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Electro-oscillographic correlation between dorsal raphe nucleus, neocortex and hippocampus during wakefulness before and after serotoninergic inactivation

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Abstract

Theta rhythm in many brain structures characterizes wakefulness and desynchronized sleep in most subprimate mammalian brains. In close relation to behaviors, theta frequency and voltage undergo a fine modulation which may involve mobilization of dorsal raphe nucleus efferent pathways. In the present study we analyzed frequency modulation (through instantaneous frequency variation) of theta waves occurring in three cortical areas, in hippocampal CA1 and in the dorsal raphe nucleus of Wistar rats during normal wakefulness and after injection of the 5-HT_{1a} receptor agonist 8-OH-DPAT into the dorsal raphe. We demonstrated that in attentive states the variation of theta frequency among the above structures is highly congruent, whereas after 8-OH-DPAT injection, although regular signals are present, the variation is much more complex and shows no relation to behaviors. Such functional uncoupling after blockade demonstrates the influence of dorsal raphe nucleus efferent serotoninergic fibers on the organization of alertness, as evaluated by electro-oscillographic analysis.

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Key words
- Theta rhythm
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- Neocortex
- Hippocampus
- Instantaneous frequency

Electrical potentials from thalamic, olfactory and septal nuclei, hippocampus, amygdala, most cortical areas and many other brain structures oscillate regularly from 6 to 11 Hz as theta rhythm during wakefulness and desynchronized sleep in rats (1). Theta wave amplitude and frequency undergo fine modulation as a function of time and of the kind of behavior occurring in those states (2). Such modulation is followed by a spectrographic correlation between central structures that is higher in attentive wakefulness than in the relaxed state and narrowing of the oscillation band also occurs, disclosing its functional coupling to wakefulness (3,4).

The serotoninergic raphe ascending projections are diffuse and reach almost all mammalian brain structures (5,6). The dorsal raphe

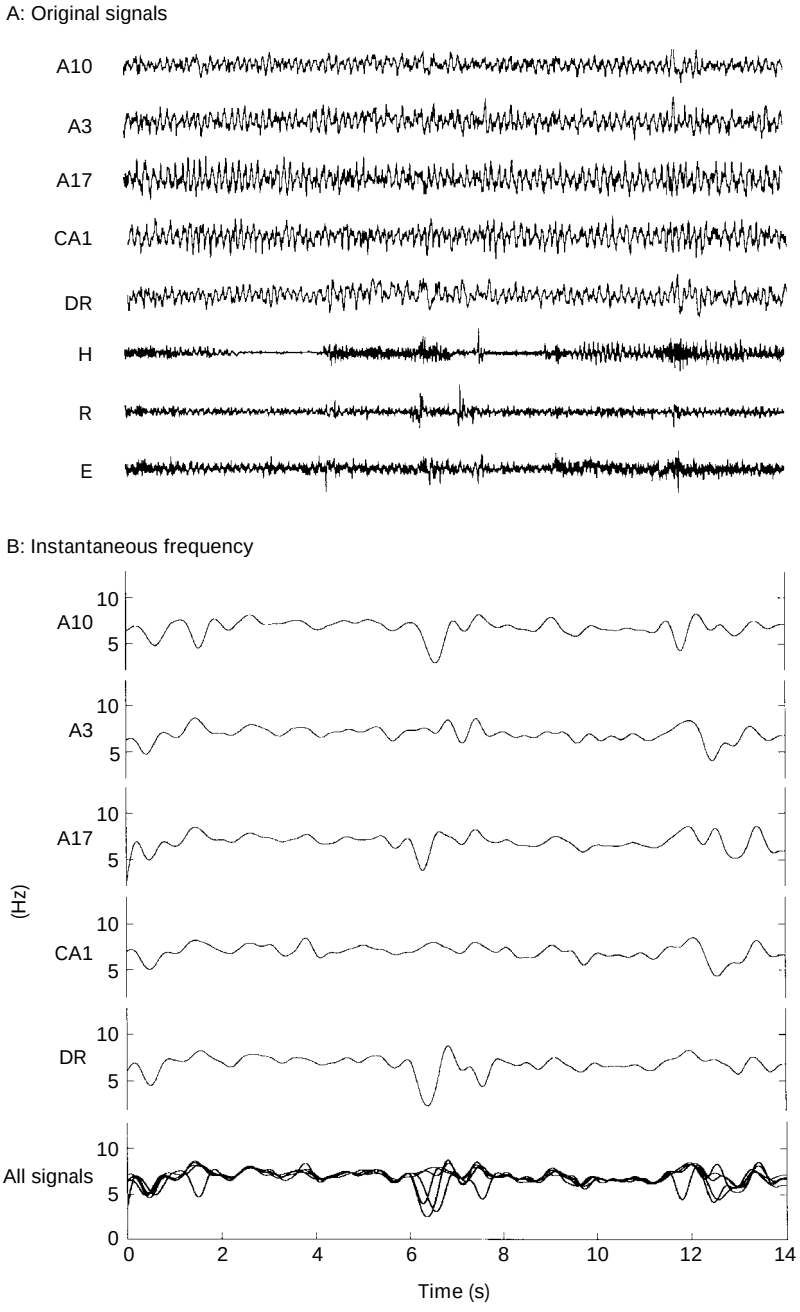

Figure 1 - *A*, Electro-oscillograms of the cortical areas 10, 3 and 17 (A10, A3 and A17), hippocampal CA1 (CA1) and dorsal raphe nucleus (DR) during attentive wakefulness with exploratory behavior, expressed as intense movements of the head (H), rostrum (R) and eyes (E). Theta rhythm appears as regular and well-modulated waves. *B*, Instantaneous frequency variation of the same brain structures as shown in A. A close coupling of frequency values occurs from 2 to 6 and from 8 to 12 s (oscillation, i.e., modulation, band near 2 Hz). Brief desynchronization episodes concomitant to the more active behaviors appear as uncoupling of frequency in the concomitant electro-oscillograms from 6 to 8 and above 12 s.

nucleus preferentially innervates the neocortex and has less dense efferent projections to the hippocampus, whose main serotoninergic afference arises from the median raphe nucleus (7,8). Physiological, chemical and clinical investigations suggest that alterations in this system may be involved in human anxiety and psychosis induced by drugs like the serotonin synthesis inhibitor parachlorophenylalanine and lysergic acid diethylamide (9,10). In such situations, the organization of behaviors is impaired.

The present study is intended to assess how theta rhythm is affected by the large fiber contingent from dorsal raphe nucleus to neocortex, which, by connecting to layers I and Va, hypothetically controls the formation and stability of spatially distributed functional modules, thus providing their temporal organization (11,12).

Nine adult Wistar rats, weighing 270 to 310 g, were anesthetized with ketamine plus diazepam and received bipolar electrodes over cortical areas 3, 10 and 17 in hippocampal CA1 and in the dorsal raphe nucleus for recording of the local electro-oscillograms. Also, bipolar electrodes were implanted into the trapezium muscles and into the muscle pad that covers the rostrum (for recording head and rostrum movements, respectively) and applied to the orbital epicanthus (to record eye movements). Furthermore, a guide cannula was implanted slightly above the dorsal raphe nucleus for microinjection of 8-OH-DPAT (a 5-HT_{1A} autoceptor agonist whose stimulation inhibits the serotoninergic efferences from the raphe nuclei) (13,14). For the microinjection procedure a 200-µm thick steel cannula was inserted into the guide cannula whose proximal end was connected to a flexible polypropylene tube. The latter was connected to a 1-µl Hamilton syringe. Either 0.5 or 1 µl of the drug in saline solution (10 mg/ml) was injected through the cannula during a period of about 10 s. In

each animal the same volume of saline solution alone was also injected for control.

Electro-oscillograms were recorded during attentive and relaxed wakefulness and sleep phases up to desynchronized sleep, both before and after injection. Selected epochs of the electro-oscillograms were digitized at a 256-Hz sampling rate and fed into a computer for off-line analysis. The potentials were visually and instrumentally analyzed. Visual analysis was necessary to identify the phases of wakefulness and sleep; instrumental analysis was performed to determine the frequency spectra as well as the instantaneous frequency of theta waves, calculated by complex demodulation. The mathematical routines were written in our laboratory for use in the MatLab® environment.

During wakefulness and desynchronized sleep theta rhythm shows great regularity (see wakefulness with exploratory behavior in Figure 1), with maximal voltage and frequency usually being observed when alertness is intensified. If behaviors become more energetic (see H, R, E in Figure 1A) such regularity may be briefly interrupted, particularly by wide oscillations or desynchronization in the dorsal raphe nucleus and area 10. Instantaneous frequency variations (Figure 1B) are concomitant and occur inside a narrow band, except between 6 to 8 and 12 to 14 s. When control injections were made, the electro-oscillograms remained the same in all leads. Between 20 to 30 s after microinjection (Figure 2) of the drug there was intense motor agitation (see H, R, E in Figure 2A) concomitant with increasing electro-oscillogram voltage and large variation of its modulation band, without synchrony between leads or relation with behaviors. Frequency varied widely. Compare the instantaneous frequency of all leads in Figure 2B with the corresponding leads in Figure 1B. Interestingly, the large variations of instantaneous frequency in the dorsal raphe nucleus caused

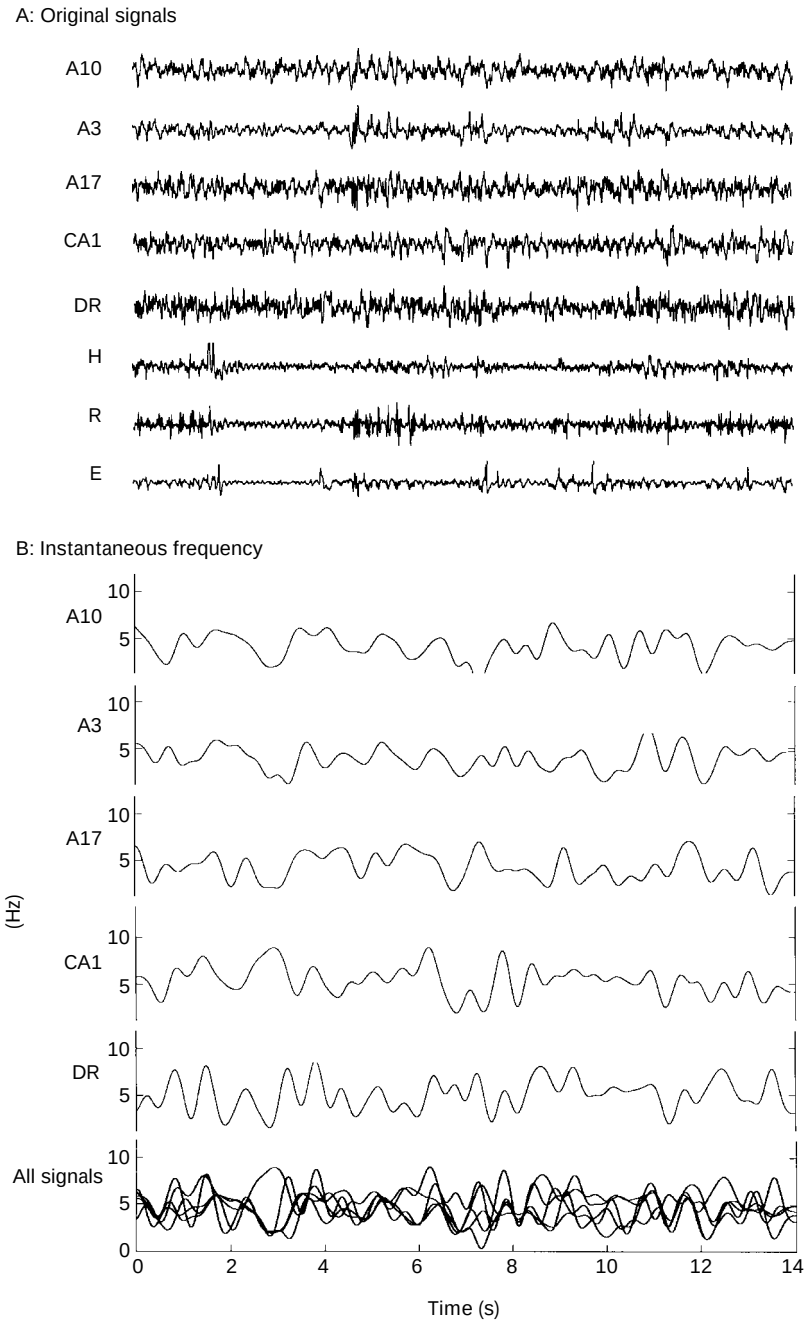

Figure 2 - *A*, Electro-oscillograms as in Figure 1A, after 8-OH-DPAT administration. The rat moved more intensely 20 to 30 s after microinjection, as shown by the larger number of high voltage muscle potentials. Despite the increase in spontaneous movements, no organized behavior can be identified. The electro-oscillograms display low entropy signals, but desynchronization is predominant and theta rhythm is not prominent and appears not to be rhythmically well organized. *B*, Instantaneous frequency variation. Only CA1, which receives its major serotoninergic afferentation from the median raphe nucleus, shows a trend to narrowing the oscillation band (segments 4 to 6 and 8 to 11 s). DR, however, oscillates more regularly (at 1 to 1.5 Hz). Abbreviations as in legend to Figure 1.

by 8-OH-DPAT were modulated by iterative and regular oscillations. CA1 recordings, however, displayed instantaneous frequency patterns closer to normal. This effect diminished progressively with the repetition of the injections (10-min intervals), but with no return to normal theta rhythm: regular potentials disappeared, with only frequencies above 20 Hz remaining after the fourth injection. Without new injections, electro-oscillograms return to the normal wakefulness pattern after 2 to 4 h.

Supporting the hypothesis of a key role for theta rhythm in alertness, the simultaneous occurrence of behavioral disorders and irregular but still present theta waves shows the importance of coordinated oscillation. The suppression of spectral coupling between the studied areas after serotoninergic inhibition indicates the influence of dorsal raphe nucleus projections to the neocortex on local genesis and synchronization of potentials.

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