Research report

Hippocampal theta rhythm synchronizes visual neurons in sleep and waking

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Abstract

The hippocampal theta rhythm (θ) was reported to be associated with movements, attention, auditory processing, autonomic functions, learning and memory and postulated as an associator of discontiguous events. Since visual information includes temporal cues, our study was centered on the correlation between hippocampal θ rhythm and lateral geniculate activity. Phase relationships between hippocampal θ and unit firing were found with both spontaneous and light evoked activity during wakefulness, slow wave and paradoxical sleep. This temporal correlation was dynamic, exhibiting changes related to the sleep–waking cycle and perhaps to attention shifts. Hippocampal θ rhythm may supply a low frequency temporal dimension to the processing of visual information. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

A rhythmic electrographic activity called theta rhythm [24]—from 4 to 10–12 cycles/s—is present in the hippocampus being prominent during active wakefulness (W), paradoxical sleep (PS) and also in slow wave sleep (SWS). It was correlated with voluntary movements [5,6,21], attention [9], learning [1], auditory processing during wakefulness–sleep cycle [17,23] and autonomic activity in PS [18]. It has been suggested that the hippocampal theta rhythm (Hipp θ), mediated by cholinergic muscarinic receptors, is related to the processing of sensory stimuli within the context of motor behavior initiation [11] and during conditioned reflexes [8]. Moreover, phase-locking of neuronal discharges with the Hipp θ was reported for motor activity [2,12], phenomenon found in many loci outside limbic regions [7,10].

The hippocampus is presumably involved in spatio-temporal learning. Thus, a deficiency of spatial memory

was observed when θ rhythm was suppressed [15,26]. Wallestein et al. [25] postulated that the hippocampus acts most critically in learning and memory tasks as an 'associator of discontiguous events' in terms of their temporal and/or spatial positioning. A temporal relationship between Hipp θ and auditory neuronal activity has been established [16,17,19]. Thus, Pedemonte and co-workers [17,19] had suggested that Hipp θ could play a role as an internal clock, adding a temporal dimension to the auditory processing at the inferior colliculus and auditory primary cortical level.

High and low frequency rhythmic activities were found in visual cortical neurons [3,20]. Since Hipp θ is a conspicuous central rhythm, it seems relevant to investigate the functional relationship of low-frequency Hipp waves, visual neuronal firing and behavioral states such as waking and sleep. It is known that the wakefulness–sleep cycle modulates sensory systems [22] and, on the other hand, a distinctive state-dependent discharge pattern in the visual neurons of the lateral geniculate nucleus (LGn) during the sleep–waking cycle has been reported [4,13,14]. Processing of visual information depends on spatial and

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temporal cues and, it is our tenet that the Hipp θ rhythm could contribute as a time giver. Our aim was to study the temporal correlation between LGn neuronal firing and the Hipp θ rhythm with the hope of providing clues as to the nature of brain functional changes and the association between causal mechanisms and behavioral shifts.

2. Materials and methods

Adult guinea-pigs (n=19), mean weight 550 g, were chronically implanted under pentobarbital anaesthesia (35 mg/kg, i.p.) with electrodes to record the bipolar parietal electrocorticogram (ECoG), bipolar hippocampus electrogram (A, 6; L, 2; H, 4) and neck electromyogram (EMG) for behavioral control. A small craniotomy over the stereotaxic parameters of the LGn (A, 5–7; L, 4–6; H, 5–9) was made and left open for glass microelectrode penetration, and protected with antibiotic and corticosteroid cream. Two light metal bars attached to the skull with screws and acrylic cement permitted a painless reconstruction of the stereotaxic position during the recording sessions. After a recovery period of at least 5 days, animals were trained on a hammock without fear, to be fed in this position and undergo sleep–waking states naturally. Each recording session was initiated at approximately the same time, preceded by a period of sleep deprivation of 2 h in a slowly revolving drum.

Visual stimuli consisted of flashes of light (Grass Photo-Stimulator TS 22; 2–8/s, 30 cm distant). Each animal was held in stereotaxic position while glass micropipettes, filled with sodium acetate and Sky blue solution, were advanced with a hydraulic micromanipulator (Narishige MO-8), searching for flash-evoked unit firing. Neuronal discharges, ECoG, EMG and hippocampal electrograms were amplified and stored in a computer using the Spike2 software (Cambridge Instruments) for off-line processing.

Single neurons were selected for quantitative study with a voltage window discriminator. The visual evoked unitary activity was routinely studied with peri-stimulus time histograms, analyzed with interval and autocorrelation histograms to be included in our study as truly visual neurons.

The hippocampal electrogram, bipolar recording, was analyzed using waveform autocorrelation and power spectrum, band-pass filtered (between 0.5–100 Hz), sampled at

500 Hz and stored. The crosscorrelation between Hipp θ field activity and spikes, to demonstrate their temporal relationship, was obtained by spike-triggered averaging considering the same number of spikes (30–50) in each situation, with both spontaneous and evoked unit firing. Thus, we were able to compare the spontaneous and evoked neuronal activity during W, SWS and PS. The crosscorrelation was considered significant when, calculated with the same data after 'shuffling' the spike series, i.e. to interchange the spike intervals at random, became flat [17].

The recording site was iontophoretically injected with Sky-blue and, after no more than three successful recordings, the animals were perfused with 10% formalin solution and the brain was removed for micro-anatomical analysis.

3. Results

The spontaneous and evoked activity of visual neurons in the guinea pig LGn was extracellularly recorded. All of them (n=55) were studied during W, 93% (n=51) were recorded during SWS and 66% (n=36) in PS. The relationship between unit firing and Hipp θ was studied using both spontaneous and evoked activity. Sixty seven percent of LGn units (n=37) showed periods with Hipp θ phase-locking, that is, the unit fired at a special phase of the θ wave. In some cases the phase relationship occurred with each θ wave, becoming a rhythmic neuronal discharge at θ frequency. This phase relationship was dynamic: in a few seconds a unit's firing pattern may pass from a rhythmic firing phase-locked to θ to an independent pattern of discharge. The same neuron could also change its dependence on the Hipp θ in different recording epochs even within the same behavioral state.

Table 1 shows the quantitative study of behavioral states/Hipp θ phase-locking in relation to spontaneous and evoked activity. The results reveal that more neurons showed phase-locking with the Hipp θ when analyzing their evoked rather than their spontaneous activity. The evoked activity produced an enhanced number of neurons phase-locked with the Hipp θ, increasing on passing from W to SWS and increasing further during the transition to PS. However, the spontaneous activity showed no significant changes in the percentage of phase-locked units

Table 1
Quantitative analysis of visual units phase-locking with hippocampal θ

	W		SWS		PS	
	Phase-locked % (n)	No phase-lock % (n)	Phase-locked % (n)	No phase-lock % (n)	Phase-locked % (n)	No phase-lock % (n)
Spontaneous	4.8 (2)	95.2 (40)	5.3 (2)	94.7 (36)	50 (5)	50 (5)
Evoked	10.9 (6)	89.1 (49)	34 (18)	66 (35)	87.5 (28)	12.5 (4)

W, wakefulness; SWS, slow wave sleep; PS, paradoxical sleep.

between W and SWS. On the other hand, ≈50% of the recorded neurons exhibited phase-locked spontaneous responses with the Hipp θ during PS. Finally, it is stressed that the phase-relationship with the Hipp θ was found with both the spontaneous and the evoked activity.

Fig. 1 shows an example of temporal correlation between LGn unit spontaneous activity and θ rhythm during W, SWS and PS. In each of the three behavioral states the unit exhibited phase-locking with the Hipp θ rhythm (bipolar recording) in selected epochs. The 'shuffling' of the spike series at random appears flat, demonstrating the phase-locking significance. The analysis of the θ rhythm (Hipp AC, autocorrelation) and their power spectra (Hipp PS, black bars) corresponding to each state is presented in the figure's lower part. The theta rhythm is usually

observed at higher frequency and power during PS as shown in the figure.

The neuronal evoked discharge exhibited epochs of phase-locking with the Hipp θ rhythm with light stimulation in all the behavioral states under study (Fig. 2). Selected epochs of visual units firing during W, SWS and PS were found phase-locked with the Hipp θ waves although during SWS the θ rhythm was not so evident in the hippocampal waveform autocorrelation (see Slow wave sleep column). In this example the Hipp θ also increases its frequency on passing from wakefulness to sleep. The 'shuffling' of the spike series at random, exhibiting a flat crosscorrelation, demonstrate the phase-locking significance. The flash stimuli rate was 4/s while the recorded θ was 8.5 cycles/s during W and SWS and 9 cycles/s in PS.
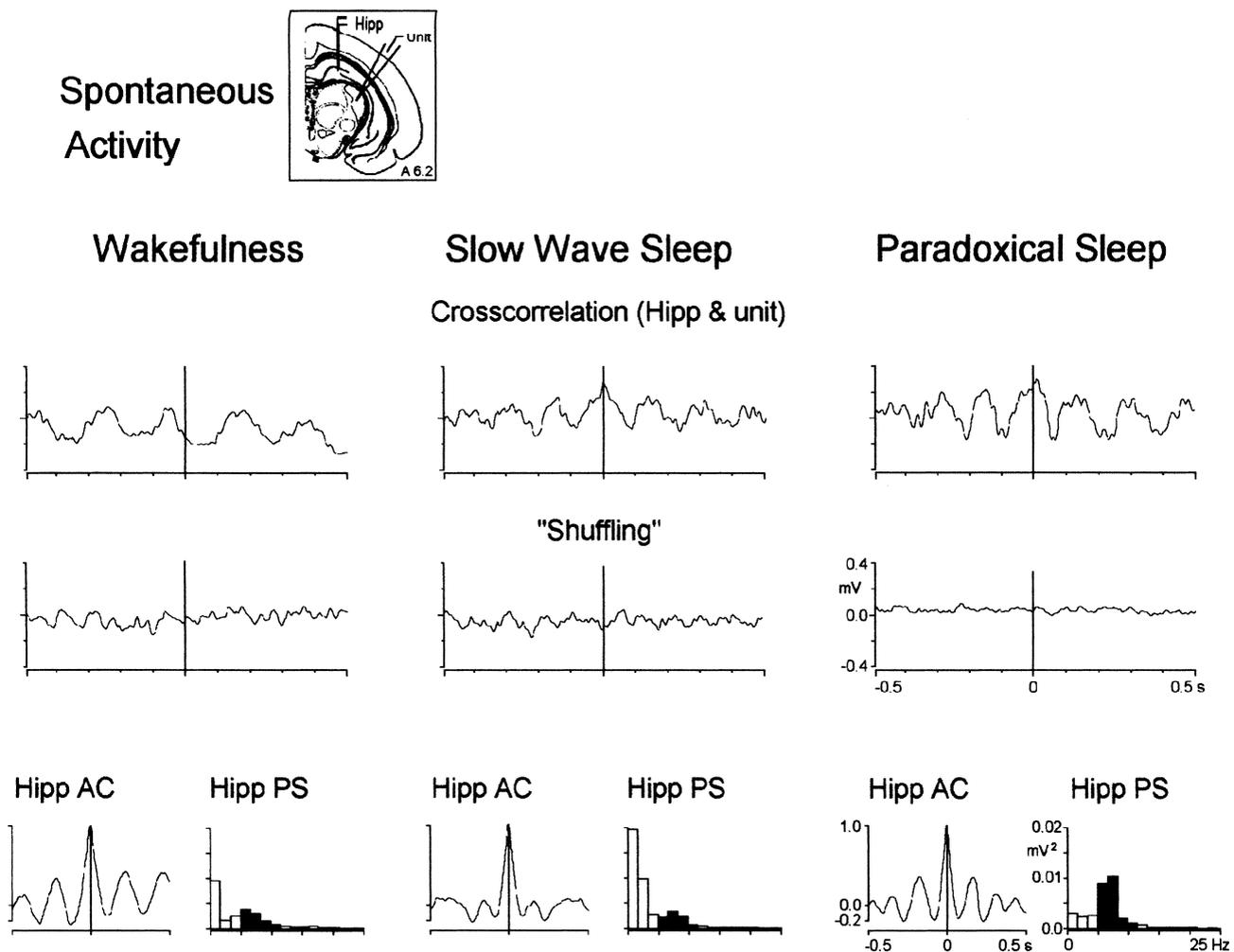
Fig. 1. Temporal relationship between neuronal spontaneous activity (#19) and hippocampal theta rhythm (bipolarly recorded) during behavior. Selected epochs of theta-unit phase-locking are shown in this figure. The cross-correlation, Hipp and unit, show phase-locking. This neuron exhibited periods of theta phase-locking in all behavioral states, even when theta power was not prominent as in slow wave sleep. The 'shuffling', cross-correlation of the spike series at random almost flat, demonstrate the phase-locking significance. Hipp AC, hippocampal electrogram waveform autocorrelation. Hipp PS, hippocampal electrogram power spectra (theta rhythm range as black bars). Although the power spectra are similar in wakefulness and slow wave sleep, a more synchronized hippocampal theta is observed during wakefulness. Paradoxical sleep shows both effects, high power in the spectrum and more synchronized theta autocorrelation. Inset: drawing showing the hippocampus (Hipp) and lateral geniculate nucleus (LGn) recording loci and the electrodes positioning.

Wakefulness

Slow Wave Sleep

Paradoxical Sleep

Crosscorrelation (Hipp & unit)

"Shuffling"

Hippocampal Wave Autocorrelation
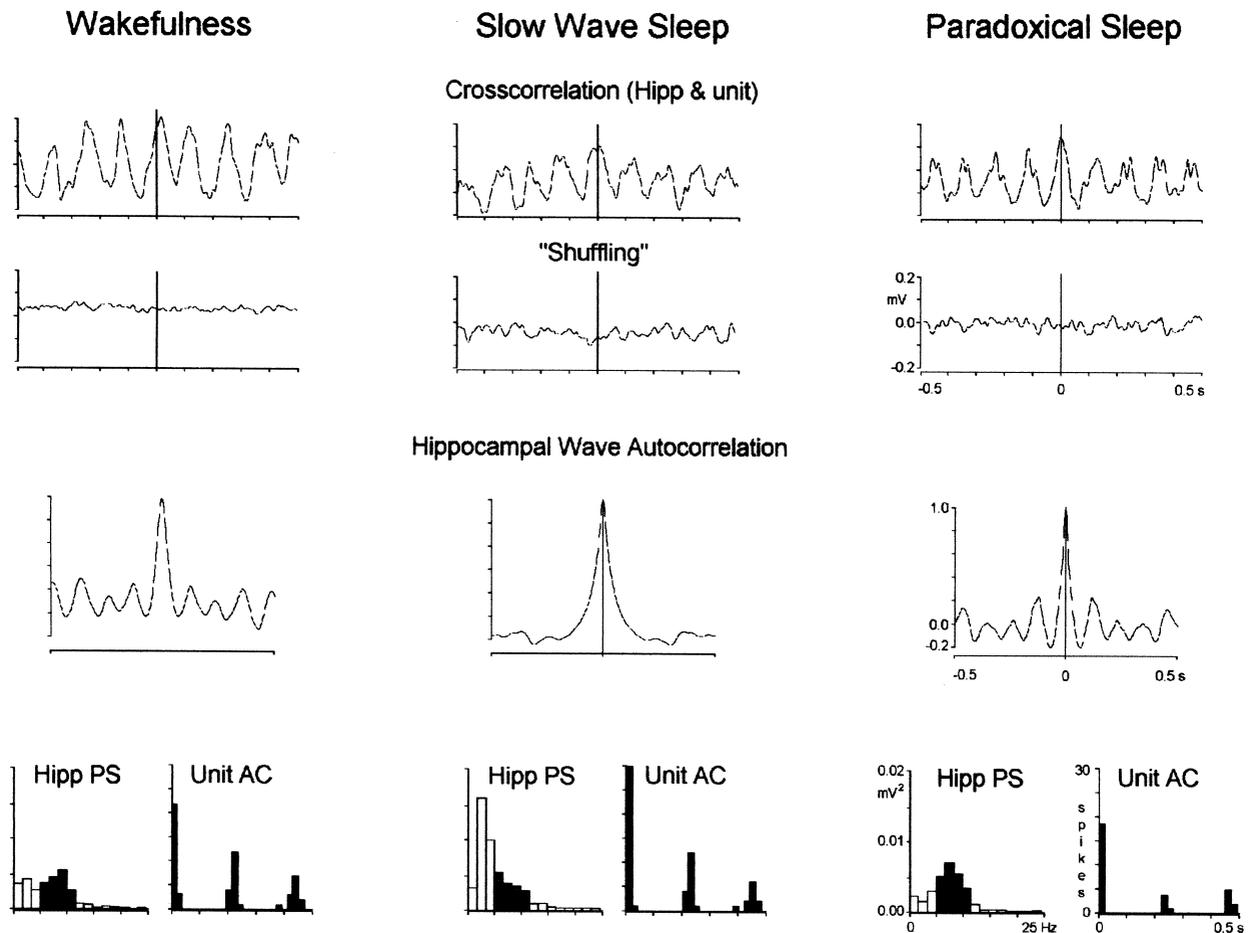
Fig. 2. Temporal relationship between neuronal evoked activity (#15) and hippocampal electrogram (bipolarly recorded) during sleep and waking. Selected epochs of theta-unit phase-locking are shown in this figure. The cross-correlation show the unit phase-locking with theta waves (8–9/s) in the three behavioral states studied. The 'shuffling' of the spike series at random, flat, is a control to demonstrate the significance of the temporal correlation. The hippocampal electrogram waveform autocorrelation shows a lower synchronization during slow wave sleep. Hipp PS, hippocampal electrogram power spectra (theta rhythm range as black bars). Unit AC, present the neuron autocorrelation showing a rhythmic discharge at the flash frequency (4/s). Inset: characterization of a visual neuron with a post-stimulus time histogram (PSTH) in response to flash stimuli (4/s).

Epochs in which the flash rate and the Hipp θ had of the same frequency were never considered.

The hippocampal power spectra (Hipp PS, black bars) shows the theta activity in each behavioral state. The unit autocorrelations (Unit AC) present the neuron discharge following the flashes of light rate.

4. Discussion

The results show that LGn neuron firing fluctuates between a state in which there is a strong significant correlation with Hipp θ rhythm (phase-locking), and other state in which the discharge pattern is independent of the Hipp θ rhythm. Moreover, there is not a strict dependence on the Hipp θ power, however, higher θ rhythm power enhances the probability of finding phase-locked neurons. These results are coincident with those reported in auditory neurons [19,23]. The two cited situations were found with

both the spontaneous and the evoked unitary activity, frequently related to transitions as stimuli rate changes or to shifts in the sleep–waking cycle. Besides, under constant behavioral conditions the phase-locking may disappear during brief epochs that could be related to attention shifts.

The θ phase-locked visual neurons may receive at least three different influences: (a) the specific sensory afferent input, (b) the Hipp θ influence, and (c) the current brain state: awake or asleep. Moreover, the interactions between points a, b and c, changing the context where the sensory processing is being carried out, is coincident with the changes reported in the sensory processing during behavior [4,13,14,22].

The hippocampal activity changes during different waking situations like motor activity [5,6,11,21], learning [1] and conditioned reflexes [8], all of them related to attention shifts [9,24]. Our results support the notion that the LGn visual neurons could use the θ rhythm as a timer during

behavioral states, with or without flashes of light stimuli, i.e. in their evoked and spontaneous activity. Sensory neurons of different modalities such as auditory neurons, have shown a similar Hipp θ-related pattern [17,23].

It is known that hippocampal activity changes are associated with behavioral shifts. Thus, the close correlation exhibited by Hipp θ and LGn unitary firing opens a new way to study sensory information processing, not only in waking periods but also during sleep when sensory information continues to bombard the CNS [22,23]. The relationship between the LGn neuronal discharges and the θ rhythm links the wakefulness/sleep cycle to visual processing.

5. Conclusion

Neurons and their action potentials are thus far the units of information processing within the CNS while a significant gap exists between the cell's activities and the brain's perceptual experience. In an attempt toward filling the gap, a temporal code is introduced, i.e. a code composed of a sensory neuronal discharge and a brain rhythm working together. Lateral geniculate visual units phase-locked with a low-frequency brain rhythm—during epochs—in every behavioral condition studied represent a different mode of sensory signal processing. Perhaps, the hippocampus as a temporary storage of sensory information could furnish a temporal order for data organization. It is our hypothesis that one role played by the Hipp θ could be to contribute, as an internal clock, giving a temporal dimension to the processing of visual sensory information in sleep and waking.

Acknowledgements

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