Endogenous Waves in Hippocampal Slices

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Kubota, Don, Laura Lee Colgin, Malcolm Casale, Fernando A. Brucher, and Gary Lynch. Endogenous waves in hippocampal slices. J Neurophysiol 89: 81–89, 2003. First published October 10, 2002; 10.1152/jn.00542.2002. Sharp waves (SPWs) are thought to play a major role in intrinsic hippocampal operations during states in which subcortical and cortical inputs to hippocampus are reduced. This study describes evidence that such activity occurs spontaneously in appropriately prepared rat hippocampal slices. Irregular waves, with an average frequency of approximately 4 Hz, were recorded from field CA3 in slices prepared from the temporal region of hippocampus. The waves persisted for hours and were not accompanied by aberrant discharges. Multi-electrode analyses established that they were locally generated within each of the subfields of CA3 and yet were coherent between subfields. The sharp waves were reversibly blocked by either cholinergic or serotonergic stimulation. Various lines of evidence indicate that they are propagated by the CA3 associational system.

INTRODUCTION

Rhythms (theta, beta, gamma) similar to those found in vivo appear in brain slices following infusion of cholinergic agonists (Fellous et al. 2000; Fisahn et al. 1998; Huerta and Lisman 1993; Konopacki et al. 1987; Shimono et al. 2000; Williams and Kauer 1997). Analyses of these in vitro oscillations have contributed significantly to the elucidation of how cholinergically dependent rhythms are generated within hippocampus. Brief episodes of rhythmic activity have also been generated using patterned afferent stimulation. The gamma (approximately 40 Hz) to beta frequency (approximately 20 Hz) sequence found in human EEG following certain types of sensory stimulation can be reproduced in hippocampal slices using high-frequency stimulation at two loci (Traub et al. 1999). Studies of this type have allowed for rigorous in vitro testing and led to explicit hypotheses about the generation of high-frequency rhythms and their potential roles in behavior.

An EEG pattern commonly recorded in hippocampus of behaving rats consists of irregular sharp waves (SPWs) with frequencies ranging from 0.01 to 3 Hz (Buzsaki 1986; Buzsaki et al. 1983). Sharp wave activity in vivo correlates with behaviors such as slow wave sleep and immobility and is antagonistic to theta rhythm both behaviorally and physiologically. Sharp waves are associated with synchronous population bursts of CA3 pyramidal neurons and may be propagated via the extensive recurrent collateral system in CA3 (Buzsaki 1986; Suzuki and Smith 1988). SPW activity has been linked to neural plasticity in vivo (King et al. 1999) and is hypothesized to be essential for memory formation (Buzsaki 1989). It would thus be advantageous to devise a means for generating SPWs in the hippocampal slice preparation such that hypotheses regarding their origins and potential functions could be more readily tested.

Although hippocampal SPWs have been recorded in a number of different species in vivo (Buzsaki et al. 1983; Frenomon et al. 1969; Frenomon and Walter 1970; Jouvet et al. 1959), they have not yet been observed in hippocampal slices. A possible explanation for this absence is that the degree of interconnectivity of pyramidal cells in typical slices is not great enough to support the recurrent activity thought to be needed for generation of SPWs. That is, while the CA3 field of hippocampus contains a remarkably dense associational system, most of its fibers are not oriented along the plane at which slices are typically prepared (Ishizuka et al. 1990). Under these circumstances, it is possible that long associational fibers are mostly cut during tissue preparation, leaving the slice without the capacity to rapidly mobilize large numbers of pyramidal neurons.

This study tested for spontaneously occurring SPWs in slices prepared from the mid-septotemporal level of hippocampus, a zone in which the associational fibers have a flatter trajectory than is the case more rostrally (Ishizuka et al. 1990). It was expected that interconnectivity, and the chances for synchronous population bursts, would be optimized under these conditions. A stable pattern of periodic slow activity resembling sharp waves was reliably obtained without external stimulation; this activity is distinct from theta rhythm and may represent an in vitro correlate of sharp waves.

METHODS

Slice preparation

Hippocampal slices were cut at a thickness of 350 μm from male Sprague-Dawley rats approximately 4 wk of age using a vibrating tissue slicer (Leica VT1000, Leica, Bannockburn, IL). Animals were anesthetized and killed by decapitation following a protocol accredited by the University of California Institutional Animal Care and Use Committee with guidelines set forth by the National Institutes of Health. The brain was quickly removed and chilled in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) of the following composition:

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composition (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 5 MgSO4, 3.4 CaCl2, 10 d-glucose, and 26 NaHCO3. Transverse slices were prepared from the level approximately two-thirds of the way down the septo-temporal arc and placed on an interface recording chamber. Oxygenated ACSF was infused at a rate of 60 ml/h. Recording ACSF was of the same composition as above, except that the CaCl2 and MgSO4 concentrations were lowered to 3 and 1 mM, respectively. Slices recovered for at least one hour prior to the start of the experiment. Additionally, warmed and humidified 95% O2-5% CO2 filled the chamber throughout the duration of the experiment.

Field potential recording

Glass electrodes filled with 2 M NaCl were used to record field potentials. Samples of 1,500 ms were recorded every 30 s for pharmacological analyses. For coherence, cross-correlation, and spike rate analyses, field potentials were continuously recorded for a period of ≥10 min. In all cases, data were recorded using a differential AC amplifier (model 1700, A-M Systems, Carlsborg, WA) and digitized at 2 kHz. Slices were maintained at physiological temperatures (32 ± 2°C) and were used for one experimental treatment only (i.e., multiple manipulations were not performed on the same slices).

Reagents

Compounds were purchased from Sigma (St. Louis, MO). Carbachol (CCh), serotonin (5-HT), and dopamine (DA) were applied to the infusion line using an injection pump. Atropine was applied via bath infusion. Solutions were prepared freshly each day.

Statistical analyses

Results are reported as means ± SE. Spectral power was estimated using the Fast Fourier Transformation function in MATLAB (MathWorks, Natick, MA). Since the frequency of the recorded activity varied across time from 1 to 10 Hz, a median band range of 4–7 Hz was chosen for calculating normalized average power.

For spike detection in CA3b striatum pyramidal, the second derivative d2(t) of the recorded data v(t) was estimated at time k using data points v(k) and its two immediate neighbors v(k − 1) and v(k + 1): d2(k) = −2v(k) + v(k − 1) + v(k + 1) from 10 min of unfiltered recording. A simple threshold (0.05) was then used to select the second derivative values positive enough to be classified as a spike.

For detection of the waves (in vitro SPWs), recordings from CA3s sylleptic were first band-pass filtered from 1 to 20 Hz. Potentials remaining above a 0 nV threshold for a duration of 40–120 ms were detected as SPWs. The maximum voltage value of each SPW was then set to be the 0-ms reference point when calculating discharge probability across time.

For every SPW event, a window starting 250 ms prior to and ending 250 ms after the peak of the SPW was used to count discharge events (spikes). The time of each spike occurrence, relative to the 0-ms reference point, was recorded for every SPW event within a slice. A histogram (5-ms bins) was then constructed, and an estimate of discharge probability was obtained by normalizing the histogram, i.e., dividing each bin by the sum of discharge events across all bins. The individual histograms, one per slice, were then averaged to produce the grand average depicted in Fig. 4B (bottom). Additionally, all detected SPWs were averaged within each slice, and the grand average was computed across all slices (shown in Fig. 4B, top).

Cross-correlations were assessed in a group of six slices with three recording electrodes placed in CA3a, CA3b, and CA3c and exhibited well-developed rhythms in CA3a. Cross-correlation coefficients were estimated from 5 min of continuous recording using the cross-correlation function in MATLAB following band-pass filtering of the data (1–55 Hz). The maximum lag time considered was ±15 ms; this lag time was chosen based on likely physiological constraints.

Coherence analysis

The coherence between two signals is a squared correlation coefficient in the spectral domain. Coherence measures the linear dependency between two processes at a particular frequency, or in other words, how much of the variance in amplitude and phase at a particular frequency in one signal is related to variance in the other signal. If the difference in phase and/or amplitude between the two signals is completely random from trial to trial, then the coherence will not be significantly different from zero. Conversely, if the phase difference between the two signals is strongly concentrated around a specific phase from trial to trial, the coherence will be relatively high.

Continuous recordings were collected simultaneously from CA3a, CA3b, and CA3c in 10 slices from eight rats for coherence analyses. A subset of 6 of the 10 slices were used in the cross-correlation study. 7 of the same slices were used for the power analysis shown in Fig. 3A, while all 10 CA3b recordings were used in the spike discharge probability analysis. Spectral power was computed for the oscillatory activity in all three regions of these 10 slices and did not differ in frequency from region to region. Additionally, spectral power estimates from 10 min of continuous recordings used for coherence analyses did not differ significantly from spectral power estimates from 1,500 ms discrete recordings used for other groups in the study.

For estimating coherence, 10 min of continuously recorded data were divided into 600 epochs. Prior to estimating coherence γXY(f) between two signals X and Y from N time epochs, the cross-spectrum CXY(f) was estimated at each frequency f using the following equation (Bendat and Piersol 1986; Srinivasan et al. 1999)

\[ C_{XY}(f) = \frac{1}{N} \sum_{n=1}^{N} X_n(f)Y_n(f) \]

where \( X_n(f) \) is the Fourier transform of the nth epoch of signal X at frequency f. The cross-spectrum was then squared and divided by the average power spectra of the individual signals to estimate coherence γXY(f) (Bendat and Piersol 1986; Srinivasan et al. 1999)

\[ \gamma_{XY}^2(f) = \left| \frac{C_{XY}(f)}{P_X(f)P_Y(f)} \right|^2 \]

where \( P_X(f) = |C_{XX}(f)| \) is the average power spectrum of signal X at frequency f. To compute the SE for each coherence estimate, based on the assumption that the signals are samples of a Gaussian random process, the following computation was performed (Bendat and Piersol 1986; Srinivasan et al. 1999)

\[ \epsilon_{XY} = \sqrt{\frac{2}{N} \left( 1 - \gamma_{XY}^2 \right)} \]

The SE was then used to construct 95% confidence intervals for the actual coherence values (\( \gamma_{XY}^2 \)) from the estimated coherence (\( \gamma_{XY}^2 \)) as follows

\[ \gamma_{XY}^2 - 2\epsilon_{XY} < \gamma_{XY}^2 < \gamma_{XY}^2 + 2\epsilon_{XY} \]

Partial coherence analysis is used to assess correlations between two signals at particular frequencies after the linear influence of a third signal is removed. Partial coherence was calculated using the equation below (Halliday et al. 1995; Mima et al. 2000; Ohara et al. 2001)

\[ |\gamma_{XY,Z}(f)|^2 = \left| \frac{C_{XY}(f) - C_{XZ}(f)C_{YZ}(f)}{C_{XX}(f) - C_{XZ}(f)C_{ZZ}(f)} \right|^2 \left( \frac{C_{YY}(f) - C_{YZ}(f)C_{ZZ}(f)}{C_{YY}(f) - C_{YX}(f)C_{XY}(f)} \right) \]

Multiple coherence γXY,Z(f) measures coherence between one signal X(f) and an optimum linear combination of the signals Y(f)
This quantity may be expressed in the following form (Jenkins and Watts 1968; Kocsis et al. 1999) for efficient calculation

\[ \gamma_{X,Y,Z}(f) = 1 - (1 - \gamma_{X,Y,Z}(f))(1 - \gamma_{X,Y,Z}(f)) \]

**RESULTS**

**Spontaneous slow wave activity in slices**

Figure 1A shows typical extracellular records of spontaneous field activity collected from the s. pyramidale of field CA3. These waves could be recorded for hours without obvious changes from the beginning of a test session (i.e., starting at 60–90 min after placing the slices in the recording chamber). The prominent feature of each cycle is a positive-going potential with a sharp rise and more gradual decay, varying from tens to hundreds of microvolts in amplitude from cycle to cycle. Power spectral analyses indicated that the frequency of the activity varied over time from approximately 1 to 10 Hz within a particular slice as described in Fig. 1C. The peak frequency recorded from field CA3b s. pyramidale was 4.0 ± 0.4 Hz, while average power in the 4–7 Hz band was 0.46 ± 0.12 mV² \((n = 23\) field recordings from 13 rats). The prevalence and amplitude of the waves were greatly reduced in field CA1 relative to CA3 and larger in slices from the mid-septotemporal level of hippocampus (Fig. 2) than in those from more rostral levels.

Recordings were taken from different CA3 subfields (Fig. 2) in an effort to identify the origins of the waves. Profiles along the “medio-lateral” dimension of CA3 showed that the largest power occurred in field CA3b with that from CA3a and CA3c being less by about 80% and 60%, respectively (Fig. 3, A and B). Similar results were obtained from seven slices in which simultaneous recordings from CA3a, CA3b, and CA3c were collected. Laminar profile analyses were carried out to further define the origins of the waves. Phase reversal of the potentials was obtained in all three CA3 subzones, indicating that activity was locally generated within each subdivision rather than being volume conducted. The waves proved to be positive at the cell bodies, positive in s. oriens and s. lucidum, and negative within the s. radiatum and s. moleculare (Fig. 3C). Interestingly, this pattern matches the depth profile analysis of sharp waves.

1 To view an animation of the rhythms described in the present work, go to http://www.colgin.net/Kubota_et_al/.
recorded from rat hippocampus in vivo (Buzsáki 1986) and is the pattern associated with stimulation of that branch of the CA3 associational collaterals traveling through the apical dendrites. Moreover, the duration of the negative going potentials from CA3b-CA3c pairs had high coherence after the correlated CA3a signal was removed (mean value: 0.57). No CA3a-CA3c cases were coherent after CA3b’s influence was partialed out, and within slices this value was the lowest of the three in all cases (average value: 0.08; Fig. 5B).

In contrast, multiple coherence estimates were generally high. CA3a was highly coherent with an optimum linear combination of CA3b and CA3c in 7 of 10 slices (mean: 0.68). CA3c coherence with CA3a and CA3b was also high in 7 of 10 slices (mean: 0.74), while CA3b with CA3a and CA3c had the highest coherence (mean: 0.84) within slices (Fig. 5C).

Cross-correlations were used to infer causal relationships between the activation of the subregions of CA3. Correlations were significant with the following pattern: CA3b versus CA3c > CA3b versus CA3a > CA3a versus CA3c, a pattern consistent with the results of coherence analysis. Despite high correlations, there were no consistent lag times between areas; that is, lag times and direction of activity flow varied randomly. This could occur if the high degree of recurrent activity in CA3 obscured directional activity conduction among subregions. Or, it is possible that the temporal resolution in this study was insufficient to determine directional activity flow.

Pharmacological sensitivity of spontaneous waves

Pharmacological studies were conducted to determine the effects of cholinergic manipulations. The cholinomimetic drug carbachol, at a concentration of 1 μM, caused a rapid suppression of the spontaneous waves (Fig. 6A), an effect that was blocked by the muscarinic receptor antagonist atropine (Fig. 6B). At higher concentrations, carbachol induces rhythmic activity in slices (Fellous et al. 2000; Fisahn et al. 1998; Huerta and Lisman 1993; Konopacki et al. 1987; Shimono et al. 2000; Williams and Kauer 1997), which could have precluded expression of the spontaneous slow activity. However, examination of power spectra over time for slices in which carbachol was infused at a concentration of 20 μM (n = 4, data not shown) showed that the disappearance of the slow activity preceded the development of the cholinergically driven rhythms, suggesting that cholinergic activation in itself suppressed the former waves at concentrations lower than those required to generate cholinergic rhythms. It is also interesting to note that infusion of atropine did not depress the ongoing waves, as would be expected if generation of the activity required muscarinic receptors (Fig. 6B). In all, the spontaneous slow activity recorded in slices was antagonized by cholinergic activation.

Ascending modulatory projections in addition to the cholinergic projections exert potent effects over hippocampal EEG in vivo (Assaf and Miller 1978; Yamamoto 1988; Yamamoto et al. 1979). Evidence that this was also the case for spontaneous periodic activity in slices is summarized in Fig. 7. Infusion of
serotonin (Fig. 7A) caused a rapid depression of the slow activity that reversed on washout. While this result resembles that obtained with cholinergic agonists, the depression obtained with serotonin differed in that it was not accompanied by the development of a new pattern of rhythmic oscillations. It appears, then, that serotonin produced a simple desynchronization. Dopamine did not cause any reliable changes in the power or the frequency of spontaneous activity in the slices (Fig. 7B).

**DISCUSSION**

The present findings demonstrate that hippocampal slices can generate spontaneous EEG patterns closely resembling sharp waves described in vivo. Endogenous SPWs have not previously been described in vitro, probably because slices are usually prepared from more rostral segments of the hippocampus where the dense associational connectivity of CA3 is less likely to be preserved within a 350-μm slice. Consistent with this idea, spontaneous slow EEG patterns have been reported in studies using mouse hippocampal slices cut at thicknesses ≥500 μm (Wu et al. 2002; Yanovsky et al. 1995), increasing the likelihood that CA3 axon collaterals will remain within the plane of the slice. Other factors such as the use of interface chambers as opposed to submerged slices and the ratio of calcium to magnesium in recording ACSF may also be important for detection of this activity.
frequency and amplitude varying widely from cycle to cycle within a slice, whereas theta rhythms exhibit a relatively uniform pattern. It is also improbable that the waves correspond to the atropine-resistant theta described by Vanderwolf and colleagues (Kramis et al. 1975; Vanderwolf 1975; Vanderwolf et al. 1977) since atropine-resistant theta in vivo depends on connections between the entorhinal cortex and hippocampus (Buzsaki 2002) that are unlikely to remain intact in the slice preparation.

The waves described in the present work more likely correspond to hippocampal sharp waves observed in vivo during slow wave sleep and immobility, which are reported to persist in isolated hippocampal grafts (Buzsaki et al. 1987). It is therefore possible that under the right conditions, SPWs could occur spontaneously in hippocampal slices.

Laminar profile analyses indicated that the waves were dipoles with a negative pole in the middle of the s. radiatum and a positive peak in the pyramidal cell layer. SPWs have an identical laminar profile pattern and are initiated by synchronous population bursts in the axon collaterals of CA3 (Buzsaki 1986). The primary component of the in vitro waves (dendritic negative/cell body positive) appeared to be a depolarizing event in the CA3 associational system as well because it was accompanied by bursts of spikes at the cell body layer. Additionally, quantification of spiking revealed that the relationship between field and unit activity during these waves matches that of pyramidal cells recorded in vivo during SPWs (see Fig. 6, Csicsvari et al. 1999); i.e., the highest discharge probability occurred during the positive-going phase of the wave (slightly before the peak) in both cases. Further evidence that the in vitro sharp waves consist of synchronous EPSPs in the CA3 associational system was obtained from coherence analyses across CA3 subfields.

Waves were locally generated, yet nonetheless coherent, across the CA3 subdivisions. The associational projections of the CA3 pyramidal cells extend across the entirety of CA3 (Ishizuka et al. 1990) and are likely responsible for propagation and coherence of the waves. Significant coherence values were obtained between all regions in several cases, with the CA3a/CA3c pairing always having the lowest values and CA3b being coherent with both CA3a and CA3c. This is consistent with the anatomical projection profiles for the CA3 associational system. That is, cells in CA3b give rise to collaterals that project densely and fairly evenly across the full extent of CA3, thereby providing the most widespread and largest contribution to the associational system (Ishizuka et al. 1990). Partial and multiple

The waves had a peak frequency (approximately 4 Hz) within the theta range but were clearly distinct from theta rhythm in several respects. First, theta rhythms are generated in vivo by ascending cholinergic inputs from the medial septum/diagonal band (Vertes and Kocsis 1997 for a review) and under some in vitro conditions by application of carbachol (Huerta and Lisman 1993; Konopacki et al. 1987), while the activity recorded in the present study occurred spontaneously in isolated hippocampus and was suppressed by cholinergic stimulation. Also, the in vitro waves had an irregular pattern, their

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**TABLE 1. Pairwise coherence**

<table>
<thead>
<tr>
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<th>CA3a-b</th>
<th>CA3a-c</th>
<th>CA3b-c</th>
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<tbody>
<tr>
<td>Slice 1</td>
<td>0.40 ± 0.04</td>
<td>0.15 ± 0.04</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>Slice 2</td>
<td>0.48 ± 0.04</td>
<td>0.24 ± 0.04</td>
<td>0.77 ± 0.02*</td>
</tr>
<tr>
<td>Slice 3</td>
<td>0.89 ± 0.01*</td>
<td>0.59 ± 0.04*</td>
<td>0.75 ± 0.03*</td>
</tr>
<tr>
<td>Slice 4</td>
<td>0.84 ± 0.02*</td>
<td>0.80 ± 0.02*</td>
<td>0.90 ± 0.01*</td>
</tr>
<tr>
<td>Slice 5</td>
<td>0.86 ± 0.02*</td>
<td>0.74 ± 0.03*</td>
<td>0.95 ± 0.01*</td>
</tr>
<tr>
<td>Slice 6</td>
<td>0.15 ± 0.04</td>
<td>0.04 ± 0.02</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>Slice 7</td>
<td>0.75 ± 0.03*</td>
<td>0.55 ± 0.04*</td>
<td>0.73 ± 0.03*</td>
</tr>
<tr>
<td>Slice 8</td>
<td>0.73 ± 0.03*</td>
<td>0.56 ± 0.04*</td>
<td>0.85 ± 0.02*</td>
</tr>
<tr>
<td>Slice 9</td>
<td>0.70 ± 0.03*</td>
<td>0.55 ± 0.04*</td>
<td>0.91 ± 0.01*</td>
</tr>
<tr>
<td>Slice 10</td>
<td>0.73 ± 0.03*</td>
<td>0.18 ± 0.04</td>
<td>0.40 ± 0.04</td>
</tr>
</tbody>
</table>

Pairwise coherence estimates with corresponding 95% confidence intervals.

* High coherence values.
Coherence results point to CA3b as the major contributor to the activity observed in the present study as well. Coherence between CA3a and CA3c declined substantially when the influence of CA3b was removed, while the highest multiple coherence values were observed for CA3b versus combined CA3a-CA3c activity. Collaterals from CA3a terminate relatively lightly in CA3c, and associational projections arising from CA3c are few in number and tend to terminate locally (Ishizuka et al. 1990; Li et al. 1994). Because CA3a and CA3c are not as strongly connected via the associational projections, it follows that coherence between these regions would be relatively low.

Both cholinergic and serotonergic stimulation were found to suppress the in vitro waves. Cholinergic activation caused a sudden cessation of slow rhythms followed by the appearance of more regular rhythms at higher concentrations of carbachol. This could be an in vitro correlate of the rhythm switching that occurs in behaving animals from hippocampal sharp waves during slow wave sleep and immobility to theta waves during REM sleep and exploration. Serotonin also reversibly suppressed the waves but in this instance there was no evidence for a substitute rhythm. In essence, then, the effect of serotonin was to desynchronize the slice. Serotonin produced a similar change in cholinergically driven oscillations (Colgin, Kubota, and Lynch, unpublished observations), and activation of ascending serotonin projections in vivo is usually reported to produce desynchronization (Assaf and Miller 1978; Yamamoto et al. 1979). Possibly relevant to this, SPW amplitude in vivo is reported to be increased by removal of subcortical afferents, as would be expected if they tonically suppress synchronization in CA3 (Buzsaki et al. 1988).

In summary, we present evidence that synchronous activity can occur in standard hippocampal slices in the absence of extrinsic input. Furthermore, our results suggest that this synchronous activity represents an in vitro equivalent of SPWs, a naturally occurring and basic operating feature of the intact hippocampus. The laminar profile resembles that of SPWs in vivo. Both in vivo and in vitro SPWs appear to be intrinsically generated in hippocampus without requiring external input. SPWs and the in vitro waves described here are presumably due to synchronous excitation in the dense associational system of CA3. The discharge probability profile of the waves closely

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**FIG. 5.** Coherence between CA3 subregions. Different symbols represent different slices (n = 10). Note that although exact values differ from slice to slice, general trends persist across slices. A: pairwise coherence. Coherence estimates are shown for the 3 pairs: CA3a vs. CA3b, CA3a vs. CA3c, and CA3b vs. CA3c for the 4- to 7-Hz frequency band. Note that the lowest coherence was observed between CA3a-CA3c. B: partial pairwise coherence. Estimated coherence values between pairs of CA3 subregions after removing the influence of the 3rd subregion are plotted. It is obvious that the lowest partial coherence in each case is between CA3a-CA3c after removing the CA3b component. C: multiple coherence. Coherence values between 1 subregion and a linear combination of the other 2 subregions tend to be relatively high; the highest multiple coherence was observed between CA3b with CA3a and CA3c.

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**FIG. 6.** Effects of cholinergic agents on slow rhythmic activity. Recordings were obtained from CA3b s. pyramidale. Power was averaged across the 4- to 7-Hz band and normalized to average power during the predrug baseline. A: CCh (1 μM) was infused for 30 min in a group of 5 slices, resulting in an approximately 70% decrease in power. B: slices (n = 7) were pretreated with atropine (10 μM) for 30 min prior to CCh infusion; additionally, atropine was infused during CCh washing and for 30 min of CCh washout. Atropine blocked the suppressive effect of CCh depicted in A and did not in itself block ongoing slow activity.
resembles that reported by Csicsvari et al. (1999) for SPWs in the behaving rat. Both SPWs and the waves of the current study are dampened by ascending neuromodulatory influences such as serotonin and acetylcholine and antagonized by the occurrence of cholinergically driven rhythms. Using the above-described preparation, it should be possible to test hypotheses regarding the function of sharp waves and to study how transitions between spontaneous SPWs and cholinergic rhythms affect a number of macroscopic operations carried out by hippocampus.

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REFERENCES


Fellous JM and Sejnowski TJ. Cholinergic induction of oscillations in the hippocampal slice in the slow (0.5–2 Hz), theta (5–12 Hz), and gamma (35–70 Hz) bands. Hippocampus 10: 187–197, 2000.


