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HIPPOCAMPAL TYPE 1 (MOVEMENT-RELATED) THETA RHYTHM POSITIVELY CORRELATES WITH SEROTONERGIC ACTIVITY

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INTRODUCTION

The classical observations by Green and Arduini (12) showed that the neocortical and hippocampal EEG are different and that an "inverse relationship ... frequently exists between the activities of the two", the hippocampal activity being "almost the reverse of that of the cerebral cortex". Green and Arduini's paper is considered "the benchmark for the beginning of the intensive study" (2) of the synchronous, high amplitude hippocampal EEG activity, which is recorded during neocortical desynchronization and is well known as hippocampal ϑ rhythm. It has been later proposed to differentiate this synchronous activity into a type 1 Rhythmical Slow Activity (RSA), observed during wakefulness (W) and, particularly, exploratory behavior and voluntary movements (such as walking, swimming, digging) and a type 2 RSA, present during desynchronized sleep (DS) and immobility (reviewed in ref. 2, 3, 22).

On the basis of pharmacological evidence, it has been proposed that in rats hippocampal type 1 (movement-related) RSA is generated by serotonergic mechanisms, whereas hippocampal type 2 (immobility-related) RSA is cholinergically mediated [reviewed in (3)]. The aim of the present work was to evaluate if there is a correlation between spontaneous hippocampal type 1 RSA and hippocampal serotonergic activity in freely behaving rats. To this purpose simultaneous recordings of: *i)* hippocampal EEG, *ii)* sleep-wake activity (polygraphically defined), and *iii)* hippocampal levels of the serotonin (5-HT) metabolite 5-hydroxyndolacetic acid (5-HIAA - measured by *in vivo* voltammetry and infrared telemetry) were performed. As previously shown the use of a telemetry system for voltammetric signals allows simultaneous neurophysiological and neurochemical recordings in the same animals (6, 14, 15). The relatively high sampling rate (2 minutes) allowed by *in vivo* voltammetry provided the basis for a detailed analysis of the relationship existing among the variables under investigation.

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METHODS

Animals and surgery. - Experiments were performed on male albino rats (CD, Charles River, Calco CO, Italy; 250-300 g). Procedures involving animals and their care were conducted in conformity with institutional guidelines that are in compliance with national (D.L. n.116, G.U. suppl. 40, 18 febbraio 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1; Dec. 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985). The animals were anaesthetized (pentobarbital 40 mg/kg + chloral hydrate 180 mg/kg, intraperitoneally), injected with a broad spectrum antibiotic (penicillin G), positioned in a stereotaxic apparatus and surgically prepared for chronic polygraphic and voltammetric recordings (8, 14, 15). Neocortical EEG electrodes (stainless steel screws) were unilaterally placed over the frontal (AP = 2 mm, L = 2.5 mm from bregma) and parietal (AP = -5 mm, L = 3.5 mm, from bregma) cortices. A third screw was placed over the cerebellum to ground the animal. Electromyogram (EMG) was recorded by means of teflon coated silver wires inserted in the neck muscles. A bipolar electrode (Rhodes Medical Instruments, Woodland Hills, CA, USA) was lowered into the hippocampal CA1 region (AP = -3.6 mm and L = 2.2 mm from bregma, V = 2.3 mm from brain surface according to Paxinos and Watson, ref. (20) to bipolarly record the hippocampal field potentials from this region, which (with the dentate region) is the main zone of ϑ generation (2). The electrode consisted of two insulated stainless steel wires (diameter = 0.2 mm), etched for 0.5 mm at their tips and separated (on a horizontal plane) by a 0.5 mm shaft. The surgical procedure for *in vivo* voltammetric measurements has been previously described (8). Briefly, a guide cannula through which the carbon fiber electrode (tip diameter = 8 μm) penetrated was stereotaxically implanted 1 mm above the CA1 region of the hippocampus contralateral to the side where the recording bipolar electrode was lowered (Fig. 1). A micromanipulator was cemented onto the skull so the electrode (protruding 1 mm beyond the guide cannula) could be changed and reliably placed in the same site. A stainless steel screw and an Ag/Cl wire, inserted between the bone and the intact dura mater, served as auxillary and reference electrodes, respectively. An integrated circuit socket was attached to the skull with dental acrylic and insulated leads were routed from this plug to the polygraphic and voltammetric electrodes, as well as to the hippocampal recording electrode. The rats were allowed one week to recover before they were connected to a flexible tether and slip ring and accustomed to the soundproof recording chambers. The animals, individually housed on a 12:12 h light-dark cycle (lights on 03.00 a.m.) at 21 ± 1 °C, were allowed at least 48 h adaptation before testing began. Food and water were available *ad libitum*.

Recording apparatus. - Signals from the EEG (both cortical and hippocampal) and EMG electrodes were fed into a Grass (Quincy, MA, USA) polygraph in the adjacent room. The EEG was amplified (factor of 2000) and bandpass filtered between 0.3 and 35 Hz (filter slope 6 dB/ octave). These signals were subjected to analog-to-digital conversion with 12-bit precision at a sampling rate of 128 Hz (NB-MIO-16; National Instruments, Austin, TX, USA). The digitized EEG waveform and integrated values for EMG recordings were stored as binary computer files until subsequent analysis.

Differential normal pulse voltammetry (Biopulse Polarograph, Tacussel, France) and carbon fiber electrodes (tip diameter: 8 μm) were used to monitor extracellular levels of the 5-HT metabolite 5-hydroxyindolacetic acid (5-HIAA) (14). Biochemical and pharmacological evidence indicates that, under the conditions described in the present study, the voltammetric peak recorded at + 260 mV corresponds to 5-HIAA (5,8) and that significant contamination by uric acid can be excluded (5). In the present study voltammetric measurements were performed every 2 min and a new electrode was used for each experimental session.

The possibility of performing simultaneous polygraphic and voltammetric measurements is usually limited by the existence of electrical cross-talk between the two settings (6,14). To solve this problem a telemetry system for voltammetric signals was used. The system consists of a wire-less connection that uses infrared diffused light (optoelectronic transmission) exploiting the diffusion of the transmitted light over walls and ceiling toward a receiver (6,14).This system

allows to physically open the ground loop for the voltammetric circuit so that no current component can flow among the voltammetric and polygraphic circuits, thus preventing tissue lesions, electron interferences and artifacts. With this system the polygraphic circuit need not be disconnected while performing voltammetric measurements, thus allowing truly simultaneous recordings and a detailed time course to be obtained.

Experimental design. - Experiments began one week after surgery. Recordings began at either dark or light onset and lasted at least 6 hours. At the end of the experiments, animals were given an overdose of chloral hydrate and an electrolytic lesion was made using the carbon fiber electrode at the recording site. Brains were then removed, immediately frozen and coronal slices (40 μm) were cut using a freezing microtome. The position of the voltammetric working electrode and of the recording electrode inside the two, contralateral hippocampal CA1 region was verified on cresyl violet stained sections (Fig. 1).

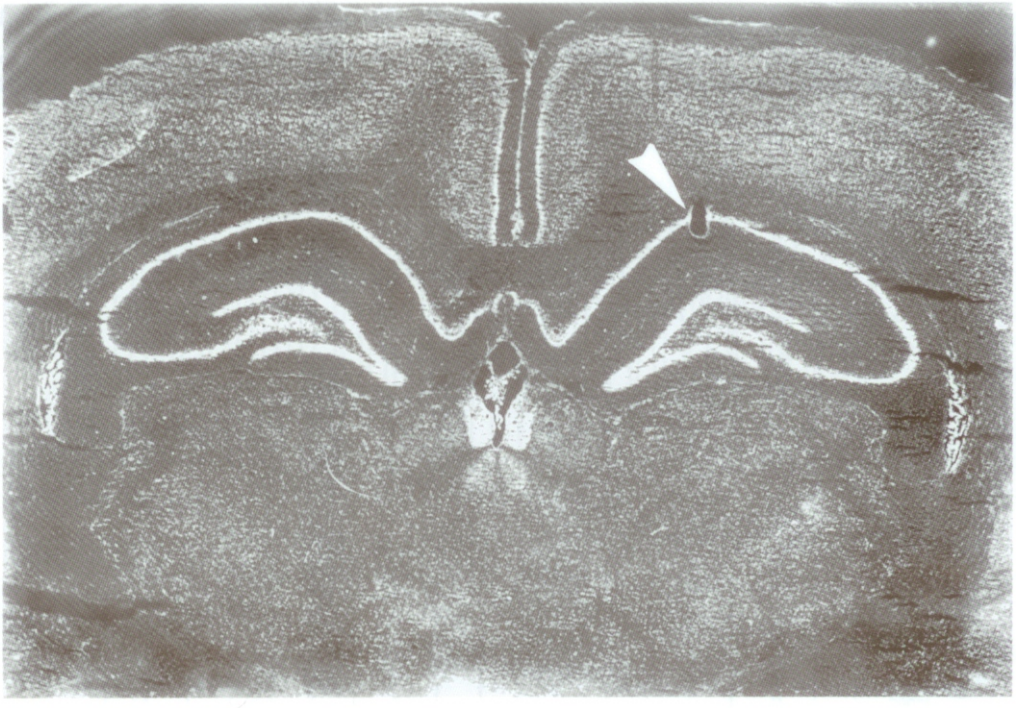

Fig. 1. - *Microphotograph of a histological, coronal section of the rat brain.*

The white arrow indicates the electrolytic lesion made by the voltammetric carbon fiber working electrode in the recording site (hippocampal CA1) at the end of the experimental sessions. For more details, please see *Methods*. Scale bar = 500 μm.

Data analysis. - Postacquisition determination of vigilance state was done by visual scoring using custom software (*Icelus*, M.R. Opp, Department of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston TX, USA) written in LabView (National Instruments, Austin, TX, USA). Twelve-s epochs of the EEG, and integrated EMG signals were

displayed on a high resolution computer monitor. EEG power density values were calculated by fast Fourier transform (FFT) on the 6 consecutive 2-s segments of the 12-s scoring epoch; this spectrum was displayed simultaneously on the monitor with the polygraphic signals to facilitate visual determination of the behavioral state. The animal behavior was classified as either wakefulness (W), slow wave (SWS) or desynchronized (DS) sleep based on the criteria published elsewhere (19).

Statistical analysis. - Univariate analysis of variance, partial correlation and stepwise multiple regression analyses were used (9, 11), to determine the correlations between the variables under investigations.

RESULTS

In the experimental conditions of this work, the hippocampal RSA recorded during W (type 1 RSA) was characterized by high amplitude, synchronous waves, with a main power spectrum peak at 3.5 Hz (Fig. 2) and a second, sub-dominant

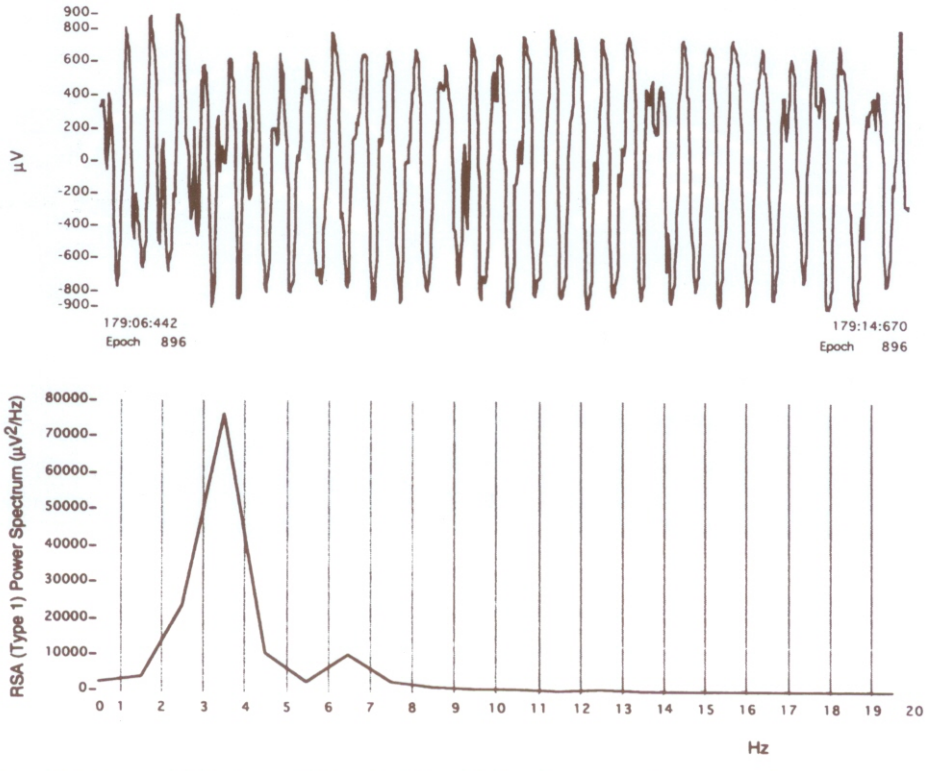

Fig. 2. - *Hippocampal Rhythmical Slow Activity (RSA - above) and corresponding power spectrum (below), recorded from the hippocampal CA1 during a phase of wakefulness.*

Time expressed in min:sec:msec from the beginning of the recording period. Please note the main power spectrum peak at 3.5 Hz.

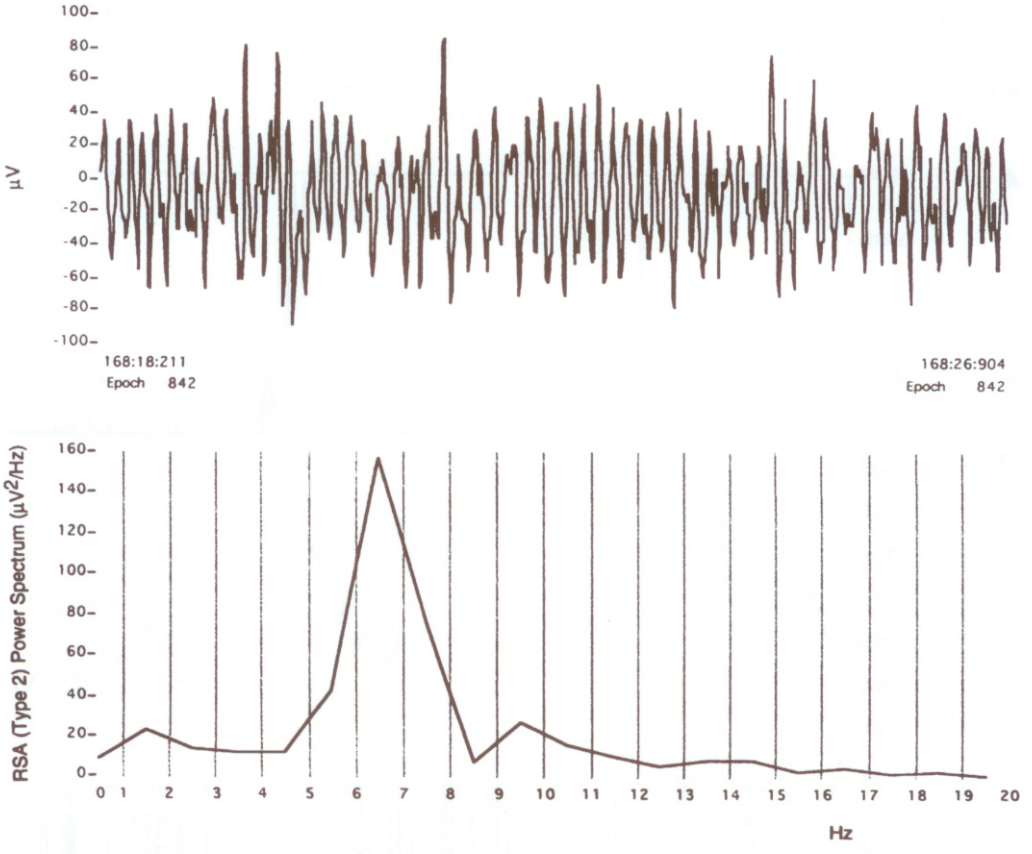

Fig. 3. - *Hippocampal Rhythmical Slow Activity (RSA - above) and corresponding power spectrum (below), recorded from the hippocampal CA1 during a phase of desynchronized sleep.*

Time expressed in min:sec:msec from the beginning of the recording period. Please note the presence of only one power spectrum peak at 6.5 Hz and the different RSA amplitude.

peak at 6.5 Hz. A completely different RSA (type 2 RSA) was present during DS: in this condition, the RSA amplitude was lower (Fig. 3), and, mainly, its power spectrum was characterized by a single peak at 6.5 Hz (Fig. 3).

Since in this experimental setting the frequencies of the type 1 RSA are included between 1.5 Hz and 7.5 Hz (Fig. 2), the hippocampal EEG activity was filtered between these values (Fig. 4, panel A). The extracellular 5-HIAA levels, simultaneously recorded in the contralateral hippocampal CA1, showed spontaneous variations (Fig. 4, panel B). When the type 1 RSA and the 5-HIAA concentrations were compared, a highly significant, positive correlation between these variables was found (Fig. 4). On the other hand, type 2 RSA was recorded during DS episodes, when 5-HIAA levels (as shown also by other studies, ref. 14, 15) reach their minumum. The variations in the 5-HIAA levels, which increase with

W and decrease with sleep (Fig. 4), were also significantly correlated to sleep-wake activity, in agreement with previous data (14, 15), as well as with neck muscle tone.

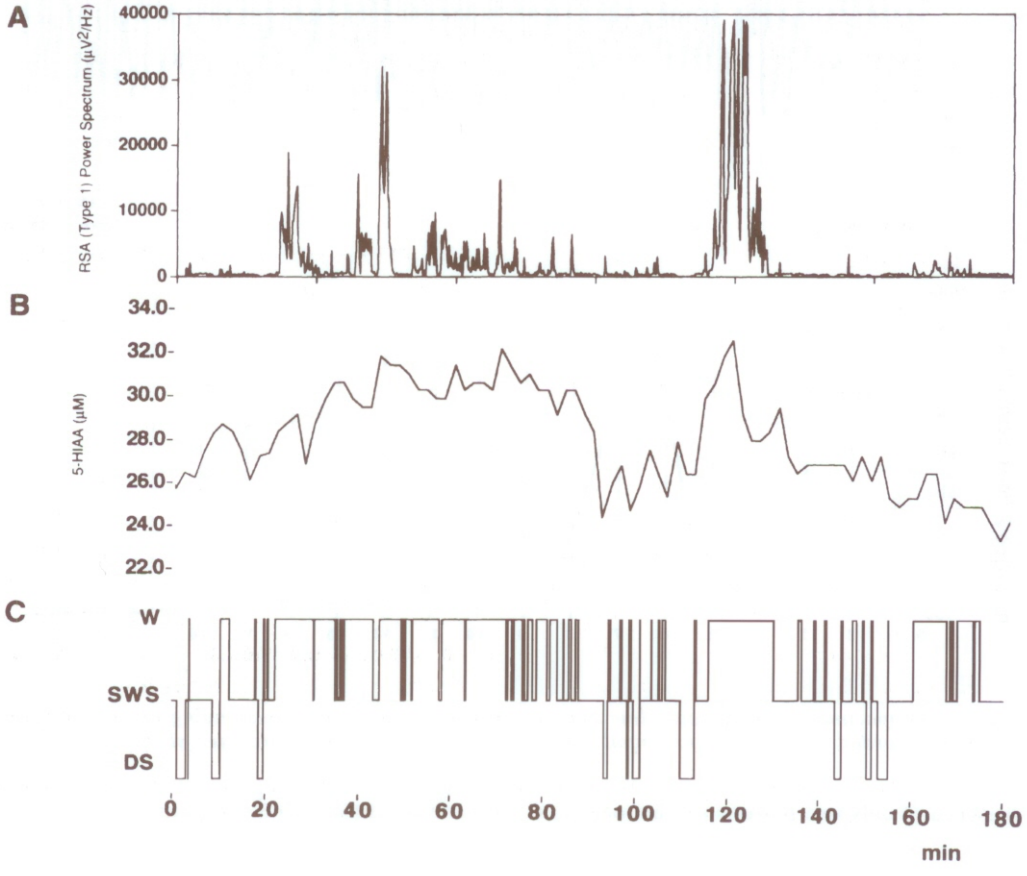

Fig. 4. - *Simultaneous recordings of the hippocampal type 1 Rhythmical Slow Activity (RSA, A), of the 5-hydroxyindolacetic acid (5-HIAA) levels in the contralateral CA1 (B) by means of* in vivo *voltammetry and infrared telemetry, and of the corresponding hypnogram (showing the sleep-wake activity of the animal).*

W: wakefulness; SWS: slow wave sleep; DS: desynchronized sleep.

DISCUSSION

The results of the present study show that hippocampal type 1 RSA, recorded during W, is positively correlated with 5-HT release, monitored at hippocampal level by means of *in vivo* voltammetry and infrared telemetry. These results, although correlative, are in agreement with the hypothesis that this type of hippoc-

ampal synchronous activity is generated by a serotonergic mechanism (3). This does not rule out a possible cholinergic contribution [reviewed in(17)]. The results also show that the hippocampal type 1 RSA and type 2 RSA are characterized, under these conditions, by two different power spectrums: while type 1 RSA power spectrum is characterized by a main peak at 3.5 Hz (and a second, sub-dominant peak at 6.5 Hz), type 2 RSA presents a single power spectrum peak at 6.5 Hz.

The existence of a close and highly significant statistical positive correlation between hippocampal type 1 RSA and neck muscle tone, while not excluding other functions for the RSA, supports the view that this type of synchronous hippocampal activity, in rats, is movement-related. In contrast to hippocampal type 1 RSA, the hippocampal type 2 RSA, characteristic during DS episodes, occurs when, as shown in this and previous studies (4, 14, 15, 21), the serotonergic activity is at its minimum. This observation of negative correlation between hippocampal type 2 RSA and serotonergic activity, is in agreement with the hypothesis that this hippocampal synchronous activity is mediated by a non-serotonergic, probably cholinergic, mechanisms [reviewed in (3)].

Serotonergic raphe nuclei can affect hippocampal activity both directly, since the hippocampus receives serotonergic innervation from these structures, especially from the median raphe (1), and indirectly via projections to the medial septum and diagonal band, which provide input to the hippocampus and may act as a pacemaker for the hippocampal ϑ rhythm (2, 26). As far as a direct serotonergic action at the hippocampal level is concerned, it has been shown that 5-HT (via 5-HT_{1A} receptors) can modulate hippocampal GABA-ergic inhibitory synapses (25) and affect hippocampal pyramidal cells (23), whose spontaneous firing is modulated by the electrical stimulation of dorsal and median raphe nuclei (13, 24).

However, the precise role of brain 5-HT in the regulation of hippocampal ϑ still needs to be fully elucidated. While some studies suggest that 5-HT may be responsible for one form of ϑ (i.e. type 1 RSA), according to other studies 5-HT would rather inhibit the generation of ϑ rhythm [reviewed in (17)]. These apparently conflicting data can be reinterpreted taking into account different aspects. For instance, an agreement about the existence of different types of hippocampal synchronous activities (pharmacologically, but also physiologically defined) would be useful. It has also been proposed that, especially in freely behaving animals, it may be difficult to differentiate between the direct effect of experimental manipulations on hippocampal EEG activity and the indirect effects of the same manipulations (e.g. induction of movement, arousal, ref. 17)]. Recent evidence suggest the existence, within the raphe nuclei, of different subpopulations of serotonergic neurons, the activity of some of which is related to waking behaviors (such as walking/running) associated with hippocampal ϑ activity (10).

A number of studies show that 5-HIAA levels measured in the extracellular space by *in vivo* voltammetry are closely linked to released 5-HT. In fact, experimental manipulations that increase 5-HT release, including dorsal raphe electrical stimulation or 5-HT releasing drugs, increase extracellular 5-HIAA levels, as measured by *in vivo* voltammetry (7, 8). Experimental manipulations that decrease

5-HT release, such as the pharmacological stimulation of presynaptic 5-HT_{1A} receptors, or manipulations that block 5-HT metabolization to 5-HIAA, such as monoaminoxidase administration, reduce 5-HIAA levels, as measured by *in vivo* voltammetry (4, 18). Moreover 5-HIAA changes measured throughout different behavioral states by means of voltammetric recordings strictly parallel the firing of serotonergic dorsal raphe cells (14, 16). The telemetry system used in this study represents a potent tool to study simultaneous neurochemical and electrophysiological recordings (6, 14). It provides a very detailed time course of neurochemical changes not obtainable with other neurochemical techniques. In fact, brain microdialysis does not allow a comparable frequent sampling. Other *in vivo* electrochemical techniques cannot be used simultaneously with electrophysiological recordings without a telemetry system, not only for the presence of artifacts on the electrophysiological recordings, but mainly for the induction of brain tissue lesion (as explained in detail in the Materials and methods section).

Taken together, the results of this study suggest that although neocortical patterns of EEG desynchronization observed during W and DS are similar, the hippocampal activity observed during these conditions may be easily differentiated at both neurophysiological and neurochemical levels. As such, hippocampal activity may subserve different functions in relation to the two different physiological states.

SUMMARY

To investigate the relationship between the hippocampal θ activity (or Rhythmical Slow Activity, RSA) and the hippocampal serotonergic activity during spontaneous behavior, simultaneous recordings of *i)* hippocampal EEG, *ii)* sleep-wake activity, and *iii)* hippocampal levels of the serotonin (5-HT) metabolite 5-hydroxyndolacetic acid (5-HIAA - measured by *in vivo* voltammetry and infrared telemetry) were performed. The results show that hippocampal type 1 RSA recorded during wakefulness and voluntary movements (such as walking), is positively correlated to hippocampal 5-HIAA levels. Since in the experimental conditions used in the study, 5-HIAA levels are a reliable index of 5-HT release, the results support the hypothesis that hippocampal type 1 RSA is generated by a serotonergic mechanism. In contrast, hippocampal type 2 RSA recorded during desynchronized sleep is negatively correlated with 5-HT release, suggesting a different neurochemical mechanism for its production. These results also show that, in the experimental condition of this study, hippocampal RSA power spectrum has a main peak frequency of 3.5 during wakefulness, and of 6.5 Hz during desynchronized sleep.

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