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Changes in hippocampal cell discharge patterns and theta rhythm spectral properties as a function of walking velocity in the guinea pig

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Abstract Dorsal hippocampal theta rhythm (\(\Theta\)) and extracellular unit activity from CA1 pyramidal layer were recorded in awake guinea pigs, both during standing and during walking on a conveyor belt at increasing speeds. Amplitude, frequency and rhythmicity of \(\Theta\) increased linearly with the movement speed. In this preparation we found the same three types of unit discharge patterns that have been described in anesthetized rats in the presence of spontaneous or induced hippocampal \(\Theta\): type 1, rhythmic at \(\Theta\) frequency and phase-locked with \(\Theta\); type 2, discharging non-rhythmically but phase-locked with \(\Theta\); and type 3, discharging at random. Furthermore, all units modified their firing pattern when the animals walked, either by increasing their rhythmicity and/or phase-locking with \(\Theta\) or by increasing their firing frequency. During walking, some type 3 units changed into type 2 or type 1, type 2 units changed to type 1, and type 1 increased their rhythmicity. Consequently, the unit discharge rhythmicity and phase-locking with \(\Theta\) increased with the speed of movement. The mean rate of neuronal discharges increased linearly as a function of walking speed. In this paper we show that the progressive spectral \(\Theta\) changes determined by the intensity of movement are concomitant with the increase in rhythmicity of hippocampal cells. Moreover, the firing rate of these cells, and the amplitude, frequency and rhythmicity of \(\Theta\), increased linearly as a function of walking speed, suggesting that neuronal excitation may be basically responsible for these changes in \(\Theta\) properties.

Key words Hippocampus · Unit discharges · Theta rhythm · Movement · Guinea pig

Introduction

Hippocampal theta rhythm (\(\Theta\)) (Green and Arduini 1954) is a physiological variable that stands in close relation to body movements and sensory inputs (Jung and Kornmüller 1938). The relation of multiple hippocampal theta cell discharge rates to slow wave theta frequency was studied in rabbits by Bland et al. (1983). Changes in \(\Theta\) spectral properties as a function of movement speed have been described (McFarland et al. 1975; Arnolds et al. 1979). Obviously, these changes in frequency, amplitude and rhythmicity of \(\Theta\) result from modifications in the electrical processes at cellular level. This led us to investigate the changes in the discharge pattern of the neurons responsible for the local generation of \(\Theta\) in the hippocampus.

In the presence of hippocampal \(\Theta\) (both spontaneous and/or induced by physostigmine injection or sensory stimulation) three types of unit discharge patterns may be observed in the hippocampus (Buño et al. 1978; García-Sánchez et al. 1978; Alonso et al. 1987): type 1 (recorded in 20% of the cells, rhythmic at \(\Theta\) frequency and phase-locked with \(\Theta\)), type 2 (40%, discharging non-rhythmically but phase-locked with \(\Theta\)), and type 3 (40%, discharging at random and not phase-locked with \(\Theta\)). It has likewise been demonstrated that when \(\Theta\) increases, under electrical stimulation of the mesencephalic reticular formation or the lateral hypothalamus, some neurons with type 2 activity change to type 1 (Buño et al. 1978; Núñez et al. 1987). In the converse situation, when \(\Theta\) decreases (spontaneously or when stimulation is discontinued), cells with type 1 activity change to type 2 or type 3 patterns as has been repeatedly reported (Macadar et al. 1970; Ranck 1973; Buño et al. 1978). Consequently, the three types of neuronal discharges may reveal different functional states, and may not really be discrete entities. It can be envisioned that if each hippocampal \(\Theta\) generator receives both rhythmic (theta) and non-rhythmic inputs, the predominance of the latter may determine the type 3 pattern of discharge, as shown by one of us in a computer simulation (Fuentes et al. 1981). In that paper,
type 2 and type 1 patterns were, in turn, simulated using increasing proportions of rhythmic versus non-rhythmic inputs.

The increase in the rhythmicity, amplitude and frequency of \( \Theta \) during relatively elementary behavioral acts such as forced walking, could be the consequence of an increase in the rhythmicity and bursting frequency of the neuronal population and/or the recruitment of new rhythmic neurons. We tested the first possibility in the guinea pig walking at different speeds.

**Material and methods**

Hippocampal \( \Theta \) and CA1 unit activity were simultaneously recorded in 25 adult guinea pigs (250–270 g) of both sexes. *Principles of laboratory animal care* (NIH publication no. 85–23, revised 1985) was followed.

Preparation and recordings

The animal was anesthetized with pentobarbital (40 mg/kg i.p.), placed in a stereotaxic frame and two parallel stainless steel tubes transversely fixed in the dorsal aspect of the skull with dental cement, together with four stainless steel screws for strengthening. The animal was then fixed again to the frame by adapted guides that fitted to the tubes, and the coordinates were calculated according to Luparello (1967).

Trophone windows (1–3 mm diameter) were drilled in the skull at pre-selected stereotaxic coordinates. An indifferent macroelectrode (150 \( \mu \)m diameter factory-insulated nichrome wire) with 2 mm of insulation removed at the tip was placed in the frontal lobe (A 10.4, L 3.0, H 2.0). A macroelectrode as above, but with only the tip end surface bare, was lowered into the left CA1 stratum oriens of the dorsal hippocampus (A 6.6, L 2.2, H 3.1) to record \( \Theta \). Both macroelectrodes were fixed to the skull with dental cement. Unitary neuronal extracellular activity was recorded with glass micropipettes (7–12 \( \Omega \)2) filled with a solution of 0.5 M sodium acetate and 2% Pontamine Sky Blue. With the aid of a micro manipulator, the micropipette was aimed at the CA1 pyramidal layer in the right dorsal hippocampus (A 6.2–7.0, L 1.5–3.5, H 2.8–3.2). Hippocampal electroencephalogram (EEG) and unit activity were AC preamplified, band-pass filtered at 0.3–31.5 Hz and 0.3–3.1 kHz, respectively, and stored on FM magnetic tape for later analysis. Filter settings did not produce relevant phase shifts of the EEG signals at frequencies within the \( \Theta \) range.

After 7 days of postoperative recovery, the animals were placed standing on the conveyor belt at rest with the head fixed in the stereotaxic apparatus as explained above; they were quiet and showed no discomfort. Then, they were trained to walk on the conveyor belt at speeds of 1.25, 2.50 and 5.00 cm/s, which they followed well without ever slipping. Five 2.0–2.5 min recordings were performed for all units: at rest, at the three speeds (generally in increasing order), and again at rest. The spikes were stored only when the recording had a signal-to-noise ratio greater than 2, and they were rejected if their amplitude decreased. Data were included in this study only when marks of Pontamine Sky Blue ejected from the recording pipette coincided with the CA1 pyramidal layer. Sixty-two units of the CA1 cellular layer were recorded simultaneously with \( \Theta \) in the five conditions described above.

Data processing

Statistical analysis of the recorded EEG included “off-line” calculations – after passing through an anti-aliasing 31.5 Hz filter – in a PDP-11 computer. At least 2 min of continuous stationary data were fed to the computer after digitizing with 4-ms sampling intervals. Spikes were transformed into time point-processes which were counted every 4 ms. The EEG autocorrelation function (ACF) and spike autocorrelation histogram (ACH) were used to assess rhythmicities. The cross-correlation (CC) between EEG and unit activity, calculated by triggering the EEG average with each successive spike, was used to determine their phase relationships with \( \Theta \). This CC was considered “positive” (i.e., above the statistical 90% confidence level) when the peak amplitudes were larger than those calculated using the same data after shuffling the interspike intervals (Fuentes et al. 1981). Siegel and Tukey's modification of the Wilcoxon test (Langley 1971) was used to compare the two distributions. On the basis of the rhythmicity of the unitary activity ACH and CC, units were classified as type 1, type 2 or type 3 (see above). Power spectra (PS) averages of 2.5 min stationary data were calculated with a Fast Fourier Transform algorithm. To quantify the rhythmicity of the EEG the coefficient of rhythmicity (CR) (Gaztelu and Bufio 1982) was calculated as the quotient between the power of the fundamental and the power of all the spectral components of the PS. The mean relative firing rate of spike discharges at each walking speed was calculated as a percentage of the mean firing rate of the unit at rest (100%). This variable, the CR and the mean value of \( \Theta \) frequency and amplitude were analyzed by simple linear regression models, with walking speed as independent variable. Regression models were performed with data from all the experiments.

![Fig. 1A–C](attachment:image) Changes in the spectral properties of hippocampal \( \Theta \) during forced walking at increasing speeds (see Methods for abbreviations). A–C Raw data, ACFs and PS, respectively. 1.5 At rest; 2–4 walking at 1.25, 2.50 and 5.00 cm/s, respectively. Numbers in B and C indicate mean \( \Theta \) frequency and CR corresponding to this experiment, respectively. Amplitude (A,C), frequency (B,C) and rhythmicity (C) of \( \Theta \) increased as a function of speed. Changes were reversed after resting for 30 s (5). In this Figure, and in the following, all data were processed during 2.5 min. Calibrations: ordinates 250 \( \mu \)V (A), 1.5x10^7 \( \mu \)V (B), 8x10^4 \( \mu \)V/Hz (C); abscissas s (A,B), Hz (C)