused a direct decrease of $I_{\text{NaP}}$. Establishing which conductances are affected by the field stimulation will require more detailed studies. Our recordings, which were done at membrane potentials at or close to rest, revealed that the direction of the initial change in somatic potential following the field pulse was not equal among the cells and the magnitude was often very small. From these observations one might speculate that the net conductance change, existing after the field was terminated, has a reversal potential close to rest, and it seems possible that more than one specific conductance is involved. Furthermore, assuming that the effect was 1) local on the pyramidal cells and 2) caused by membrane polarization during the 10-ms field pulse, the polarity independence suggests that the conductance(s) could be activated from two cellular compartments, i.e., the apical dendritic as well as the somatic/basal dendritic membrane. The possibility that the conductance change is due to field-induced breakdown of the lipid membrane (electroporation) is very unlikely, as the maximal TMP reached in the ends of a pyramidal cell during an electric field of 114 mV/mm (average intensity used in our experiments with $R_{\text{in}}$ measurement) will be lower than required for reaching the threshold for electroporation (TMP 500–1,000 mV; for review, see Gehl 2003; Neuman et al. 1999). Furthermore, the recovery time of $R_{\text{in}}$ was ~1 s, as opposed to membrane resealing after electroporation, which takes several minutes (Gehl 2003).

To account for the observed changes in response to the applied field, other possibilities than direct effects of the field on pyramidal cells should be considered. In particular, the slow decay of the response would seem compatible with a "humoral signal," involving the binding of a ligand to membrane receptors. Here we found no evidence for such mechanism, as antagonists of adenosine and ATP receptors were without effect and the induced changes in membrane properties, as well as the suppressive effect, persisted in the presence of 500 μM Cd²⁺, making it unlikely that calcium-dependent
transmitter release is involved. It should also be noted that the orientation dependence is not easily explained by effects of the field on cell populations or cellular elements without preferential geometric orientation, e.g., glial cells, interneurons, or axons.

Perspectives and future studies. The present study shows a suppressive effect of an applied electric field on epileptiform activity, which is correlated with conductance changes in pyramidal cell membranes. These novel findings expand our knowledge of the impact of electrical stimulation in brain tissue. Significantly, the effect included a decrement in voltage signals associated with the \( I_{\text{NaP}} \). At present it is uncertain to what extent the results can be generalized, as we cannot exclude that the susceptibility toward the field is enhanced by factors specific to the model. Future studies should therefore seek to explore the impact and applicability of this mechanism in different models and types of epileptiform activity, including long-lasting, ictalike events. Although still speculative, utilizing it as target for brain stimulation in vivo might be feasible. The need to use only short-duration stimuli and the polarity independence of the effect may allow for the use of intermittent and biphasic current protocols, which are associated with less risk of inducing tissue damage than constant, intermittent and biphasic current protocols, which are associated with less risk of inducing tissue damage than constant, mono- or biphasic current (Merrill et al. 2005). Alignment of an electric field with respect to the soma-dendrite axis of neurons is a more difficult task in vivo than in vitro. However, evidence suggests that, at least for some hippocampal pyramidal neurons, this challenge can be met by using a strategically positioned radial electric field (Richardson et al. 2003; Sunderam et al. 2009).

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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