Relationship Between Membrane Potential Oscillations and Rhythmic Discharges in Identified Hippocampal Theta-Related Cells

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Received 24 April 2002; accepted in final form 9 August 2002

Bland, Brian H., Jan Konopacki, and Richard H. Dyck. Relationship between membrane potential oscillations and rhythmic discharges in identified hippocampal theta-related cells. J Neurophysiol 88: 3046–3066, 2002; 10.1152/jn.00315.2002. Intracellular recordings of cells, classified according to the criteria of Colom and Bland as phasic theta-ON or phasic theta-OFF cells, were carried out in the dorsal region of the hippocampal formation in urethane-anesthetized rats. Cells were studied during two spontaneously occurring hippocampal field conditions, asynchrony, termed large-amplitude irregular activity, and synchrony, termed theta. During the spontaneous cycling between these two field states, the effect of four levels of intracellular depolarizing and hyperpolarizing constant current injections on the amplitude and phase of membrane potential oscillations (MPOs) and the rate and pattern of cell discharges was assessed. Labeled CA1 pyramidal cells and bistratified cells met the criteria for classification as phasic theta-ON cells and labeled CA1 pyramidal layer basket cells, mossy hilar cells, and granule cells met the criteria for classification as phasic theta-OFF cells. MPOs were recorded in CA1 pyramidal cells, CA1 layer basket cells, mossy interneurons, and granule cells only during theta field activity, their onset in theta-ON cells signaled by a depolarizing shift of 5–10 mV and in theta-OFF cells by a hyperpolarizing shift of 5–10 mV, in membrane potential. The effect of current injections in phasic theta-ON and theta-OFF cells during the theta field condition revealed that MPO amplitude was voltage dependent and frequency was voltage independent. There were no phase changes observed in phasic theta-ON cells during current injections; however, amplitude analysis revealed an inverted U-shaped curve asymmetrically distributed around the average value of the membrane potential occurring during the spontaneous theta (no current) control condition. The occurrence and rate of rhythmic cell discharges in CA1 pyramidal phasic theta-ON cells during the theta condition was precisely controlled within a critical range of membrane potential values from approximately −57 to −68 mV, corresponding to a range of MPO amplitudes of −4–7 mV. Outside the critical range, rhythmic cell discharges were abolished. Membrane potential oscillations in CA1 pyramidal layer basket cells underwent an approximate 180° phase reversal when the membrane potential was depolarized above −65 mV. The occurrence and rate of rhythmic cell discharges in CA1 pyramidal layer basket cell phasic theta-OFF cells during the theta condition was precisely controlled within a critical range of membrane potential values from approximately −62 to −60 mV, corresponding to a range of MPO amplitudes of −7–7.5 mV. Outside the critical range, cell discharges were absent or occurred singly.

INTRODUCTION

Brain rhythms occurring at various frequencies appear to represent a basic mode of operation of large neural networks, allowing for the selective processing of information related to behavioral and perceptual states. Theta-band oscillation and synchrony in the hippocampal formation (HPC) and related limbic structures is recorded as an extracellular field potential consisting of a sinusoidal-like waveform with an amplitude ≤2 mV and a narrowband frequency range of 3–12 Hz in mammals. The asynchronous activity termed large-amplitude irregular activity (LIA) is an irregular waveform with a broadband frequency range of 0.5–25 Hz (Leung et al. 1982). Many populations of cells in the HPC and related structures exhibit discharge properties that are precisely related to hippocampal theta field activity. Such theta-related cells comprised two distinct populations termed theta-ON and theta-OFF, first described in acute preparations using extracellular recordings by Colom et al. (1987), followed by a detailed cell classification paper by Colom and Bland (1987) and subsequently used to classify theta-related cells in the HPC in a number of studies (Bland and Colom 1988, 1989; Bland et al. 1996; Colom et al. 1991; Konopacki et al. 1992; Smythe et al. 1991). Theta-ON and -OFF cells have also been recorded in the medial septal nucleus and nucleus of the diagonal band of Broca (MS/vDBB) (Bland et al. 1990, 1994; Colom and Bland 1991; Ford et al. 1989), the entorhinal cortex (Dickson et al. 1994, 1995), cingulate cortex (Colom et al. 1988), caudal diencephalon (Bland et al. 1995; Kirk et al. 1996), rostral pontine region (Hanada et al. 1999), the superior colliculus (Natsume et al. 1999), the basal ganglia (Hallworth and Bland 1999), the red nucleus (A. Dypvik and B. H. Bland, unpublished data), and the neocortex (Lukatch and MacIver 1996; but see review by Buzsáki 2002 that does not acknowledge the literature on theta-ON and theta-OFF cells). The preceding data suggest, because many regions of the brain from the lower brain stem to the cerebral cortex display “theta-related” neuronal activity, that theta-ON and -OFF cells may represent a general organization of the cellular mechanisms underlying “theta band” oscillation and synchrony (Bland 2000; Bland and Oddie 1998). The morphological identity of theta-ON and -OFF cells is therefore crucial to the understanding of the cellular interactions involved in the generation of theta field activity. Earlier studies by Fox and Ranck...
(1975, 1981) provided indirect evidence that theta cells (theta-ON cells in our scheme) in the HPC were interneurons, a view supported by some recent work on identified cells (see review by Buzsaki 2002). On the other hand, Bland and Colom (reviewed in Bland and Colom 1993) have proposed, also based on indirect evidence, that a subpopulation of HPC projection cells (pyramidal and granule cells) were theta-ON cells and a subpopulation of HPC interneurons were theta-OFF cells.

Theta-band oscillations may also be recorded intracellularly in some populations of cells in the HPC, during the simultaneous occurrence of the extracellular theta field oscillations. In agreement with previous work (Leung and Yim 1991), we have adopted the term membrane potential oscillations (MPOs) to designate the slow intracellular oscillations that occur at theta frequencies in subsets of hippocampal cells. The occurrence of MPOs in HPC pyramidal cells, dentate granule cells, and interneurons has been well documented (Bland et al. 1988; Chapman and Lacaille 1999; Fox 1989; Fox et al. 1983; Fujita and Sato 1964; Konopacki et al. 1992 Leung and Yim 1986, 1988, 1991; Leung and Yu 1998; MacVicar and Tse 1989; Munoz et al. 1990; Nunez et al. 1987, 1990a–c; Ylinen et al. 1995). At present, there is little agreement on how intracellular theta (MPOs) is generated, some arguing that inhibitory postsynaptic potentials (IPSPs) make the major contribution, whereas others argue the case for excitatory postsynaptic potentials (EPSPs) or intrinsic mechanisms (Bland and Colom 1993). As well, there is a lack of understanding of the relationship between MPOs and the rhythmic discharge properties of HPC cells. Do the mechanisms underlying the rhythmic discharge properties of the cell produce the MPOs or do the mechanisms underlying MPOs control the rhythmic cell discharges? The objectives of the present study were to determine the contributions made by MPOs in the control of the rhythmic discharge properties of HPC cells meeting the criteria for classification as phasic theta-ON or phasic theta-OFF were selected for subsequent analysis. Theta-ON cells were defined as cells that increased their activity during theta field activity as reflected by an overall mean increase in discharge rate or as a linear positive increase in discharge rate in relation to increasing frequencies of simultaneously-recorded theta field activity. Theta-OFF cells were defined as cells that decreased their activity during theta field activity as reflected by an overall mean decrease in discharge rate (to 0 in many cases) or as a linear negative increase in discharge rate as theta frequency declines. A further criterion relates to the pattern of cell discharges. A given theta-related cell discharges in one of two characteristic patterns during theta field activity. A rhythmic discharge pattern was defined as two or more cell discharges occurring per extracellular theta wave and was termed phasic because the discharges occurred with a consistent phase relation to each cycle of theta field activity. The second pattern is either regular or irregular discharges termed tonic because they consisted of a nonrhythmic discharge pattern with no observable phase relation to theta field activity. Further criteria were developed on the basis of intracellular recordings of theta-related cells in the hippocampal formation. At theta field onset, phasic theta-ON cells underwent a depolarizing shift of the membrane potential along with prominent theta-related MPOs that were highly coherent with the field oscillations. Rhythmic cell discharges occurred on the positive peaks of the MPOs. At theta field onset, phasic theta-OFF cells underwent a hyperpolarizing shift of the membrane potential, prominent MPOs appeared that were highly coherent with the field oscillations, and cell discharges ceased. As theta frequency slowed, the cell would discharge single spikes, gradually making a brief transition to rhythmic cell discharges (Bland et al. 1988; Konopacki et al. 1992). Signals were lead through an active bridge circuit (Axon Instruments, Axoclamp 2A) allowing simultaneous injection of current and measurement of membrane potential (V_m). The bridge balance was monitored and adjusted as necessary throughout the recording procedures. These and all other signals were displayed on a digital oscilloscope (Tektronix TDS 420).

**Methods**

**Subjects and surgical procedures**

The data were obtained from 38 male black-hooded rats (125–130g) supplied by the Life and Environmental Sciences Animal Care Facility at the University of Calgary. The rats were initially anesthetized with Halothane while tracheal and jugular cannulae were inserted. Halothane was then discontinued, and urethan was administered with Halothane while tracheal and jugular cannulae were inserted. Halothane was then discontinued, and urethan was administered with Halothane while tracheal and jugular cannulae were inserted. Halothane was then discontinued, and urethan was administered with Halothane while tracheal and jugular cannulae were inserted. Halothane was then discontinued, and urethan was administered with Halothane while tracheal and jugular cannulae were inserted. The rats were placed in the stereotaxic instrument with the plane of the bone fragment was drilled out using a 1/4 carbide drill bit, the dimensions ~3.0 mm long by 2.0 mm wide. The well was filled with glycerine to keep the brain moist. Using the same carbide drill bit and holding the bone fragment down with a pair of forceps, a hole was drilled in the center of the bone and rough edges removed from the bone fragment by polishing with the drill bit. Dura was removed using a No. 26 syringe with a small hook in the end and a pair of fine forceps, and the position of the large blood vessels observed because this determined the exact location of the bone fragment. With the layer of glycerine in the pool and using fine forceps, the bone fragment was placed upside down in the hole in the skull with the long side oriented to the midline and gently pushed under the skull at the midline enough to allow it to be pulled back under the lateral edge. The location of the bone fragment was finessed with respect to blood vessels, as discussed in the preceding text.

**Electrophysiological recordings**

Intracellular recordings in HPC cells were made with glass microelectrodes (80–120 MΩ) filled with 2 M potassium acetate and 2% Neurobiotin (Vector Laboratories). Details of these procedures have been published previously (Bland and Konopacki 2000; Konopacki et al. 1992). Cells meeting the criteria for classification as phasic theta-ON or phasic theta-OFF were selected for subsequent analysis. Theta-ON cells were defined as cells that increased their activity during theta field activity as reflected by an overall mean increase in discharge rate or as a linear positive increase in discharge rate in relation to increasing frequencies of simultaneously-recorded theta field activity. Theta-OFF cells were defined as cells that decreased their activity during theta field activity as reflected by an overall mean decrease in discharge rate (to 0 in many cases) or as a linear negative increase in discharge rate as theta frequency declines. A further criterion relates to the pattern of cell discharges. A given theta-related cell discharges in one of two characteristic patterns during theta field activity. A rhythmic discharge pattern was defined as two or more cell discharges occurring per extracellular theta wave and was termed phasic because the discharges occurred with a consistent phase relation to each cycle of theta field activity. The second pattern is either regular or irregular discharges termed tonic because they consisted of a nonrhythmic discharge pattern with no observable phase relation to theta field activity. Further criteria were developed on the basis of intracellular recordings of theta-related cells in the hippocampal formation. At theta field onset, phasic theta-ON cells underwent a depolarizing shift of the membrane potential along with prominent theta-related MPOs that were highly coherent with the field oscillations. Rhythmic cell discharges occurred on the positive peaks of the MPOs. At theta field onset, phasic theta-OFF cells underwent a hyperpolarizing shift of the membrane potential, prominent MPOs appeared that were highly coherent with the field oscillations, and cell discharges ceased. As theta frequency slowed, the cell would discharge single spikes, gradually making a brief transition to rhythmic cell discharges (Bland et al. 1988; Konopacki et al. 1992). Signals were lead through an active bridge circuit (Axon Instruments, Axoclamp 2A) allowing simultaneous injection of current and measurement of membrane potential (V_m). The bridge balance was monitored and adjusted as necessary throughout the recording procedures. These and all other signals were displayed on a digital oscilloscope (Tektronix TDS 420).
and stored on an FM tape recorder (Teac XR-30) for subsequent off-line data analysis. Once a recording was considered stabilized the experimental protocol was initiated. First, recordings were taken during the spontaneously occurring HPC field activities of synchrony (theta) and asynchrony or large-amplitude irregular activity (LIA), ensuring that ≥1 min in duration of each type was accumulated. Next a series of hyperpolarizing (−100, −200, −300, and −400 pA) and depolarizing (100, 200, 300, and 400 pA) constant current pulses were administered during ≥30 s of each of the spontaneously occurring HPC field activities of theta and LIA. In our first such series of experiments, the current pulses were administered in random order. In later experiments, the protocol consisted of administering the hyperpolarizing pulses in ascending magnitude, followed by the depolarizing pulses in ascending magnitude. Analysis revealed no differences between the two stimulation protocols in terms of their effects on MPOs and cell discharge rate and pattern. After completion of this part of the protocol, a series of short-duration hyperpolarizing current pulses (100-ms duration, −100, −200, −300, and −400 pA) and depolarizing current pulses (100, 200, 300, and 400 pA) were administered in ascending magnitude, respectively, for purposes of carrying out standard electrophysiological measurements. Procedures for intra-cellular staining were modified slightly from the procedures previously described (Kita and Armstrong 1991). Positive pulses were applied (5 nA, 2 Hz, 100-ms duration) for 1–10 min and the electrode was left inside the cell for 2–3 min before it was withdrawn.

**Data analysis**

Data were analyzed using the Axon Instruments Axoscope 7, Microcal Origin 4.1, and Data Waves 6.1 software. Spike height and duration was determined from the first action potential evoked at threshold levels of depolarization. Resting membrane potentials were defined as an average of a series of a minimum of 30 membrane potentials measured between the spike discharges occurring during the LIA field conditions. The membrane potential values for the spontaneous theta control (no current) condition and the spontaneous theta condition plus intracellular current injections were defined as average membrane potentials measured at the midpoint of the MPOs when present. Input resistance was provided as the slope of the linear regression line fitted through the linear portion of the current-voltage plots derived from the family of hyperpolarizing and depolarizing current pulse injections. MPOs, defined as the slow intracellular membrane potential oscillations occurring at extracellular theta frequencies, were measured at the positive and negative peaks (see Fig. 2A) using the cursor facility in the Axoscope 7 program, and calculated as the statistical average of a minimum of 50–60 individual MPOs measured in each experimental condition. Additional data analyses included: fast Fourier transforms of the field activities of theta and LIA, autocorrelation histograms and first-order interval spike histograms of the cell discharges during theta and LIA, cross-correlation’s between MPOs and theta field activity, and quantification and statistical analysis using t-tests of the spike discharges occurring during each experimental condition.

**Imaging techniques and cellular identification**

At the termination of a recording session, all animals were administered an overdose of pentobarbital sodium and perfused transcardially with 50 ml phosphate-buffered saline (PBS, 0.1 M, pH 7.4) followed by 300 ml of 4% paraformaldehyde in PBS. The brain was removed and immersed in the fixative for an additional 4 h and then cryoprotected, overnight, in a solution of 30% sucrose in PBS. The brains were frozen, and coronal sections were cut at 50 μm on a sliding microtome. Every section through the hippocampal formation was collected in PBS and then incubated for 2 h in a 1:500 solution of avidin-HRP in PBS. After three washes in PBS, the Neurobiotin-avidin-HRP complex was visualized by incubating the sections in a chromogen solution consisting of 10 ml 0.1 M Tris-buffered saline containing 5 mg diaminobenzidine, 40 μg nickel ammonium sulfate, and 10 μl 30% hydrogen peroxide. When the reaction was complete, the sections were washed three times in PBS and then mounted onto gelatin-coated glass slides and allowed to dry. The sections were dehydrated in an ascending series of ethanol, cleared in xylene and cover slipped using Permount. The labeled cells were identified using a Zeiss Axioplan 2 microscope at ×20, and digital images were captured and serially reconstructed using Open lab (v. 3.0, Improvison) and Adobe Photoshop (v. 6.0, Adobe) running on an Apple G4.

**RESULTS**

**Cells classified as phasic theta-on cells**

**CA1 PYRAMIDAL CELLS.** We describe here the results of intra-cellular recordings of cells (n = 18) in the HPC of urethane-anesthetized rats, classified as phasic theta-on cells (comparison of group mean discharge rates for theta vs. LIA significant at P < 0.05, paired t-test). These cells discharged in a rhythmic pattern during theta but not during LIA, and MPOs were recorded only during the theta field condition, their onset signaled by a 5- to 10-mV depolarizing shift in the membrane potential. Each rhythmic discharge occurred on the depolarizing phase of the MPO and was phase locked to the simultaneously occurring extracellular theta waves. The average resting membrane potential was 62.6 ± 0.91 (SE) mV, average spike height 62.5 ± 1.8 mV, and average input resistance 31.3 ± 1.6 MΩ. Of the 18 cells classified as phasic theta-on cells, 7 were successfully labeled and morphologically identified as CA1 pyramidal cells. Unlabeled cells were inferred to be pyramidal cells as well based on their anatomical location in the CA1 pyramidal layer and their electrophysiological characteristics, which did not differ from those of the labeled cells.

Figure 1A shows an example of a CA1 pyramidal cell (102) recorded during the simultaneous occurrence of either HPC theta (left half) or LIA (right half). MPOs were recorded during the theta field condition only and their onset was signaled by a 5- to 10-mV depolarizing shift in membrane potential. Gamma oscillations were recorded in all cells during both theta and LIA (average frequency = 70 Hz). During theta field activity, the cell discharged in a rhythmic pattern, had a higher discharge rate (12.4 ± 0.7 Hz) compared with the LIA condition (10.3 ± 0.5 Hz), and cell discharges occurred on the depolarizing phase of MPOs with an average amplitude of 9.7 ± 0.3 mV. The MPOs occurred at the same frequency (2.9 ± 0.04 Hz) and phase of the extracellular theta. The positive peak of the MPO corresponded to the positive peak of the theta recorded from the dentate. This would correspond to the negative peak of theta recorded from the CA1 cell layer since the theta field activity recorded from these two regions is ~180° out of phase (Bland and Whishaw 1976). During LIA, the cell discharges were irregular and MPOs were absent. Average spike amplitude during both the LIA and theta conditions was 60 mV. Figure 1B shows the fast Fourier transform (FFT) of the theta field seen in A, top left, which revealed a peak in power at 2.9 Hz. Figure 1C shows the autocorrelation histogram of the rhythmic cell discharges associated with the theta field seen in A, top left. The intervals of regular peaks in the histogram (340 ms) coincided with the theta field frequency. The bimodal distribution of the interspike interval histogram of cell discharges during theta field activity (Fig. 1D) shows the within rhythmic discharge and between rhythmic discharge intervals.
respectively, indicating a rhythmic discharge pattern. Figure 1E shows the FFT of the LIA field activity seen in A, top right, indicating the absence of a peak in power at any narrowband frequency. The autocorrelation histogram (Fig. 1F) of the cell discharges associated with the LIA field seen in A, top right, shows the absence of peaks, thus indicating an irregular cell discharge pattern. This is also illustrated by the interspike interval histogram (Fig. 1G) of cell discharges during LIA field activity showing the absence of a bimodal distribution.

Figure 2A shows a segment of spontaneous theta with the cell trace amplified to illustrate the MPOs and the cursor method used to measure MPO amplitude, whereas Fig. 2B is a segment of LIA illustrating the absence of MPOs in the cell trace. Figure 2C shows the response of this cell to a 200-pA depolarizing intracellular current pulse, applied during the occurrence of spontaneous theta field activity. All cells responded in this simple spike pattern of stimulus-graded trains of independent action potentials (Jensen et al. 1996). Figure 2D presents the current-voltage plot along with the linear regression curve (input resistance = 27.2 MΩ).

Figure 3 shows a CA1 pyramidal cell (154; spikes truncated) during the control (no current) condition and the cell’s responses to hyperpolarizing (left) and depolarizing (right) intracellular constant current injections administered during spontaneously occurring HPC theta field activity, highlighting the effects on cell discharge pattern and MPO amplitude. Phase measurements (data not shown) made comparing the simultaneously occurring extracellular theta field and MPOs revealed maximal phase shifts of <5° occurred during any level of the hyperpolarizing or depolarizing constant current injections. The HPC theta field frequency remained stationary throughout the administration of all current levels (3.9 ± 0.04 Hz). Figure 3, top middle, shows that during the no current control condition the cell exhibited rhythmic discharges with average MPO amplitudes of 6.5 ± 0.4 mV occurring at an average membrane potential of −61 mV. Figure 3 (left) shows that during the injection of −100 pA the cell retained a rhythmic discharge pattern and average MPO amplitudes were reduced (5.7 ± 0.6 mV) at an average membrane potential of −64.7 mV. Figure 3 (left) shows that during the injection of −200 pA, the rhythmic discharge pattern was abolished (although single spikes were still phase-locked to the extracellular theta
(field) and average MPO amplitudes were reduced (3.4 ± 0.4 mV) at an average membrane potential of −67.3 mV. Figure 3 (left) shows that during the injection of ~300 pA all cell discharges were abolished and average MPO amplitudes were reduced (2.4 ± 0.4 mV) at an average membrane potential of −73.3 mV. Figure 3 (left) shows that during the injection of ~400 pA all cell discharges were abolished and average MPO amplitudes were reduced to zero with an average membrane

**FIG. 2.**  A: segment of spontaneous theta from left half of Fig. 1A with cell trace amplified to show the slow MPO and method of measurement using cursors (MPO = 8.6 mV; positivity up in all traces). B: segment of spontaneous LIA from Fig. 1A, right half, showing absence of MPOs. C: intracellular depolarizing current pulse (200-pA, 100-ms duration) applied to cell shown in Fig. 1A during spontaneously occurring theta. Note the simple spike pattern. D: current-voltage plot of the cell shown in Fig. 1A, input resistance = 22.5 MΩ.
potential of $-80.9 \text{ mV}$. Figure 3 (right) shows that during the injection of 100 pA, the cell exhibited rhythmic discharges. The MPOs decreased in amplitude (compared with the no current condition; $4.9 \pm 0.7 \text{ mV}$) at an average membrane potential of $-58.4 \text{ mV}$. Figure 3 (right) shows that during the injection of 200 pA the cell exhibited rhythmic discharges and the MPOs decreased in amplitude ($4.9 \pm 0.5 \text{ mV}$) at an average membrane potential of $-55.4 \text{ mV}$. Figure 3 (right) shows that during the injection of 300-pA rhythmic discharges were abolished and the MPOs decreased in amplitude ($3.4 \pm 0.3 \text{ mV}$) at an average membrane potential of $-54 \text{ mV}$. Figure 3 (right) shows that during the injection of 400-pA cell rhythmicity was abolished at an average membrane potential of $-51 \text{ mV}$, and there were no longer any measurable MPOs.

Data in both Fig. 4, A and B, is plotted against the average membrane potential values produced by the current injections and the average membrane potential value occurring during the control (no applied current) condition for cell 154. Figure 4A graphically summarizes the effects of depolarizing constant current injections and the control (no applied current) condition on the spike discharge pattern and rate during the theta and LIA conditions for the cell shown in Fig. 3. In the control (no current) condition, the rhythmic cell discharge pattern occurred at a mean rate of $6.5 \pm 0.4 \text{ Hz}$, whereas during LIA cell discharge pattern was irregular at a mean rate of $5.0 \pm 0.7 \text{ Hz}$. As the membrane potential was more depolarized from the value of the membrane potential during the spontaneous (no current) control condition, the number of cell discharges increased during both the theta and LIA conditions. The discharge rate remained significantly higher during theta compared with LIA ($P < 0.005$) and rhythmic cell discharges occurred only during the theta condition. Within the theta condition, rhythmic discharges were abolished at membrane potential values depolarized above $-55.4 \text{ mV}$. Cell amplitude during the 200- and 300-pA conditions decreased to 55 mV and decreased further to 42 mV during the 400-pA condition.

As the membrane potential was hyperpolarized from the value of the membrane potential during the spontaneous (no current) control condition, the number of cell discharges decreased during both the theta and LIA conditions. Rhythmic cell discharges occurring during the theta condition only were
completely abolished at membrane potentials hyperpolarized below –64.7 mV. In the –200-pA current condition, rhythmic discharges were abolished during theta; however, when single discharges did occur, they remained phase-locked to HPC theta waves. In the –300-pA current condition, rhythmic discharges were also abolished during theta; but again, when single discharges occurred they remained phase-locked to HPC theta.

Figure 4B graphically summarizes the effects of hyperpolarizing and depolarizing constant current injections and the control (no applied current) condition on the MPO amplitudes during the theta condition for the cell (154) shown in Fig. 3. The effects of current injections during the theta field condition on MPO amplitudes revealed an inverted U-shaped curve. The curve was asymmetrical and distributed around the average value of the membrane potential occurring during the spontaneous theta (no current) control condition. A comparison of the graph in Fig. 4A with the graph in B revealed that rhythmical cell discharges during the theta condition occurred in a critical range of membrane potential values from –55.4 to –64.7 mV. This corresponded to a range of MPO amplitudes of 4–7 mV. The 4- to 7-mV amplitude range was skewed such that more of the larger MPO amplitudes occurred at membrane potentials depolarized above the average value in the control condition compared with the MPO amplitudes that occurred when the membrane potential was hyperpolarized.
The group data for all 18 CA1 phasic theta-on cells are summarized in Fig. 5, presented as means and standard errors of the mean. In this figure, each level of constant current injection has again been converted along the abscissa to the average value of the membrane potential produced by the current injection for each cell, and then averaged across all 18 cells (SEs across all levels of current injections ranged from \( \pm 1.0 \) to \( \pm 1.4 \) mV, whereas the range for \( +400 \) pA was \(-45 \) to \(-55 \) mV and the range for \(-400 \) pA was \(-73 \) to \(-90 \) mV). The effects of constant current injections during the theta field condition on MPO amplitudes, averaged across all cells, again revealed an inverted U-shaped curve. The curve was asymmetrically distributed around the maximal amplitude (6.9 ± 0.3 mV, range: 5.4–9.9 mV) of the MPOs occurring during the average membrane potential (–64 mV) associated with the spontaneous theta no current condition. Phase measurements (data not shown) revealed no significant phase shifts occurring in any of the 18 CA1 phasic theta-on cells.

The effects of current injections during the theta field condition on MPO amplitudes, averaged across all cells, again revealed an inverted U-shaped curve. The curve was asymmetrically distributed around the maximal amplitude (6.9 ± 0.3 mV, range: 5.4–9.9 mV) of the MPOs occurring during the average membrane potential (–64 mV) associated with the spontaneous theta no current condition. Phase measurements (data not shown) revealed no significant phase shifts occurring in any of the 18 CA1 phasic theta-on cells.

The effects of current injections during the theta field condition on the rate of spike discharges averaged across all cells revealed an increase from near 0 Hz (12 of the 18 cells had cell discharge rates reduced to 0) at the maximum hyperpolarized membrane potential of −81 mV to 18.1 Hz at the maximally depolarized membrane potential of −49 mV. The effects of current injections during the LIA field condition on the rate of cell discharges revealed an increase from 0 to 13.7 ± 0.5 Hz over the range of membrane potentials from −81 to −49 mV, respectively. With the exception of the two most hyperpolarized values of −77 and −81 mV where cell discharges were reduced to zero in 12 of the 18 cells, the discharge rates during LIA were significantly lower than those occurring during the theta condition \((P < 0.005)\). Also, unlike the theta condition, rhythmic discharges did not occur at any value of the membrane potential during the LIA condition.

A comparison of the graphs revealed that rhythmic cell discharges during the theta condition occurred in a critical range of membrane potential values from approximately −57 to −68 mV. This corresponded to a range of MPO amplitudes of −4–7 mV. The 4- to 7-mV amplitude range was skewed such that more of the critical range of MPO amplitudes occurred at membrane potentials depolarized above the average value in the control (no current) condition compared with the MPO amplitudes that occurred when the membrane potential was hyperpolarized. Thus the group data for all 18 cells did not differ from the single cell data shown in Fig. 4.

Figure 6, left, shows an example of a CA1 pyramidal cell (226) recorded during the simultaneous spontaneous (no current) occurrence of either HPC theta (left half of the panel) or LIA (right half of the panel). MPOs were recorded during the theta field condition only. The Fig. 6, right,
shows that injecting Neurobiotin into cell 226 resulted in the labeling of 2 CA1 pyramidal cells. Of 7 labeled cells, 57% \((n = 4)\) resulted in double labels (2 pyramidal cells) and 3 were single labels.

**BISTRATIFIED INTERNEURONS.** The left side panel in Fig. 7 shows an example of one of two bistratified cells (216) recorded during the simultaneous occurrence of either HPC theta (left half of the panel) or LIA (right half of the panel). We were unable to measure MPOs of these cells during the occurrence of either theta or LIA field activity. Both cells had a pronounced afterhyperpolarization (see Fig. 7) and showed the highest number of rhythmic cell discharges per theta wave (mean = 6.5 ± 1.2 Hz) compared with all other cells in the study. The mean discharge rate during theta field activity was 24 ± 3.1 Hz and during LIA was 20 ± 2.7 Hz. The right side panel shows that cell 216 was located in the CA1 pyramidal layer (as was the other cell) and was identified as a bistratified interneuron.

Figure 8 shows cell 216 (spikes truncated) during the control (no current) condition (top middle) and the cell’s responses to hyperpolarizing (left) and depolarizing (right) intracellular constant current injections administered during spontaneously occurring HPC theta field activity, highlighting the effects on cell discharge pattern and MPO amplitude. The effects of these manipulations on cell discharge pattern and frequency of the two bistratified cells were different in some respects from those reported in the preceding text for CA1 pyramidal phasic theta-ON cells (see Fig. 9).

Figure 9 reveals that the bistratified cell 216 responded overall to depolarizing current injections with more cell discharges during theta field activity compared with LIA field activity similar to CA1 pyramidal phasic theta-ON cells. However, during theta and LIA, increasing levels of depolarizing current injections resulted in a steady decline in the number of cell discharges, the opposite to the response of CA1 pyramidal phasic theta-ON cells. The response of the bistratified cells to increasing levels of hyperpolarizing current injections was
similar to that of CA1 pyramidal theta-ON cells in that increasing levels of hyperpolarizing current resulted in decreasing cell discharge rates, with complete cessation of discharges at \(-300\) and \(-400\) pA.

**Cells classified as phasic theta-OFF cells**

We describe here the results of intracellular recordings of cells (\(n = 22\)) in the HPC of urethan-anesthetized rats classified as phasic theta-OFF cells. These cells were mostly silent
during theta and discharged during LIA while MPOs were recorded only during the theta field condition, their onset signaled by a 5- to 10-mV hyperpolarizing shift in membrane potential. The cell discharges that did occur during low-frequency theta discharged on the depolarizing phase of the MPOs and were phase locked to the simultaneously occurring extracellular theta waves. Nine of the phasic theta-off cells were recorded from the stratum pyramidale, 11 were recorded from the dentate granule cell layer, and 2 were recorded in the hilus. The average resting membrane potential of the CA1 layer basket cells was $-66.6 \pm 0.91$ mV, average spike height $60.5 \pm 1.8$ mV, and average input resistance $29.4 \pm 6.1 \, \text{M} \Omega$. Of the nine cells recorded in the CA1 pyramidal cell layer and classified as phasic theta-off cells, one was successfully labeled and morphologically identified as a CA1 pyramidal layer basket cell. Unlabeled cells were inferred to be CA1 pyramidal layer basket cells as well based on their anatomical location in the CA1 pyramidal layer, electrophysiological characteristics, and responses to current manipulations, which did not differ from that of the labeled cell. The average resting membrane potential of the dentate layer cells was $-65 \pm 4.91$ mV, average spike height $58 \pm 1.8$ mV, and average input resistance $32 \pm 3.1 \, \text{M} \Omega$. Of the 11 cells recorded in the dentate layer, 3 were labeled and identified as dentate granule cells. Unlabeled cells were inferred to be granule cells as well, based on their anatomical location in the dentate granule layer and their electrophysiological characteristics, which did not differ from that of the labeled cells. Of the two cells recorded in the hilus, one was labeled and identified as a mossy cell interneuron.
CA1 PYRAMIDAL LAYER BASKET CELLS. Figure 10, A and B, shows examples of a CA1 layer basket cell (229) recorded during the simultaneous occurrence of either LIA (left half of the panel) or HPC theta (right half of the panel). MPOs were recorded during the theta field condition only. In Fig. 10A, theta frequency was slightly higher (4.3 Hz) and the cell failed to discharge. In Fig. 10B, theta frequency was lower (3.4 Hz) and the cell began to discharge, first single discharges and then a doublet. Figure 10C shows the response of this cell to a 200-pA depolarizing intracellular current pulse, applied during the occurrence of spontaneous theta field activity. Figure 10D reveals that the intracellular injection of Neurobiotin into cell (229) resulted in the labeling of a cell identified as a basket cell interneuron. The top panel is a low-power magnification showing the location of the cell in the CA1 cell pyramidal layer. The bottom panel is a higher-power magnification showing the details of cell morphology (axons not drawn). The axons of the basket cell were visually confirmed to form a plexus restricted to the CA1 pyramidal layer. Figure 10E presents the current-voltage plot along with the linear regression curve (input resistance = 29.4 MΩ).

Figure 11 shows CA1 layer basket cell (229; spikes truncated) during the control (no current) condition and the cell’s responses to hyperpolarizing (left) and depolarizing (right) intracellular constant current injections administered during spontaneously occurring HPC theta field activity, highlighting the effects on cell discharge pattern and MPO amplitude. Phase measurements (data not shown) made comparing the simultaneously occurring extracellular theta field and MPOs revealed that during the control (no current) condition, the positive peak of the MPOs corresponded with the negative peak of the extracellular theta field activity recorded from the dentate region. This was exactly opposite for the phase relations described in the preceding text for the CA1 pyramidal phasic theta-on cells. As discussed in the preceding text, the positive peak of the MPO would correspond to the positive peak of theta recorded from the CA1 cell layer because the theta field activity recorded from these two regions is ~180° out of phase (Bland and Whishaw 1976). A maximal phase shift of ~90° began to occur during the injection of a 100-pA depolarizing constant current injection and shifted ~180° to become in phase with the extracellular theta field activity during the injection of 200, 300, and 400 pA (Fig. 11, right). The MPOs remained ~180° out of phase with the extracellular theta field activity during the injection of ~100, ~200, ~300, and ~400 pA (Fig. 11, left). The HPC theta field frequency remained stationary throughout the administration of all current levels (4.1 ± 0.06 Hz). Figure 11, top middle, shows the cell failed to
FIG. 10.  A and B: relationships between spontaneously occurring HPC field activity and the cell discharges of a CA1 layer basket cell (229) classified as a phasic theta-OFF cell, in the no current control condition. Top trace in each panel is the HPC field activity recorded from the molecular layer of the dentate region; bottom trace is the discharge pattern of the cell (positivity up in all traces). The 1st half of the panels in A and B show the irregular cell discharge pattern occurring during HPC LIA. Note the absence of MPOs. The 2nd half of the panel in A shows the complete cessation of cell discharges during theta field activity with a higher frequency (4.3 Hz) and the occurrence of MPOs. The 2nd half of the panel in B shows that as theta field frequency slowed to 3.4 Hz, phase-locked cell discharges began to occur. Again, MPOs were recorded during theta field activity. C: intracellular depolarizing current pulse (200-pA, 100-ms duration) applied to the cell during spontaneously occurring theta. D: the intracellular injection of Neurobiotin into cell 229 resulted in the labeling of a cell identified as a CA1 pyramidal layer basket cell. Top: a low-power magnification showing the location of the cell in the CA1 cell pyramidal layer. Bottom: a higher-power magnification showing the details of cell morphology. Calibration bar = 50 μ. E: current-voltage plot of the cell shown in Fig. 1A, input resistance = 29.4 MΩ.
discharge during the no current control condition with average MPO amplitudes of $3.9 \pm 0.5$ mV occurring at an average membrane potential of $-66.4$ mV. Figure 11 (left) shows that during the injection of $-100$, $-200$, $-300$, and $-400$ pA the cell failed to discharge. Figure 11 (right) shows that during the injection of 100 pA, the cell exhibited single cell discharges occurring on average just prior to extracellular theta peak positivity. During the injection of 200 pA, the cell exhibited cell discharges now occurring at peak positivity. Figure 11 (right) shows that during the injection of 300-pA rhythmical discharges phase-locked to the extracellular theta field began to occur. During the injection of 400 pA, cell phase-locked rhythmical discharges continued with an increase in the number of cells per burst. Note this is exactly opposite for the results of these levels of depolarizing current injections in CA1 pyramidal phasic theta-ON cells (i.e., rhythmicity was abolished in these cells).

Data in Fig. 12, A and B, are plotted against the average membrane potential values produced by the current injections and the average membrane potential value occurring during the control no applied current condition for cell 229. Figure 12A graphically summarizes the effects of hyperpolarizing and depolarizing constant current injections and the control (no applied current) condition on the cell discharge pattern and rate during the theta and LIA conditions for the cell shown in Figs. 10 and 11. In the control (no current) condition, the cell failed to discharge while during LIA cell discharge pattern was irregular at a mean rate of $0.8 \pm 0.1$ Hz. As the membrane potential was more depolarized from the value of the membrane potential during the spontaneous (no current) control condition, the number of cell discharges increased during both the theta and LIA conditions. Interestingly, cell discharges increased more during the LIA condition compared with the theta condition, the exact opposite of the data for CA1 pyramidal phasic theta-ON cells. Also, in contrast to the data for CA1 pyramidal phasic theta-ON cells, rhythmic cell discharges occurred only during the theta condition and then only at the most depolarized membrane potential values (300 and 400
pA). In the 100- and 200-pA current conditions, the single-cell discharges that occurred were phase-locked to HPC theta. At all hyperpolarized membrane potential values and during the spontaneous (no current) control condition, there was a total failure of cell discharges.

Figure 12B graphically summarizes the effects of hyperpolarizing and depolarizing constant current injections and the control (no applied current) condition on the membrane potential values produced by the current injections and the average membrane potential value occurring during the control (no applied current) condition. SE bars showing positive half only in A.

FIG. 12. A: graph summarizing the effects of hyperpolarizing and depolarizing constant current injections and the control (no applied current) condition on the cell discharge pattern and rate during the theta and LIA conditions for cell 229 shown in Figs. 11 and 12. B: graph summarizing the effects of hyperpolarizing and depolarizing constant current injections and the control (no applied current) condition on the MPO amplitudes during the theta condition for cell 154. Data in both A and B are plotted against the average membrane potential values produced by the current injections and the average membrane potential value occurring during the control (no applied current) condition. SE bars showing positive half only in A.

The data presented in the preceding text for basket cell 229 was representative of all the data collected for the remaining eight basket cells.
MOSSY CELL INTERNEURONS. Figure 13 (left side panel) shows a transition from LIA to theta field activity (top trace) and the accompanying cell discharges of a labeled mossy cell (203) located in the hilus (right side panel). The cell discharged at a mean rate of 7.0 ± 1.1 Hz during LIA and no MPOs were recorded. The beginning of theta was marked by a hyperpolarizing shift in the membrane potential along with the presence of clear MPOs and the cessation of cell discharges. As the membrane potential became more depolarized, a few cell discharges occurred. In the interest of identifying the cell, the stimulation protocol was not carried out. Figure 13, right, shows that injecting Neurobiotin into cell 203 resulted in the labeling of a mossy cell interneuron in the hilus of the dentate gyrus. Similar relations between cell discharges and spontaneous theta and LIA were observed for one other unlabeled cell recorded from the hilar region.

GRANULE CELLS. Figure 14 (left side panel) shows a transition from LIA to theta field activity (top trace) and the accompanying cell discharges of a labeled granule cell located in the lower blade of the dentate granule layer (right side panel). The cell discharged at a mean rate of 3.0 ± 4.1 Hz during LIA and no MPOs were recorded. The beginning of theta was marked by a hyperpolarizing shift of 5.2 mV in the membrane potential along with the presence of clear MPOs and the cessation of cell discharges. These data are representative of all 11 granule cells recorded. We found these cells very difficult to hold for long periods so in the interest of identifying the cell, the stimulation protocol was not carried out. Figure 14, right, shows that injecting Neurobiotin into cell 190 resulted in the labeling of three granule cells in the lower blade of the granule cell layer. The injection of Neurobiotin into granule cells 218 and 220 both resulted in the labeling of two granule cells (data not shown).

DISCUSSION

Cells classified as phasic theta-on cells

In the present study, all 18 CA1 pyramidal cells responded with a simple cell discharge pattern to depolarizing current pulses. Jensen et al. (1996) demonstrated that CA1 pyramidal cells fell into two categories according to their response to depolarizing current pulses: nonbursters (simple spikes) and bursters (complex spikes). Nonbursters generated stimulus-graded trains of independent action potentials with nondecrementing amplitudes, whereas bursters generated clusters of three or more closely spaced spikes of descending amplitude riding on a distinct depolarizing envelope.

The present study has provided evidence supporting the following conclusions concerning cells classified as phasic theta-on cells: 1) morphologically identified hippocampal CA1 pyramidal cells formed a subset of cells meeting the criteria for classification as phasic theta-on cells. Previous studies (Fujita and Sato 1964; Leung and Yim 1988, 1991; Nunez et al. 1987, 1990a) have clearly demonstrated the rhythmical discharge properties of CA1 pyramidal cells; 2) MPOs occurred only during theta field activity, their onset signaled by a 5- to 10-mV depolarizing shift in membrane potential; 3) the amplitude of membrane potential oscillations in CA1 pyramidal phasic theta-on cells was voltage dependent and frequency was voltage independent; 4) there were no phase changes observed during current injections; however, amplitude analysis of MPOs revealed an inverted U-shaped curve asymmetrically distributed
around the average value of the membrane potential occurring during the spontaneous theta (no current) control condition; 5) the rate of rhythmic cell discharges in the CA1 pyramidal phasic theta-on cells during the theta condition was precisely controlled within a critical range of membrane potential values from approximately $-57$ to $-68$ mV, corresponding to a range of MPO amplitudes of $\sim 4-7$ mV. Outside the critical range, rhythmic discharges were abolished. And 6) there appear to be some interneurons that meet our criteria for being phasic theta-on cells. These are the bistralitified cells first described by Buhl et al. (1994). However, MPOs were not recorded in these cells during theta field activity and they responded in a different manner to depolarizing constant current injection.

Mechanisms underlying the generation of MPOs in identified CA1 pyramidal cells

The present study demonstrated that MPOs occurred only during spontaneous theta field activity and not during the spontaneous occurrence of LIA field activity. Previous data on the mechanisms underlying the generation of MPOs may be summarized as supporting three main conclusions: they are generated by inhibitory postsynaptic potentials (IPSPs); they are generated by excitatory postsynaptic potentials (EPSPs); and they are generated by intrinsic properties of the cell membrane. Evidence supporting the IPSP hypothesis came from observations that the laminar profile of antidromically evoked IPSPs in the CA1 region was similar to that of the theta rhythm of anesthetized animals (Artemenko 1973; Fox et al. 1983). Leung and Yim (1986) provided stronger support by demonstrating that MPOs recorded in CA1 pyramidal cells reversed in phase with respect to extracellularly recorded theta at a reversal potential corresponding to GABA-mediated IPSPs. Further support for the importance of IPSPs has come from studies by Fox (1989), Soltesz and Deschenes (1993), and Ylinen et al. (1995). The main support for the EPSP hypothesis has derived from two main observations: MPOs do not reverse with membrane voltage or the intracellular injection of Cl$^{-}$ and the MPO was larger with hyperpolarization. (Fujita and Sato 1964; Nunez et al. 1987, 1990a). The main support for the intrinsic generation hypothesis has come from studies using in vitro hippocampal preparations. Leung and Yim (1988, 1991) demonstrated theta frequency MPOs could be induced in hippocampal cells solely by depolarization and even when synaptic transmission was blocked by low Ca$^{2+}$, low Na$^{+}$, and tetrodotoxin. In the present study, the demonstration of an inverted U-shaped function following the injection of depolarizing and hyperpolarizing constant current pulses also argued against the dependence of MPOs on synaptic activity. There were no phase reversals observed between the MPOs and the extracellularly recorded theta field activity and hyperpolarization of the membrane potential did not increase the size of the MPOs. Nunez et al. (1990a) also reported that the injection of hyperpolarizing current pulses into CA1 pyramidal cells recorded in vivo failed to produce any phase reversals, although they did report that the amplitude of the intracellular theta (MPOs) increased. In the present study, we did not observe an increase in amplitude during hyperpolarizing current injections; in fact, increasing hyperpolarization eventually totally abolished MPOs (as did increasing levels of depolarization). On the other hand, Soltesz and Deschenes (1993) did observe an $\sim 180^\circ$ phase shift in CA1 cells that were hyperpolarized from $-65$ to $-85$ mV, and similar phase shifts were reported by Ylinen et al. (1995). In the present study, we failed to see a

FIG. 14. Relationships between spontaneously occurring HPC field activity and cell discharges of a dentate granule cell (190) classified as a phasic theta-off cell in the no current control condition. Top trace: the HPC field activity recorded from the molecular layer of the dentate region; bottom trace: the discharge pattern of the cell (positivity up in all traces). The 1st half of the panel shows the irregular cell discharge pattern occurring during HPC LIA. Note the absence of MPOs. The 2nd half of the panel shows the complete cessation of cell discharges during theta field activity. MPOs occurred along with theta field activity. Right: the intracellular injection of Neurobiotin into the cell resulted in the labeling of 3 dentate granule cells. Right, top: a low-power magnification showing the location of the cells in the lower blade of the dentate gyrus. Bottom: a higher-power magnification showing the details of the cells morphology. Calibration bar = 50 µ.
phase reversal in three cells hyperpolarized in the range from $-85$ to $-90$ mV. A possible explanation for the difference between these two studies and the present study may lie in the way the CA1 pyramidal cells were selected for data analysis. Indeed, in both the Soltesz and Deschenes and Ylinen et al. studies only low-firing (<1 and 2 Hz, respectively) CA1 pyramidal cell were selected for analysis compared with discharge rates of 10–12 Hz during theta in the present study. Soltesz and Deschenes (1993) and Ylinen et al. (1995) also reported that the frequency of intracellular theta rhythm in CA1 pyramidal cells was independent of membrane potential, a result supported by the present study. The absence of any measurable MPOs during the 300- and 400-pA applied current conditions could be due to the fact that the membrane potential would be largely determined by the spike conductances, which may short-circuit those responsible for the generation of MPOs. If this was the case, then rhythmic spike discharges would be abolished due to the large current-induced depolarization and not the abolishment of the MPOs. In the current study, we had no direct experimental evidence to determine which explanation was correct. It is also of interest to note that in the present study the four levels of depolarizing constant current failed to produce MPOs during spontaneously occurring LIA field activity, in agreement with the results of Ylinen et al. (1995). On the other hand, in the in vitro studies of Leung and Yim (1998), they were able to manipulate MPO frequency with intracellular current injections. We have no explanation for the differences except to note that our experiments were carried out in vivo. In addition, it’s possible that our current steps were too large and thus missed producing the appropriate membrane potential for activating the membrane currents underlying the generation of MPOs.

**Relationship between MPOs and rhythmic cell discharges at theta frequencies in identified CA1 pyramidal cells**

The present study demonstrated that the occurrence of MPOs in a critical range of amplitudes and the rhythmic cell discharges at theta frequencies was highly correlated. These data provide evidence that once extrinsic synaptic inputs enable the theta state, voltage-dependent intrinsic MPOs functioned to control the rhythmic discharge properties of CA1 hippocampal pyramidal cells classified as phasic theta-on cells. Thus intrinsically generated MPOs allow CA1 phasic theta-on cells to be tuned selectively to theta-band frequencies. The question remains as to whether MPOs control the rhythmic discharge properties or vice versa. We believe our data provide evidence that once synaptic inputs enable the theta state, voltage-dependent MPOs control the rhythmic discharge properties of phasic theta-on cells morphologically identified as CA1 pyramidal cells. We are aware of only one other published study in which the data suggested that MPOs actually modulate rhythmic CA1 pyramidal cell discharges rather than vice versa (Leung and Yim 1998). Although the present data are not unequivocal, there were several observations supporting our contention: 1) we observed on occasions during the control (no current) spontaneous theta condition that MPOs may occur without cell discharges but never observed rhythmic cell discharges in the absence of an MPO; 2) in some phasic theta-on cells, we observed MPOs persisting at lower levels of current produced hyperpolarizations along with a complete inhibition of cell discharges; 3) nonrhythmic cell discharges still occurred when MPOs were very small or totally abolished; 4) nonrhythmic cell discharges occurred during the LIA field condition in the complete absence of MPOs; and 5) the application of depolarizing current during the LIA field state was not sufficient to produce MPOs or rhythmic cell discharges. We therefore concluded that MPOs in a critical range of amplitudes between 4 and 7 mV serve to entrain cell discharges into a rhythmic pattern.

The present research had the advantage of studying spontaneously occurring field activities in the whole animal. Thus all experimental manipulations occurred during the spontaneous cycling between “physiologically occurring” theta and LIA. Furthermore, theta field frequency in each animal was essentially clamped as a variable. That is, in a given experiment, theta frequency was stationary, varying <0.5 Hz for the entire duration of the experiment. Across all experiments, theta field frequencies ranged from 3 to 4.5 Hz. MPOs occurred only during theta field activity and at the same frequency as the extracellular theta frequency. Previous work using extracellular recording techniques demonstrated that phasic theta-on cells in both the CA1 and dentate regions of the hippocampal formation increased the number of rhythmic discharges per theta wave as theta field frequency increased in both acute (Colom and Bland 1987; Colom et al. 1987) and freely moving animals (Bland et al. 1983; Sinclair et al. 1982). In the present experiments, despite the fact that theta frequency remained constant, CA1 phasic theta-on cells increased the number of rhythmic cell discharges in response to increases in membrane depolarization in a range from approximately $-67$ to $-58$ mV, corresponding to MPO amplitudes from 4 to 7 mV. Thus while “physiologically occurring” synaptic inputs were driving the cells at a given theta frequency, injecting depolarizing or hyperpolarizing current was capable of modulating the rate of rhythmic spike discharges within a critical range of MPO amplitudes. In contrast, while “physiologically occurring” synaptic inputs were producing LIA field activity with no accompanying MPOs, injecting depolarizing or hyperpolarizing current modulated the rate of irregular spike discharges. The present data therefore indicated that the relationship between the intrinsic membrane properties and extrinsic theta-related synaptic inputs is a complex one. It would be of interest to repeat the present experiments with the addition of stimulating the ascending brain stem hippocampal synchronizing pathways to produce a range of theta frequencies.

Our finding that the injection of Neurobiotin in 57% of our recorded cells resulted in the labeling of two pyramidal cells was very similar to the value of 63% of Lucifer yellow-coupled CA1 pyramidal cells recorded during theta field activity (Munoz et al. 1990).

**Cells classified as phasic theta-off cells**

In the present study, 22 cells met the criteria for classification as phasic theta-off cells and provided evidence to support the following conclusions: 1) morphologically identified CA1 pyramidal layer basket cells, mossy hilar cells, and granule cells formed a subset of cells meeting the criteria for classification as phasic theta-off cells; 2) MPOs occurred only during theta field activity, their onset signaled by a hyperpolarizing shift of 5–10 mV in membrane potential; 3) the amplitude of...
membrane potential oscillations in CA1 pyramidal layer basket cells was voltage dependent and frequency was voltage independent; 4) the phase of membrane potential oscillations in CA1 pyramidal layer basket cells underwent an ~180° phase reversal when the membrane potential was depolarized to around ~65 mV; and 5) the occurrence and rate of rhythmic cell discharges in the CA1 pyramidal layer basket phasic theta-off cells during the theta condition was precisely controlled within a critical range of membrane potential values from approximately ~62 to ~60 mV, corresponding to a range of MPO amplitudes of ~7–7.5 mV. Outside the critical range, cell discharges were absent or occurred singly.

Mechanisms underlying the generation of MPOs in identified CA1 pyramidal layer basket cells

The present study demonstrated that MPOs occurred in phasic theta-off cells only during spontaneous theta field activity and not during the spontaneous occurrence of LIA field activity, the same findings reported above for phasic theta-on cells. However, in contrast to phasic theta-on cells, the onset of MPOs in all cells identified as phasic theta-off cells was signaled by a hyperpolarizing shift of ~5–10 mV in the membrane potential. These data support our earlier paper (Bland et al. 1988) that was the first to our knowledge to report this finding in cells recorded in hippocampal slices and subsequently in acute in vivo preparations (Konopacki et al. 1992). Furthermore, the amplitude of the MPO was voltage dependent, increasing in amplitude and reversing in phase when the membrane was depolarized below the average membrane potential occurring during spontaneous theta field activity. Contrary to the findings for phasic theta-on cells, these observations support the view that MPOs in basket cells act as synaptic mediators and that chloride-dependent IPSPs play a role in their generation. This, along with the finding that the frequency of MPOs was voltage independent in basket cells, supports the previous findings of Ylinen et al. (1995). Ylinen et al. also reported that basket cells discharged in a manner related to theta that would meet our criteria of being theta-on cells, exactly the opposite of our findings but consistent with the earlier inferences of Buzsaki et al. (1983) and Fox and Ranck (1975) that interneurons discharge at higher rates during theta compared with LIA and theta-off cells were often silent during theta and discharged during LIA. Further study extended these findings and resulted in a classification scheme for theta-related cells (Colom and Bland 1987). Fox and Ranck (1975, 1981) presented evidence that theta (theta-on) cells were interneurons while Bland et al. (1980) argued that projection cells were theta (theta-on) cells. Based on several lines of evidence, Bland and Colom (1989) proposed that theta-off cells were inhibitory interneurons that received inhibitory GABAergic projections from the medial septum (see also Smyth et al. 1992). Ylinen et al. (1995) have provided evidence that CA1 layer basket cells are related to theta in the manner that we would classify as theta-on cells and that granule cells are related to theta in a manner we would classify as theta-off cells. The findings of the present paper serve to illustrate that the relationship of cells in the hippocampal formation to the field states of theta and LIA are more complex than was previously envisioned by theta researchers. Indeed, as we previously hypothesized, some interneuron subtypes behave as theta-off cells (basket cells and mossy interneurons) but some behave as theta-on cells (bistratified cells). On the other hand, contrary to our previous proposal, some projection cells (granule cells) behave as theta-off cells while at least a subset of others (CA1 pyramidal cells) behave as theta-on cells.

Relationship between theta-on and theta-off cells

As discussed in the preceding text, MPOs in basket cells occurred during theta field activity only and begin with a hyperpolarizing shift in the membrane potential. Thus for much of the time, cell discharges are prevented from reaching firing threshold. However, as the membrane becomes depolarized, correlated with the brief transition between slow frequency theta to LIA, theta-off cells begin to show phase locked single cell discharges, followed by an increasing number of rhythmic discharges per theta wave. These results are compatible with earlier findings that phasic theta-off cells code the declining theta field frequency (Colom and Bland 1987). The cell discharges under these conditions were correlated with the MPOs in that they occurred on the depolarizing phase of the MPOs and increased the number of rhythmic discharges per MPO in relation to the increasing amplitude of the MPOs.

Mossy cells and granule cells classified as theta-off cells

Because of the great difficulties we experienced in being able to maintain lengthy stable recordings in these cells, we made the decision to collect enough data during the spontaneous field conditions to classify the cells and then attempt to stain them for identification. The 2 mossy cells and 11 granule cells classified as theta-off cells all behaved in the same manner at the transition from LIA to theta field activity as the basket cells described in the preceding text. That is, MPOs in mossy cells and granule cells occurred during theta field activity only and began with a hyperpolarizing shift in the membrane potential. Cell discharges only occurred as the membrane potential began to depolarize. These findings are in essential agreement with those reported by Soltesz et al. (1993) for mossy cell interneurons and Ylinen et al. (1995) for granule cells although these authors did not refer to them as theta-off cells.

General discussion

Identification of theta-related cells. Colom et al. (1987) first introduced the nomenclature of theta-on and -off to distinguish two types of HPC cells that were essentially reciprocally related to theta field activity. As the name implies, theta-on (theta) cells discharged at higher rates during theta compared with LIA and theta-off cells were often silent during theta and discharged during LIA. Further study extended these findings and resulted in a classification scheme for theta-related cells (Colom and Bland 1987). Fox and Ranck (1975, 1981) presented evidence that theta (theta-on) cells were interneurons while Bland et al. (1980) argued that projection cells were theta (theta-on) cells. Based on several lines of evidence, Bland and Colom (1989) proposed that theta-off cells were inhibitory interneurons that received inhibitory GABAergic projections from the medial septum (see also Smyth et al. 1992). Ylinen et al. (1995) have provided evidence that CA1 layer basket cells are related to theta in the manner that we would classify as theta-on cells and that granule cells are related to theta in a manner we would classify as theta-off cells. The findings of the present paper serve to illustrate that the relationship of cells in the hippocampal formation to the field states of theta and LIA are more complex than was previously envisioned by theta researchers. Indeed, as we previously hypothesized, some interneuron subtypes behave as theta-off cells (basket cells and mossy interneurons) but some behave as theta-on cells (bistratified cells). On the other hand, contrary to our previous proposal, some projection cells (granule cells) behave as theta-off cells while at least a subset of others (CA1 pyramidal cells) behave as theta-on cells.

Relationships between theta-on and theta-off cells

Colom et al. (1987) emphasized the reciprocal relationship between the discharge rates of phasic theta-on and phasic theta-off cells that occurred over a range of theta field frequen-
cies. That is, phasic theta-on cells increased their discharge rates as theta frequency increased and phasic theta-off cells decreased their discharge rates as theta frequency increased (to 0 in many cases). These observations were based on extracellular unit recordings. The intracellular data in the present paper provides further evidence in support of this reciprocal relationship (see also Cobb et al. 1995). Increasing levels of membrane depolarization in both CA1 pyramidal phasic theta-on cells and CA1 layer basket phasic theta-off cells resulted in increasing discharge rates during theta and LIA field activity. However, in phasic theta-on cells, discharge rates were higher during theta compared with LIA and in phasic theta-off cells discharge rates were higher during LIA compared with theta. Furthermore, at the highest levels of membrane depolarization, phasic theta-on cell discharges became arrhythmic while phasic theta-off cell discharges became rhythmic. Finally, the data supported the view that the main currents underlying the voltage-dependent MPOs in CA1 pyramidal theta-on cells are intrinsic, while those underlying the MPOs, at least in CA1 layer basket phasic theta-off cells, were mainly synaptic, mediated by chloride currents. In conclusion, the findings of the present paper support the model of theta generation proposed by Bland and Colom (1993).

This research was supported by Natural Sciences and Engineering Research Council Grants A9935 to B. H. Bland and A217322 to R. H. Dyck.

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