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Research article

Inactivation of the medial mammillary nucleus attenuates theta rhythm activity in the hippocampus in urethane-anesthetized rats

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HIGHLIGHTS

- Procaine injection to the medial mammillary nucleus of anesthetized rats was made.
- The pharmacological inactivation suppressed theta rhythm in the hippocampus.
- Procaine injection decreased the EEG signal power of low theta frequency bands.
- The mammillary body may regulate theta-rhythm in the extended hippocampal system.

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ABSTRACT

Although the importance of the mammillary body for memory and learning processes is well known, its exact role has remained vague. The fact, that many neurons in one nucleus of the mammillary body in rats, i.e. the medial mammillary nucleus (MM), fires according with hippocampal theta rhythm, makes this structure crucial for a theta rhythm signaling in so-called extended hippocampal system. These neurons are driven by descending projections from the hippocampal formation, but it is still unknown whether the mammillary body only conveys theta rhythm or may also modulate it. In the present study, we investigated the effect of pharmacological inactivation (local infusion of 0.5 μl of 20% procaine hydrochloride solution) of the MM on hippocampal theta rhythm in urethane-anesthetized rats. We found that intra-MM procaine microinjections suppress sensory-elicited theta rhythm in the hippocampus by reduction of its amplitude, but not the frequency. Procaine infusion decreased the EEG signal power of low theta frequency bands, i.e. 3–5 Hz, down to 9.2% in 3–4 Hz band in comparison to pre-injection conditions. After water infusion (control group) no changes of hippocampal EEG signal power were observed. Our findings showed for the first time that inactivation of the MM leads to a disruption of hippocampal theta rhythm in the rat, which may suggest that the mammillary body can regulate theta rhythm signaling in the extended hippocampal system.

1. Introduction

Theta rhythm is the most synchronized local field oscillation in the hippocampus, which frequency can range from 3 to 12 Hz depending on species and behavioral conditions. In rats, theta rhythm is prominent during exploratory motor behavior and paradoxical sleep. Two types of hippocampal theta rhythm have been distinguished on the basis of frequency, behavior, and pharmacological sensitivity. Type I (7–12 Hz) occurs in awake animals during voluntary movement (movement-related theta [1]) and is abolished by anesthetics such as urethane. Type II (3–7 Hz) occurs during immobility and REM sleep (sensory processing theta [1]) and is resistant to urethane, but sensitive to muscarinic receptor antagonists such as atropine [2–5]. Many studies have revealed that theta rhythm is involved in various types of memory and learning processes in humans and animals [e.g. 6–10].

The generation and synchronization of hippocampal theta rhythm is dependent on many brain structures, e.g. the brainstem reticular formation, posterior hypothalamic area, supramammillary nucleus, medial septum/diagonal band of Broca, ventral tegmental area [11–14]. Exiting the hippocampus theta-rhythmic

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signals reach other structures, mainly these constituting so-called extended hippocampal system, such as the mammillary body (MB) and anterior thalamic nuclei. Both the structures have direct afferent projections from the hippocampus, but the MB reaches the hippocampus indirectly via the anterior thalamus [15]. The overwhelming body of evidence suggests that these two structures are critical for memory processes (for review see [16,17]). In the MB, nerve cells which fire according with hippocampal theta rhythm have been found mostly in the medial mammillary nucleus (MM). It has been shown that these neurons are driven by descending projections from the hippocampal formation, which suggests that the MB is involved in relaying theta-rhythmical activity to the anterior thalamus and back to the hippocampus [18–21]. In our studies we postulate that the MB not only relays theta signal, but may also modulate it. Sharp and Koester [22] have also tried to verify this idea by lesioning the MB and measuring changes of theta frequency in the hippocampal formation in rats during pellet-chasing task in an open field. In result, the lesions caused an approximately 1 Hz reduction in the frequency of theta rhythm in neuron populations of the hippocampal formation. Unfortunately, the MB lesions used in their work included destruction of the supramammillary nucleus as well, which may suggest that the damage of this adjacent nucleus was responsible for the theta changes observed – the supramammillary nucleus is well known to be involved in the modulation of hippocampal theta frequency in both anesthetized and awake rats [21,23,24]. In the present study we have performed more precise "silencing" of the MB by injecting procaine into the MM. Our aim was to examine whether such temporal inactivation of the MM would affect sensory-elicited theta rhythm in the hippocampus in urethane-anesthetized rats.

2. Material and methods

2.1. Animals

All experiments were performed on male, adult (5–6 months old) Wistar rats (371 ± 24 g body weight), carried out in compliance with the guidelines of the European Communities Council Directive (2010/63/UE) and approved by the Local Ethics Committee in Gdańsk. Animals were kept in conditions of regular light-dark cycles (12 h day/12 h night), constant temperature (22° C), and with access to food (standard pellets) and water *ad libitum*. All effort was made to minimize both animals' discomfort and the number of animals used. Overall, 20 rats were used in the study, however due to incorrect cannula implantations only 11 animals were included in statistical analysis.

2.2. Surgery

Surgery and EEG recordings were performed under deep urethane anesthesia (Urethane, Sigma-Aldrich, Germany, 1.5 g/kg, i.p.) whose level was controlled by monitoring the frequency of breathing and by checking for withdrawal reflex. The animals were implanted using stereotaxic frame (Kopf Instruments, USA) with a recording bipolar electrode (200 μm dia., teflon-coated twisted stainless steel wire, SS-5T; Science Products, Germany) in the dorsal hippocampus (AP: 3.4 mm, L: ± 2.4 mm, D: 3.1 mm), and a guide cannula (28 gauge, PlasticsOne, USA) for an injection cannula above the lateral part of the MM (at an angle of 16°). The tip of the injection cannula (22 gauge, PlasticsOne, USA) was located 2.5 mm below the implanted guide cannula, i.e. in the lateral part of the MM (AP: 4.5 mm, L: ± 0.6 mm, D: 9.6 mm) (Fig. 1). The implantations were conducted in accordance with the standard stereotaxic procedure and coordinates [25]. A silver wire (99.99%, 250 μm dia., AG-10W, Science Products, Germany) connected to a screw mounted anteriorly to bregma was used as a reference electrode. Electrodes were connected to a 3-pin connector (MillMax, USA) and cemented to the skull with dental acrylic.

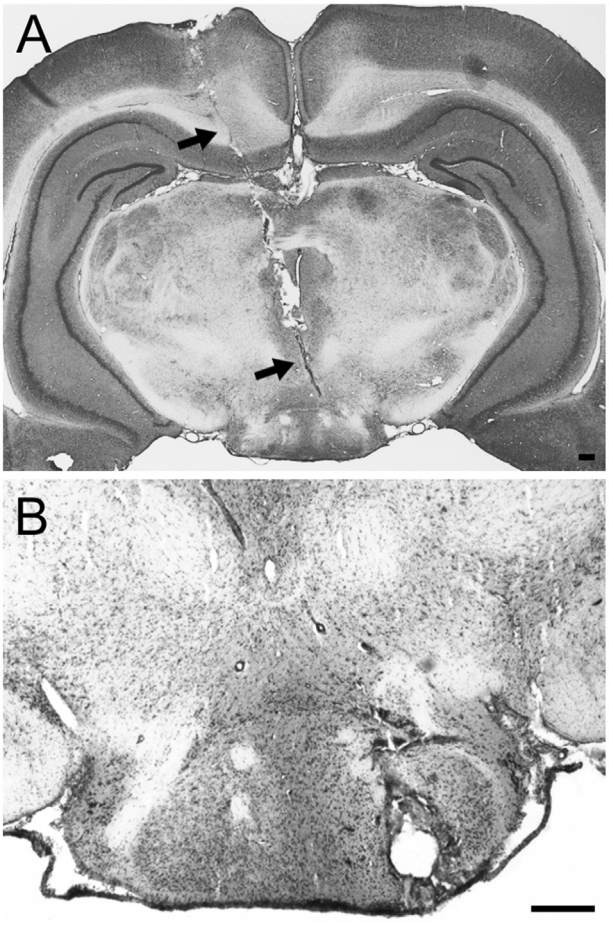

Fig. 1. Microphotographs showing the trajectory of the guide cannula (black arrows) to the mammillary body (A) (4.8 mm posterior to bregma according to [25]) and the injection cannula tip (B) in the lateral part of the medial mammillary nucleus (4.92 mm posterior to bregma according to [25]) in the representative rat. Scale bar: 500 μm.

2.3. Experimental procedure

EEG was recorded from the hippocampal electrode during the whole experiment using EEG DigiTrack computer system (ELMIKO, Poland), which serves to amplify and store signal on a hard drive (bandpass 0–70 Hz, sampling rate 250 Hz). The animals were maintained at the level of anesthesia at which spontaneous theta rhythm was not present in the hippocampal EEG but could be elicited by sensory stimulation (tail pinch lasting 1 min, by using a plastic clamp positioned at the tail base). For the injection, an injection cannula connected to a 10 μl Hamilton syringe placed in the microinjection unit (Model 5000, Kopf Instruments, USA) was used. Sensory stimulation was applied 2–4 times before injection cannula insertion to the MM and after its insertion, but before the drug injection, to verify proper position of the hippocampal electrode and proper parameters of the recorded EEG signal. Then, animals were unilaterally infused into the MM (0.5 μl volume, infusion lasted for 1 min) with 20% solution of procaine hydrochloride (n = 5; Polpharma, Poland), or water (n = 6; drug vehicle). After the infusion, the injection cannula was left in the MM for 1 min. Hippocampal EEG was recorded continuously and the tail pinch was applied at