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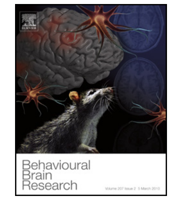

Research report

Theta activity in local field potential of the ventral tegmental area in sleeping and waking rats

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HIGHLIGHTS

- Local field potential of the VTA was studied in sleep and wakefulness.
- Theta activity dominated in the VTA LFP in active waking and in PS.
- F_{max} in theta band correlated between the hippocampus and the VTA during WT and PS.
- Cross-correlation was detected between the hippocampus and the VTA in delta and theta.

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ABSTRACT

Hippocampal theta rhythm appears in two vigilance states: active waking and paradoxical sleep. The ventral tegmental area (VTA) is active in sleep and waking and is connected to the hippocampus. We assessed the relationship between local field potential (LFP) of the VTA and sleep–waking stages in freely moving rats. Electrical activity of the VTA was divided into: quiet waking (W), waking with theta (WT), slow wave sleep (SWS) and paradoxical sleep (PS), depending on the hippocampal signal and the animal's behavior. We analyzed total power in the VTA signal and we also extracted peak power (P_{max}) and corresponding frequency (F_{max}) in theta and delta bands from both the VTA and hippocampal recording. In the VTA the 6–9 Hz band had the highest power during PS, and the ratio of the 6–9 to 3–6 Hz power was highest during both PS and WT, which accentuated P_{max} of this particular theta sub-band. During W, a very slight increase (or plateau) in signal power was seen in theta range. P_{max} and F_{max} of theta were higher in PS than in both WT and W, and these parameters did not differ between W and WT. During WT and PS, F_{max} in the 6–9 Hz band was greatly correlated between the VTA and hippocampus signal. We also detected high cross-correlation in power spectra between the hippocampus and the VTA (for delta and theta, during WT and PS). The results suggest that the VTA may belong to the broad network involved in theta rhythm induction.

1. Introduction

Rhythmic slow activity (RSA), also called theta rhythm, is a high voltage and low frequency (3–12 Hz) electrical activity of the hippocampus and it has a clear, easily recognizable pattern, especially in the rodent brain. A body of evidence emphasizes involvement of theta oscillations in the encoding of information particularly during active exploratory movements and spatial navigation, orientation, learning and working memory [1–5]. In rats, theta in waking state is associated with voluntary locomotor activities [6,7], and in sleep it appears in episodes of REM sleep [8–10]. Theta rhythm of waking and sleep is considered to be generated by different systems of neurotransmitters [11].

The ventral tegmental area (VTA), composed of dopaminergic and GABAergic neurons [12] and a small subpopulation of glutamatergic cells [13], belongs to the structures involved in the regulation of theta. Inactivation of the VTA in urethane-anesthetized rats, either by procaine microinjection or by electrolytic lesion, caused disruptions in generation of hippocampal theta rhythm [14]. Local microinjections of bicuculline [15], phaclofen, flupenthixol and amphetamine [15,16] induced regular theta rhythm in hippocampal EEG. Dopaminergic fibers of the VTA activate the hippocampus via the forebrain septum [12,17,18]. Early studies indicated invariable discharges of dopaminergic neurons across the sleep–wake cycle [19–21], while GABAergic neuronal activity was shown to change depending on the arousal level

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[19,22]. Later it was found that the VTA dopaminergic cells also change firing pattern depending on the vigilance state, decreasing discharges during SWS and increasing significantly in the PS [23]. The impact of the VTA on its target, the septum, is the outcome of at least three mechanisms: the activity of VTA afferents, the influence of internal GABAergic interneurons and somatodendritic dopamine acting on projecting dopaminergic neurons [12,24,25]. Therefore, analyzing the local field potential (LFP) seems to be an appropriate method of investigating the relationship between the VTA and the hippocampal signal. LFP recording allows to study brain structures by assessing activity of larger groups of neurons without assessing their transmitter characteristics. Estimation of the relationship between the VTA field activity and the hippocampal theta could confirm the involvement of the VTA in the induction of the rhythm. When theta rhythm appears in the hippocampus, power at this frequency range can be expected to increase also in other centers related to theta induction.

So far the research into the influence of the VTA on hippocampal theta has been conducted in rats under anesthesia with theta induced with sensory or pharmacological stimulation [14–16]. In the current experiments we used freely moving animals, in which theta rhythm appeared spontaneously, according to the animals' behavior. The aim of the study was to describe the LFP pattern of the VTA in the basic types of behavior distinguished in rats (waking - W, active waking with hippocampal theta - WT, slow wave sleep - SWS and paradoxical sleep - PS), with particular attention given to those states that are accompanied with hippocampal theta rhythm.

2. Material and methods

2.1. Animals

Male Wistar rats ($n = 7$; 280–350 g) were maintained in a room with controlled temperature and humidity, with 12:12 h. light/dark cycle (lights on at 6:00 a.m.) and ad libitum access to food and water. The animals were handled before the procedures and the experiments were carried out in compliance with regulations guiding the use of experimental animals for scientific investigation.

2.2. Surgery

For the purpose of surgery, the animals were anesthetized with nembutal (50 mg/kg i.p.) and positioned in the frame of stereotaxic apparatus. Bilateral recording electrodes were implanted in the rostral part of the VTA and in the dentate gyrus of the hippocampus according to the standard stereotaxic procedure. The electrodes were monopolar and made of stainless steel wire of 0.2 mm diameter insulated on the entire length except for the flat-cut tip.

The following Paxinos and Watson [26] stereotaxic coordinates (skull leveled) were used for the VTA implantation: 4.9 mm posterior to bregma, 1.0 mm lateral to midline and 8.0 mm ventral to skull surface. Stereotaxic coordinates for the hippocampus were: 3.1 mm posterior to bregma, 2.0 mm lateral to midline and 3.3 mm ventral to skull surface. A silver wire electrode was implanted in the neck muscles for EMG recording. All recording electrodes were fixed to skull surface with dental acrylic. Stainless steel screws screwed into the skull bone in the parietal area and over the olfactory bulb (where electrical activity is minimal) served as ground and reference electrodes respectively.

2.3. Experimental design

VTA and hippocampal LFP was recorded using EEG head unit (Braintronics B.V., The Netherlands) as the preamplifier (band pass 0.3–70 Hz) and EEG Digi Track system 6.55 (ELMIKO, Poland) to record the signal (sampling rate 240 Hz) on a hard drive. During the recording the animals were held in 260 × 260 × 400 mm glass cages placed in a sound attenuating chamber where they could move freely. Each recording session was preceded by an hour adaptation of animals to the experimental conditions. Recordings were held in the morning and lasted about 1 h. During each session a detailed account of an animal's behavior was prepared, which allowed to match LFP samples to particular vigilance states. Each rat was subjected to recording of the VTA electrical activity several times over a period of 3 weeks, always in the same morning hours.

2.4. Data analysis

At the end of the experiments, the LFP recordings were viewed in the EEG Viewer version 4.00 standard. Periods of sleep (SWS and PS) and waking (W and WT) were diagnosed on the basis of the hippocampal pattern, which was analyzed visually and verified by Elmiko software function for analysis of signals; they were also confirmed with the use of detailed protocol of behavioral observation. EEG behavioral state identification was based on the high-amplitude theta oscillations characteristic of REM sleep, the high-amplitude delta characteristic of SWS, relatively low power without marked maxima in successive frequency bands characteristic of quiet waking and additionally, high-amplitude theta oscillations characteristic of waking with theta. The EEG epochs which represented transitions between waking and sleep states were excluded from samples selected for further analysis.

The spectral analysis of bilateral VTA signal (pass band 0.5–30 Hz) was performed off line by fast Fourier transformation (FFT) on ten to twenty 5-s artifact-free samples for each behavioral state from each recording day. Power distribution in 0.15-Hz bands was analyzed with the Elmiko software, then averaged for each rat and for the day of recording and logarithmically transformed. Afterwards we calculated signal power in 3-Hz frequency bands: 0.5–3, 3–6, 6–9, 9–12, 12–15, 15–18, 18–21, 21–24, 24–27 and 27–30 Hz. Two parameters of the power spectrum, maximal peak power (FFT peak magnitude, P_{max} [μV^2]) and the frequency corresponding to the maximal peak power (FFT peak frequency, F_{max} [Hz]), were assessed for 6–9 Hz (middle theta) and delta band. These data were subsequently submitted to statistical analysis with two- or one way ANOVA with behavioral state factor (W, WT, SWS and PS) and post hoc Tukey's test (SPSS 21.0 software). Since the statistical analysis of power in consecutive frequency bands did not show interhemispheric differences, the power spectra of both hemispheres are presented together. Theta and delta F_{max} of hippocampal signal were compared with the corresponding parameter of ipsilateral VTA LFP obtained from the same time points (Spike software, duration of a signal sample: 6.83 s; statistic tests: correlation (rho Spearman) and analysis and estimation of linear regression). On the basis of the samples, cross-correlation between the VTA and hippocampus power was calculated during WT and PS in the following four frequency bands: delta (1–3 Hz) and three sub-bands of theta (3–6, 6–9 and 9–12 Hz). Correlation values were averaged over the periods of the same type of behavior for each subject. In order to approximate a normal distribution, the power values were transformed into logarithms (SPSS 21.0 software).

Afterwards, 2-sec epochs (three epochs from each sample) were cut from the same LFP samples to calculate time-resolved cross-correlation between the VTA LFP and hippocampus signal in delta (1–3 Hz) and middle theta (6–9 Hz) band (i.e. the sub-band that contained the maximal peak of power within the whole 3–12 Hz range) during WT and PS. For this purpose LFP signal was filtered in the range of 1–3 and 6–9 Hz (Spike software) and analyzed using SPSS software.