Septo-Hippocampal Networks in Chronically Epileptic Rats: Potential Antiepileptic Effects of Theta Rhythm Generation

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INTRODUCTION

Epilepsy is a chronic disorder of the brain with seizures intermittently arising. The incidence in developed countries is ~50–100 per 100,000 individuals per year with total lifetime prevalence around 5–8% (Shorvon 1996). The cure of chronic epilepsy frequently relies on pharmacological approaches or surgical resection of epileptogenic area. The mechanisms underlying seizure development and the relationship between brain activity and seizure manifestation are yet poorly understood. The understanding of these mechanisms may result in novel antiepileptic approaches dedicated to protect the brain against abnormal excitability states.


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which hippocampal theta rhythm occurs (Montplaisir et al. 1987). Thus the theta rhythm appears to indicate a hippocampal functional state in which seizure production is inhibited. Microinjections of the muscarinic agonist carbachol in the medial septum produce hippocampal theta activity and inhibit both pentyleneetetrazol-induced seizures and electrically kindled limbic status epilepticus (Miller et al. 1994). It also decreases electroencephalographic (EEG) spiking rates in both epilepsy models. Medial septal electrical stimulation at theta frequencies had similar effects. In contrast, electrolytic medial septal lesions abolished hippocampal theta activity and lowered seizure thresholds (Miller et al. 1994). These results suggest that the hippocampal theta rhythm is part of a seizure-resistant functional state. However, these studies did not examine the cellular mechanisms underlying the hippocampal theta rhythm anti-epileptic effect. Furthermore, septo-hippocampal neuronal populations and networks have not been investigated in animals with chronic epilepsy.

The present study examines the firing repertoires of medial septal neurons in the pilocarpine model of chronic epilepsy. The hypothesis underlying our work is that basal forebrain synchronizing inputs, including the septo-hippocampal projection, exert a powerful control on hippocampal epileptic discharges and modulation of those inputs constitutes an appropriate target for the design of new and effective therapeutic approaches dedicated to control brain hyperexcitability states and seizure production.

**METHODS**

**Model of temporal lobe epilepsy induced by systemic pilocarpine administration**

Twenty-one adult Sprague–Dawley rats (200–350 g at the beginning of the experiment) were used for this study. Rats were maintained in controlled conditions 12 h/12 h light/dark cycle with food and water ad libitum. All animal experimentation was conducted in accordance with IACUC guidelines and with The National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. Animals were assigned to control (n = 9) and experimental (epileptic) groups (n = 12). Age-matched control rats were injected with methyl-scopolamine but received systemic physiological saline injection instead of pilocarpine. Chronic epileptic animals were obtained using the pilocarpine model of epilepsy that was developed according to previous protocols (Cavalheiro 1995). Rats were injected subcutaneously with a single dose of methyl-scopolamine (1 mg/kg) 30 min prior pilocarpine administration to minimize peripheral cholinergic effect. Pilocarpine hydrochloride (Sigma, St. Louis, MO) was then intraperitoneally injected as a single bolus of 350 mg/kg diluted in physiological saline. Pilocarpine-treated rats exhibited oral automatisms and two to three episodes of generalized tonic-clonic seizures that rapidly evolved to a sustained convulsive behavior of status epilepticus. Convulsive manifestations of status epilepticus were interrupted by subcutaneous injections of diazepam (2 mg/kg) administered 3 h after status epilepticus onset. Diazepam administration significantly increases survival of animals and also serves to standardize the amount of seizure-triggered lesions (Leite and Cavalheiro 1995; Lemos and Cavalheiro 1995). Several supporting measures were additionally undertaken immediately after status epilepticus termination and during the next 2 days. Life-saving measures include reposition of water and electrolytes using glucose-based Ringer lactate injection (5 ml/100 g body wt ip) and offering diet supplements (e.g., Gatorade wet-chow pellets and fresh apples and bananas) for ≥48 h. These supportive measures increased the welfare of animals experiencing status epilepticus. Animals suffering status epilepticus remain asymptomatic for a period of 7–20 days (silent period) before experiencing spontaneous recurrent seizures (3–5 per week) as previously described (Leite et al. 1990; Lemos and Cavalheiro 1995). Pilocarpine-treated rats were placed individually in transparent Plexiglas cages and observed for 5–6 h/d to determine seizure frequency. Animals were observed by a trained researcher. Only chronic epileptic animals exhibiting three to five seizures per week were assigned to the experimental group. In the present study, we limited the duration of status epilepticus to 3 h, which is slightly above the threshold to induced chronic epileptogenesis (Lemos and Cavalheiro 1995). To minimize variability in our data, electrophysiological experimentation was undertaken in animals that have suffered 30–60 days of chronic epileptic condition.

**Electrophysiology**

Data were obtained from 12 pilocarpine-treated and 9 control Sprague Dawley male rats (300–450 g). The rats were initially anesthetized with isoflurane (Butler, Dublin, OH) while a jugular cannula was inserted. Isoflurane was then discontinued and urethan (Sigma-Aldrich), 0.8 g/ml, was administered via the jugular cannula to maintain an appropriate level of anesthesia during the remaining surgical and experimental procedures. The rats were placed in an animal stereotoxic instrument (David Kopf Instruments, Tujunga, CA) with the plane between bregma and lambda leveled to horizontal. Body temperature was maintained at 37°C with a self-regulating heating pad (Fine Science Tools, Foster City, CA). An un-insulated silver wire (Sigma-Aldrich) placed in the cortex, anterior to bregma served as an indifferent electrode. Another insulated stainless steel wire for recording hippocampal field activity was placed in the right dorsal hippocampal formation in the dentate molecular layer (3.8 mm posterior to bregma, 2 mm lateral to the midline and 2.5 mm ventral to the dorsal surface). Medial septal vertical diagonal band of Broca (MS-VDBB) recordings were made 0.5 mm anterior to bregma, 0.0–0.5 mm lateral to the midline, and ventral 5.2–7.2 mm from the dorsal surface. Cells were recorded with glass microelectrodes (15–30 MΩ) filled with 0.5 M sodium acetate. Hippocampal and septal microelectrodes were carried in independent microdrives, Electrode Manipulator Model 960 (David Kopf Instruments) and a CMA-12CC actuator (Newport, Irvine, CA) respectively. Histological confirmation of electrode positioning (hippocampal electrodes, septal cannula) was assessed after perfusing the rat at the end of the electrophysiological experiments. Briefly, deeply anesthetized rats were intracardially perfused with cold 0.1 M phosphate buffer saline (PBS) solution followed by a fixative (PBS- buffered 4% paraformaldehyde). After perfusion the brain was removed, cryoprotected in 30% sucrose solution followed by a fixative (PBS- buffered 4% paraformaldehyde). After perfusion the brain was removed, cryoprotected in 30% sucrose and sliced in 40-μm sections using a cryostat (Microm). Sections corresponding to medial septum and hippocampus were mounted on glass slides, dried and processed for Nissl’s staining. Then, sections were dehydrated in graded alcohols, cleared in xylene, and coverslipped for microscopic analysis.

**Data acquisition and analysis**

Brain signals were displayed, digitized, sampled at a frequency of 10 kHz with a 12-bit DT-2839 A/D board and SciWorks 3.0 SP1 (DataWave Technologies, Longmont, CO), and recorded for offline analysis. EEG signals were amplified and filtered on-line (low-pass at 100 Hz) using an AC/DC amplifier (3000 model, A-M Systems, Carlsborg, WA). Cell recordings were amplified and filtered on-line (low-pass at 500 Hz) using a NEURODATA IR-183A recording amplifier and a FLA-01 filter/amplifier (Cygnus Technology, Delaware Water Gap, PA). Hippocampal field potentials and septal cell discharges were simultaneously recorded during four hippocampal field conditions: LIA only, transition from LIA to theta, theta only,
and transition from theta to LIA. Stable cell recordings were made for an average of 30 min to insure that a minimum of 5–10 transitions were acquired for analysis. Each EEG was subjected to a fast Fourier analysis, Clampfit 9.2 (Molecular Devices), and classified as either theta or LIA by the following criteria: the theta rhythm functional state was defined as a sinusoidal-like waveform with a peak frequency of 3–8 Hz and a small bandwidth and the “LIA” functional state was defined as a large-amplitude irregular activity with a broad frequency band (0.5–25.0 Hz) (Leung et al. 1982). Hippocampal epileptic discharges (interictal spikes) were defined as abnormal EEG activity in the hippocampal recordings and consisted of high-amplitude biphasic sharp transients (amplitude: ≥2.5 mV) and a duration >50 ms. Epileptic discharges were distinct from sharp waves present in the normal hippocampus EEG (<2 mV) (Bragin et al. 1999a, b).

Statistical analysis was performed using Student’s t-test. Differences were considered significant at P < 0.05. Analysis of cell recordings (30 s) using Clampfit 9.2 software (Molecular Devices) provided the mean, firing frequency (Hz), action potential duration (ms), and amplitude (mV). Autocorrelation (AC) analysis (SciWorks 3.0 SP1) produced a histogram of the discharge pattern of the cell, and a cross-correlation (X-CORR) analysis produced a histogram indicating the strength of the relationship between the discharge of the cell versus the hippocampal field during the occurrence of hippocampal theta or LIA. Signal frequency analysis was done using MATLAB’s Signal Processing Toolbox (MATLAB, Natick, MA). The spectrogram was used to extract the short-time Fourier transform from a signal. Information was displayed as the magnitude of the time-dependent Fourier transform versus time in a color gradient graph. Peristimulus time histograms (SciWorks 3.0 SP1) produced a histogram indicating the increase or decrease of the discharge rate of the cell (Hz), before (500 ms), and after (500 ms) an epileptic discharge.

**Chemicals**

Carbachol 0.005 M (Sigma-Aldrich) was injected into the septum (0.5 mm anterior to bregma, 0.0–0.5 mm lateral to the midline, and ventral 5.2–7.2 mm from the dural surface) at 0.5 ml/min using a 1-ml syringe positioned into a Pico Plus Syringe Pump (Harvard Apparatus, Holliston, MA).

**RESULTS**

**Hippocampal theta rhythm is abnormal in the pilocarpine model of chronic epilepsy**

Power Spectrum analysis from the EEG recordings in the hippocampus of epileptic rats showed that both theta rhythm amplitude and frequency were altered in the epileptic group. Theta rhythm amplitude was reduced 80% (14375 mV²/Hz in controls to 2859 mV²/Hz in epileptic animals, Student’s t-test: P < 0.05; Fig. 1, inset). Reduction of theta amplitude was accompanied by changes in theta frequencies. In this regard, the peak of the theta frequency was shifted from 3.38 ± 0.09 Hz in controls to 4.25 ± 0.23 Hz in epileptic animals (Fig. 1, inset). This change was statistically significant (Student’s t-test: P < 0.05). To prove that this effect was not produced by inappropriate positioning of the recording electrode due to anatomical changes subsequent to cell death in the brain of epileptic animals, the point of maximum theta amplitude was found as described in previous work in each experiment (Bland and Colom 1993; Bland et al. 1999). Furthermore, in certain experiments, the position of the tract was verified to assess the location of the electrode in the antero-posterior axis. These experiments demonstrated that reduction in theta amplitude was not dependent on electrode positioning. Although changes in power may be explained by alterations restricted to the generator level (e.g., hippocampus), frequency changes usually reflect alterations at the pacemaker level (e.g., septum). Thus the decrease in power and the frequency shift suggests that generator and pacemaker structures for theta rhythm production are both altered during the epileptic process.

**Neuronal firing repertoires are altered in the pilocarpine model of chronic epilepsy**

Septal neurons were classified as slow-firing (putative cholinergic) and fast-firing (putative GABAergic or glutamatergic) (Colom et al. 2005; Sotty et al. 2003). This classification is in agreement with studies showing two major types of neurons in the medial septal region distinguished by intracellular or extracellular recordings in vivo on the basis of the durations of their action potentials, firing rates, phase-relation to the hippocampal theta rhythm, and sensitivity of their rhythmicity to blockade of muscarinic transmission (Brazhnik and Fox 1997, 1999). In this study, septal units having <12 Hz were considered slow-firing neurons and units having ≥12 Hz were considered fast-firing neurons. Differences in action potential duration support this classification (slow firing units: 0.59 ± 0.03 ms, fast firing units: 0.44 ± 0.05 ms). Firing periodicity (rhythmicity) was examined using autocorrelation analysis. Rhythmic units showed periodicity in their autocorrelations (Bland and Colom 1993). Neurons showing periods of high-frequency firing separated by periods of silence were considered bursting units. Cross-correlograms were used to determine whether unit and field rhythmicity were interrelated. Phase-relation to the hippocampal theta rhythm and sensitivity of their rhythmicity to blockade of muscarinic transmission were not explored in this study.
Twenty-eight septal neurons from control rats were recorded. In control animals, 17 neurons showed slow firing properties. From this group, 3 showed rhythmic firing correlated to hippocampal theta rhythm and 14 showed nonrhythmic firing properties. From this group, one showed rhythmic firing correlated with the hippocampal theta rhythm. The remaining 18 showed nonrhythmic firing patterns. Slow firing units from epileptic rats had an average firing rate of 4.67 ± 0.64 spikes/s during theta and an average of 4.44 ± 0.62 spikes/s during LIA. The only rhythmic slow firing unit had a frequency of 11.22 spikes/s during theta and 11.11 spikes/s during LIA. Eighteen nonrhythmic units had an average frequency of 4.31 ± 0.55 spikes/s during theta and 4.07 ± 0.52 spikes/s during LIA.

Five neurons showed fast firing properties. Fast firing neurons from epileptic animals had an average firing rate of 30.76 ± 6.8 spikes/s during theta and 21.76 ± 6.43 spikes/s during LIA. In this group, four neurons showed rhythmic bursting firing that correlated well with the hippocampal theta rhythm (34.71 ± 7.16 spikes/s during theta, 23.58 ± 7.97 spikes/s during theta, 16.45 ± 2.79 spikes/s during LIA). This group had shorter duration spikes than slow firing neurons. Differences in spike duration were statistically significant (Student’s t-test: \( P < 0.05 \)).

Twenty-four neurons from epileptic rats were recorded (Figs. 2B and 3A). In epileptic rats, neurons showed slow firing properties. From this group, one showed rhythmic firing correlated with the hippocampal theta rhythm. The remaining 18 showed nonrhythmic firing patterns. Slow firing units from epileptic rats had an average firing rate of 4.67 ± 0.64 spikes/s during theta and an average of 4.44 ± 0.62 spikes/s during LIA. The only rhythmic slow firing unit had a frequency of 11.22 spikes/s during theta and 11.11 spikes/s during LIA. Eighteen nonrhythmic units had an average frequency of 4.31 ± 0.55 spikes/s during theta and 4.07 ± 0.52 spikes/s during LIA.

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spikes/s during LIA) and only one fast firing neuron did not fire rhythmically (14.97 spikes/s during theta vs. 14.5 spikes/s during LIA). Spike duration in both slow and fast firing neurons was not significantly altered in epileptic animals.

Although the number of fast firing neurons recorded from epileptic rats was reduced when compared with controls, this difference was not statistically significant. When frequency rates were analyzed over 30-s periods that included theta and LIA states, epileptic rats overall frequencies were unchanged (8.97 spikes/s in control rats vs. 9.32 spikes/s in epileptic rats, $P > 0.05$). However, in epileptic rats the group of rhythmical bursting fast firing neurons showed a significant increase in frequency rates (13.86 spikes/s in control rats and 29.14 spikes/s in epileptic rats, Student’s $t$-test: $P < 0.05$; Figs. 2B and 3B). Thus the firing repertoire of neurons that burst rhythmically at the theta frequency is altered in chronic epileptic rats.

**Theta rhythm inhibits the occurrence of epileptic discharges in the hippocampal formation**

The presence of theta rhythm in the hippocampal EEG produced a strong reduction in the frequency of hippocampal epileptic discharges. This reduction was observed under three conditions: spontaneous theta, theta produced by sensory stimulation, and chemical stimulation. Spontaneous theta was produced by regulating anesthesia to a level that electroencephalographic activity spontaneously oscillated between trains of theta and periods of irregular activity. Sensory stimulation was produced by tail pinch (Fig. 4, C and H). Chemical stimulation was produced by intraseptal injection of the cholinergic agonist carbachol (Fig. 5). During these three conditions, the occurrence of epileptic discharges was reduced to 7–14% of the number observed during LIA (Fig. 6). Reduction in the occurrence of epileptic events was statistically significant for each of the three conditions producing hippocampal theta rhythm (Student’s $t$-test: $P < 0.05$). Notice in Fig. 4, D—F, how tail pinch stimulation induces hippocampal theta rhythm and produces a concomitant suppression of epileptic discharges. Thus sensory stimulation through theta generation produces a profound antiepileptic effect. Intraseptal injection of carbachol produced theta, probably through activation of muscarinic receptors from septal GABAergic neurons (Alreja et al. 2000), dramatically reducing the number of epileptic discharges (Fig. 5). Further studies are necessary to precisely assess the respective contributions of the theta rhythm functional state and the sensory input to the antiepileptic effect. However, the fact that similar reductions in epileptic discharge rates were recorded during three different experimental conditions that only have the production of theta rhythm in common (Figs. 4 – 6) suggests that the main contributor to the antiepileptic effect is the

![Figure 4](image-url)
Inhibited by hippocampal epileptic discharges. This finding is illustrated in the composite histograms of Fig. 7, C and D. Those composite histograms also show that septal neurons are already inhibited 100 ms before hippocampal epileptic activity. Thus the hippocampo-septal influence during hippocampal epileptic discharges has mostly an inhibitory nature.

Cross-correlations of septal units are not altered by hippocampal epileptic discharges

During our recording sessions with epileptic animals, four pairs of units were recorded. Individual units from every pair were clearly separated by action potential size (Colom and Bland 1991). Each recorded pair of units presented properties of slow firing neurons. Cross-correlations between pair units were performed during time periods with no epileptic discharges (e.g., theta rhythm) and time periods that included abundant discharges (e.g., LIA) to determine if their cross correlations were altered during or immediately after epileptic discharges. No significant changes were observed in any of the cross correlations (data not shown). These data suggest that some of the functional properties of septal networks including slow firing neurons are not acutely altered by the epileptic discharges.

Discussion

Our results show that the hippocampal theta rhythm is altered in the pilocarpine model of chronic epilepsy. The observed reduction in the power spectrum at the theta peak and the shift in theta frequency suggests that mechanisms underlying hippocampal theta rhythm generation and neuronal network activity of pacemaker structures are all affected during the epileptogenic process. Lesions provide evidence of the importance of the septal region in hippocampal theta rhythm generation (Bland and Colom 1993; Winson 1978). In freely moving rats subjected to electrolytic lesions of the septal area or surgical transaction of the fimbria-fornix, it was confirmed that theta is dependent on the integrity of the MS (Rawlins et al. 1979). Thus the septal region constitutes a nodal point in the ascending synchronizing systems responsible for hippocampal oscillatory functional state at theta frequencies and not the specific way employed to induce it.

Manipulations that resulted in the modulation of epileptic discharges (e.g., tail pinch and carbachol) did not produce significant alterations in the power spectrum of the hippocampal field activity other than the induction of theta.

Most medial septal neurons reduce their firing rate after hippocampal epileptic discharges

The discharge properties of medial septal cells neurons during hippocampal epileptic discharges were assessed using peristimulus histograms. For this study, the peak of hippocampal sharp waves was considered the stimulus and, thus the time 0 of the histogram. Whereas all fast firing neurons showed a decrease in firing rates following hippocampal epileptic discharges, 58% of slow firing neurons reduced firing rates, 21% were not affected by hippocampal epileptic discharges, and 21% increased firing rates following hippocampal discharges (Fig. 7F). Overall, fast firing and slow firing neurons were inhibited by hippocampal epileptic discharges. This finding is illustrated in the composite histograms of Fig. 7, C and D. Those composite histograms also show that septal neurons are already inhibited 100 ms before hippocampal epileptic activity. Thus the hippocampo-septal influence during hippocampal epileptic discharges has mostly an inhibitory nature.

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theta rhythm generation. Furthermore, the frequency of the hippocampal theta rhythm is modulated by the rhythmic bursting of medial septal neurons (Bland and Colom 1993; Jackson and Bland 2005; Vinogradova 1995). Thus our findings suggest that septal structures pacing the hippocampal theta rhythm are affected in the pilocarpine model of chronic epilepsy. Increased firing rates in the population of rhythmically fast firing neurons indicate that the septal function is chronically altered during epileptogenesis. Fast-firing rhythmically bursting septal neurons are probably GABAergic or glutamatergic (Colom et al. 2005; Sotty et al. 2003). Thus those septal neuronal populations are the most probable targets of hippocampal axons. This assumption is supported by anatomical work showing a direct hippocampo-medial septal GABAergic projection (Toth et al. 1993). The finding that most recorded medial septal neurons and, in particular all fast firing neurons, decreased their firing rates following hippocampal epileptic discharges is in agreement with the concept of a direct hippocampo-septal projection originating in nonprincipal neurons and producing inhibition, probably through GABA release, on medial septal GABAergic neurons (Toth et al. 1993). Septal glutamatergic neurons, which may also have a fast firing phenotype (Sotty et al. 2003), may constitute a second possible target of hippocampo-septal axons. However, anatomical evidence has yet to be found in support of this notion. Slow firing neurons are probably cholinergic, but they also may be glutamatergic (Sotty et al. 2003). Thus they may be diversely positioned in relation to the hippocampo-septal projection. This assumption may explain their varied firing changes after hippocampal epileptic discharges. The finding of a mainly inhibitory hippocampo-septal effect after hippocampal epileptic discharges may indicate the following. 1) The excitatory hippocampo-lateral septal projection does not strongly affect medial septal neurons. Anatomical studies that put in doubt the presence of a well-defined lateral-medial septal connection (Leranth et al. 1992) support this interpretation of the data. 2) The excitatory hippocampo-lateral septal projection mainly activates GABAergic inhibitory neurons in the lateral septum. These inhibitory neurons may in turn project to the medial septal region producing or contributing to the postepileptic discharge inhibition of medial septal neurons. The work of Risold and Swanson (1997) shows the presence of an important latero-medial septal projection and supports the possibility of a latero-medial septal influence during the hippocampal epileptic discharges. Thus medial septal neurons may receive directly and/or indirectly (through the lateral septum) inhibitory hip-
hippocampal influences during epileptic discharges. Inhibitory influences are already activated 100 ms before hippocampal epileptic activity (Fig. 7, C and D). Thus the hippocampo-septal projection needs to be activated early in the process conducing to the hippocampal interictal discharge. Early inhibition of septal neurons may be necessary for the occurrence of hippocampal epileptogenesis. Further work combining electrophysiology and restricted lesions is needed to clarify this issue. It remains to be explained how septal circuits are affected during epileptogenesis. Two possibilities should be analyzed here. One explanation is that the hippocampal alteration, through the hippocampo-septal projection, changes the extrinsic properties of septal neurons. Thus the hippocampal alteration may be sufficient to explain the observed changes in septal electrophysiology. However, a second possibility needs to be analyzed here. It is plausible that the repetitive activation of inhibitory hippocampo-septal projections leads to their degeneration, leaving septal neurons discharging at abnormally high-frequency levels (e.g., fast firing neurons). Some of these neurons may not tolerate chronic increases in firing rates and will then degenerate during epileptogenesis. Anatomopathological data suggest the septum is affected by hippocampal seizures and that neuronal degeneration occurs at the septal level (Covolan and Mello 2000; Turski et al. 1986). Thus anatomopathological data support the concept that alterations in sepal electrophysiology may be produced by both anatomical alterations at the septal level and alterations of the hippocampo-septal input.

Several lines of evidence support an anti-epileptic role for medial septal neurons. Widespread lesions of basal forebrain cholinergic systems by intraventricular administration of 192 IgG-saporin accelerate epileptogenesis produced by hippocampal kindling. To investigate the contribution of different basal forebrain cholinergic systems to its seizure-suppressant action in hippocampal kindling, Ferencz and collaborators (2001) injected 192 IgG-saporin into the MS or into the nucleus basalis, leading to selective hippocampal or cortical cholinergic deafferentation, respectively. Hippocampal denervation facilitated kindling similar to the extensive lesion caused by intraventricular 192 IgG-saporin, whereas the cortical lesion had no effect. Thus septo-hippocampal neurons are responsible for the antiepileptogenic effect of the cholinergic system in hippocampal kindling, whereas the cortical projection is not significantly involved (Ferencz et al. 2001). Furthermore, the theta rhythm functional state, perhaps through the septo-hippocampal cholinergic projection, has an antiepileptic function in acute (pentyleneetrazol injection) and chronic (kindling) models of epilepsy (Miller et al. 1994). Our work extends those findings to a pilocarpine model of chronic temporal lobe epilepsy. It also shows that reduction of epileptic discharges is facilitated kindling similar to the extensive lesion caused by intraventricular 192 IgG-saporin, whereas the cortical lesion had no effect. Thus septo-hippocampal neurons are responsible for the antiepileptogenic effect of the cholinergic system in hippocampal kindling, whereas the cortical projection is not significantly involved (Ferencz et al. 2001). Furthermore, the theta rhythm functional state, perhaps through the septo-hippocampal cholinergic projection, has an antiepileptic function in acute (pentyleneetrazol injection) and chronic (kindling) models of epilepsy (Miller et al. 1994). Our work extends those findings to a pilocarpine model of chronic temporal lobe epilepsy. It also shows that reduction of epileptic discharges is produced by the presence of a “theta rhythm functional state” regardless of whether it is induced by electrical (Miller et al. 1994), chemical, or sensory stimulation. Therefore synchronization of brain neuronal networks at the theta frequency is sufficient to ameliorate epileptic discharges. At the cellular level, it is tempting to speculate that the interplay between inhibitory GABAAergic interneurons and hippocampal principal cells produces a refractory state that blocks the propagation of the epileptic phenomena. In those hippocampal networks, interneuron activation by septal cholinergic afferents may provide the critical level of inhibition needed to stop the epileptic discharges. The role of septo-hippocampal GABAAergic axons is more difficult to speculate due to their exclusive termination on hippocampal inhibitory interneurons and their disinhibitory role in hippocampal functions (Freund and Antal 1988).

A general model for septal control of hippocampal excitability has been recently proposed (Colom 2006). The following model attempts to explain our findings on septo-hippocampal function in the pilocarpine model of chronic epilepsy (Fig. 8). GABAAergic hippocampo-medial septum and latero-medial septum inputs contribute to the predominantly inhibitory hippocampo-medial septal influences around interictal discharges. Substantial destruction of septal GABAAergic neurons results in a reduction of inhibitory influences on the remaining GABAAergic septal neurons, explaining the firing rate increases observed in the group of fast firing (putative GABAAergic) neurons. Some of the remaining glutamatergic or GABAAergic interneurons (Colom et al. 2005) are now losing connectivity due to the surrounding cell death. Those circuit alterations, in conjunction with abundant hippocampal cell death, explain the observed changes in theta rhythm. Additionally, we postulate that the antiepileptic effect of the theta rhythm functional state is due to the powerful excitation of inhibitory hippocampal interneurons by septal cholinergic and glutamatergic afferents. Sprouting at the septal and hippocampal levels has been demonstrated (Ma et al. 2006). Thus sprouting is incorporated to our diagram.

In conclusion, both septal neuronal firing repertoires and hippocampal theta rhythm are altered in the pilocarpine model of chronic epilepsy. Those alterations suggest that both generator and pacemaker structures are affected by the epileptic process. Nonetheless, the theta rhythm functional state appears to have a profound antiepileptic action. This action is observed during spontaneous, sensory- or chemically induced theta. The understanding of this effect at the cellular and molecular levels may result in new therapeutic approaches dedicated to reduce hippocampal hyperexcitability and thus improve the quality of life of epileptic patients.

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R E F E R E N C E S


