

# Intrinsic Theta-Frequency Membrane Potential Oscillations in Hippocampal CA1 Interneurons of Stratum Lacunosum-Moleculare

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**Chapman, C. Andrew and Jean-Claude Lacaille.** Intrinsic theta-frequency membrane potential oscillations in hippocampal CA1 interneurons of stratum lacunosum-moleculare. *J. Neurophysiol.* 81: 1296–1307, 1999. The ionic conductances underlying membrane potential oscillations of hippocampal CA1 interneurons located near the border between stratum lacunosum-moleculare and stratum radiatum (LM) were investigated using whole cell current-clamp recordings in rat hippocampal slices. At 22°C, when LM cells were depolarized near spike threshold by current injection, 91% of cells displayed 2–5 Hz oscillations in membrane potential, which caused rhythmic firing. At 32°C, mean oscillation frequency increased to 7.1 Hz. Oscillations were voltage dependent and were eliminated by hyperpolarizing cells 6–10 mV below spike threshold. Blockade of ionotropic glutamate and GABA synaptic transmission did not affect oscillations, indicating that they were not synaptically driven. Oscillations were eliminated by tetrodotoxin, suggesting that Na<sup>+</sup> currents generate the depolarizing phase of oscillations. Oscillations were not affected by blocking Ca<sup>2+</sup> currents with Cd<sup>2+</sup> or Ca<sup>2+</sup>-free ACSF or by blocking the hyperpolarization-activated current (I<sub>h</sub>) with Cs<sup>+</sup>. Both Ba<sup>2+</sup> and a low concentration of 4-aminopyridine (4-AP) reduced oscillations but TEA did not. Theta-frequency oscillations were much less common in interneurons located in stratum oriens. Intrinsic membrane potential oscillations in LM cells of the CA1 region thus involve an interplay between inward Na<sup>+</sup> currents and outward K<sup>+</sup> currents sensitive to Ba<sup>2+</sup> and 4-AP. These oscillations may participate in rhythmic inhibition and synchronization of pyramidal neurons during theta activity in vivo.

## INTRODUCTION

Rhythmic synchronization of principal neurons is thought to contribute to mnemonic and information-processing capabilities of the hippocampal formation. Rhythmic synchronization may enhance cooperativity among efferents and transmission through polysynaptic pathways (Jones 1993; Yeckel and Berger 1990) and may promote potentiation or depression of synaptic inputs by generating alternate states of postsynaptic depolarization and hyperpolarization (Chapman and Becker 1995; Chapman and Racine 1997; Huerta and Lisman 1995; Larson et al. 1986; Pavlides et al. 1988; Singer 1993). Both extrinsic inputs (Petsche et al. 1962) and intrinsic conductances of principal neurons (Garcia-Munoz et al. 1993; Leung and Yim 1991; Nuñez et al. 1987) contribute to hippocampal theta-frequency (4–12 Hz) activity (Bland and Colom 1993). The extrinsic input that drives both hippocampal and entorhinal cortex theta rhythms in vivo is derived from the medial septum

(Alonso and Garcia-Austt 1987; Mitchell et al. 1982; Petsche et al. 1962), and theta-like activity also can be induced in vitro by cholinergic agonism (Konopacki et al. 1987; MacVicar and Tse 1989).

Hippocampal interneurons powerfully inhibit large numbers of pyramidal neurons (Freund and Buzsáki 1996; Lacaille et al. 1987) and contribute to theta activity by rhythmically inhibiting pyramidal cells (Fox 1989; Leung 1984; Tóth et al. 1997; Ylinen et al. 1995). Each theta cycle is associated with activation of GABA<sub>A</sub> chloride conductances proximal to the soma of CA1 pyramidal cells (Buzsáki et al. 1986; Brankack et al. 1993; Fox et al. 1983; Leung and Yim 1986; Soltesz and Deschênes 1993; Ylinen et al. 1995). Further, theta-frequency membrane potential oscillations in pyramidal cells in vitro can be synchronized by rhythmic stimulation of basket cells (Cobb et al. 1995). Interneurons also may mediate much of the septal contribution to hippocampal theta because septal cholinergic inputs contact both interneurons and pyramidal cells (Léránth and Frotscher 1987) and GABAergic inputs target predominantly interneurons (Freund and Antal 1988; Gulyás et al. 1990).

Although the physiological and morphological diversity of hippocampal interneurons suggests that subtypes differ in their computational roles, it is not known which interneuronal subtype(s) contribute most critically to theta activity. Basket and axo-axonic cells can pace theta-frequency membrane potential oscillations in pyramidal cells (Cobb et al. 1995), but the high-frequency of spontaneous synaptic activity and firing in these interneurons (Schwartzkroin and Mathers 1978) suggest that they in turn must be paced by other neurons. Interneurons located near the border of stratum radiatum and stratum lacunosum-moleculare (LM) display much less spontaneous synaptic activity, fire at slower rates, and show intrinsic membrane potential oscillations (Lacaille and Schwartzkroin 1988; Williams et al. 1994). Therefore in addition to modulating the activity of other interneuron subtypes (Hajos and Mody 1997; Vida et al. 1998), LM cells may provide rhythmic inhibition of CA1 pyramidal neurons and may mediate some of the influence of the septum and entorhinal cortex on hippocampal theta activity (Freund and Antal 1988; Kunkel et al. 1988; Witter et al. 1988).

In the present experiments, we have characterized membrane potential oscillations in LM interneurons using whole cell current-clamp recordings in slices and have found that oscillations were generated by Na<sup>+</sup> and K<sup>+</sup> conductances. Using biocytin cell labeling we found that oscillations were

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expressed preferentially in interneurons in LM and occurred less often in interneurons located in stratum oriens.

## METHODS

Methods were similar to those reported previously (Williams et al. 1994), and all chemicals were obtained from Sigma (St. Louis, MO) unless otherwise indicated.

### *Hippocampal slices*

Hippocampal slices were obtained from 4- to 6-wk-old Sprague Dawley rats (Charles River, Montréal) anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ) and decapitated. The brain was removed quickly from the skull and placed in cold (4°C) artificial cerebrospinal fluid (ACSF) that contained (in mM) 124 NaCl, 5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 2 MgSO<sub>4</sub>, 2.4 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, and 10 dextrose saturated with 95% O<sub>2</sub>-5% CO<sub>2</sub>. Transverse hippocampal slices (300- $\mu$ m thick) were cut in cold oxygenated ACSF using a vibratome and then kept at room temperature.

After  $\geq$ 1 h, slices were held submerged in a recording chamber and viewed with an upright microscope (Carl Zeiss Axioskop, Jena, Germany) equipped with Hoffman optics (Modulation Optics, Greenvale NY), a long-range water immersion objective ( $\times$ 40), and an infrared video camera (Cohu 6500, San Diego, CA). The chamber was perfused with oxygenated ACSF at room temperature (22°C) at a rate of 3 ml/min. Temperature was increased to 32  $\pm$  1°C in experiments with five LM interneurons to monitor oscillations at more physiological temperatures (Dagan Corp. TC-10, Minneapolis, MN).

### *Whole cell recording*

Patch pipettes for whole cell current-clamp recordings were pulled from borosilicate glass (1.0 mm OD, 4–7 M $\Omega$ ) using a horizontal puller (Sutter Instruments, P87, Novato, CA). Patch pipettes were filled with a solution containing (in mM) 140 K-gluconate, 5 NaCl, 2 MgCl<sub>2</sub>, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.5 ethylene glycol-bis( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 2 ATP-Tris, 0.4 GTP-Tris, and 0.1% biocytin (pH adjusted to 7.2–7.3 with KOH). Patch pipettes were placed in contact with soma of interneurons under visual guidance and using gentle positive pressure. Tight seals (2–12 G $\Omega$ ) were obtained under voltage clamp with the aid of gentle suction, and stronger suction was used to obtain whole cell configuration. Current-clamp recordings were begun after a 5-min period to allow the patch solution and cell interior to equilibrate. Membrane potential recordings (DC–3 kHz) were obtained with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA), displayed on a digital oscilloscope (Gould 1604, Ilford, UK), and digitized for storage on video cassette (Neuro Data Instruments, NeuroCorder DR-886, New York, NY). Recordings also were analogue filtered at 2 kHz (8-pole Bessel, Frequency Devices 900, Haverville, MA), and digitized at 10 kHz (Axon, TL-1) for storage on computer hard disk. Recordings were accepted if the series resistance was <50 M $\Omega$  (mean = 34  $\pm$  1.3 M $\Omega$ ) and if input resistance and mean resting membrane potential were stable. Series resistance was monitored repeatedly during each experiment.

The voltage dependence of membrane potential oscillations and their effect on cell discharge patterns were assessed by varying membrane potential relative to spike threshold with steady current injection. Cell discharge was monitored at spike threshold and at slightly more depolarized levels. Recordings were repeated at the same potentials after application of pharmacological agents, and membrane conductance blocks were assessed by monitoring changes in action potential and voltage response (at rest) to positive and negative current pulses (500-ms duration).

### *Pharmacology*

Stock solutions were stored frozen and diluted in ACSF on the day of experiments. Ionotropic glutamate and GABA<sub>A</sub> synaptic transmission were blocked with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20  $\mu$ M), ( $\pm$ )-2-amino-5-phosphonopentanoic acid (AP-5, 50  $\mu$ M; Research Biochemicals International, Natick, MA) and bicuculline methiodide (25  $\mu$ M). Sodium currents were blocked with tetrodotoxin (TTX, 0.5  $\mu$ M). The hyperpolarization-activated cationic current *I*<sub>h</sub> (Hotson et al. 1979; Maccaferri and McBain 1996a) was blocked with CsCl (1 mM). Calcium- and Ca<sup>2+</sup>-dependent currents were blocked by perfusing slices with Ca<sup>2+</sup>-free ACSF in which Ca<sup>2+</sup> was replaced by Mg<sup>2+</sup> or by applying CdCl<sub>2</sub> (50  $\mu$ M).

The K<sup>+</sup> channel blockers Ba<sup>2+</sup> (1 mM), 4-aminopyridine (4-AP, 50  $\mu$ M or 10 mM), and tetraethylammonium (TEA, 30 mM) were applied in the presence of CNQX (20  $\mu$ M), AP-5 (50  $\mu$ M) and bicuculline (25  $\mu$ M). A low concentration of TEA (100  $\mu$ M) was applied in the absence of these antagonists. When millimolar concentrations of 4-AP and TEA were used, the GABA<sub>B</sub> receptor blocker CGP 55845A (1.0  $\mu$ M; Ciba Geigy, Basel, Switzerland) also was added, and the ACSF osmolarity was held constant (300–310 mOsm) by reducing Na<sup>+</sup> and replacing it in control ACSF with choline (ICN Biomedicals, Aurora, OH). When Ba<sup>2+</sup> and Cd<sup>2+</sup> were used PO<sub>4</sub> and SO<sub>4</sub> were removed.

### *Analysis*

Samples of membrane potential (15-s duration) were prepared for routine spectral analysis by low-pass filtering at 40 Hz and reducing the effective sampling rate to 1 kHz. Average power spectra were calculated as the square of the magnitude of the FFT using the software package Origin (Microcal, Northampton, MA) based on three 2.048-s duration segments selected to contain no action potentials. The low-pass filter was set to 100 Hz instead of 40 Hz for preliminary assessment of oscillations in LM cells and for analysis of oscillations in interneurons in stratum oriens. Cells without a clear peak in the power spectrum were considered to be non oscillatory. Changes in peak frequency and total power between 2.0 and 5.4 Hz after pharmacological manipulations were assessed using matched samples *t*-tests or repeated measures ANOVAs when appropriate.

Electrophysiological properties of LM cells were analyzed using the software package pClamp 6.0 (Axon). Action potential height was measured from resting membrane potential and action potential duration was measured at the base. Amplitude of afterhyperpolarizations was measured relative to the base of action potentials. Input resistance was determined from the peak voltage response to –100-pA current pulses (500-ms duration). Inward rectification was quantified by expressing peak input resistance as a proportion of the steady-state resistance measured at the end of the current pulse (rectification ratio). Membrane time constant was measured by fitting an exponential function to the transient voltage response evoked by small hyperpolarizing current pulses that did not evoke hyperpolarization-activated rectification. Data were expressed as means  $\pm$  SE.

### *Histology*

Slices were fixed between two filter papers in 4% paraformaldehyde in 0.1 M phosphate buffer for 2–4 h. Slices then were rinsed in 0.1 M phosphate buffer and kept at 4°C. Slices were embedded in 1% agarose and cut on a vibratome in 60  $\mu$ m sections. Sections were treated with 1% H<sub>2</sub>O<sub>2</sub> for 30 min to eliminate endogenous peroxidases and then washed (4  $\times$  5 min) in 2.5% dimethyl sulfoxide and 0.1% Triton X in 0.1 M phosphate buffer. Sections then were incubated in avidin-biotin complex (ABC kit, Vectors Lab, Burlingame, CA; dilution 1:200) for 24 h. After rinsing in 0.05 M Tris buffer, sections were incubated in a solution of 0.05% 3',3'-diaminobenzidine 4 HCl, 0.02% NiSO<sub>4</sub>, 0.1 M imidazole, and 0.001% H<sub>2</sub>O<sub>2</sub> in Tris-buffered

saline (0.9% NaCl). Sections then were rinsed in Tris-buffered saline and cleared in xylene. Axonal and dendritic arborizations of well-filled cells were traced with a camera lucida.

## RESULTS

Stable recordings were obtained from 92 cells in LM and 8 cells in stratum oriens. The passive electrical properties and firing patterns of LM cells were similar to those recorded in previous whole cell studies (Khazipov et al. 1995; Williams et al. 1994). They had a mean resting membrane potential of  $-56.5 \pm 0.5$  mV, a high-input resistance ( $280 \pm 10$  M $\Omega$ ), and a long membrane time constant ( $\tau_m = 25.7 \pm 1.3$  ms). Action potentials (amplitude,  $92.0 \pm 1.3$  mV; duration,  $2.7 \pm 0.1$  ms) typically were followed by biphasic fast (fAHP; amplitude,  $9.0 \pm 0.4$  mV) and medium duration (mAHP; amplitude,  $8.7 \pm 0.4$  mV) afterhyperpolarizations. Hyperpolarization-activated inward rectification was observed in 51 of 92 cells (e.g., Fig. 6B) and mean rectification ratio among these cells was  $1.33 \pm 0.03$ . The low incidence of spontaneous synaptic potentials in LM cells (Lacaille and Schwartzkroin 1988) allowed membrane potential oscillations to be studied routinely in the absence of glutamate and GABA antagonists.

### *Intrinsic voltage-dependent oscillations*

LM cells usually did not fire action potentials at rest. When cells were depolarized near spike threshold by positive current injection (usually  $<50$  pA), almost all LM cells (84 of 92 cells) demonstrated oscillations in membrane potential at a frequency of 2–5 Hz reflected by clear peaks in the power spectrum (Fig. 1). When oscillations reached suprathreshold voltage levels, they caused firing which varied from single spikes to clusters of two to five spikes evoked at the frequency of oscillations (e.g., Fig. 3). Oscillations were sensitive to membrane potential and were eliminated by hyperpolarizing cells 6–10 mV below spike threshold (Fig. 1, A and B). Oscillation frequency increased from  $2.3 \pm 0.1$  Hz to  $3.7 \pm 0.3$  Hz when mean membrane potential was increased from 4 mV below threshold to above spike threshold ( $t_5 = 4.4$ ,  $P < 0.01$ ) and oscillation power was increased from  $0.81 \pm 0.20$  mV<sup>2</sup>/Hz to  $2.84 \pm 0.14$  mV<sup>2</sup>/Hz at depolarized membrane potentials (Fig. 1C;  $t_5 = 8.4$ ,  $P < 0.01$ ). Peak-to-peak amplitude of oscillations was increased from  $1.3 \pm 0.1$  to  $3.6 \pm 0.4$  mV.

To verify that oscillations in LM cells were not dependent on glutamate or GABA<sub>A</sub> synaptic inputs from other cells, recordings were repeated during blockade of non-N-methyl-D-aspartate (non-NMDA), NMDA, and GABA<sub>A</sub> synaptic transmission with CNQX (20  $\mu$ M), AP5 (50  $\mu$ M), and bicuculline (25  $\mu$ M), respectively. Oscillations were not reduced in power or frequency in the presence of these antagonists ( $n = 12$ ; Fig. 2) ( $2.03 \pm 0.30$  mV<sup>2</sup>/Hz and  $3.2 \pm 0.3$  Hz in ACSF,  $1.86 \pm 0.25$  mV<sup>2</sup>/Hz and  $3.5 \pm 0.3$  Hz in antagonists), indicating that the generation of oscillations does not require glutamate or GABA<sub>A</sub> synaptic input from other cells.

To determine if the frequency of oscillations approaches that of the endogenous theta (4–12 Hz) EEG rhythm at more physiological temperatures, recordings were repeated with the temperature of the bath increased from 22 to 32°C ( $n = 5$ ; Fig. 3). Raising temperature reduced spike duration ( $2.4 \pm 0.3$  to  $1.2 \pm 0.1$  ms) and amplitude ( $91 \pm 2$  to  $76 \pm 3$  mV), consistent with an increase in the kinetics of K<sup>+</sup> conductances

(Shen and Schwartzkroin 1988; Thompson et al. 1985). Raising temperature significantly increased oscillation frequency from  $3.2 \pm 0.3$  Hz to  $7.1 \pm 0.7$  Hz ( $t_4 = -4.9$ ,  $P < 0.01$ ) at membrane potentials near threshold while having no significant effect on the power of oscillations ( $1.92 \pm 0.22$  mV<sup>2</sup>/Hz at 22°C,  $2.08 \pm 0.50$  mV<sup>2</sup>/Hz at 32°C; Fig. 3C). Action potentials maintained a clustered firing pattern at 32°C, and additional modest current injection induced sustained patterns of firing at frequencies between 9 and 14 Hz. At more physiological temperatures, the frequency of oscillations is therefore similar to that of the endogenous 4–12 Hz theta rhythm (Petsche et al. 1962).

### *Oscillations do not require inward Ca<sup>2+</sup> currents*

Low-threshold Ca<sup>2+</sup> spikes contribute to membrane potential oscillations in thalamic and mammillary complex neurons (Alonso and Llinás 1992; Jahnsen and Llinás 1984), and low-threshold transient Ca<sup>2+</sup> currents have been reported in LM interneurons (Fraser and MacVicar 1991). The potential role of Ca<sup>2+</sup> and Ca<sup>2+</sup>-dependent K<sup>+</sup> conductances (Sah 1996) in the generation of oscillations in LM cells was tested by monitoring the effects of the Ca<sup>2+</sup> channel blocker Cd<sup>2+</sup> (50  $\mu$ M;  $n = 4$ ) and Ca<sup>2+</sup>-free ACSF ( $n = 6$ ; Fig. 4). Amplitudes of afterhyperpolarizations, which are dependent on Ca<sup>2+</sup>-activated K<sup>+</sup> conductances, were reduced by Cd<sup>2+</sup> (fAHP,  $8.6 \pm 1.4$  mV to  $6.4 \pm 0.9$  mV; mAHP,  $5.0 \pm 1.7$  mV to  $2.3 \pm 1.5$  mV; Fig. 4B) and by Ca<sup>2+</sup>-free ACSF (fAHP,  $7.0 \pm 1.7$  mV to  $1.4 \pm 0.5$  mV; mAHP,  $9.7 \pm 1.8$  mV to  $4.2 \pm 1.4$  mV). There was a small increase in action potential duration in Ca<sup>2+</sup>-free ACSF ( $2.9 \pm 0.2$  ms to  $4.1 \pm 0.3$  ms) but not in Cd<sup>2+</sup> ( $2.6 \pm 0.3$  ms to  $2.5 \pm 0.3$  ms). The frequency and amplitude of membrane potential oscillations, however, were unaffected by either Cd<sup>2+</sup> or Ca<sup>2+</sup>-free ACSF (Fig. 4C). Neither treatment caused significant changes in either the frequency ( $112 \pm 16\%$  of control in Cd<sup>2+</sup>;  $101 \pm 7\%$  of control in Ca<sup>2+</sup>-free ACSF) or power ( $105 \pm 4\%$  of control in Cd<sup>2+</sup>;  $89 \pm 28\%$  of control in Ca<sup>2+</sup>-free ACSF) of oscillations. Therefore neither Ca<sup>2+</sup> currents nor Ca<sup>2+</sup>-dependent K<sup>+</sup> currents appear to be required for membrane potential oscillations in LM cells.

### *Oscillations require sodium currents*

Inward Na<sup>+</sup> currents play an essential role in generating theta-frequency oscillations in the entorhinal cortex (Alonso and Llinás 1989; Klink and Alonso 1993). The role of Na<sup>+</sup> conductances in oscillations in LM cells therefore was tested using the Na<sup>+</sup> channel blocker TTX. Bath application of TTX (0.5  $\mu$ M) eliminated Na<sup>+</sup>-dependent action potentials evoked by depolarizing current pulses and also totally eliminated membrane potential oscillations in LM cells ( $n = 7$ ; Fig. 5). Membrane potential oscillations in LM cells therefore are dependent on inward Na<sup>+</sup> currents for the generation of the depolarizing phase of the oscillations. Further, because oscillations in LM cells are observed at subthreshold membrane potentials in the absence of sustained repetitive spiking, they appear dependent on a persistent (noninactivating) Na<sup>+</sup> current (Klink and Alonso 1993).

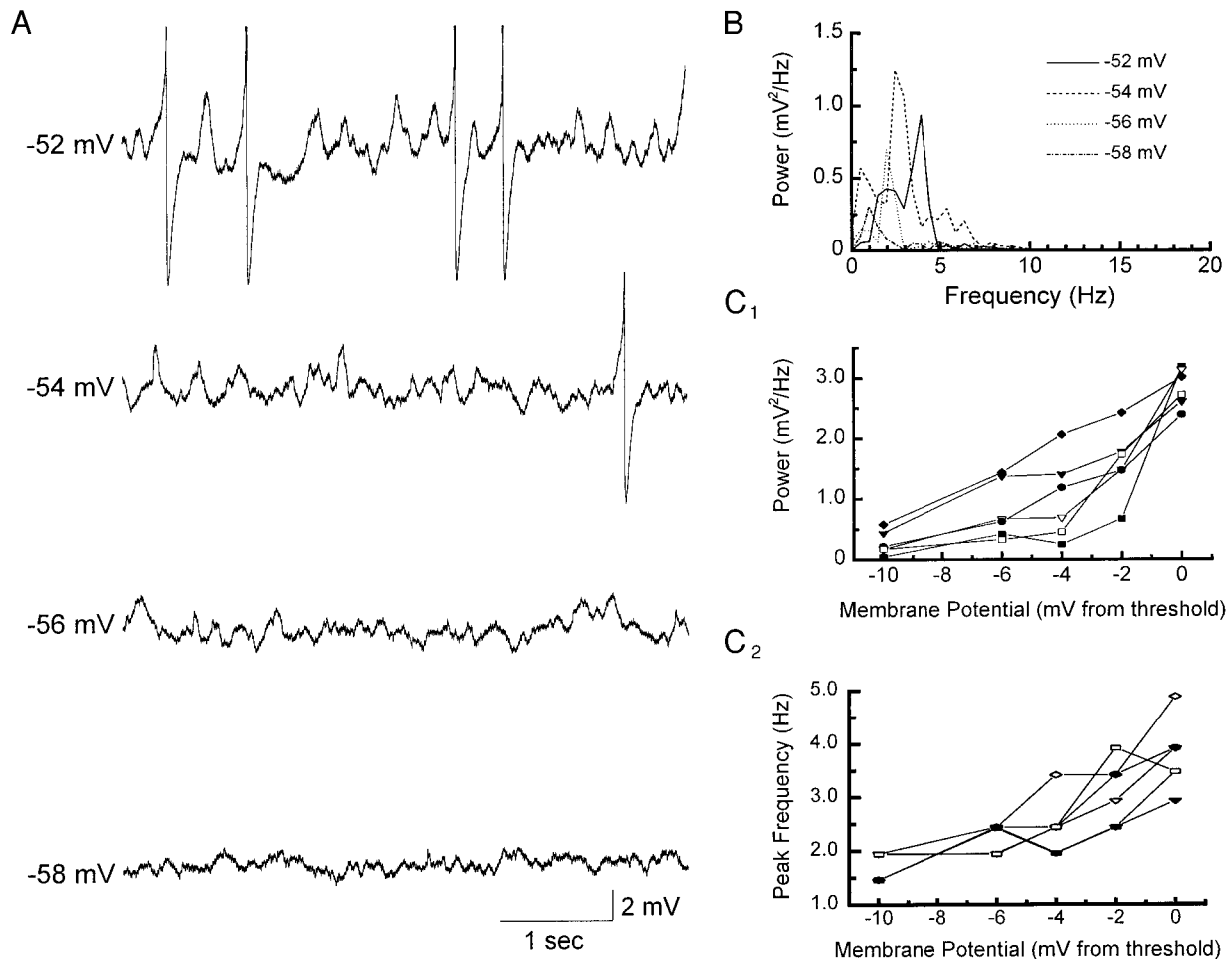


FIG. 1. Amplitude and frequency of membrane potential oscillations in stratum lacunosum-moleculare (LM) interneurons are voltage sensitive. *A*: whole cell current clamp recordings in a representative LM cell at the membrane potentials indicated at *left*. When the cell was held near spike threshold by positive current injection, membrane potential oscillations occurred at a frequency of 2–5 Hz and generated low-frequency repetitive spiking. In this and following figures, action potentials are truncated. *B*: power spectra of records taken at the indicated membrane potentials during periods without spiking for the cell shown in *A*. Peak power increased in the 2- to 5-Hz range at depolarized membrane potentials. *C*: power (*C*<sub>1</sub>) and frequency (*C*<sub>2</sub>) of oscillations are shown as a function of membrane potential relative to spike threshold for a sample of 6 LM interneurons. Oscillations were barely discernible at 6–10 mV below spike threshold, and oscillation power (*C*<sub>1</sub>) and frequency (*C*<sub>2</sub>) increased at more depolarized membrane potentials. Individual cells are represented by the same symbols in *C*<sub>1</sub> and *C*<sub>2</sub>. Mean spike threshold was  $-48.7 \pm 0.6$  mV and resting potential was  $-56.0 \pm 0.8$  mV.

#### Oscillations do not require the hyperpolarization-activated current $I_h$

Regular, repetitive firing properties of interneurons in stratum oriens-alevis of the CA1 region (Maccaferri and McBain 1996a) and of neurons in the inferior olive (Bal and McCormick 1997) are determined in part by the hyperpolarization-activated mixed cationic current  $I_h$  (Hotson et al. 1979). To determine if  $I_h$  is involved in membrane potential oscillations in LM interneurons, recordings were conducted in the presence of 1 mM  $\text{Cs}^+$  to block  $I_h$  ( $n = 4$ ; Fig. 6). The voltage- and time-dependent inward rectification of responses to hyperpolarizing current pulses was eliminated by bath application of  $\text{Cs}^+$ , and the steady-state input resistance measured at the end of current pulses was increased from  $231 \pm 7$  to  $431 \pm 131$  M $\Omega$  (Fig. 6B). Action potentials and afterhyperpolarizations were unaffected by  $\text{Cs}^+$ . Although  $\text{Cs}^+$  effectively blocked inward rectification in LM cells, it had no significant effect on either the frequency ( $3.7 \pm 0.6$  Hz in ACSF vs.  $3.4 \pm 0.2$  Hz

in  $\text{Cs}^+$ ) or power ( $1.48 \pm 0.08$  mV<sup>2</sup>/Hz in ACSF vs.  $1.86 \pm 0.34$  mV<sup>2</sup>/Hz in  $\text{Cs}^+$ ) of membrane potential oscillations (Fig. 6A). These results, coupled with the observation of oscillations in LM cells that did not display significant inward rectification (data not shown), indicate that  $I_h$  is not necessary for the oscillations and does not contribute significantly to their pacing.

#### Potassium currents

Theta-frequency membrane potential oscillations in entorhinal cortex stellate cells are generated by an interaction between a persistent  $\text{Na}^+$  current and outward  $\text{K}^+$  currents (Klink and Alonso 1993), and oscillations in LM cells occur within a voltage range in which many  $\text{K}^+$  currents are activated. The role of  $\text{K}^+$  conductances in oscillations in LM cells therefore was tested using known  $\text{K}^+$  channel blockers. First, the effects of the widely acting  $\text{K}^+$  channel blocker  $\text{Ba}^{2+}$  were examined (Fig. 7). Block of  $\text{K}^+$  conductances by  $\text{Ba}^{2+}$  (1 mM;  $n = 7$ ) led

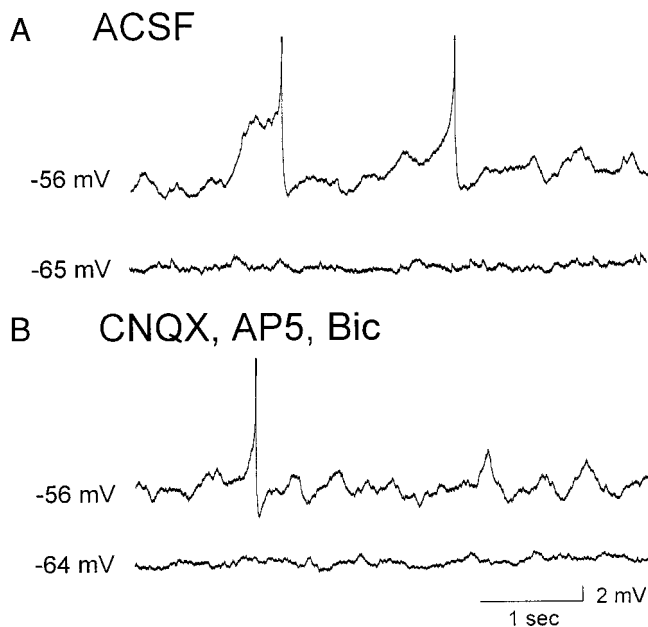


FIG. 2. Intrinsic conductances mediate membrane potential oscillations in LM interneurons. Recordings from an LM cell at different membrane potentials (*left*) before (A) and after (B) blockade of ionotropic glutamatergic and GABAergic synaptic transmission with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20  $\mu$ M), 2-amino-5-phosphonopentanoic acid (AP5, 50  $\mu$ M), and bicuculline (25  $\mu$ M). Voltage-sensitive oscillations were present and unaffected during blockade of synaptic transmission ( $n = 12$ ), indicating that synaptic inputs from other neurons were not necessary to generate the oscillations.

to membrane potential depolarization, and negative current injection was used to test cells at the same membrane potentials. Action potential duration was increased from  $3.1 \pm 0.3$  to

$4.7 \pm 0.5$  ms, and amplitude was reduced for both fAHPs ( $7.4 \pm 1.6$  to  $2.9 \pm 1.5$  mV) and mAHPs ( $8.1 \pm 0.5$  to  $4.5 \pm 1.1$  mV) in  $\text{Ba}^{2+}$  (Fig. 7B). Barium also caused significant reductions in oscillation power ( $45 \pm 10\%$  of control;  $t_6 = 4.4$ ,  $P < 0.01$ ) and frequency ( $58 \pm 7\%$  of control;  $t_6 = 4.8$ ,  $P < 0.01$ ) at membrane potentials near spike threshold (e.g., Fig. 7A). The hyperpolarizing phase of oscillations in LM cells therefore may result from the activation of voltage-dependent  $\text{K}^+$  currents sensitive to  $\text{Ba}^{2+}$ .

To characterize in more detail the  $\text{K}^+$  currents that mediate the repolarizing phase of oscillations, the effects of high and low doses of the  $\text{K}^+$  channel blocker 4-AP (10 mM,  $n = 5$ ; 50  $\mu$ M,  $n = 6$ ) were examined. A slowly inactivating delayed-rectifying  $\text{K}^+$  current in cultured neonatal LM cells (Chikwendu and McBain 1996) and the slowly inactivating  $\text{K}^+$  current  $I_D$  (Storm 1990) are sensitive to low doses of 4-AP, whereas high doses of 4-AP block the transient current  $I_A$  (Storm 1990). Both high and low doses of 4-AP had strong effects on oscillations and electrophysiological properties of LM cells (Fig. 8). The low concentration of 4-AP (50  $\mu$ M) increased spike duration ( $2.9 \pm 0.3$  to  $5.0 \pm 0.6$  ms) and reduced the amplitude of fAHPs ( $9.9 \pm 0.4$  to  $5.0 \pm 1.8$  mV) and mAHPs ( $8.7 \pm 2.2$  to  $3.1 \pm 1.3$  mV) (e.g., Fig. 8B). The high dose of 4-AP (10 mM) also reduced action potential repolarization and eliminated afterhyperpolarizations (data not shown). Both high and low doses of 4-AP significantly reduced the frequency ( $57 \pm 11\%$  of control for 10 mM;  $63 \pm 6\%$  of control for 50  $\mu$ M;  $F_1 = 29.8$ ,  $P < 0.001$ ) and power ( $51 \pm 9\%$  of control for 10 mM;  $26 \pm 4\%$  of control for 50  $\mu$ M;  $F_1 = 17.0$ ,  $P < 0.01$ ) of oscillations in LM cells at membrane potentials near threshold. Effects of 10 mM 4-AP were not significantly different from those of 50  $\mu$ M 4-AP

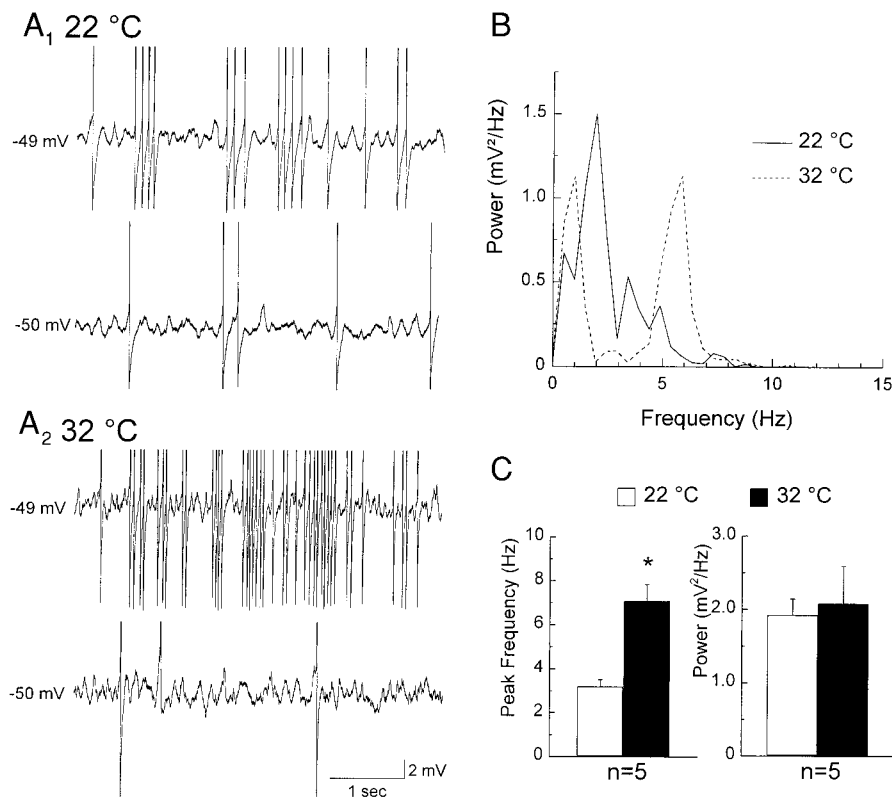


FIG. 3. Oscillations occur within the theta-frequency range at 32°C. Frequency of membrane potential oscillations in LM interneurons increased when bath temperature was raised to 32°C ( $n = 5$ ). A: recordings from a representative cell at membrane potentials near threshold at 22°C (A1) and 32°C (A2). Note the maintained clustering of action potentials and the accelerated kinetics of afterhyperpolarizations at 32°C. B: power spectra of records of the cell shown in A at  $-50$  mV. C: histograms of average frequency and power of oscillations near spike threshold ( $-51.2 \pm 0.7$  mV) at 22 and 32°C in the same cells ( $n = 5$ ). Mean frequency of oscillations was increased from 3.2 to 7.1 Hz by raising temperature, but the amplitude of oscillations was unaffected.

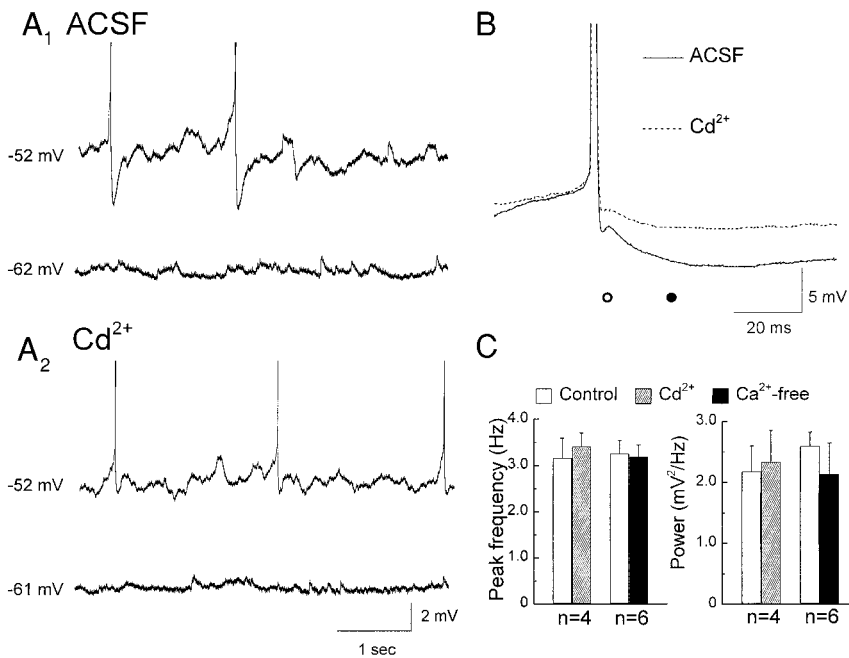


FIG. 4. Oscillations do not require inward Ca<sup>2+</sup> currents. Contribution of Ca<sup>2+</sup> currents to membrane potential oscillations in LM cells was tested by blocking Ca<sup>2+</sup> currents using Cd<sup>2+</sup> (50 μM) or Ca<sup>2+</sup>-free ACSF. *A*: voltage-dependent oscillations (*A1*) were not affected by the addition of Cd<sup>2+</sup> (*A2*, *n* = 6). *B*: superimposed action potentials taken from traces in *A1* and *A2*. Cadmium effectively blocked Ca<sup>2+</sup> conductances as shown by the reduction in the amplitude of fast (○) and slow (●) afterhyperpolarizations. Afterhyperpolarizations also were reduced in Ca<sup>2+</sup>-free ACSF (see RESULTS). *C*: histograms of average frequency and power of oscillations near spike threshold before (control) and after application of either 50 μM Cd<sup>2+</sup> or Ca<sup>2+</sup>-free ACSF. Oscillation frequency and power were unaffected by both manipulations indicating that inward Ca<sup>2+</sup> currents and Ca<sup>2+</sup>-dependent K<sup>+</sup> conductances do not contribute to membrane potential oscillations in LM cells.

(frequency,  $F_{1,1} = 1.6$ ,  $P = 0.24$ ; power,  $F_{1,1} = 4.1$ ,  $P = 0.07$ ; Fig. 8C).

The contributions of K<sup>+</sup> conductances sensitive to TEA to the oscillations were examined using high (30 mM) and low (100 μM) doses of TEA. Low doses of TEA preferentially block a sustained delayed-rectifier K<sup>+</sup> current in neonatal LM cells, whereas high doses of TEA block in addition a slowly inactivating delayed-rectifier current (Chikwendu and McBain 1996). Although both high and low doses of TEA had strong effects on electrophysiological properties of LM cells, membrane potential oscillations persisted in the presence of both concentrations of TEA (Fig. 8). Thirty millimolar TEA increased spike duration ( $3.1 \pm 0.2$  to  $18.5 \pm 5.4$  ms), eliminated fAHPs, and reduced the ampli-

tude of mAHPs ( $9.7 \pm 1.3$  to  $1.7 \pm 1.1$  mV) (Fig. 9B). The 100 μM dose of TEA reduced the amplitude of fAHPs ( $12.9 \pm 2.3$  to  $10.1 \pm 2.6$  mV) and mAHPs ( $11.1 \pm 1.6$  to  $8.4 \pm 1.8$  mV) but did not significantly increase spike duration ( $2.1 \pm 0.1$  to  $2.7 \pm 0.5$  ms). Oscillations in membrane potential were observed reliably in the presence of both 30 mM and 100 μM TEA at membrane potentials near threshold, and neither dose caused a significant reduction in either the frequency (108 ± 10% of control in 100 μM TEA; 85 ± 7% of control in 30 mM TEA) or power (109 ± 24% of control in 100 μM TEA; 132 ± 12% of control in 30 mM TEA) of oscillations (Fig. 9, A and C). The K<sup>+</sup> conductances involved in oscillations in LM cells therefore appear insensitive to high and low doses of TEA.

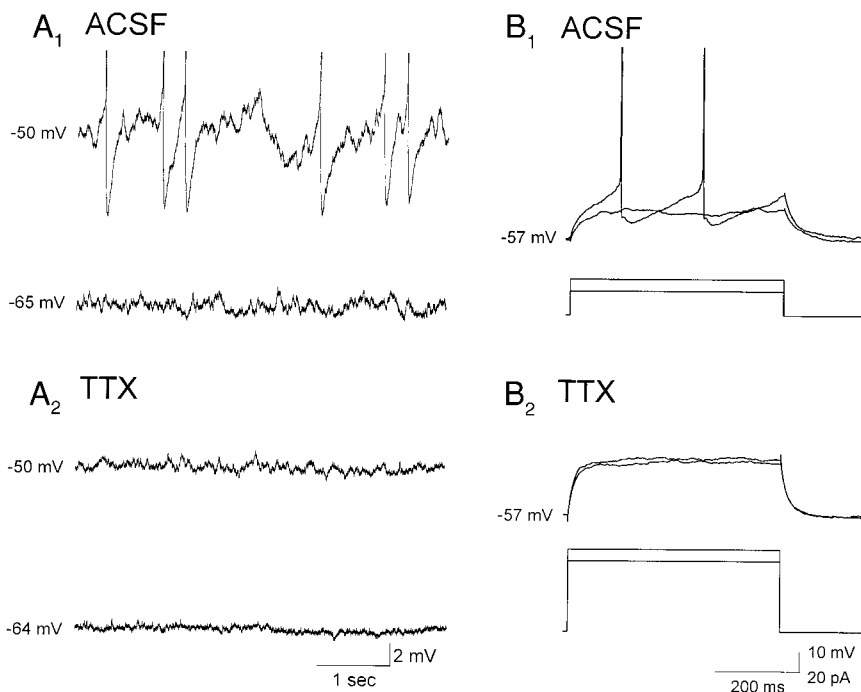


FIG. 5. Oscillations require inward Na<sup>+</sup> currents. Role of Na<sup>+</sup> currents in membrane potential oscillations was tested by using TTX (0.5 μM; *n* = 7). *A*: voltage-dependent oscillations recorded from an LM cell (*A1*) were eliminated by TTX (*A2*). Membrane potentials are indicated (*left*). *B*: responses in the same cell to positive current pulses at resting membrane potential in normal ACSF (*B1*) and in the presence of TTX (*B2*). TTX effectively blocked Na<sup>+</sup>-dependent action potentials. Block of oscillations by TTX indicates that inward Na<sup>+</sup> currents are necessary to generate the depolarizing phase of the oscillations.

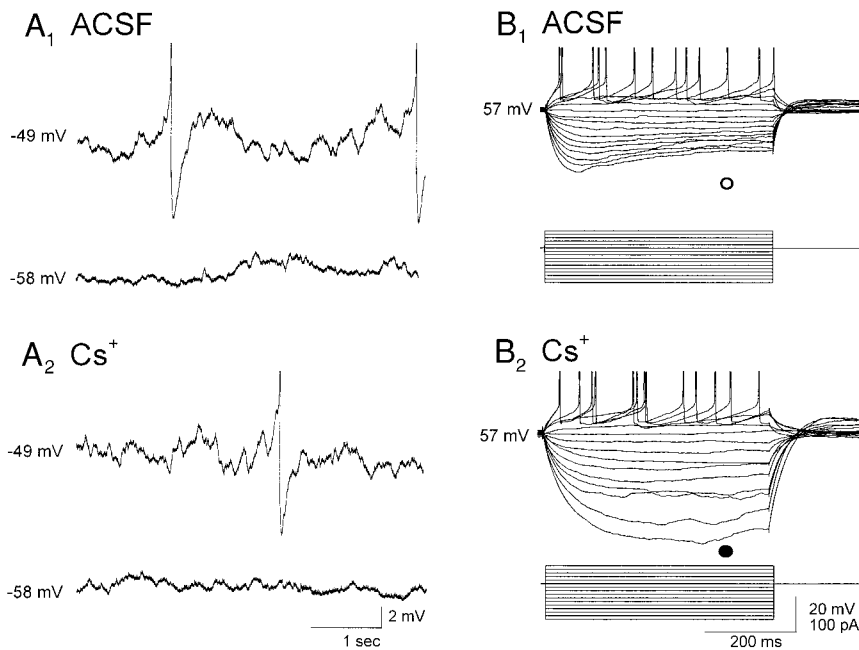


FIG. 6. Blocking  $I_h$  with  $\text{Cs}^+$  does not affect the oscillations. Contribution of the hyperpolarization-activated inward current  $I_h$  to membrane potential oscillations in LM cells was tested during block of  $I_h$  with  $\text{Cs}^+$  (1 mM;  $n = 4$ ). **A**: voltage-dependent oscillations recorded from an LM cell (**A1**) were unaffected by  $\text{Cs}^+$  (**A2**). Membrane potentials are indicated (*left*). **B**: membrane potential responses to hyperpolarizing and depolarizing current pulses delivered at resting membrane potential in normal ACSF (**B1**) and in the presence of  $\text{Cs}^+$  (**B2**) for the same cell as in **A**. Note the voltage- and time-dependent inward rectification in voltage responses to large hyperpolarizing current pulses in normal ACSF, resulting in a sag (**B1**, ○), which follows the peak voltage response. Cesium effectively blocked  $I_h$  as shown by the absence of inward rectification and the increased steady-state input resistance at the end of the voltage response (**B2**, ●). Lack of an effect of  $\text{Cs}^+$  on membrane potential oscillations indicates that  $I_h$  is not required for oscillations in LM cells.

#### Membrane potential oscillations in stratum oriens interneurons

To examine if membrane potential oscillations similar to those found in LM interneurons also were present in other types of interneurons, cells in stratum oriens were examined under similar conditions. The electrophysiological properties of interneurons located in stratum oriens were more heterogeneous than those of LM interneurons (Freund and Buzsáki 1996; Lacaille and Williams 1990; Maccaferri and McBain 1995, 1996b) (e.g., Fig. 10A), and only one of eight cells showed membrane potential oscillations clearly in the same frequency range as LM cells. Two cells had action potentials of short-duration (1.1 and 1.3 ms) and prominent fAHPs (8.4 and 14.8 mV; e.g., Fig. 10A2). Both of these cells showed regular, voltage-dependent oscillations, but the

frequency of the oscillations was higher than that of LM cells (5.9 and 42 Hz at 22°C, e.g., Fig. 10B2). A third interneuron with long-duration action potentials (2.7 ms) showed oscillations that also were higher in frequency (7.3 Hz) than in LM cells and that persisted in the presence of CNQX, AP5, and bicuculline. Of five remaining interneurons tested in the presence of these antagonists (action potential duration, 2.0–3.3 ms), only one showed regular membrane potential oscillations similar to those observed in LM cells (not shown). The membrane potential of the other four interneurons fluctuated more near threshold than at hyperpolarized levels, but regular, rhythmic oscillations were not observed (compare Fig. 10B, 1 and 3).

Morphological information was obtained from 65 biocytin-filled interneurons in LM and 7 in stratum oriens. The mor-

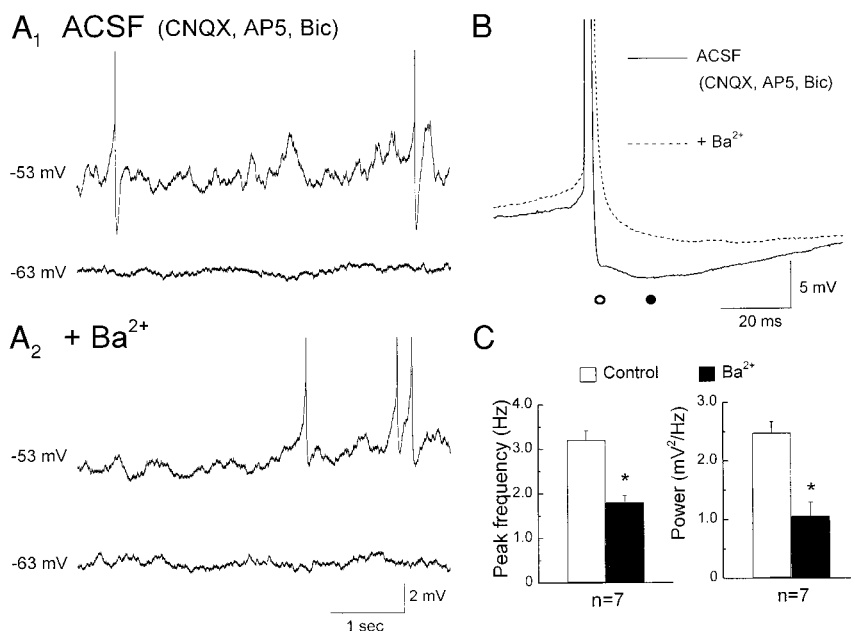


FIG. 7. Potassium conductances sensitive to barium contribute to the oscillations. To determine if the hyperpolarizing phase of oscillations is generated by outward  $\text{K}^+$  conductances, oscillations were recorded before and after application of the broad-action  $\text{K}^+$  channel blocker  $\text{Ba}^{2+}$  (1 mM;  $n = 7$ ). **A**: voltage-dependent oscillations recorded in the presence of antagonists of non- $N$ -methyl-D-aspartate (non-NMDA), NMDA, and  $\text{GABA}_A$  receptors (**A1**) were reduced in amplitude and frequency by  $\text{Ba}^{2+}$  (**A2**). Membrane potentials are indicated (*left*). **B**: superimposed action potentials taken from upper traces in **A1** and **A2**.  $\text{K}^+$  conductance block by  $\text{Ba}^{2+}$  produced an increase in spike duration and reductions in amplitude of fast afterhyperpolarizations (fAHPs, ○) and mAHPs (medium AHPs, ●). **C**: histograms of average frequency and power of oscillations near spike threshold before (control) and after application of  $\text{Ba}^{2+}$ . Oscillation frequency and power were reduced significantly indicating that outward  $\text{K}^+$  currents sensitive to  $\text{Ba}^{2+}$  contribute to the hyperpolarizing phase of oscillations.

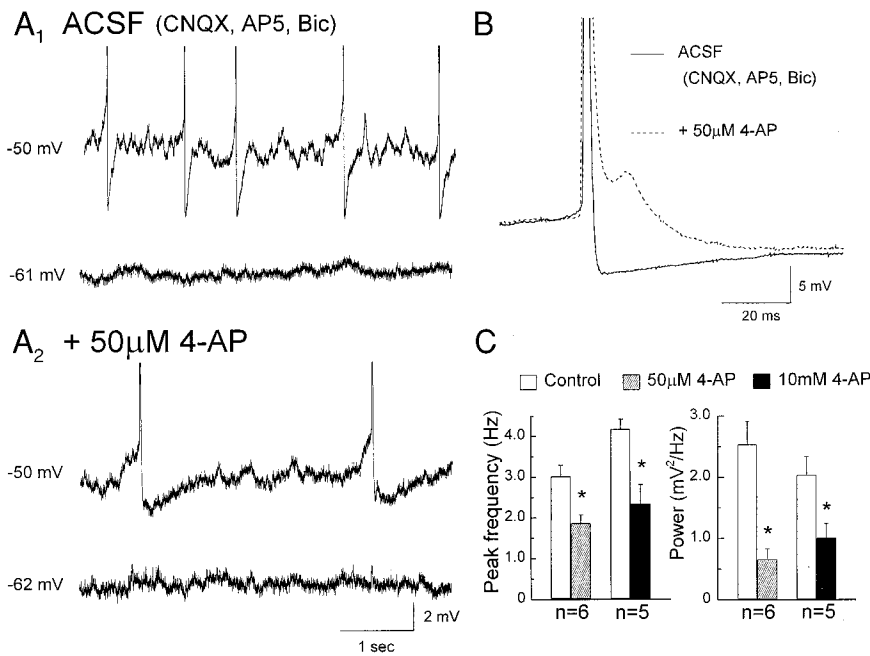


FIG. 8. Potassium conductances sensitive to 4-aminopyridine (4-AP) contribute to the oscillations. Because of the differential sensitivity of K<sup>+</sup> currents to low (50 μM) and high (10 mM) doses of 4-AP, the 2 concentrations were used to investigate the K<sup>+</sup> conductances involved in membrane potential oscillations in LM cells. A: voltage-dependent oscillations recorded in the presence of antagonists of non-NMDA, NMDA, and GABA<sub>A</sub> receptors (A1) were reduced in amplitude and frequency by the low doses of 4-AP (50 μM; A2). Membrane potentials are indicated (left). B: superimposed action potentials taken from A, top. Action potential duration was increased, and afterhyperpolarizations were reduced by 50 μM 4-AP. C: histograms of mean frequency and power of oscillations at membrane potentials near threshold before (control; □) and after application of either 50 μM (shaded bars) or 10 mM (■) 4-AP. Both low and high doses of 4-AP caused similar significant reductions in oscillation frequency and power, indicating that K<sup>+</sup> conductances sensitive to low doses of 4-AP contribute significantly to the oscillations, whereas those sensitive only to the higher doses of 4-AP may not.

phology of cells in LM was similar to that reported previously (Kunkel et al. 1988; Morin et al. 1996; Williams et al. 1994) (Fig. 10C). Somata of LM cells (10–25 μm diameter) were usually fusiform (n = 42 cells) and oriented parallel to the pyramidal cell layer (35 of 42 cells), but some cells with multipolar somata also were observed (n = 12 cells). They usually had two to three primary dendrites, which bifurcated close to the soma and arborized in LM and stratum radiatum (n = 64 cells). Axons of LM cells tended to originate from a primary dendrite and arborized mostly in LM and stratum radiatum (n = 38 cells) but also were observed in stratum pyramidale and stratum oriens (n = 11 cells). Somata of interneurons located in stratum oriens were fusiform (n = 4 cells) or multipolar (n = 2 cells) with two to six primary

dendrites which arborized in stratum oriens and the alveus (n = 7 cells), and also sometimes in stratum radiatum (n = 3 cells; Fig. 10D). Axon collaterals were observed in stratum oriens (n = 3 cells) and also projected to stratum radiatum in one cell.

DISCUSSION

Membrane potential oscillations in LM interneurons of the CA1 region were characterized and shown to arise from intrinsic voltage-dependent conductances. Oscillations regulate the timing of cell firing when LM cells are depolarized near threshold. Raising temperature from 22 to 32°C increased oscillation frequency from 3.2 to 7.1 Hz, which falls within the

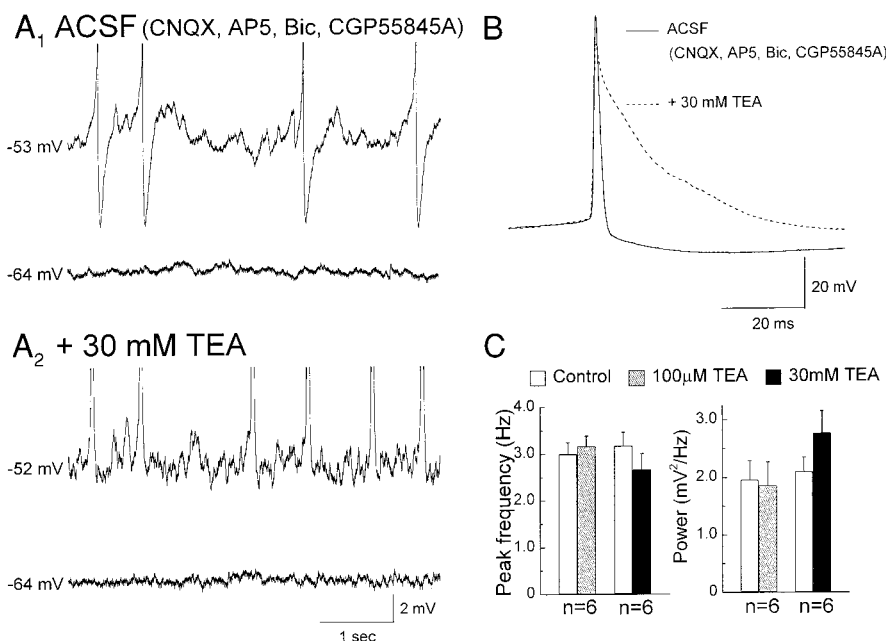


FIG. 9. Potassium conductances sensitive to TEA are not required for oscillations. Because of the differential sensitivity of K<sup>+</sup> currents to low (100 μM) and high (30 mM) doses of TEA, the effects of both concentrations on membrane potential oscillations were examined in LM cells. A: voltage-dependent oscillations recorded in the presence of antagonists of non-NMDA, NMDA, GABA<sub>A</sub>, and GABA<sub>B</sub> receptors (A1) were not reduced by 30 mM TEA (A2, n = 6). Membrane potentials are indicated (left). B: superimposed action potentials taken from A, top. Action potential duration was increased greatly, and afterhyperpolarizations were reduced greatly by 30 mM TEA. C: histograms of mean frequency and power of oscillations at membrane potentials near threshold before (control; □) and after application of 100 μM (shaded bars) and 30 mM (■) TEA. Oscillation frequency and power were unaffected by both doses of TEA, indicating that K<sup>+</sup> currents sensitive to TEA are not necessary and do not contribute to membrane potential oscillations in LM cells.



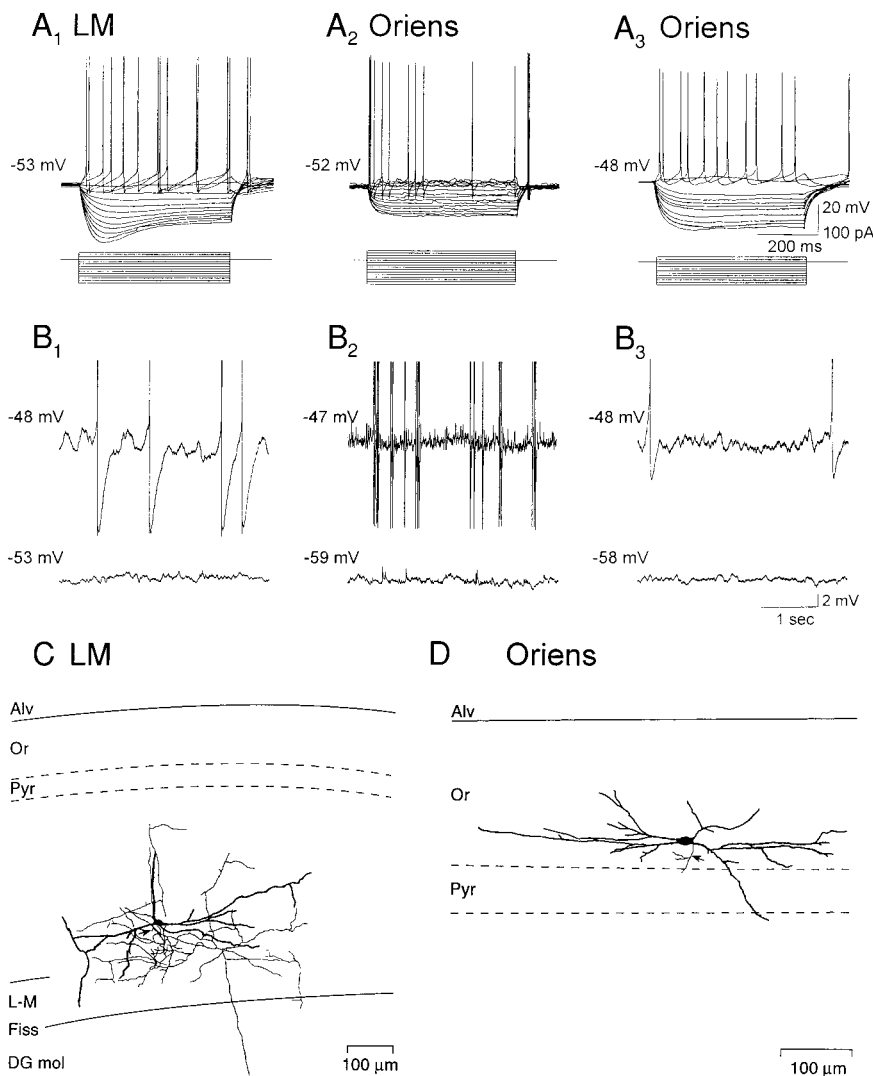


FIG. 10. Membrane potential oscillations in stratum oriens interneurons. Comparison of electrophysiological and morphological characteristics of interneurons near stratum lacunosum-moleculare and in stratum oriens. *A*: responses to hyperpolarizing and depolarizing current pulses recorded in an interneuron in LM (*A1*) and in 2 interneurons located in stratum oriens (*A2* and *3*), at resting membrane potentials indicated (*left*). Action potentials recorded from the cell in *A2* were brief in duration (1.1 ms) and were followed by a prominent afterhyperpolarization. Afterhyperpolarizations of the cell in *A3* were much smaller. Action potentials in these panels are not truncated. *B*: recordings from the same cells as in *A* (LM interneuron, *B1*; interneurons in stratum oriens, *B2* and *3*) at the membrane potentials indicated at left. Typical voltage-dependent membrane potential oscillations in the 2- to 5-Hz range were observed in the LM cell (*B1*) but not in stratum oriens interneurons. Voltage-dependent oscillations in the interneuron of stratum oriens in *B2* had a faster peak frequency of 39 Hz. Interneuron in *B3* had more fluctuations at membrane potentials near threshold but did not show regular oscillations as did LM cells (*B2* vs. *1*). *C* and *D*: camera lucida tracings of the same LM interneuron as in *A1* and *B1* (*C*) and of the same interneuron in stratum oriens as in *A3* and *B3* (*D*). ←, axonal processes. Axons and dendrites of LM interneurons arborized mainly in stratum lacunosum-moleculare and stratum radiatum. Axonal projections also crossed the hippocampal fissure and coursed into stratum moleculare of the dentate gyrus (*C*). Dendrites of the horizontally oriented interneuron in stratum oriens (*D*) extended into both the stratum oriens and alveus, and the initial portion of the axon extended toward stratum pyramidale. Abbreviations: alveus, Alv; stratum oriens, OR; stratum pyramidale, PYR; stratum lacunosum-moleculare, LM.

range of the hippocampal theta rhythm (Petsche et al. 1962). Oscillations were not dependent on synaptic inputs and were eliminated by holding cells 6–10 mV below threshold, indicating that oscillations are generated by intrinsic voltage-dependent conductances. Oscillations were not affected by blocking either  $\text{Ca}^{2+}$  currents or  $I_h$  (Alonso and Llinás 1992; Maccaferri and McBain 1996a) but were blocked by TTX and reduced by the  $\text{K}^+$  channel blockers  $\text{Ba}^{2+}$  and 4-AP. Oscillations therefore likely involve an interplay between a persistent voltage-gated  $\text{Na}^+$  current and outward  $\text{K}^+$  currents, similar to that which generates theta-frequency oscillations in stellate cells of the entorhinal cortex (Alonso and Llinás 1989; Klink and Alonso 1993). Membrane potential oscillations generated by these conductances provide a mechanism through which depolarization of LM interneurons can lead to rhythmic inhibition of pyramidal neurons during theta activity.

A high-proportion of LM interneurons displayed theta-frequency membrane potential oscillations, but these were much less common in interneurons in stratum oriens. Most interneurons in stratum oriens either did not show oscillations in membrane potential or displayed oscillations that occurred at higher frequencies than in LM cells. The tendency of stratum oriens interneurons to oscillate at higher peak frequencies than

LM interneurons suggests that the two classes of interneurons may contribute preferentially to different frequencies of rhythmic hippocampal population activity. For example, the higher-frequency oscillations in interneurons in stratum oriens may contribute to network interactions thought to underlie gamma frequency ( $\approx 40$  Hz) activity in the CA1 region (Wang and Buzsáki 1996; Whittington et al. 1995). Further,  $\text{GABA}_A$ -mediated synaptic inhibition of pyramidal cells by LM interneurons decays more slowly than inhibition mediated by other interneuron subtypes ( $\tau_d \approx 40$  ms) (Banks et al. 1998; Ouardouz and Lacaille 1997) and may allow LM cells to contribute most effectively to slower frequencies of postsynaptic rhythmic activity. Therefore both intrinsic conductances underlying interneuron membrane potential oscillations and different time courses of synaptic inhibition may be important for generating different frequencies of rhythmic population activity.

#### Conductances generating oscillations

The persistence of membrane potential oscillations in blockers of ionotropic glutamatergic and  $\text{GABA}_A$ ergic synaptic transmission indicates that intrinsic membrane conductances gen-

erate oscillations in LM cells. Oscillations were preserved in  $\text{Ca}^{2+}$ -free ACSF and in  $\text{Cd}^{2+}$  that blocked voltage-dependent  $\text{Ca}^{2+}$  currents (Fig. 4). Calcium currents and  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  currents (Fraser and MacVicar 1991; Sah 1996) that contribute to oscillations in the thalamus and mammillary complex (Alonso and Llinás 1992; Jahnsen and Llinás 1984) are therefore not required for the generation of theta-frequency oscillations in LM cells. Many LM cells have inwardly rectifying voltage responses to hyperpolarizing current pulses; this suggested that the hyperpolarization-activated cationic current ( $I_h$ ), which paces cell firing in CA1 oriens/alveus interneurons (Macafferri and McBain 1996a) and inferior olive neurons (Bal and McCormick 1997), might contribute. However,  $I_h$  normally was activated at membrane potentials more negative than the voltage range in which oscillations were observed (see also Macafferri and McBain 1996a) and oscillations were not affected by blocking  $I_h$  with  $\text{Cs}^+$ . Further, oscillations also were observed consistently in cells without inwardly rectifying  $I-V$  responses. Therefore  $I_h$  does not contribute significantly to the pacing of membrane potential oscillations in LM interneurons.

The effects of  $\text{Na}^+$  and  $\text{K}^+$  channel blockers on oscillations in LM cells indicate that oscillations result from an interplay between voltage-dependent  $\text{Na}^+$  and  $\text{K}^+$  conductances. Oscillations were eliminated by the  $\text{Na}^+$  channel blocker TTX and strongly reduced in amplitude and frequency by the  $\text{K}^+$  channel blockers  $\text{Ba}^{2+}$  and 4-AP. Each oscillation cycle, which begins when cells are depolarized near spike threshold, therefore likely is generated by activation of a persistent  $\text{Na}^+$  current ( $I_{\text{Na(p)}}$ ) followed by the activation of repolarizing  $\text{K}^+$  currents. Similar mechanisms mediate theta-frequency oscillations in stellate cells of the entorhinal cortex (Klink and Alonso 1993). Sodium (Garcia-Munoz et al. 1993; Leung and Yim 1991) and potassium (Leung and Yim 1991) conductances also are implicated in intrinsic membrane potential oscillations in CA1 pyramidal neurons, which, like oscillations in LM cells, are not sensitive to  $\text{Ca}^{2+}$ -free ACSF,  $\text{Cs}^+$ , or  $\text{Cd}^{2+}$  (Garcia-Munoz et al. 1993; Leung and Yim 1991). Because  $I_{\text{Na(p)}}$  is noninactivating, oscillation frequency in LM cells may be governed by the inactivation time constant of the  $\text{K}^+$  current. Therefore the voltage-dependent  $\text{K}^+$  current must inactivate over roughly one-half of the period of the theta rhythm. Increased  $\text{K}^+$  channel inactivation kinetics likely account for the increase in oscillation frequency from 3 to 7 Hz when temperature was raised from 22 to 32°C.

The present results indicate that the  $\text{K}^+$  conductances underlying oscillations in LM cells are sensitive to  $\text{Ba}^{2+}$  and 4-AP but not TEA. The classic transient  $\text{K}^+$  current  $I_A$  (Storm 1990) has an appropriate inactivation time constant ( $\tau_d = 50$  ms) but is not blocked by 50  $\mu\text{M}$  4-AP and therefore cannot alone support oscillations. Transient  $\text{K}^+$  currents have been observed in LM cells, but they require strong hyperpolarization to deinactivate (Fan and Wong 1996). Potassium conductances generating oscillations in LM cells must inactivate and recover from inactivation rapidly in the absence of strong hyperpolarization because oscillations were observed regularly in the absence of afterhyperpolarizations evoked by cell firing (e.g., Figs. 1 and 3). The delay current  $I_D$  is sensitive to low concentrations of 4-AP (Storm 1990) similar to those that blocked oscillations, but  $I_D$  inactivation requires seconds and is too slow to mediate theta-frequency oscillations. Two voltage-dependent delayed-rectifying  $\text{K}^+$  currents have been identified

in cultured neonatal LM cells: a slowly inactivating current sensitive to low concentrations of 4-AP and a sustained current sensitive to low concentrations of TEA (Chikwendu and McBain 1996). Oscillations cannot be mediated by the sustained current because they persisted in TEA and were blocked by 4-AP which does not reduce the sustained current. The sensitivity of the slowly inactivating current to low concentrations of 4-AP (Chikwendu and McBain 1996) suggested that it may contribute significantly to oscillations, but most of this current also is blocked by high doses of TEA (Chikwendu and McBain 1996), which did not block oscillations. These findings suggest that  $\text{K}^+$  currents in LM interneurons of the mature hippocampus may have different pharmacological sensitivities than those described for neonatal LM cells (Chikwendu and McBain 1996) and that  $\text{K}^+$  conductances mediating theta-frequency oscillations in the adult may not be present in neonatal LM interneurons. The less frequent observation of membrane potential oscillations in LM cells of younger rats (Williams et al. 1994) is consistent with a developmental regulation of  $\text{K}^+$  conductances mediating oscillatory activity in LM interneurons.

#### *Genesis and phase-locking of oscillations*

LM interneurons are typically silent at resting membrane potential and must be depolarized near spike threshold for membrane potential oscillations to be expressed. The medial septum is likely to play a central role in the depolarization and induction of membrane potential oscillations in LM cells. Cholinergic afferents from the medial septum synapse onto LM interneurons (Frotscher and Léránth 1985) and CA1 interneurons are depolarized by cholinergic agonists (Behrends and Ten Bruggencate 1993; Reece and Schwartzkroin 1991). In the entorhinal cortex, membrane potential oscillations in stellate cells are induced by cholinergic depolarization (Klink and Alonso 1997).

To contribute significantly to rhythmic inhibition of pyramidal cells during theta activity, the repetitive firing of a large number of LM cells must be closely phase-locked; randomly phased LM cell firing would result in tonic hyperpolarization of pyramidal neurons. LM cells mediate primarily feedforward inhibition in CA1 (Lacaille and Schwartzkroin 1988) so that feedback excitation by pyramidal neurons during theta activity is likely insufficient for their synchronization. However, excitatory input to LM interneurons from CA3 pyramids or contralateral CA1 region (Kunkel et al. 1988), rhythmic inhibitory GABAergic inputs from the medial septum (Petsche et al. 1962; Tóth et al. 1997), and mutual inhibition among interneurons (Atzori 1996) also may synchronize the phase of LM cell oscillations with respect to theta activity in pyramidal cells. Finally, because direct inputs to stratum lacunosum-moleculare from the entorhinal cortex terminate on both interneurons and pyramidal cells (Desmond et al. 1994; Kunkel et al. 1988), theta-frequency input from entorhinal cortex neurons (Mitchell and Ranck 1980) might rhythmically excite and synchronize many LM cells. Thus entorhinal cortex afferents to LM cells indirectly may control the phase of output of CA1 pyramidal cells to parahippocampal areas, including the entorhinal cortex.

In conclusion, LM cells of the CA1 region possess intrinsic  $\text{Na}^+$  and  $\text{K}^+$  conductances that generate theta-frequency oscillations in membrane potential near threshold. LM interneurons

therefore likely contribute to hippocampal theta activity by rhythmically inhibiting and synchronizing pyramidal neurons. Local hippocampal circuits and extrahippocampal afferents may thus contribute to hippocampal theta activity by an action on LM interneurons.

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