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Septo-hippocampal networks in chronic epilepsy

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Abstract

The medial septum inhibits the appearance of interictal spikes and seizures through theta rhythm generation. We have determined that medial septal neurons increase their firing rates during chronic epilepsy and that the GABAergic neurons from both medial and lateral septal regions are highly and selectively vulnerable to the epilepsy process. Since the lateral septal region receives a strong projection from the hippocampus and its neurons are vulnerable to epilepsy, their functional properties are probably altered by this disorder. Using the pilocarpine model of temporal lobe epilepsy we examined the pilocarpine-induced functional alterations of lateral septal neurons and provided additional observations on the pilocarpine-induced functional alterations of medial septal neurons. Simultaneous extracellular recordings of septal neurons and hippocampal field potentials were obtained from chronic epileptic rats under urethane anesthesia. Our results show that: (1) the firing rates of lateral septal neurons were chronically decreased by epilepsy, (2) a subset of lateral septal neurons increased their firing rates before and during hippocampal interictal spikes, (3) the discharges of those lateral septal neurons were well correlated to the hippocampal interictal spikes, (4) in contrast, the discharges of medial septal neurons were not correlated with the hippocampal interictal spikes. We conclude that epilepsy creates dysfunctional and uncoupled septo-hippocampal networks. The elucidation of the roles of altered septo-hippocampal neuronal populations and networks during temporal lobe epilepsy will help design new and effective interventions dedicated to reduce or suppress epileptic activity.

Keywords

theta rhythm; pilocarpine; interictal spikes; extracellular recordings; hippocampal field potentials; cross correlation

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Introduction

Temporal lobe epilepsy is the most frequent type of epilepsy in humans (Engel, 2001). Due to the scarcity and variability of human tissue from epileptic patients, the investigation of mechanisms underlying the generation of epileptic activity mostly relies on the use of animal models. The pilocarpine animal model is one the most widely used and accepted models of temporal lobe epilepsy (Cavalheiro, 1995; Leite *et al.*, 1990; Leite and Cavalheiro 1995; Lemos and Cavalheiro 1995).

The elucidation of the pathophysiology of epilepsy requires the identification of the neurons involved in both the production of epileptic activity and its control. We have established that the medial septal region, through its role in hippocampal theta rhythm (θ) production, exerts a strong antiepileptic effect in chronic epileptic rats produced by systemic pilocarpine injection (Colom *et al.*, 2006). The work of Lerma and Miller using penicillin, pentylenetetrazol and electrically induced epilepsy (kindling) demonstrates that the hippocampal theta rhythm state corresponded to a seizure- and interictal spike-resistant condition (Lerma *et al.*, 1984; Miller *et al.*, 1994). The recent work of Kitchiniga and Butuzova shows that theta oscillations, either spontaneous or evoked by sensory stimulation, abolish epileptiform activity in kindled rabbits (Kitchiniga and Butuzova, 2009). Experimental evidence also indicates that septal cholinergic neurons exert an antiepileptic effect in hippocampal kindled rats (Ferencz *et al.*, 2001). However, the septo-hippocampal networks participating in epilepsy production and control remain to be established. This determination of epileptic networks and control mechanisms is important to create (1) new therapeutic targets and (2) computer and mathematical models that can be used to predict the evolution of the disease (Lytton 2008).

The medial septum sends cholinergic, GABAergic and glutamatergic projections to the hippocampus (Colom *et al.*, 2005; Colom, 2006). In turn, the hippocampus projects back to the medial and lateral septal structures. While a small hippocampo-medial septal projection appears to be GABAergic (Toth *et al.*, 1993), the main hippocampo-septal projection terminates on lateral septal neurons, is excitatory, and uses glutamate as neurotransmitter (Meibach and Seigel, 1977; Stevens and Cotman, 1986). Typically it has been considered that the septo-hippocampal loop is closed by a latero-medial septal projection. While the presence of this projection has been questioned (Leranth *et al.*, 1992), more recent work clearly identified latero-medial septal projections (Risold and Swanson, 1997). Thus, the lateral septum is probably affected by the hippocampal epileptic discharges and transmits the epilepsy-induced changes to the medial septal region. We have found that in chronic epileptic rats the group of rhythmically bursting firing medial septal neurons significantly increased their firing rates in relation to controls (from 13.86 to 29.14 spikes/s; Colom *et al.*, 2006). This finding could be explained by decreases in the firing rates of the lateral septal GABAergic neurons that project to the medial septal region. In this study, we examined the epilepsy-induced alterations of lateral septal neuron firing repertoires and the relationship between medial septal, lateral septal and hippocampal changes.

Material and methods

Model of temporal lobe epilepsy induced by systemic pilocarpine administration

Twelve young Sprague–Dawley rats (100–150 g at the beginning of this experiment) were used for this study. Additionally, medial septum recordings obtained from twenty four pilocarpine treated rats, used in previous work (Colom *et al*., 2006), were subjected to further analysis. 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 = 5) and experimental (epileptic) groups (n = 7). Age-matched control rats were injected with methyl-scopolamine and received systemic physiological saline injection instead of pilocarpine. Experimental 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 intraperitonealy 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). 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 induce chronic epileptogenesis (Limos and Cavalier 1995). To minimize variability in our data, electrophysiological experimentation was undertaken in animals that have suffered 30–60 days in a chronic epileptic condition.

Electrophysiology

Rats were initially anesthetized with Isoflurane (The Butler Company, Dublin, OH) while a jugular cannula was inserted. Isoflurane was then discontinued and Urethane (Sigma-Aldrich, St. Louis, MO), 0.80 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 stereotaxic instrument (David Kopf Instruments, Tujunga, CA) with the plane between bregma and lambda leveled horizontally. Body temperature was maintained at 37°C with a self regulating heating pad (Fine Science Tools Inc., Foster City, CA). An un-insulated silver wire (Sigma-Aldrich, St. Louis, MO) placed in the cortex, anterior to bregma served as an indifferent electrode. Another insulated stainless steel wire (0.20 mm diameter) for recording hippocampal field activity was placed in the right dorsal hippocampal formation in the dentate molecular layer (3.8 mm posterior to bregma, 2mm lateral to the midline and 2.6 mm ventral to the dural surface). Lateral Septum (LS) recordings were made 0.5mm anterior to bregma, 0.6-0.7 mm lateral to the midline, and ventral 4.0-7.0 mm from the dural surface. Medial Septum (MS) recordings were made 0.5 mm anterior to bregma, 0.0-0.2 mm lateral to the midline, and ventral 5.0-7.0 mm from the dural surface. Cells were recorded with glass microelectrodes (15-30 MΩ) filled with 0.5 M sodium acetate. Septal microelectrodes were carried in a microdrive (CMA-12CC, Newport, Irvine, CA) mounted on a manipulator of the stereotaxic instrument (model 900, David Kopf Instruments, Tujunga, California). At the end of some experiments, anesthesia levels were deepened and rats were perfused intracardially with 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde in 0.1 M PBS. Brains were removed, post-fixed overnight in the same solution and cryoprotected in 30% sucrose. Forty μm slices were produced using a cryostat and processed for histochemistry to confirm septal and hippocampal tracks locations. All visualized tracks were located within the targeted structures.

Data acquisition and analysis

Brain signals were displayed, digitized and 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 digitally recorded in a computer for off-line analysis. Electroencephalographic (EEG) signals were amplified and filtered on-line (low-pass at 100 Hz) using an AC/DC amplifier (3000 model, A-M Systems, Inc., 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, Inc. Delaware Water Gap, PA). Hippocampal field potentials and septal cell discharges were simultaneously recorded during four hippocampal field conditions: (1) LIA only, (2) transition from LIA to theta, (3) theta only, and (4) transition from theta to LIA. Theta was produced spontaneously or by tail pinch. Stable cell recordings were made for an average of 6.0 min to insure that a minimum of 2-3 transitions were acquired for analysis. Each EEG was subjected to a fast Fourier analysis, Clampfit 9.2 (Molecular Devices, CA), and classified as either theta or LIA by the following criteria: (1) 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 (2) 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 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 msec. Interictal spikes 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 (15 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 software) produced a histogram of the discharge pattern of the cell, and a cross-correlation (X-CORR) analysis produced a histogram indicating the strength of any relationship between the discharge of the cell versus the hippocampal field during the occurrence of hippocampal theta or LIA. In the same way, cross-correlation analysis produced a histogram indicating the strength of any relationship between the discharges of the cell *versus* the hippocampal interictal spikes. The cross-correlation histograms were divided in two groups: the ones that showed a cross-correlation with the hippocampal interictal spikes and the ones that did not showa cross-correlation. To determine whether correlated and non-correlated lateral septal neurons changed their firing rates around hippocampal interictal spikes, peri-stimulus histograms were carried out in the group of neurons that show strong cross-correlations to the hippocampal interictal spikes and in the group that showed weak or no correlations with the hippocampal interictal spikes. A fifth degree polynomial regression was applied to the first and second part of every grouped histogram ($P < 0.05$; $R = 0.95$). The first and second derivative was taken from the obtained polynomials and solved to zero in order to find the time at which neurons started to increase their firing rates prior to the occurrence of the hippocampal interictal spikes. The polynomial regression and the derivatives were performed using MATLAB® 7.0.4.365 R(14) Service Pack 2 (MATLAB, Natick, MA) software.

Statistical analysis

Mean cell firing frequencies and hippocampal theta rhythm power spectrum values were compared between the control and epileptic group using the Student's T-test; to find if there was any statistically significant reduction in the numbers of recorded neurons with different characteristics in the two groups, the Difference in Proportions Test for Two Independent Proportions was used. Significant differences for all statistical testing were defined by a *p* value of less than 0.05. All the neuronal firing rates are reported as the median plus or minus its standard error. Statistical tests were performed using StatsDirect 2.7.2 (StatsDirect LTD, Cheshire, UK) statistical software.

Results

Hippocampal theta rhythm

Septo-hippocampal synchronizing mechanisms were weakened but still functional in chronic epileptic rats. All epileptic animals (7 rats) showed a hippocampal theta rhythm with reduced amplitude but still clearly detectable (compare Figure 1A to Figure 1B). These epilepsy-induced theta rhythm alterations have been previously described (Colom *et al.*, 2006).

Lateral septal neurons

Forty-one lateral septal neurons were recorded from each group (control and chronic epileptic rats). Lateral septal neurons recorded from chronic epileptic rats showed mean discharge rates of 3.51 ±0.76 action potentials/s (AP/s) during LIA and 3.23 ±0.75 AP/s during tetha. Controls showed mean discharge rates during LIA of 5.03 ±1.09 AP/s and 7.53 ±1.34 AP/s during tetha. Thus, in the epileptic group the average firing frequencies were decreased. The reduction of discharges rates during hippocampal theta rhythm was statistically significant but not during LIA (Figure 2, Theta: $p< 0.05$; LIA: $p< 0.30$). Most neurons recorded from both controls (36 of 41) and epileptic rats (41 of 41) exhibit non-rhythmical firing repertoires (Figure 1). Five of 41 recorded from controls (Figure 3) and 0 of 41 from epileptic rats showed rhythmical firings that were correlated to the hippocampal theta rhythm. The Difference in Proportions Test for two independent proportions showed statistically significant reduction in the numbers of recorded rhythmical neurons between the control an epileptic group ($p<0.05$).

Interictal spikes, as defined in the methods section, were detected in all the epileptic rats; however, not all neuronal recordings had enough interictal spikes (and/or action potentials) in order to construct a histogram or a cross-correlogram. To determine whether the occurrence of action potentials in a given lateral septal neuron was correlated to the hippocampal interictal spikes, cross-correlation analyses between those two events were carried out. Of the 41 recorded neurons 19 did not fire enough action potentials to be subjected to cross-correlation analysis. The remaining 22 neurons were successfully analyzed. While many lateral septal neurons (n = 16/41) did not show or showed weak cross-correlations (Figure 4A), a subset of neurons (n = 6/41) (Figure 4B) showed strong positive correlations with the interictal spikes. Thus, a subset of lateral septal neurons fired action potentials before, during and after the occurrence of the hippocampal interictal spikes and those action potentials were timed to the hippocampal spikes. Lateral septal neurons with firings correlated to the hippocampal interictal spikes exhibit the following action potentials characteristics: mean discharge rate 1.98 AP/s, 1.53 mV amplitude, 0.67 msec. half-width during LIA; and mean discharge rate 1.98 AP/s, 1.47 mV amplitude, 0.58 ms half width during theta. Lateral septal neurons with firings non-correlated to the interictal spikes exhibit the following action potential characteristics: mean discharge rate 4.97 AP/s, 2.05 mV amplitude, 0.87 msec. half-width during LIA; and mean discharge rate 3.64 AP/s, 2.54 mV amplitude, 0.67 ms half width during theta. Differences between the electrophysiological characteristics of neurons correlated to interictal spikes and neurons non-correlated to interictal spikes were not statistically significant. To determine whether lateral septal neurons changed their firing rates around the hippocampal interictal spikes, peri-stimulus histograms were carried out. The 6 neurons that showed strong cross-correlations with the interictal spikes noticeably increased their firing rates ≅ 500 ms before the appearance of interictal spikes in the hippocampal EEG (Figure 5) (mean discharge rates 1.35±0.30 AP/s during the -1000 to -500 ms period and 7.80±1.74 AP/s during the -500 to 0 ms period, $p<0.05$). This increase continued for 500 ms after the interictal spike (9.05±2.02 AP/s during the 0 to 500 ms period and 0.95±0.21 AP/s during the 500-1000 ms period,

p<0.05). The remaining 16 neurons showed robust, moderate or no increases around the interictal spikes (Figure 5). However, in this group of neurons the overall discharge rate was increased around the interictal spikes. Discharges in the period of 500 ms prior and 500 ms after the interictal spike were more frequent than in the preceding and following 500 ms (mean discharge rates 17.05±0.96 AP/s during the -1000 to -500 ms and 21.7±1.33 AP/s during the -500 to 0 ms period, p<0.05) (mean discharge rates 21.5±0.89 AP/s during the 0 to 500 ms period and 14.8±0.82 AP/s during the period 500 to 100 ms, p<0.05).

Medial septal neurons

Medial septal neurons receive projections, probably of inhibitory nature, from lateral septal and hippocampal neurons. The lateral septum contains mostly GABAergic neurons (Panula *et al.*, 1984). A subpopulation of lateral septal neurons projects to the medial septum (Risold and Swanson, 1997). In addition, medial septal neurons receive a GABAergic projection from the hippocampus (Toth *et al.*, 1993). Thus, we investigated whether medial septal neuron discharges were influenced by hippocampal interictal spikes (directly or via lateral septal neurons). We were able to carry out cross-correlograms in 13 of the 24 neurons recorded from epileptic rats. Our results show that the firing of medial septal neurons was not correlated to the hippocampal interictal spikes (Figure 4C).

Discussion and conclusions

The frequent failure to successfully treat temporal lobe epilepsy is probably due to our lack of understanding of: (1) epileptogenesis, (2) epilepsy-induced alterations of brain networks and (3) roles of altered neuronal populations and circuits in both epilepsy generation and control. This work and previous studies from our laboratory aimed to clarify those issues (Colom *et al*., 2006; Garrido-Sanabria *et al.*, 2006).

Septal neurons modulate the activity of hippocampal and neocortical circuits necessary for the occurrence of rhythmical cortical activity, which controls the processing of sensory information and memory functions (Colom, 2006). Septal networks may also play a role in maintaining oscillation of the septo-hippocampal system within its normal limits, preventing the occurrence of abnormal excitability states (Lerma *et al.* 1984; Miller *et al.*, 1994). We have previously shown, using the rat pilocarpine model of temporal lobe epilepsy, that the epileptic process significantly reduces the number of septal neurons. However, septal neurons are not affected uniformly. While GABAergic neurons are injured in both medial and lateral septal regions and their density is dramatically reduced, other septal neurons are spared (Garrido-Sanabria et al., 2006). We have also determined that rhythmically bursting medial septal neurons (putative GABAergic; Bassant *et al.*, 2005) significantly increase their firing rates in relation to controls (Colom *et al.*, 2006). Those firing increases are not exclusive of the pilocarpine model; they have been observed during hippocampal kindling (Kitchigina and Butuzova, 2009). Altered septal networks, nevertheless, retain the ability to contribute to hippocampal theta rhythm generation. The presence of this rhythm in pilocarpine-induced chronic epileptic rats reduced the frequency of hippocampal interictal spikes to 7-14% of the number observed during its absence (Colom *et al.*, 2006). In kindled rabbits, theta oscillations, either spontaneous or evoked by sensory stimulation, also abolished the epileptiform events (Kitchigina and Butuzova, 2009). Since the lateral septal region receives a strong projection from the hippocampus and its neurons are vulnerable to epilepsy, their functional properties are probably altered by this disorder.

Our results show that pilocarpine-induced chronic epilepsy in rats produced strong functional modifications in the lateral septum. While the firing rates of lateral septal neurons were chronically reduced in comparison to controls, a subpopulation of lateral septal neurons transiently increased their firing rates around the interictal spikes. The positive

cross-correlations between hippocampal interictal spikes and lateral septal neuron discharges suggested that epileptic activity was successfully transferred from hippocampal circuits to lateral septal neurons using the well known hippocampo-lateral septal excitatory projection (Alonso and Frotscher, 1989).

Firing increases appeared ≈ 500 ms before hippocampal interictal spikes, suggesting that the interictal spikes detected by the dentate molecular layer electrode were generated outside this hippocampal region. This is in agreement with *in vitro* experiments showing always a CA3 origin of epileptiform activity when K^+, K^+ channel blockers or $GABA_A$ receptors blockers were added to the bath (Colom and Saggau, 1994). It is also possible that epileptic activity originated in extrahippocampal structures, as postulated by others (Nagao *et al.*, 1996; Cohen *et al.*, 2002). The large extent of extrahippocampal lesions observed in the pilocarpine model may also support an extrahippocampal site for epileptic activity generation (Sloviter, 2008). Further, experimentation and modeling is necessary to clarify this issue (Lytton 2008).

While our current work shows that lateral septal neurons decrease their firing rates during chronic epilepsy, our previous work showed that medial septal neurons increased their discharge rates under the same condition (Colom *et al.*, 2006). These results suggest that medial septum increased firing rates may be the effect of decreased firing rates in lateral septal GABAergic neurons that send axons to the medial septum. In this study we show a lack of correlation between interictal discharges and the firing of medial septal neurons in chronic epileptic rats. These results suggest that in chronic epileptic rats the latero-medial septal and hippocampo-medial septal pathways do not transfer interictal spikes from hippocampal/parahippocampal structures directly, or through lateral septal regions, to the medial septum. This disconnection may be consequence of the vast GABAergic neuronal loss observed in the pilocarpine model of temporal lobe epilepsy (Garrido-Sanabria *et al.*, 2006; Wang *et al.*, 2008). We may further speculate that a reduced septal GABAergic neuronal population (10-20% of controls, Garrido-Sanabria *et al.*, 2006) may be adequate to transfer certain neuronal impulses but not suitable to transfer the highly synchronized epileptic activity.

Rhythmically bursting, theta-related, septal neurons are assumed to be GABAergic. This has been confirmed in the medial septal region (Simon *et al.*, 2006) but not in the lateral septal region. Medial septal rhythmically bursting neurons appear to play a key role in theta rhythm generation (Hangya *et al.*, 2009). The role of lateral septal neurons, including the subpopulation of rhythmically bursting neurons, in theta generation is still under discussion. Rhythmically bursting, theta-related, neurons were found in the lateral septum of control rats. In contrast, they were not present in chronic epileptic rats. The loss of these neurons may be part of the extensive GABAergic neuronal death found across the septum in chronically epileptic rats (Garrido-Sanabria *et al.*, 2006) and is in agreement with the reduction of the larger population of medial septal rhythmically bursting neurons observed in the chronic epileptic rat (Colom *et al.*, 2006). Thus, rhythmically bursting neurons may be vulnerable to epilepsy and subjected to epilepsy-induced neuronal death in both medial and lateral septal regions. It is also possible that the reduced connectivity between lateral and medial septal regions observed in the chronic epileptic rat contribute to the disappearance of rhythmically bursting firing patterns in the lateral septum. Further work is necessary to clarify this point.

In conclusion, in chronic epileptic rats lateral septal neurons exhibit an overall reduction in mean firing rates. However, most lateral septal neurons increased their discharges before and during hippocampal interictal spikes. In addition, the discharges of some lateral septal neurons showed strong positive correlations with the hippocampal interictal spikes. In

contrast, medial septal neurons discharges did not correlate with hippocampal epileptic activity. Our work provides strong evidence that both medial and lateral septal regions are functionally altered by the epileptic process and that the medial septum of chronic epileptic rats is functionally disconnected from the hippocampal/parahippocampal epileptic activity.

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References

Alonso JR, Frotscher M. Hippocampo-septal fibers terminate on identified spiny neurons in the lateral septum: a combined Golgi/electron-microscopic and degeneration study in the rat. Cell Tissue Res. 1989; 258(2):243–246. [PubMed: 2479479]

Bassant MH, Simon A, Poindessous-Jazat F, Csaba Z, Epelbaum J, Dournaud P. Medial septal GABAergic neurons express the somatostatin sst2A receptor: functional consequences on unit firing and hippocampal theta. J Neurosci. 2005; 25(8):2032–2041. [PubMed: 15728843]

Bragin A, Engel J Jr, Wilson CL, Fried I, Mathern GW. Hippocampal and entorhinal cortex high-frequency oscillations (100–500 Hz) in human epileptic brain and in kainic acid-treated rats with chronic seizures. Epilepsia. 1999a; 40:127–137. [PubMed: 9952257]

Bragin A, Engel J Jr, Wilson CL, Vizentin E, Mathern GW. Electrophysiologic analysis of a chronic seizure model after unilateral hippocampal KA injection. Epilepsia. 1999b; 40:1210–1221. [PubMed: 10487183]

Cavalheiro EA. The pilocarpine model of epilepsy. Ital J Neurol Sci. 1995; 16:33–37. [PubMed: 7642349]

Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science. 2002; 298(5597):1418–1421. [PubMed: 12434059]

Colom LV, Saggau P. Spontaneous interictal-like activity originates in multiple areas of the CA2-CA3 region of hippocampal slices. J Neurophysiol. 1994; 71(4):1574–1585. [PubMed: 8035236]

Colom LV, Castaneda MT, Reyna T, Hernandez S, Garrido-Sanabria E. Characterization of medial septal glutamatergic neurons and their projection to the hippocampus. Synapse. 2005; 58(3):151–164. [PubMed: 16108008]

Colom LV, García-Hernández A, Castañeda MT, Perez-Cordova MG, Garrido-Sanabria ER. Septo-hippocampal networks in chronically epileptic rats: potential antiepileptic effects of theta rhythm generation. J Neurophysiol. 2006; 95(6):3645–3653. [PubMed: 16554504]

Colom LV. Septal networks: relevance to theta rhythm, epilepsy and Alzheimer's disease. J Neurochem. 2006; 96(3):609–623. [PubMed: 16405497]

Engel J Jr. Mesial temporal lobe epilepsy: what have we learned? Neuroscientist. 2001; 7(4):340–352. [PubMed: 11488399]

Ferencz I, Leanza G, Nanobashvili A, Kokaia Z, Kokaia M, Lindvall O. Septal cholinergic neurons suppress seizure development in hippocampal kindling in rats: comparison with noradrenergic neurons. Neuroscience. 2001; 102(4):819–832. [PubMed: 11182245]

Garrido Sanabria ER, Castañeda MT, Banuelos C, Perez-Cordova MG, Hernandez S, Colom LV. Septal GABAergic neurons are selectively vulnerable to pilocarpine-induced status epilepticus and chronic spontaneous seizures. Neuroscience. 2006; 142(3):871–883. [PubMed: 16934946]

Hangya B, Borhegyi Z, Szilágyi N, Freund TF, Varga V. GABAergic neurons of the medial septum lead the hippocampal network during theta activity. J Neurosci. 2009; 29(25):8094–8102. [PubMed: 19553449]

Kitchigina VF, Butuzova MV. Theta activity of septal neurons during different epileptic phases: the same frequency but different significance? Exp Neurol. 2009; 216(2):449–458. [PubMed: 19168062]

Leite JP, Bortolotto ZA, Cavalheiro EA. Spontaneous recurrent seizures in rats: an experimental model of partial epilepsy. Neurosci Biobehav Rev. 1990; 14:511–517. [PubMed: 2287490]

Leite JP, Cavalheiro EA. Effects of conventional antiepileptic drugs in a model of spontaneous recurrent seizures in rats. Epilepsy Res. 1995; 20:93–104. [PubMed: 7750514]

Lemos T, Cavalheiro EA. Suppression of pilocarpine-induced status epilepticus and the late development of epilepsy in rats. Exp Brain Res. 1995; 102:423–428. [PubMed: 7737389]

Leranth C, Deller T, Buzsáki G. Intraseptal connections redefined: lack of a lateral septum to medial septum path. Brain Res. 1992; 583(1-2):1–11. [PubMed: 1380395]

Lerma J, Herreras O, Muñoz D, Solís JM. Interactions between hippocampal penicillin spikes and theta rhythm. Electroencephalogr Clin Neurophysiol. 1984; 57(6):532–540. [PubMed: 6202484]

Leung LS, Harvey GC, Vanderwolf CH. Combined video and computer analysis of the relation between the hemispheric response and behavior. Behav Brain Res. 1982; 6:195–200. [PubMed: 7138646]

Lytton WW. Computer modelling of epilepsy. Nat Rev Neurosci. 2008; 9(8):626–37. [PubMed: 18594562]

Meibach RC, Siegel A. Efferent connections of the hippocampal formation in the rat. Brain Res. 1977; 124(2):197–224. [PubMed: 402984]

Miller JW, Turner GM, Gray BC. Anticonvulsant effects of the experimental induction of hippocampal theta activity. Epilepsy Res. 1994; 18(3):195–204. [PubMed: 7805641]

Nagao T, Alonso A, Avoli M. Epileptiform activity induced by pilocarpine in the rat hippocampal-entorhinal slice preparation. Neuroscience. 1996; 72(2):399–408. [PubMed: 8737410]

Panula P, Revuelta AV, Cheney DL, Wu JY, Costa E. An immunohistochemical study on the location of GABAergic neurons in rat septum. J Comp Neurol. 1984; 222(1):69–80. [PubMed: 6365983]

Risold PY, Swanson LW. Chemoarchitecture of the rat lateral septal nucleus. Brain Res Brain Res Rev. 1997; 24(2-3):91–113. [PubMed: 9385453]

Simon AP, Poindessous-Jazat F, Dutar P, Epelbaum J, Bassant MH. Firing properties of anatomically identified neurons in the medial septum of anesthetized and unanesthetized restrained rats. J Neurosci. 2006; 26(35):9038–9046. [PubMed: 16943562]

Stevens DR, Cotman CW. Excitatory amino acid antagonists depress transmission in hippocampal projections to the lateral septum. Brain Res. 1986; 382(2):437–440. [PubMed: 2875769]

Tóth K, Borhegyi Z, Freund TF. Postsynaptic targets of GABAergic hippocampal neurons in the medial septum-diagonal band of Broca complex. J Neurosci. 1993; 13(9):3712–3724. [PubMed: 7690065]

Wang L, Liu YH, Huang YG, Chen LW. Time-course of neuronal death in the mouse pilocarpine model of chronic epilepsy using Fluoro-Jade C staining. Brain Res. 2008; 1241:157–167. [PubMed: 18708038]

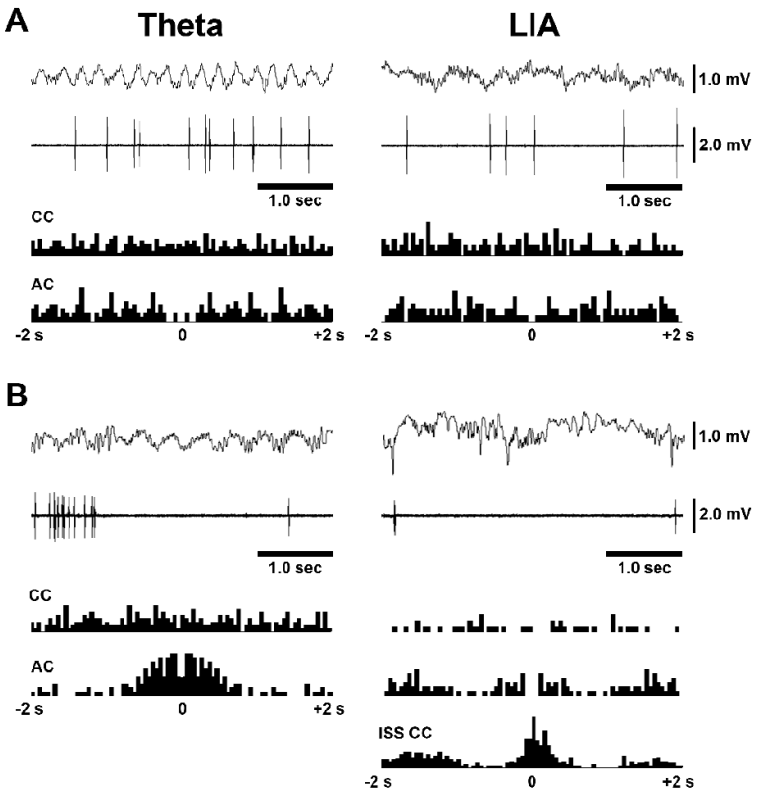

Figure 1.

Comparisons of hippocampal field potentials (upper trace) and firing patterns of two lateral septal neurons (lower trace) from control and epileptic animals. The discharges of both neurons were non-correlated to the hippocampal theta rhythm. Tail pinch-induced robust field potential oscillations in the control rat (A) but altered (low amplitude, higher frequency) theta oscillations in the epileptic rat (B). A: Control neuron during theta oscillations (left panel), mean discharge rate 2.4 action potentials/s (AP/s). Unit firing pattern discharge did not correlate to theta field oscillations as presented in the crosscorrelogram (CC) below. In the absence of theta rhythm (periods of large irregular amplitude activity, LIA) (right panel), the unit maintained its firing rate to 2.1 AP/s and exhibited no rhythmical firing as shown in AC and CC below. B: Epileptic neuron during abnormal theta oscillations (left panel). The unit fired non-rhythmically with theta oscillations as shown in the autocorrelation (AC). Mean firing frequency in epileptic unit during theta was 2.6 AP/s. In the absence of theta rhythm (LIA) (right panel), the unit decreased its firing rate to 0.50 AP/s and did not show rhythmical discharges as confirmed in AC and CC below. The arrows in figure 1B show epileptic interictal spikes in the EEG recording of the epileptic rat. This unit showed positive cross-correlation with the interictal spikes as confirmed in IIS CC crosscorrelogram at the bottom of panel 1B.

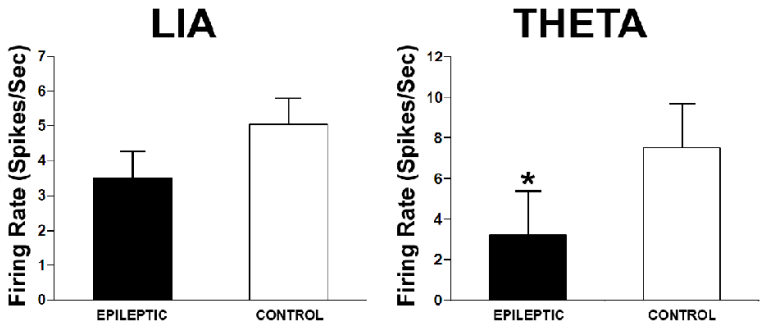

Figure 2.

Forty one lateral septal neurons were recorded from controls (Average firing frequencies, LIA: 5.03 ±1.09 action potentials/s (AP/s); theta: 7.53 ±1.34 AP/s) and 41 were recorded from chronic epileptic rats (Average firing frequencies, LIA: 3.51 ±0.76 AP/s; theta: 3.23 ±0.75 AP/s). During theta, lateral septal neurons recorded from chronic epileptic rats showed a significant decrease in firing rate when compared to controls.

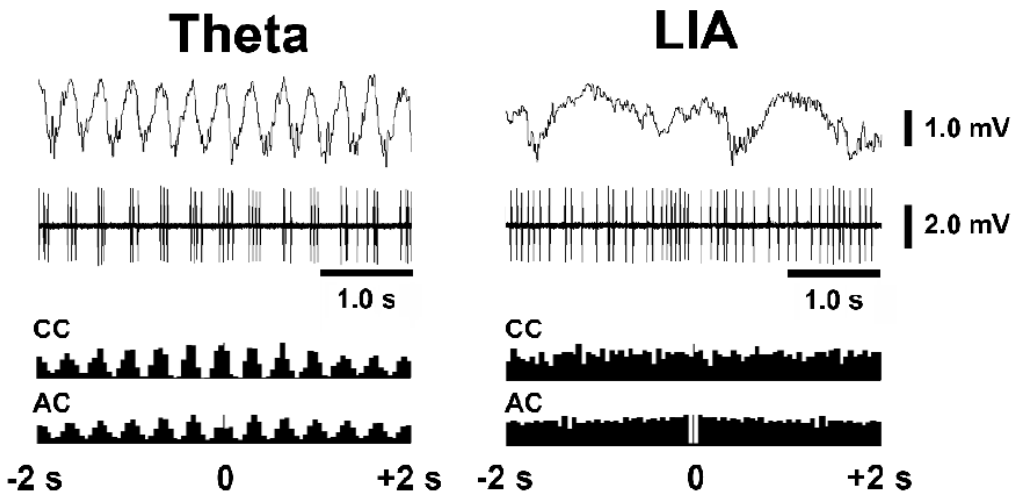

Figure 3.

Five of 41 neurons recorded from controls and 0 of 41 from epileptic rats showed rhythmical firings that were correlated to the hippocampal theta rhythm (Average firing frequencies, LIA: 7.28 AP/s, theta: 11.72 AP/s). The Difference in Proportions Test for two independent proportions showed statistically significant reduction in the numbers of recorded rhythmical neurons between the control and epileptic group(($p<0.05$).

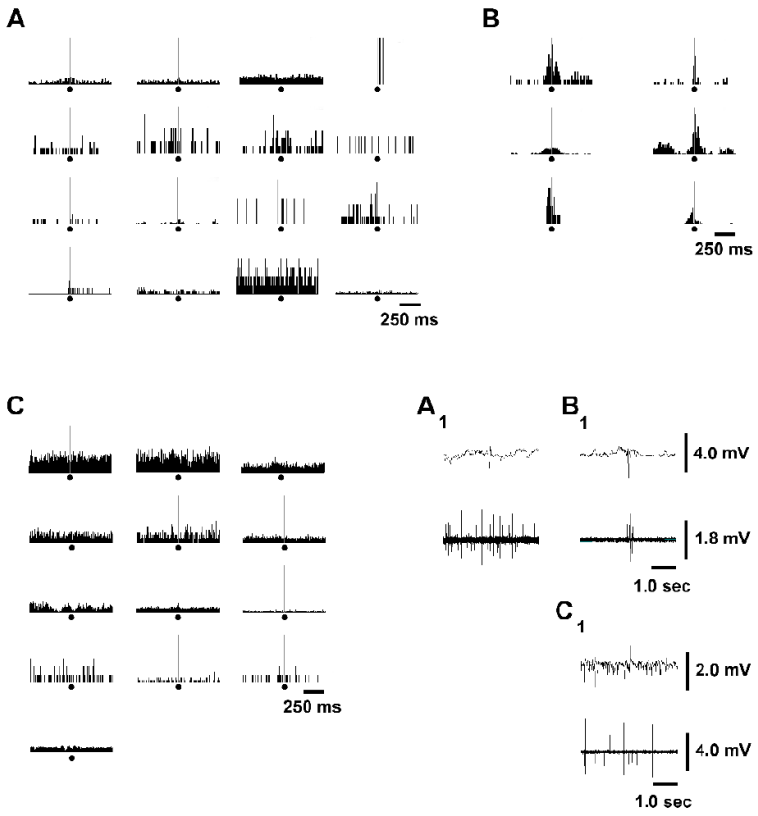

Figure 4.

Cross-correlation analyses show that the firing rate of medial septal neurons was not correlated to the hippocampal interictal spikes. A: Numerous lateral septal neurons (n = 16/41) either were not cross-correlated with the interictal spikes or showed weak cross-correlations with interictal spikes. B: A subset of neurons (n = 6/41) showed strong positive correlations with the interictal spikes. Thus, a subset of lateral septal neurons fired action potentials before, during and after the occurrence of the hippocampal interictal spikes and those action potentials were timed to the hippocampal spikes. C: The discharges of medial septal neurons were not influenced by hippocampal interictal spikes (directly or via lateral septal neurons). Twenty four medial septal neurons were recorded from epileptic rats. Cross-correlation analyses show that the firing rate of medial septal neurons was not correlated to the hippocampal interictal spikes. A1, B1, and C1: Representative recordings from lateral (A1 and B1) and medial septal neurons (C1) and hippocampal interictal spikes. A black circle (●) denotes the center for every histogram.

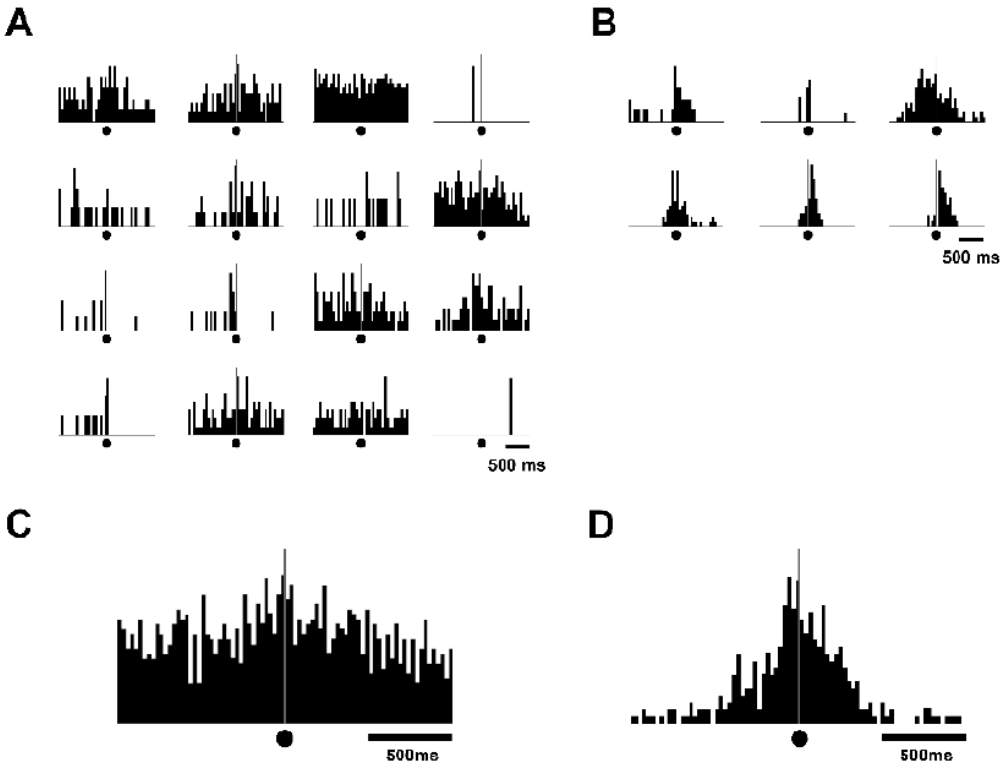

Figure 5.

To determine whether lateral septal neurons changed their firing rates around the hippocampal interictal spikes, peri-stimulus histograms were carried out. A: Peri-stimulus histograms of the 16 lateral septal neurons that showed no cross-correlation with hippocampal interictal spikes (see figure 4A and $4A_1$). These neurons showed robust, moderate or no increase in firing activity around the interictal spikes. B: Peri-stimulus histograms of the 6 lateral septal neurons that showed positive cross-correlations with the hippocampal interictal spikes (see figure 4B and $4B_1$). These neurons showed strong increases in firing activity around the interictal spikes. Firing rate increases were observed ≅ 500 ms before the appearance of interictal spikes; C: Group data histogram from all 16 neurons analyzed in part A. D: Group data histogram from all 6 neurons analyzed in part B. A black circle (●) denotes the center for every histogram.