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Synaptic impairment induced by paroxysmal ionic conditions in neocortex

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Summary

Purpose—Seizures are associated with a reduction in extracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_o$) and an increase in extracellular K^+ concentration ($[\text{K}^+]_o$). The long-range synchrony observed between distant electrodes during seizures is weak. We hypothesized that changes in extracellular ionic conditions during seizures are sufficient to alter synaptic neuronal responses and synchrony in the neocortex.

Methods—We obtained in vivo and in vitro electrophysiologic recordings combined with microstimulation from cat/rat neocortical neurons during seizures and seizure-like ionic conditions. In vitro the $[\text{K}^+]_o$ was 2.8, 6.25, 8.0, and 12 mM and the $[\text{Ca}^{2+}]_o$ was 1.2 and 0.6 mM.

Key Findings—During seizures recorded in vivo, we observed abolition of evoked synaptic responses. In vitro, the membrane potential of both regular-spiking and fast-spiking neurons was depolarized in high $[\text{K}^+]_o$ conditions and hyperpolarized in high $[\text{Ca}^{2+}]_o$ conditions. During high $[\text{K}^+]_o$ conditions, changes in $[\text{Ca}^{2+}]_o$ did not affect membrane potential. The synaptic responsiveness of both regular-spiking and fast-spiking neurons was reduced during seizure-like ionic conditions. A reduction in $[\text{Ca}^{2+}]_o$ to 0.6 mM increased failure rates but did not abolish responses. However, an increase in $[\text{K}^+]_o$ to 12 mM abolished postsynaptic responses, which depended on a blockade in axonal spike propagation.

Significance—We conclude that concomitant changes in $[\text{K}^+]_o$ and $[\text{Ca}^{2+}]_o$ observed during seizures contribute largely to the alterations of synaptic neuronal responses and to the decrease in long-range synchrony during neocortical seizures.

Keywords

Cortex; Ions; Epilepsy; Excitability; Synaptic

Cortically generated seizures are accompanied by a simultaneous and dramatic increase in potassium ($[\text{K}^+]_o$) as well as a decrease in calcium ($[\text{Ca}^{2+}]_o$) extracellular ion concentration (Heinemann & Lux, 1977; Heinemann et al., 1977; Pumain et al., 1983; Somjen, 2002). These ionic changes are found commonly in different cortical structures, including neocortex and hippocampus (Heinemann et al., 1977; Avoli et al., 1996; Jensen & Yaari,

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Disclosure

None of the authors has any conflict of interest to disclose.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

1997; Amzica et al., 2002). The $[K^+]_o$ increase reaches 16 mM during 4-aminopyridine-induced epileptiform discharges in the hippocampus (Avoli et al., 1996) and 7–18 mM during spontaneous electrographic seizures in neocortex (Prince et al., 1973; Moody et al., 1974; Amzica et al., 2002). During paroxysmal discharges, the $[Ca^{2+}]_o$ decreases to 0.4–0.7 mM (Heinemann et al., 1977; Pumain et al., 1983; Amzica et al., 2002). The most dramatic alterations in ionic concentrations during paroxysmal discharges take place in the pyramidal cell layer in the hippocampus (Krnjevic et al., 1982; Yaari et al., 1986) and approximately 1 mm from the cortical surface in neocortex (Moody et al., 1974).

An increase in $[K^+]_o$ to 8–16 mM in vitro (Traynelis & Dingledine, 1988) was shown to lead to the generation of paroxysmal activities in the hippocampal formation. However, in vivo experiments have shown that seizures develop when perfusion with high potassium medium occurs for at least 10 min (Zuckermann & Glaser, 1968). In low $[Ca^{2+}]_o$ solutions in hippocampal slices, seizures also develop after tens of minutes (Jefferys & Haas, 1982; Konnerth et al., 1986).

Cortically generated seizures evolve from slow oscillation (Steriade et al., 1998; Boucetta et al., 2008). Similar to seizures, the ionic fluctuations were found also during cortically generated slow oscillation, and they affect synaptic responsiveness. During a depth positive electroencephalography (EEG) wave, the synaptic response increases in parallel with an increase in $[Ca^{2+}]_o$; in contrast, during an EEG depth-negative wave, the synaptic response decreases in parallel with a decrease in $[Ca^{2+}]_o$ (Crochet et al., 2005). Even a slight decrease in $[Ca^{2+}]_o$ from 1.2–1.0 mM reduces the field excitatory postsynaptic potential (fEPSP) amplitude by one third (Somjen, 2002), and may be due to a decrease in synaptic release probability (Zucker & Regehr, 2002; Silver et al., 2003). $[K^+]_o$ also fluctuates during network activities. During brief periods of waking, $[K^+]_o$ is relatively stable (around 3 mM) and remains at this value during depth-positive EEG waves of sleep; however, during depth-negative EEG waves, $[K^+]_o$ increases (approximately 4 mM) (Seigneur et al., 2006).

During the transition from the normal slow oscillation to an electrographic seizure, the steady increase in $[K^+]_o$ is added to the existing oscillatory behavior of $[K^+]_o$ (Amzica & Steriade, 2000). In hippocampal slices, when $[K^+]_o$ was increased above 6.0 mM, the field potential responses in CA1 region were reduced (Poolos et al., 1987), which may be explained by conduction failure in axons of this region (Meeks et al., 2005). Precise measurements of synchrony during these seizures suggest that long-range synchrony analyzed on a wave-by-wave basis is rather weak (Meeren et al., 2002; Timofeev & Steriade, 2004; Derchansky et al., 2006; Boucetta et al., 2008).

Here we hypothesized that alterations in $[K^+]_o$ and $[Ca^{2+}]_o$ during seizures are a sufficient factor altering synaptic neuronal responses and, therefore, synchrony during neocortical seizures.

Methods

Experiments were carried out in accordance with the guideline of the Canadian Council on Animal Care and approved by the Laval University Committee on Ethics and Animal Research. Both the cats and the rats for these experiments were purchased from the reputable animal supplier in good health conditions determined by physical examination.

In vivo preparation

Single and pairs of neurons were recorded intracellularly from the undercut suprasylvian gyrus of cats under ketamine-xylazine (10–15 and 2–3 mg/kg, i.m., respectively) anesthesia, as described previously (Topolnik et al., 2003). The EEG was monitored continuously

during the experiments, and additional doses of the same anesthetic were given at the slightest tendency toward an activated EEG pattern. All pressure points and the tissues to be incised were infiltrated with lidocaine (0.5%). The animals were paralyzed with gallamine triethiodide (20 mg/kg) and artificially ventilated. The end-tidal CO₂ concentration at 3.5–3.8% and electrocardiography (ECG) (acceptable range, 90–110 beats min⁻¹) were monitored. Body temperature was maintained at 37–38°C. The stability of intracellular recordings was reinforced by cisternal drainage, bilateral pneumothorax, and by filling the hole made for recordings with a solution of 4% agar.

In vivo stimulation and recording

A pair of tungsten microelectrodes was inserted in the cortex at an angle of 45 degrees in the vicinity (<0.5 mm) of the recording intracellular micropipette to deliver microstimuli (0.03–0.08 mA, 0.01–0.02 ms). The intracellular recordings were obtained using glass micropipettes filled with 2.5 M potassium acetate (DC resistance, 30–70 MΩ). Local field potentials were recorded by means of a high impedance tungsten electrode positioned near the recorded neurons. Direct current local field potentials were recorded using glass microelectrode filled with 1 M NaCl. All electrical signals were digitized online at a sample rate of 20 kHz.

Slice preparation

Sprague Dawley P21-P30 rats [Charles River Laboratories (CD)] were first anesthetized with ketamine (100 mg/kg) and then with pentobarbital (40 mg/kg) to be decapitated. The brain was quickly dissected and maintained in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 2.8, CaCl₂ 1.2, MgSO₄ 2, NaH₂PO₄ 1.25, NaHCO₃ 26 and D-glucose 10 (Sigma-Aldrich Canada, Canada), pH 7.4, aerated with 95% O₂, 5% CO₂. Osmolarity was 300 ± 5 mOsm. Coronal slices (400 μm) were cut to obtain complete sections containing the somatosensory cortex. The brain slices were transferred into a submerged recording chamber maintained at room temperature, containing the perfusion ACSF at a rate of 4 ml/min. The perfusion solution was identical to the cutting solution. All control recordings were obtained in this solution.

Modified ACSF solution

To replicate ionic conditions found during paroxysmal states, we used modified perfusing solutions with low [Ca²⁺]_o to 0.6 mM and/or high [K⁺]_o concentration to 6.25, 8.0, or 12.0 mM.

In vitro electrophysiologic recordings and stimulation

Neurons in layers II/III were preselected using an infrared differential interference contrast camera-microscopy on an upright microscope. We obtained somatic whole-cell current-clamp recordings (10–20 MΩ access resistances) with patch pipettes (resistance between 3 and 7 MΩ) containing (mM): potassium D-gluconate 130, HEPES 10, KCl 10, MgCl₂ 2, ATP 2 and GTP 2 (Sigma-Aldrich Canada) at pH 7.2 and 280 mOsm. Neurobiotin 0.2% (Vector Laboratories, Inc., Burlingame, CA, U.S.A.) or Lucifer Yellow 0.2% (LY, Sigma Aldrich Canada) was added to the patch solution for following morphologic identification of recorded neurons.

Two tungsten electrodes were placed in layers II/III for extracellular electrical stimulation. Extracellular pulses (0.01–0.02 ms, 0.01–0.15 mA) were given every 5 sec, corresponding to minimal stimulation intensity from which synaptic responses and failures were obtained. To elicit antidromic spikes, the stimulating electrodes were placed in layers V–VI and the intensity of stimuli was set to 0.1–1.5 mA.

All details of methods are described in supplementary materials.

Results

Synaptic responses during electrographic seizures in vivo

In two cats, showing spontaneous electrographic seizures, we used intracellular recordings ($n = 5$) and minimal intensity stimuli of nearby tissue to investigate synaptic responses of cortical neurons during seizures. As we previously reported, 30% of cats anesthetized with ketamine-xylazine developed electrographic seizures (Boucetta et al., 2008). The intensity of stimuli was adjusted to obtain a combination of responses and failures. In agreement with our previous study (Crochet et al., 2005), during slow oscillation prior to seizures, the responses were of variable amplitude (Fig. 1). The failure rate during silent states was 20% (Fig. 1B) and increased to 60% during active network states (not shown). However, during seizures the responses to minimal intensity stimuli were absent, even during silent network states corresponding to wave component of spike-and-wave complexes (Fig. 1C).

This observation suggests that seizure-related factors could affect synaptic release. The change in $[K^+]_o$ and $[Ca^{2+}]_o$ occurring during seizures may contribute to a modification in synaptic responsiveness. Therefore, we investigated in vitro the extent to which seizure-like ionic conditions modify synaptic responses.

Membrane potential alterations to ionic changes in vitro

As we stated in the methods section, our control extracellular solution contained 2.8 mM $[K^+]_o$, 1.2 mM $[Ca^{2+}]_o$, and 2 mM $[Mg^{2+}]_o$. In these conditions, none of the recorded neurons fired spontaneously (Fig. 2) and both regular-spiking (RS) and fast-spiking (FS) neurons had similar membrane potential (RS -67.99 ± 3.87 mV ($n = 27$), FS -69.60 ± 5.49 mV ($n = 14$) (Fig. S1). The membrane potential of recorded neurons was stable in control conditions. When $[K^+]_o$ was increased to 6.25 mM, recorded neurons were depolarized and fired action potentials (Fig. 2A). Upon returning to control extracellular conditions, the membrane potential of both neurons returned to initial values. A similar behavior of membrane potential was observed when both $[K^+]_o$ was increased to 6.25 mM and $[Ca^{2+}]_o$ was reduced to 0.6 mM in ACSF (Fig. 2B). In the population of RS neurons, increasing $[K^+]_o$ to 6.25, 8.0, or 12 mM led to a significant depolarization ($p < 0.05$) of recorded neurons (Fig. 2C). In 12 mM $[K^+]_o$, the firing occurred during the transition period, but 2–3 min after perfusion with 12 mM $[K^+]_o$, we observed a depolarizing block, and no spikes were generated (not shown). As expected, in 2.8 mM K^+ conditions, an increase in $[Ca^{2+}]_o$ to 2.0 mM led to hyperpolarization and a decrease in $[Ca^{2+}]_o$ to 0.6 mM resulted in depolarization of pyramidal cells (Fig. 2C). However, when $[K^+]_o$ was 6.25 mM and higher, the changes in $[Ca^{2+}]_o$ did not affect the membrane potential significantly (Fig. 2C). Therefore, the progressive increase in $[Ca^{2+}]_o$ from 0.6–2.0 mM led to neuronal hyperpolarization only in the condition of 2.8 mM $[K^+]_o$ (Fig. 2). The observed changes in the membrane potential of FS neurons were similar to the changes of RS neuron in all tested conditions (Fig. S1). In high K^+ conditions, the FS neurons fired at high frequencies and the recording rapidly deteriorated; therefore, the majority of FS neurons were not included for statistical analysis.

In the experiments described above, $[Mg^{2+}]_o$ was kept at 2.0 mM. There is no evidence that extracellular Mg^{2+} concentration in interstitial neocortical space is altered during seizures (Somjen, 2004). In some experiments, in order to keep the ratio of divalent ions constant, we also decreased $[Mg^{2+}]_o$ to 0.5 mM. When both $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ were lowered, the change in the membrane potential depended linearly on $[K^+]_o$ (Fig. 2C, left, dashed blue line).

In these experiments, we never observed self-sustained seizure-like activity. However, in 7 of 90 investigated slices kept in ACSF containing 6.25–12 mM $[K^+]$ and 0.6 $[Ca^{2+}]$ for >20 min, neurons generated paroxysmal depolarizing shifts (PDSs) during or upon return to control conditions (Fig. 2B, inset). In two simultaneous dual whole-cell recordings, we observed that PDSs started in some preferential place and propagated within local networks (Fig. 2B), demonstrating that it was a network and not a single-neuron phenomenon.

Synaptic responses to minimal intensity stimulation

To investigate an alteration in synaptic responsiveness induced by seizure-like ACSF composition, we used microstimulation (see Methods section). Initially we estimated the maximal frequency of stimulation that does not induce changes in response. We found that if stimuli were applied once every 5 s, the response of the cortical neuron was variable in amplitude from 0–2.5 mV and the majority of responses were <1 mV. The histogram of responses was usually bimodal, with one peak corresponding to responses and another peak corresponding to failures. The noise had a Gaussian distribution centered on 0 mV and the confident interval at 95% was ± 0.3 mV. The failures had a similar distribution as noise (Fig. S2B). Because of synaptic failures and due to the presence of noise, some measurements obtained after a stimulus had negative values. The observed variability in response amplitude did not change over very long periods of recordings (Fig. S2) and it was similar with lower frequency (one every 10 s) stimulation. With higher frequency of stimuli (2.5 Hz), we observed either synaptic facilitation or depression for different neurons (not shown).

During high $[K^+]_o$ and low $[Ca^{2+}]_o$, the mean response amplitudes were reduced or the response was abolished. The decrease in mean amplitude could occur due to (1) an increase in failure rates and/or (2) a decrease in response amplitude. We found that the failure of synaptic responses was the primary cause of decrease in the mean response amplitude. In control conditions, the failure rate was around 25% [23.3% for RS ($n = 27$) and 26.3% for FS ($n = 14$), Fig. 3]. A decrease in $[Ca^{2+}]_o$ to 0.6 mM increased the failure rate to about 55% (RS $n = 7$, FS $n = 3$, Fig. 3C), which was similar to that in 0.5 or 2.0 mM Mg^{2+} conditions (not shown). An increase in $[K^+]_o$ to 6.25 mM did not increase failure rates significantly, and did not affect large-amplitude responses, although RS neurons showed a tendency to increased failure rates in these conditions. The failure rates were significantly increased in 8.0 and 12 mM K^+ conditions (Fig. 3C). When $[Ca^{2+}]_o$ was 0.6 mM and $[K^+]_o$ was 12 mM the failure rates were close to 100%.

Ca^{2+} is the primary ion responsible for presynaptic mediator release, which easily explains the increase in failure rates in low Ca^{2+} conditions. The role of K^+ in the increase in failures of synaptic responses was less clear. We hypothesized that high $[K^+]_o$ depolarizes axons to the state of depolarizing block, and action potentials do not propagate through the axons.

Axonal responses in different ionic conditions

To test the hypothesis that axonal conductance is impaired in high K^+ conditions, we initially performed dual-patch experiments from single neurons having one patch pipette in the soma and another pipette in the axon. Unfortunately, using thick slices from adult animals we were unable to see axons further than 20–25 μm from the soma. Therefore, the second pipette was placed on axonal initial segment within first 15–20 μm from the soma ($n = 5$, Fig. 4B) for juxtacellular recording. In all occasions, an increase in $[K^+]_o$ to 12 mM induced a remarkable (around 60%) decrease in spike amplitude in both somatic and periaxonal recordings, and we did not encounter failures of spike propagation in the initial segment. Therefore, we used an alternative experimental paradigm. We placed stimulating electrodes in layers IV/V to elicit antidromic action potentials (Fig 4A). The antidromic spikes started directly from the baseline and had stable and short latencies. Antidromic

spikes followed high-frequency stimuli (usually 50 Hz) and failed during the collision test (Lipski, 1981). When 8 mM K^+ (four neurons) or 12 mM K^+ (5 neurons) was applied to the bath, the antidromic spikes were not recorded in the soma (Fig. 4A) regardless of the membrane potential of the recorded neuron. This suggests a block in axonal conductance by 8 mM and higher $[K^+]_o$. When 6.25 mM $[K^+]_o$ was used, we observed only a slight increase in failure of antidromic spike propagation. These experiments demonstrate that the increase in $[K^+]_o$ above 6.25 mM produces a block of axonal conductance.

Discussion

In this study, we demonstrated that simple changes in extracellular ion concentration reproduce major alterations in synaptic responses during seizures. (1) We show here that an increase in $[K^+]_o$ or decrease in $[Ca^{2+}]_o$ led to neuronal depolarization [as reviewed in (Somjen, 2002)]. (2) In high K^+ conditions (above 6.25 mM), changes in $[Ca^{2+}]_o$ did not influence membrane potential and in low Ca^{2+} conditions, changes in $[K^+]_o$ had only a moderate influence on the membrane potential. (3) A reduction of $[Ca^{2+}]_o$ to 0.6 mM doubled failure rates of synaptic transmission. (4) An increase in $[K^+]_o$ to 8.0 mM or higher increased the failures of axonal conductance. (5) Similar to in vivo observations, the synaptic responses were absent in a condition of 0.6 mM $[Ca^{2+}]_o$ and 12 mM $[K^+]_o$ in vitro.

Ionic changes and synchronization during seizures

A large number of intrinsic currents, chloride-dependent inhibitory events mediate PDS, the main depolarizing event accompanying spike and wave complexes of seizures, and only to minor extent is it mediated by excitatory synaptic events (Timofeev et al., 2002b, 2004; Timofeev & Steriade, 2004). In cerebrospinal fluid, the normal level of $[K^+]_o$ is between 2.7 and 3.5 mM and the normal level of $[Ca^{2+}]_o$ is between 1.0 and 1.5 mM [reviewed in (Somjen, 2002)]. As we described in the introduction, during paroxysmal activities, there is an increase in $[K^+]_o$ and a decrease in $[Ca^{2+}]_o$. These changes affect the dynamics of intrinsic currents mediated by these ions (Timofeev et al., 2002a). Our results demonstrate that these changes reduce the efficacy of synaptic interactions and, therefore, may play a role in network synchronization during seizures.

During seizures, the long-range synchrony of the cortical network is impaired (see introduction). Synaptic responses are decreased for minutes after the end of seizure (Nita et al., 2008). On the other hand, seizures are associated with large-amplitude field potentials generated from nearly simultaneously occurring depolarizing and hyperpolarizing events in a large number of neighboring cells (Lopes da Silva & Van Rotterdam, 2005). The synchronization results from the following major types of interactions between brain cells (neurons and glia). (1) Chemical synaptic transmission takes place when the presynaptic neuron fires an action potential and invades the nerve terminal, allowing Ca^{2+} entry into the presynaptic terminal. This triggers the neurotransmitter release, causing depolarization or hyperpolarization of the postsynaptic neuron (Eccles, 1964; Katz & Miledi, 1968). (2) The cells interact via electrical synapses mediated by gap junctions (Bennett, 1997; Galarreta & Hestrin, 2001). (3) A third type of interaction is ephaptic, which is mediated by large extracellular currents and potential fields generated by cortical structures in the absence of specialized contacts (Taylor & Dudek, 1982). Some authors separate ephaptic transmission from field-effect interactions (Jefferys, 1995). (4) A last type of interaction is achieved by alteration in the extracellular ionic balance caused by activity of brain cells (Somjen, 2002; Crochet et al., 2005). We showed here that synaptic responses during cortical seizures and in seizure-like ionic conditions are largely impaired.

All these interactions (1–4) play a role in local synchrony, but only chemical synaptic transmission can effectively synchronize the network over long distances. The chemical synaptic interactions become inefficient because the intra-cortical axonal conductance is blocked in high $[K^+]_o$ as reached during seizures. This phenomenon is enhanced by a decrease in $[Ca^{2+}]_o$ that reduces the synaptic release of neurotransmitter. This is likely a sufficient factor to reduce long-range synchronization during seizures.

A network of inhibitory interneurons in the neocortex is connected by electrical synapses (Galarreta & Hestrin, 1999; Gibson et al., 1999). Glial cells are also connected via gap junctions (Giaume & McCarthy, 1996; Giaume et al., 2010). Low levels of $[Ca^{2+}]_o$ (below 1.4 mM) promote opening of hemichannels, forming electrical synapses (Thimm et al., 2005) that might increase local synchronization (Perez Velazquez & Carlen, 2000). Gap junctions work as a low-pass filter with a coupling coefficient near 100% for frequencies below 2–3 Hz (Galarreta & Hestrin, 2001), which can efficiently synchronize the cortical network. Paroxysmal discharges during seizures are accompanied with the generation of high-amplitude EEG signals, suggesting high local synchrony. However, long-range synchrony during cortically generated seizures is largely impaired (Meeren et al., 2002; Derchansky et al., 2006; Boucetta et al., 2008). Activities of inhibitory neurons effectively depolarize postsynaptic neurons during spike and wave components of seizures (Timofeev et al., 2002b). A pharmacologic block of gap junctions with halothane stopped the generation of recurrent electrographic seizures (Grenier et al., 2003), suggesting that similar to other types of oscillations, the inhibitory interneurons (Lytton & Sejnowski, 1991; Whittington et al., 1995; Somogyi et al., 1998), and glial cells (Fellin et al., 2009; Halassa et al., 2009) play a critical role in synchronization during seizure activity.

Possible role of ions in the onset and termination of seizures

Do ionic changes play a role in the onset and/or termination of seizures? The current study as well as previous reports (Zuckermann & Glaser, 1968; Jefferys & Haas, 1982; Konnerth et al., 1986) demonstrate that paroxysmal activities can develop when $[K^+]_o$ is increased for tens of minutes. In the case when the $[K^+]_o$ was high and $[Ca^{2+}]_o$ was low, paroxysmal activities developed within minutes. This is sufficient time to alter the synthesis and/or the membrane insertion of K^+ channels (Hille, 2001). At least a part of this process might be Ca^{2+} dependent. In addition, an altered regulation of some K^+ channels may lead to seizure generation. Chronic changes in ion metabolism may contribute to epileptogenesis, and some acute changes in ion concentration lasting from minutes to hours may lead to an increase in likelihood of seizure generation.

Several recent reviews described a number of possible mechanisms of seizure termination (Timofeev & Steriade, 2004; Lado & Moshé, 2008). In light of this study, we propose an additional mechanism. A small increase in $[K^+]_o$ and decrease in $[Ca^{2+}]_o$ will reduce the efficacy of chemical synapses. However, given that (1) the neurons discharge more (2) the membrane potential of neurons is closer to firing threshold, and (3) the electrical coupling increases the propensity for seizure development. A major change in the extracellular ion concentration (12 mM of K^+ and 0.6 mM of Ca^{2+}), in turn, will lead to a strong depolarization of neurons, often reaching a state of depolarizing block, a block of axon conductances that altogether prevent synaptic communication between neurons. Moreover, the increased activation of intrinsic hyperpolarizing currents (Na^+ - and Ca^{2+} -activated K^+ currents) would outweigh the synchronizing effects achieved via electrical synapses that blocks the development of seizures (Timofeev & Steriade, 2004).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Synaptic responses during silent periods of slow oscillation and electrographic seizure in anesthetized cat in vivo. **(A)** Simultaneous field potential and intracellular recordings during slow oscillation and electrographic seizure. Small negative deflections in intracellular trace are artifacts of electrical stimuli. **B** and **C** upper panels show expanded periods from panel **A** as indicated by arrows; lower panels show superposition of 30 traces showing responses to stimuli applied during silent periods of slow oscillation (**B**) and silent periods of seizure (**C**). *Epilepsia* ILAE

Figure 2.

Responses of neocortical neurons on changes in $[K^+]_o$, $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ in ACSF solution in vitro. (A) Two simultaneously recorded pyramidal neurons separated by 80 μ m from layer III were depolarized in the presence of 1.2 mM Ca^{2+} and 6.25 mM K^+ . Arrows indicate wash-in and washout of modified solution. (B) Similar experiment but 0.6 mM $[Ca^{2+}]_o$. Paroxysmal depolarizing shifts (PDSs) appear spontaneously upon recovery to control ACSF. The left and right parts of the panel B are separated by 20 min. PDSs from the traces are expanded as indicated by arrow. (C) Membrane potential changes resulting from a change in $[K^+]_o$ (left) and $[Ca^{2+}]_o$ (right). Solid lines for solutions containing 2.0 mM of magnesium. Dotted lines for solutions containing 0.5 mM of magnesium.

Epilepsia ILAE

Figure 3.

Different ionic conditions modulate efficiency of synaptic responses. Synaptic responses to ionic changes in a RS neuron **A** and FS neuron **B**. Averaged EPSPs responses ($n = 100$ each) are shown in the top panels. Tested ionic concentrations are indicated on the top of each panel. The middle panels show the histograms of synaptic responses and noise in the same neurons in the different ionic conditions. The bottom panels show the amplitude variation of all synaptic responses in the different ionic conditions from the neurons shown in the upper panels. The intensity stimuli have been adjusted in control solution to obtain a failure rate close to 20% (16% for **A** and 27% for **B**). (**C**) Mean failure rates of synaptic responses for all recorded neurons.

Epilepsia ILAE

Figure 4.

Axonal spike conductance impairment in high K^+ conditions. **(A)** High-intensity cortical stimulation induces EPSPs, and orthodromic and antidromic spikes as indicated by small arrows. Early responses of middle left panel are indicated at leftmost panel as indicated by large arrow. Antidromic spikes follow high-frequency trains with stable amplitude and fail during a collision test (see arrow in middle right panel). Both antidromic spikes and synaptic responses were completely abolished in 12.0 mM K^+ . **(B)** Whole-cell recording from soma and juxtacellular recording from axon initial segment (approximately 15 μ m from soma) in 2.8 mM K^+ (left panel) and in 12.0 mM (right panel). Action potentials were elicited by depolarizing current injected to soma.

Epilepsia ILAE