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Characterization of spontaneous recurrent epileptiform discharges in hippocampal–entorhinal cortical slices prepared from chronic epileptic animals

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ABSTRACT

Epilepsy, a common neurological disorder, is characterized by the occurrence of spontaneous recurrent epileptiform discharges (SREDs). Acquired epilepsy is associated with long-term neuronal plasticity changes in the hippocampus resulting in the expression of spontaneous recurrent seizures. The purpose of this study is to evaluate and characterize endogenous epileptiform activity in hippocampal–entorhinal cortical (HEC) slices from epileptic animals. This study employed HEC slices isolated from a large series of control and epileptic animals to evaluate and compare the presence, degree and localization of endogenous SREDs using extracellular and whole cell current clamp recordings. Animals were made epileptic using the pilocarpine model of epilepsy. Extracellular field potentials were recorded simultaneously from areas CA1, CA3, dentate gyrus, and entorhinal cortex and whole cell current clamp recordings were obtained from CA3 neurons. All regions from epileptic HEC slices ($n = 53$) expressed SREDs, with an average frequency of 1.3 Hz. In contrast, control slices ($n = 24$) did not manifest any SREDs. Epileptic HEC slices demonstrated slow and fast firing patterns of SREDs. Whole cell current clamp recordings from epileptic HEC slices showed that CA3 neurons exhibited paroxysmal depolarizing shifts associated with these SREDs. To our knowledge this is the first significant demonstration of endogenous SREDs in a large series of HEC slices from epileptic animals in comparison to controls. Epileptiform discharges were found to propagate around hippocampal circuits. HEC slices from epileptic animals that manifest SREDs provide a novel model to study in vitro seizure activity in tissue prepared from epileptic animals.

1. Introduction

Epilepsy is a common neurological disorder affecting over 1% of the adult population[1] and is associated with long lasting neuronal plasticity changes that have been well documented in humans and replicated in a variety of animal models.[2,3] To localize the focus of seizure onset, the identification of localized interictal or ictal spike discharges (ISD) has been employed. The mechanism underlying ictal and interictal activity involves an interplay of membrane properties intrinsic to epileptic neurons and a balance between local inhibitory and excitatory synaptic activity, however the progression from interictal to ictal events is not entirely clear.[4] Because epileptic brain tissue is unique and manifests long lasting alterations it has been recommended that models of epileptic tissue be employed for testing anticonvulsant agents and studying the mechanisms of epileptogenesis.[5] Thus, it is important to develop in vitro models of endogenous spontaneous recurrent epileptiform discharges (SREDs) derived from epileptic brain tissue.

The pilocarpine model of acquired epilepsy (AE) in the rat manifests spontaneous recurrent seizures (SRSs), epileptiform discharges, and continuous expression of ISDs.[6] The seizures in this model of epilepsy resemble complex partial seizures in humans[7] and this model has been well established as a model of AE.[8] Following pilocarpine induced status epilepticus (SE), animals develop recurrent electrographic seizures with associated behavioral manifestations of SRSs.[6,9] The hippocampus has been noted to originate or enhance complex partial seizures.[10] The majority of studies on hippocampal slices have produced SREDs using

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electrical stimulation techniques,[11–19] pharmacological interventions, such direct application of pilocarpine or 4-aminopyridine to the slice[20–22] or removal of Mg^{2+} from the bath.[23,24] However, it would be very important to evaluate endogenous hippocampal epileptiform activity in hippocampal–entorhinal cortical (HEC) slices prepared from epileptic animals. To our knowledge there have been no controlled studies evaluating prolonged endogenous epileptiform activity in isolated HEC slices from a large series of epileptic animals. If HEC slices from epileptic animals consistently manifested endogenous SREDs in vitro, this would provide a novel and useful model to study AE.

The present study was initiated to evaluate the degree and localization of endogenous hippocampal SREDs in HEC slice preparations from a larger series of control and epileptic animals employing established electrophysiological procedures.[15] The in vitro HEC slice preparation has the advantage of retaining a closed hippocampal–parahippocampal circuit loop with intact synaptic organization.[15] Long-term electrophysiological studies were conducted on HEC slices in order to perform simultaneous extracellular and whole cell current clamp recordings throughout the hippocampus and to characterize the expression of epileptiform discharges in this chronic seizure model. Simultaneous recordings were obtained from the CA3, CA1 regions, granule cell layer of the dentate gyrus (DG), and the entorhinal cortex (EC). The results demonstrate that SREDs were expressed throughout the hippocampal–parahippocampal circuit in HEC slices from epileptic but not control animals. These studies indicate that the HEC slice preparation from animals manifesting pilocarpine-induced AE provide a unique resource to study endogenous SREDs in vitro and provide a novel model for the testing and development of novel antiepileptic agents.

2. Materials and methods

All the drugs and reagents were purchased from Sigma Chemical Co (St. Louis, MO) unless otherwise noted.

2.1. Pilocarpine treatment

The pilocarpine model of AE routinely used in our laboratory[25,26] is a modification of the protocol developed from the Cavalheiro's group.[6] It uses an episode of pilocarpine-induced SE to induce epilepsy in rats. All treatments used were approved and are in accordance with the National Institutes of Health guide for the care and use of laboratory animals and approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee, and designed to minimize pain to the animals (IACUC approved protocol# AM10147 and AM10039). Adult male Sprague Dawley rats (Harlan, Fredrick, MD) received an initial scopolamine methyl nitrate (1 mg/kg, i.p.) injection to minimize the peripheral effects of pilocarpine. Thirty minutes after scopolamine administration, rats were injected with pilocarpine (350 mg/kg, i.p.). Typically the onset of SE was within 20–40 min following pilocarpine injection. One hour after the onset of SE, diazepam (4 mg/kg, i.p., in 50% propylene glycol) was administered to control seizure activity.[9] Paired controls received the same injection protocol as the experimental group, except that injections contained saline in place of pilocarpine. Animals were video monitored beginning 2 weeks post-SE for the occurrence of SRSs and monitoring was continued for 8 weeks. The resultant seizures were scored on a scale from 1 to 5, using the Racine criteria for seizure evaluation.[27] Pilocarpine-treated animals were considered epileptic after at least two class 3 or greater SRSs were observed. For experiments evaluating the contribution of the NMDA receptor on the development of AE and the presence of SREDs in the HEC slices, MK-801 (4 mg/kg; i.p.) was injected 20 min prior pilocarpine injection.[9,28]

2.2. Slice preparation for electrophysiology

HEC slices were prepared as described previously.[15] Briefly, rats were decapitated following halothane anesthesia. The whole brain was removed and placed in cold, oxygenated sucrose based artificial cerebrospinal fluid (ACSF) containing in mM: 200 sucrose, 3 KCl, 1.25 Na_2PO_4, 26 $NaHCO_3$, 10 glucose, 0.9 $MgCl_2$, and 2 $CaCl_2$. By using this equiosmolar substitution of NaCl with sucrose we observed an increased viability of the slices.[15] Following a 2 min incubation each hemisphere of the brain was individually blocked and sectioned (450 μm thickness) with a vibratome (Lancet 1000, St Louis, MO) along a 12° inclined transverse plane.[29] Each slice was further dissected manually with a scalpel to isolate the hippocampus with adjoining entorhinal and temporal cortices.[15] Slices employed corresponded approximately to the range of bregma −6.60 to −4.60 mm stereotaxic coordinates.[30] These HEC slices were then incubated for 1–2 h in ACSF containing in mM: 130 NaCl, 3 KCl, 1.25 Na_2PO_4, 26 $NaHCO_3$, 10 glucose, 0.5 $MgCl_2$, and 2 $CaCl_2$; bubbled with 95% O_2–5% CO_2 and warmed to 33 °C. In this study we employed a 0.5 mM $MgCl_2$ concentration for our data collection after demonstrating that it gave the same outcome as the 1 mM concentration, but offered a much more rapid recording period. Using 0.5 mM $MgCl_2$ concentrations resulted in more frequent epileptiform discharges in all of the pilocarpine animal slices with no epileptiform discharges in slices from the control group. Using higher $MgCl_2$ concentrations up to 1.0 mM resulted in less frequent epileptiform discharges in the pilocarpine slices, requiring much longer recording times and no activity in the control group. However, neither concentration of $MgCl_2$ changed the outcome of the experiment. This incubation media has been noted to promote polysynaptic interactions in HEC slices and lower $MgCl_2$ recording solutions have been routinely employed in many laboratories to maintain hippocampal slices in vitro for electrophysiological studies.[31,32] Following this recovery incubation, HEC slices were placed in an interface-type chamber (Medical Systems, Greenvale, NY) and perfused with warmed (35 °C) ACSF at 0.5–1.0 ml/min for electrophysiological recording experiments.

2.3. Extracellular recording

Extracellular field potentials were recorded as described previously[15] with use of insulated tungsten electrodes (A-M Systems Inc, Everett, WA). Briefly, simultaneous recordings were carried out throughout the hippocampal–parahippocampal circuit by placing electrodes in the pyramidal cell body layer of area CA1, CA3, DG and EC. This technique allowed for the controlled observation of discharges in all major regions of the HEC slices and provided a model that can observe the initiation and propagation of discharges around the hippocampal circuits. Amplified signals were digitized with a PCM device at 8 kHz per channel (NeuroData Instruments, New York, NY) and recorded onto videotape with playback on an AstroMed Dash IV chart recorder (AstroMed-Grass Inc., West Warwick, RI). Additional analysis was carried out with pClamp software (Molecular Devices, Foster City, CA). Initial assessment of slice viability was accomplished with single stimulus pulses of minimal voltage to Schaeffer collaterals in order to acquire an orthodromic population spike of 0.5 mV in amplitude. Once a slice was accepted as being viable, extracellular electrophysiological recordings were carried out for the occurrence of endogenous SREDs.

2.4. Whole cell current clamp recordings

While recording extracellular field potentials, we simultaneously obtained whole cell current clamp recordings from CA3 neurons within the HEC slices using the "blind slice patch"

technique.[33] Patch electrodes of 2–4 mΩ in resistance were pulled on a Brown-Flaming P-80C electrode puller (Sutter Instruments, Novato, CA) and filled with electrode solution. The electrode solution composition consists of (in mM) 140 K^+ gluconate, 1 $MgCl_2$, and 10 Na-HEPES, pH 7.2, osmolarity adjusted to 310 ± 5 mOsm with sucrose. Recordings were made in whole cell current-clamp recording mode with an Axopatch-1A amplifier (Molecular Devices, Foster City, CA) and recorded on videotape as stated above.

2.5. Intracellular staining and histological techniques

Pyramidal cells undergoing whole cell current clamp recordings in region CA3 exhibiting epileptiform discharges were stained with biocytin during recordings to display their morphology. Staining was performed with tips of the glass electrodes filled with a solution of 2% biocytin dissolved in 2 M potassium acetate.[34] These neurons were filled with biocytin for 15–30 min. For light microscopy slices were embedded in 3% agar and re-sectioned on a vibratome (100 μM thickness). The slices containing biocytin filled cells were processed by immersion-fixing the tissue overnight at 4 °C with 100 mM phosphate-buffered solution (PBS, pH 7.4) containing 1% paraformaldehyde and 2.5% glutaraldehyde. Sections were then rinsed several times with 100 mM PBS and then transferred to phosphate-buffered 3% H_2O_2 for 30 min. Slice were rinsed again several times in 100 mM PBS and processed in ABC solution (1:25 Vectastain Elite). Additional rinses were conducted with PBS followed by air-drying for 1 h and eventual counter stain with Nissl solution for 15 s. After this reaction, slices were dehydrated through an ascending series of ethanol and finally embedded in Hypermount (Life Sciences) and analyzed with a Zeiss inverted microscope. This technique allowed us to morphologically identify the neurons subjected to whole cell current clamp recording studies.

2.6. Data analysis

For creation of the histogram of SRED frequencies, SigmaPlot 8.02 was used (SPSS Inc., Chicago, IL).

3. Results

3.1. Characterization of endogenous SREDs in HEC slices from control and epileptic animals

This study was initiated to evaluate and characterize the presence or absence of endogenous SREDs in HEC slices prepared from control and chronic epileptic animals. None of the control animals demonstrated SRSs. Approximately 70% of pilocarpine treated animals developed SRS. Clinical seizures appeared 10–14 days after SE. Animals were used for electrophysiology experiments 14-weeks after SE.

Simultaneous extracellular recordings were taken from the CA3, CA1, DG, and EC for a minimum of 30 min from each slice. None of the HEC slices from control animals ($n = 24$) manifested SREDs. Conversely, all HEC slices from epileptic animals ($n = 53$) manifested SREDs. With ACSF perfusion, epileptiform activity was recorded immediately upon placement of recording electrodes in HEC slices from epileptic animals. Quantification of the frequency of epileptiform discharges from epileptic HEC slices revealed that the mean frequency of SREDs in all HEC slices from 53 epileptic animals was 1.3 Hz.

A representative recording from a control HEC slice is shown in Fig. 1 indicating the absence of epileptiform activity from any of the recorded regions. In some control preparations, HEC slices were monitored for more than an hour without the presence of any

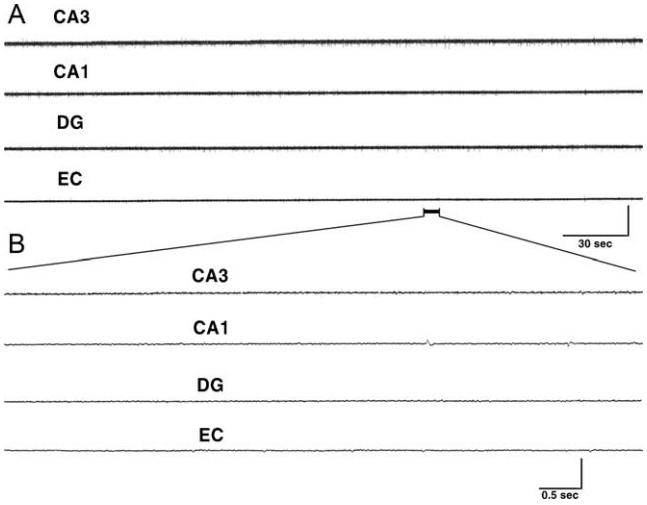

Fig. 1. Extracellular recordings from a control HEC slice. (A) Simultaneous recordings were taken from the CA3, CA1, DG, and EC. No SREDs were observed in any of the recorded regions of HEC slice. (B) Section of the playback (indicated by bar) enlarged at a faster speed. Calibration: 1.0 mV CA3, 0.5 mV CA1, and DG.

SREDs. HEC slices from epileptic animals consistently and reliably expressed epileptiform activity that was synchronized within all recorded regions (Figs. 2 and 3). Two major variants of SREDs were observed: a slow and a rapid rate. A recording from a typical HEC slice from an epileptic animal with the slow SRED pattern is shown in Fig. 2. The spontaneous population spike activity in HEC slices from pilocarpine treated animals was observed to have a polyspiking pattern that was observed to occur in all recorded regions of the epileptic HEC slice. This pattern was more complex in the CA3 region as compared to the other recorded regions. The slower SRED variant was found to have an average frequency of 0.25 Hz. The amplitude of these population spikes was smaller in the EC than in the other regions. However, the amplitudes of activity in the DG, CA3, and CA1 regions were equivalent. This spiking activity continued unabated in all recorded regions for recording periods of greater than 30 min. A recording from a typical HEC slice from an epileptic animal with the fast SRED variant is shown in Fig. 3. The faster frequency of epileptiform

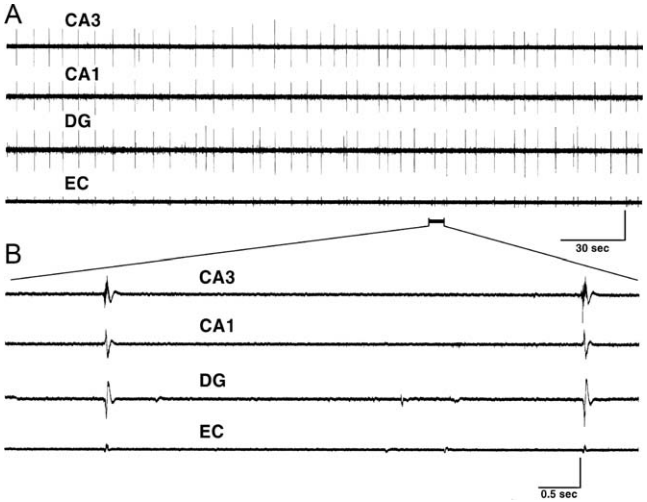

Fig. 2. Extracellular recordings from a HEC slice from epileptic rat displayed SREDs in vitro. (A) Simultaneous extracellular recordings taken from the CA3, CA1, DG, and EC. SREDs are synchronized in all recorded regions of HEC slice. This type of SRED activity constituted the first or the slow-type (0.25 Hz) of SREDs. (B) Section of the playback (indicated by bar) is enlarged at a faster speed. Calibration: 1.0 mV CA3, 0.5 mV CA1, and DG.

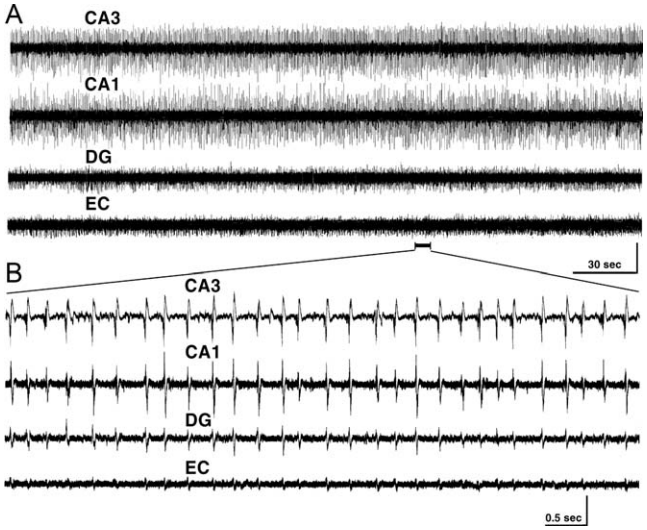

Fig. 3. Second or the fast-type (3.2 Hz) variant of SREDs in HEC slices from epileptic rats. (A) Simultaneous extracellular recordings taken from the CA3, CA1, DG, and EC. SREDs are synchronized in all recorded regions of HEC slice. (B) Section of the playback (indicated by bar) is enlarged at a faster speed. Calibration: 1.0 mV CA3, 0.5 mV CA1, and DG.

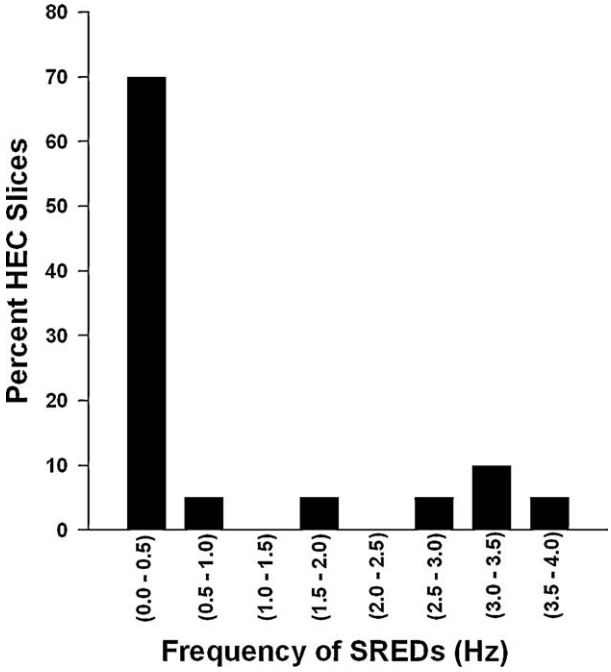

Fig. 4. Frequency distribution of SREDs in HEC slices from epileptic rats. Cumulative frequency histogram plotting the distribution of SRED frequency recorded for >30 min. Two major types of SRED frequencies are noted. The first category averaged a frequency of 0.32 Hz, with 80% of the HEC slices demonstrating a SRED frequency of less than 2.0 Hz. The second group (20% of the HEC slices) expressed an average SRED frequency of 3.2 Hz.

activity pattern was found to have an average frequency of 3.2 Hz. The spontaneous epileptiform discharges of this pattern had a more complex spiking pattern in each of the recorded regions as compared to the slow SRED variant. Additionally, the discharges from the CA3 and CA1 regions manifested larger amplitudes as compared to both DG and EC. In this subset of epileptiform discharges the EC also had the smallest amplitude. In comparison to the slow variant this spontaneous activity was also generalized throughout the hippocampal–parahippocampal circuit with each of the recorded regions having a synchronous pattern of activity. These epileptiform discharges also continued unaltered for the life of the slice in the monitoring chamber (as long as 4 h).

We characterized the frequency of SREDs in the 53 epileptic animals evaluated. The overall epileptiform activity was found to have a double peak distribution in frequency. The histogram in Fig. 4 shows the breakdown of epileptic HEC slices exhibiting SREDs over a range of various frequencies. Of the 53 epileptic HEC slices sampled, approximately 80% ($n = 43$) were noted to exhibit SREDs at a frequency less than 2.0 Hz, with bursting patterns corresponding to the slow SRED variant. The remaining 20% ($n = 10$) of HEC slices exhibited SRED frequencies greater than 2.0 Hz, with bursting patterns corresponding to the fast SRED variant. This property of variance in frequency and duration of interictal epileptiform discharges has also been noted with direct application of pilocarpine to hippocampal slices.[21] Rats subjected to the kindling paradigm have also been noted to display epileptiform discharges with variable frequencies in hippocampal slices.[22] To our knowledge this data provides direct evidence from a large series of epileptic animals that the endogenous SREDs observed in HEC slices obtained from epileptic animals are also observed to present in both slow or fast frequency patterns.

3.2. NMDA receptor involvement in the induction of SREDs

Previous studies in our laboratory have demonstrated that blocking the NMDA receptor with MK-801 prior to pilocarpine injection prevented epileptogenesis in rats.[9,28] Therefore, for this study, we wanted to evaluate the effect of NMDA receptor antagonism on blocking the development of endogenous SREDs in HEC slices prepared from MK-801 and pilocarpine-treated animals. MK-801 was administered 20 min prior to pilocarpine. SE was observed in the MK-801-treated animals and was treated with diazepam as described in the Methods. Animals were monitored for the presence of SRSs to determine the development of AE. In agreement with our previous observations,[9,28] 84% of the animals treated with pilocarpine alone developed AE, whereas none of the animals treated with MK-801 and pilocarpine developed AE. None of the sham controls developed AE. All of the HEC slices prepared from pilocarpine treated epileptic animals manifested SREDs, whereas none of the HEC slices from the animals treated with MK-801 and pilocarpine had SREDs. These results provide a direct correlation with the development of SRSs in epileptic animals and the appearance of SREDs in HEC slices from epileptic animals, demonstrating that the appearances of both SRSs and SREDs in epileptic animals in the pilocarpine model of AE are mediated by NMDA receptor activation during SE and epileptogenesis.

3.3. Whole cell current clamp recordings of SREDs from CA3 neurons in HEC slices

Whole cell current clamp recordings were conducted to identify the cellular correlates of the epileptiform discharges from pyramidal cells in the region CA3 with simultaneous extracellular recordings from CA3, CA1 and DG in HEC slices from epileptic rats. CA3 pyramidal cells were identified first electrophysiologically as having an intracellular firing pattern of broad action potentials[35] and then morphologically by injection with biocytin following the recording of epileptiform activity. A representative neuron from the CA3 region is portrayed with its corresponding whole cell current clamp recording (Fig. 5A and B). The electrode was filled with biocytin to allow simultaneous staining and recording. This technique allowed us to identify individual neurons recorded with whole cell current clamp methods.

Within the first type of HEC slices, exhibiting the slower 0.25 Hz firing pattern, CA3 pyramidal neurons were noted to express

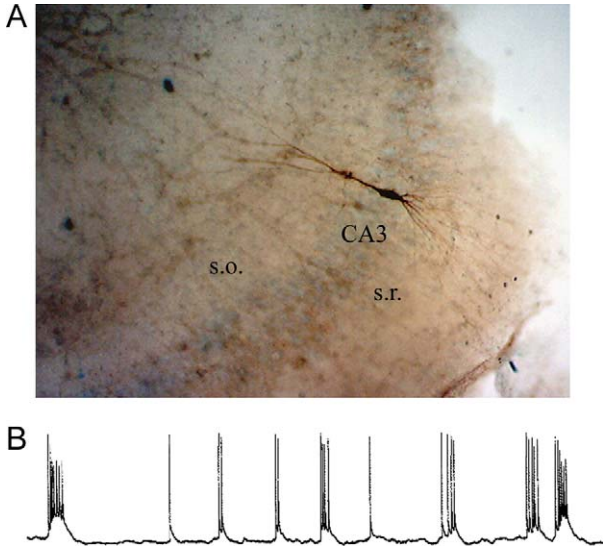

Fig. 5. Morphological characteristics of biocytin-filled CA3 pyramidal neuron with corresponding electrophysiological properties. (A) A representative example of pyramidal neuron in area CA3 is illustrated showing morphology of cell recorded in a slice from an epileptic animal. Note numerous dendritic branches with characteristic arborization of the axon typical of pyramidal cells. s.r. represents stratus radiatum and s.o. represents stratus oriens. (B) Whole cell current clamp recording again displaying the cyclical pattern of action potentials during expression of SREDs (3.2 Hz). All data are obtained from the same neuron.

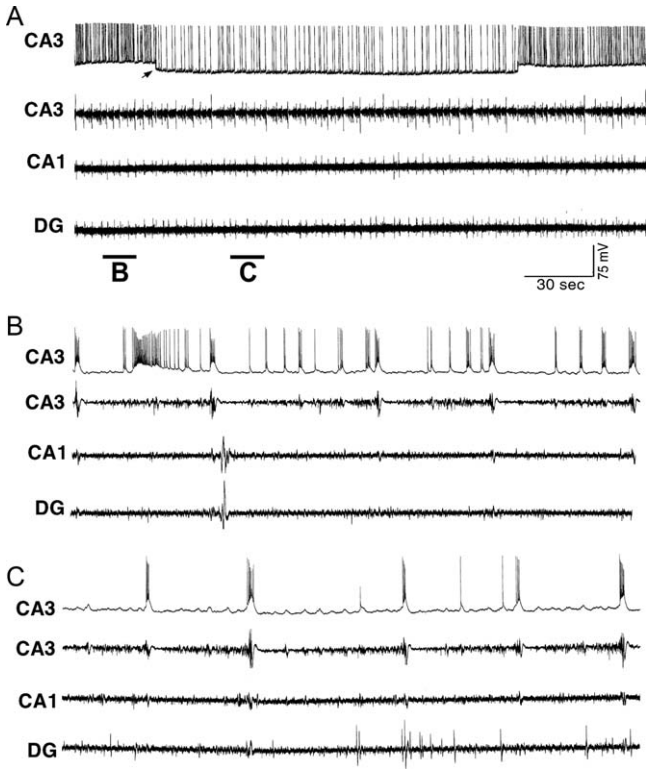

Fig. 6. (A) Simultaneous whole cell current clamp recordings in area CA3 (top trace) and multiple site extracellular field potential recordings of SREDs (0.25 Hz). The membrane potential was hyperpolarized from −68 to −98 (arrow) to reveal the underlying excitatory waves. There was no change in frequency in the corresponding extracellular burstings. Segments of the playback (indicated by bars) are enlarged at a faster speed in panels B and C. (B) Faster playback of intracellular and extracellular recording segments labeled B. CA3 patch recordings (top trace) illustrate the progression of increasing intensity of epileptiform bursts leading to onset of interictal event with synchronized extracellular recordings. Calibration: intracellular 75 mV. C. Faster playback of whole cell current clamp and extracellular recording segments labeled C. CA3 neuron bursting pattern during hyperpolarized membrane potential to illustrate gradual increase of depolarizing waves culminating into a PDS burst that corresponds to the extracellular interictal spike.

robust paroxysmal depolarizing shifts (PDS) as the cellular contributions to burst activity (Fig. 6A). A segment of the recordings shown in Fig. 6A was expanded for further evaluation (Fig. 6B and C). The expanded scale of these intracellular recordings illustrates the spontaneous activity that initially presents with a depolarization with a minimal number of spikes and then gradually progresses to a larger depolarization wave with multiple spikes. On several occasions a prolonged PDS was observed (Fig. 6B). This spontaneous activity was followed by a brief quiescent phase before the expression of the next PDS. The large-wave PDSs were noted to be synchronous with the extracellular recordings of epileptiform discharges throughout the HEC slices. Using the whole cell current clamp technique it was possible to hyperpolarize the membrane potential of the cell being recorded from the slice. Hyperpolarization of the membrane potential from −68 to −98 mV (Fig. 6A, arrow) caused a drastic reduction in the number of spikes without affecting the overall firing frequency exhibited in the extracellular activity, indicating network property. Hyperpolarization also allowed the underlying excitatory waves to become more evident and appeared to increase in frequency because smaller excitatory post-synaptic potentials (EPSPs) were more visible. A rhythmic component was noted in the spontaneous expression of the SREDs and they showed a gradual increase in degree of depolarization in both intracellular and extracellular recordings (Fig. 6C). A trend toward progressive depolarization was noted with a gradual increasing intensity culminating in an eventual expression of a large wave PDS. Thus, a tendency toward self-generation of epileptic bursting was clearly evident.

Whole cell current clamp recordings from CA3 pyramidal cells in HEC slices with the faster frequency of epileptiform activity (3.2 Hz) exhibited progression of PDS activity in a less gradual fashion (Fig. 7). In this instance the PDS activity built up immediately from an event containing a single spike to a series of PDSs with a multispike bursting pattern. This cyclical pattern developed rapidly and retained the level of multispike bursting activity for several events. This intracellular occurrence of faster activity was also synchronized with extracellular recordings

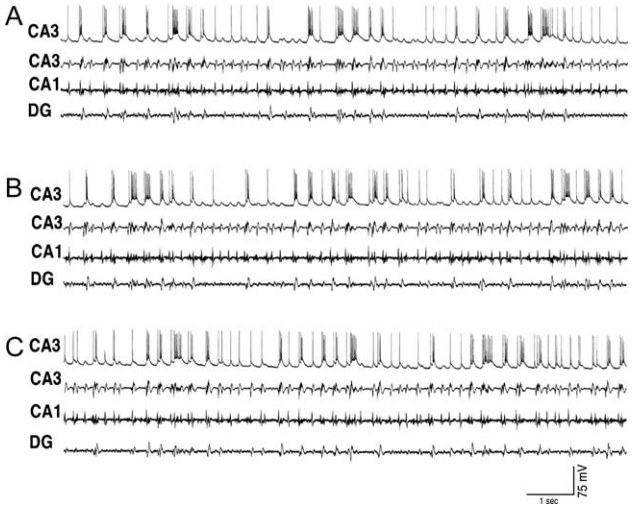

Fig. 7. Whole cell current clamp recordings of second variant of SREDs (3.2 Hz) in HEC slice from an epileptic animal. Traces A, B, and C are continuous and depict the long duration (>30 s) of SRED activity with both intracellular (top trace) and extracellular recordings. The activity is synchronized and generalized throughout the HEC slice.

throughout the HEC slice. These recordings demonstrate that the HEC slices from all epileptic animals were capable of generating endogenous SREDs without any external stimulation or pharmacological interventions.

4. Discussion

The results of this study demonstrate the presence of endogenous SREDs in HEC slices prepared from a large group of epileptic animals. While all HEC slices isolated from epileptic animals exhibit SREDs, HEC slices from control animals had no evidence of SREDs. We have shown that there are two main variants of SREDs that occur naturally in the HEC slices prepared from epileptic animals; one with a slow (0.25 Hz) bursting frequency, and the other with a much faster (3.2 Hz) bursting frequency. Both patterns of activity persisted uninterrupted for >30 min, and SREDs were synchronous throughout the hippocampal–parahippocampal circuit. Longer recordings in selected HEC slices from epileptic animals demonstrated that the SREDs persisted indefinitely and were recorded for up to 4 h of recording time. Animals with fast SRED bursting were from animals with a higher seizure frequency of greater than three seizures per day. Animals with slow SRED bursting were from animals with less than one seizure per day. Although this suggests that the seizure frequency in epileptic animals relates to the presence of slow vs. fast SRED bursting, it is not possible to clearly establish this relationship between the in vitro and in vivo situations. The fact that hippocampal slices from control animals did not manifest SREDs indicate that these epileptiform events in the slices from epileptic animals represent the altered "epileptic phenotype". With simultaneous whole cell current clamp recordings in CA3 pyramidal cells, it was evident that the pattern of depolarization varied between the two types of recording patterns. The patch slice recordings indicated that the slower type of epileptiform activity was characterized by having a gradual buildup in the degree of spontaneous depolarization, while the faster type expressed a recurrent increase in number of spikes with each PDS event. The results of this study provide a methodology to conduct continuous long-term monitoring of HEC slices in vitro prepared from epileptic animals and provides a unique model to study endogenous epileptiform activity.

Hippocampal slices in normal Mg^{2+} concentrations have similar findings as the slices in the slightly lower concentrations except that the frequency of SREDs and SRSs is reduced by threefold. The concentration of Mg^{2+} used in this study was the same concentration used by our laboratory and others to conduct hippocampal slice physiology.[36] The presence or absence of SREDs and SRSs was not affected by the concentration of Mg^{2+}, only the frequency of discharges. Since hippocampal slices can only be maintained for limited periods of time in the recording chamber, the concentrations of Mg^{2+} used provide optimal electrographic activity to conduct sophisticated experiments to evaluate epileptiform discharges in vitro. This model offers a viable alternative to intact animal models to study epileptiform activity in vitro from control and epileptic animals.

The data reported here provide an analysis of the presentations of endogenous SREDs in epileptic HEC slices and demonstrate the existence of two major variants of epileptiform activity with regards to the frequency of SREDs. In all recordings of SREDs, the extracellular field discharge recordings correlated with whole cell current clamp recordings. These results indicate that large populations of neurons are interacting and participating to generate the spontaneous bursting activity observed during SREDs in HEC slices from epileptic animals. Earlier studies also demonstrated two types of interictal activity (slow and fast) in hippocampal slices treated with acute application of either pilocarpine or 4-aminopyridine.[21,37] In addition, previously published studies have noted that hippocampal slice preparations from chronically seizing pilocarpine-treated rats can express epileptiform activity upon electrical stimulation.[38] The data presented in this study confirm the presence of endogenous epileptiform discharges at a chronic stage of seizure expression and provide direct evidence that essentially all HEC slices from epileptic animals in this model manifest SREDs in vitro. Our data also demonstrate that the expression of all types of interictal activity is generalized throughout the hippocampal–parahippocampal circuit. This is suggestive of a recruitment phenomenon among the interconnecting regions of the hippocampus in this model of endogenous epileptiform discharges from chronic epileptic animals.

The high frequency pattern of SREDs observed in this study had a different rhythmic bursting pattern in comparison to the slower pattern of SREDs. In the high frequency pattern the whole cell current clamp recordings of CA3 pyramidal cells presented a larger number of PDS events, each with multiple action potentials that were preceded by spiking events consisting of a minimal number of bursts, progressing to multiple complex PDS events. Immediately following the expression of a complex PDS there was an immediate return to single spike PDS events (Fig. 7). This is in contrast to studies with acutely induced epileptic events. In such instances interictal spikes have been followed by refractory periods suggestive of inhibitions preventing a transition to ictal activity.[39] The use of the epileptic HEC slice preparation to evaluate and study the properties of epileptic events offers a powerful tool to investigate the basis for neuronal excitability.

Sobieszek[40] has suggested that following kindling there are three components contributing to the epileptiform activity: (1) clusters of localized bursts, (2) synchronous discharges of high amplitude spikes and (3) random components. However, extracellular recordings have pointed to enhanced EPSPs and population spikes as being the underlying mechanism.[41] The SREDs in HEC slices from pilocarpine-induced epileptic animals provide evidence that there is a reciprocal interaction between the regions of the intact hippocampal–parahippocampal circuit. These results from our study indicate that in the chronic epilepsy condition the SREDs are not exclusive to any one region of the hippocampus. One might argue about site of origin of discharges in this preparation. Using titanic stimulation of Schaeffer collaterals in the hippocampal–EC slice, our previous study demonstrated that DG lead in generating individual bursts within the secondary discharge, followed by CA3, CA1, and EC. Similarly, using lesion studies, we also showed that severing the mossy fibers between DG and CA3 abolished the secondary event without affecting the immediate after-discharge. This data along with in vivo observations that DG may have its "gate" function reduced following maximal activation[42] implicate DG as an important area in the generation of discharges.

One might argue that to prove NMDA involvement in epileptogenesis, the ideal experiment would be to block this receptor system after the course of SE and then monitor for development of SRS. We have dealt with this issue in depth in our previous study.[9] Similar to this study, NMDA receptor blockade prior to or during SE prevented development of epilepsy. However, we have routinely observed that MK-801 administered after pilocarpine-SE does not prevent development of epilepsy (unpublished observations). In fact, it has also been shown that NMDA receptor blockade after SE protects against limbic brain damage but not against epilepsy in the kainate model of temporal lobe epilepsy.[43] Thus, treating animals with MK-801 during SE prevents the development of SRSs and SREDs. However, giving MK-801 after SE in a single dose or over a 2-week treatment regimen does not prevent the development of SRSs or SREDs. MK-801 administration after the injury has occurred does not block epileptogenesis.

In summary, these results demonstrate that HEC slices isolated from epileptic animals manifested SREDs and indicate that the epileptic phenotype is associated with hyperexcitability in the hippocampus that can be recorded and studied using continuous recording techniques in HEC slice preparations. The use of epileptic HEC slices for studying the pathophysiology and electrophysiological properties of the epileptic hippocampus offers a unique approach to studying the epileptic phenotype. When developing models for anticonvulsant efficacy and for studying the pathophysiology of epilepsy, it is important to employ epileptic tissue.[5] The use of the HEC slice preparation from epileptic animals characterized in this study provides a novel model for studying epileptic tissue in vitro and offers a potent tool for developing new therapeutic agents for the treatment of epilepsy and for the study of the epileptic condition.

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