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Research article

Hippocampal theta rhythm after local administration of procaine or amphetamine into the ventral tegmental area in fear conditioned rats

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HIGHLIGHTS

- VTA injection of procaine suppressed avoidance response in fear conditioned animals.
- VTA inactivation affects hippocampal theta rhythm linked with fear conditioned immobility.
- Administration of amphetamine has no effect on the behavior and hippocampal LFP.

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ABSTRACT

The ventral tegmental area (VTA) is thought to be an important component in the mesocorticolimbic system involved in the regulation of theta rhythm in the hippocampus. In this study we investigate the effect of pharmacological inactivation (local procaine infusion) or activation (local amphetamine infusion) of the VTA on theta rhythm parameters during task specific behavior in fear conditioned, freely moving rats. Animals were implanted with bilateral recording electrodes into the dorsal hippocampus (CA1) and bilateral injection cannulas into the VTA. Behavioral activities and hippocampal local field potentials (LFP) were recorded throughout the experiment, in pre- and post-injection conditions. We found that intra-VTA injection of procaine temporarily suppressed fear conditioned avoidance response (escape from the foot-shock arena) and also influenced hippocampal theta rhythm parameters during immobility linked with arousal and/or attention. Procaine infusion decreased the signal power (P_{max}) of theta rhythm during immobility behavior, in comparison to the control group (water infusion), whereas administration of amphetamine had no effect on the behavior and hippocampal LFP. Our results indicate that temporal inactivation of neuronal activity in the VTA affects hippocampal theta rhythm linked with attentional immobility and suppresses avoidance response in fear conditioned animals.

1. Introduction

The theta activity is the one of the most regular rhythms of the brain and can be easily recorded in rats, rabbits, cats, and other small mammals. Depending on behavioral conditions and species, theta frequency can range from 3 to 12 Hz [1,2]. There are two types of hippocampal theta rhythm in freely moving rats, distinguished on the basis of differences in the characteristics of EEG frequency and the type of accompanying behavioral activity. Type I (translation movement-theta) occurs in awake animals in a frequency range between 6 and 12 Hz (or 8–14 Hz). In the experimental conditions episodes of type I theta can be recorded in rats during spontaneous locomotion or induced locomotor responses [3–7]. This type is thought to be related with the serotonergic system activity [8]. Type II (attentional-theta) is recorded in awake animals during immobility, as well as during paradoxical sleep episodes, and its frequency range is between 3 and 9 Hz (or 4–8 Hz). This type is thought to be related with the activity of cholinergic system [1,2,9]. In awake animals, the theta rhythm is also recorded during freezing behavior induced by an aversive conditioning procedure [10,11]. The two components of theta, present during translational movements or arousal, are not mutually incompatible and their main frequency ranges partially overlap.

The theta rhythm synchronization system is composed of different brain structures forming together the brainstem-diencephalo-septohippocampal system [12–15]. The midbrain

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ventral tegmental area (VTA), composed mainly of dopaminergic cells forming the A10 group [16–18], is also believed to be involved in the processes of theta regulation or modulation. Our previous studies have shown that unilateral ibotenate lesions of the VTA in freely moving rats, temporary pharmacological inactivation or permanent lesion by electrocoagulation of the VTA in urethanized rats is capable of reducing the power of hippocampal theta activity [19,20]. On the other hand, electrical stimulation of the VTA induces a regular theta rhythm in hippocampal EEG [21].

Local regulatory mechanisms within the VTA were assessed with a microinjection of amphetamine, a dopaminergic agonist of indirect activity, which enhances dopamine release from presynaptic endings and blocks its reuptake and metabolism [22,23]. Intra-VTA amphetamine administration in uretanized rats elicited theta rhythm [24]. Available evidence suggests a role of VTA-hippocampus pathways in mediating locomotor activity, learning and hippocampal-dependent memory processes [25,26]. Lisman and Otmakhova [27] also suggest that the functional VTA-hippocampal loop is involved in detection of novelty and in incorporation of novel information depending on the hippocampal processing.

The present study aimed to examine whether temporal pharmacological inactivation (procaine) or activation (amphetamine) of the VTA would affect the hippocampal theta rhythm induced during task specific behavior (attentional immobility) as well as avoidance response in fear conditioned rats.

2. Materials and method

2.1. Animals

All experiments were performed on male, adult (4–6 months old) Wistar rats (345 ± 16.7 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 Gdansk. Animals were kept in separate cages (after surgery), 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.

2.2. Stereotaxic implantation

The surgery was performed under isoflurane (1–2.5% in oxygen; Aerrane; Baxter, UK) anesthesia (combi-vet system, Rothacher Medical, Switzerland) and butorphanol analgesia (1.5 mg/300 g b.w.; Butomidor; Richter Pharma AG, Austria), Rats were implanted with the use of stereotaxic frame (Kopf, USA) with bilateral hippocampal (AP: −3.7, L: ±2.4, D: −3.1 mm) recording electrodes (125 μm diameter, teflon-coated twisted stainless steel wire (SS-5T; Science Products, Germany), and bilaterally with standard pedestal guides for infusions (PlasticsOne, USA) to the VTA (AP: −5.1, L: ±0.9, D: −8.1 mm). A silver wire connected to a screw mounted anteriorly to bregma was used as a ground/reference electrode. Electrodes were connected to a 6-pin connector (MillMax, USA) and cemented to the skull with dental acrylic.

2.3. Conditioning procedure

A setup for fear conditioning and EEG recordings in freely moving animals was assembled (PC with the SPIKE-2 software (CED, UK), a signal converter unit – MICRO-1410-3 (CED, UK), a commutator (Crist Instruments, USA), signal amplifier (AM Systems, USA) and an electrical stimulator (model A320, WPI, USA) combined with two Plexiglas cages (40 × 40 × 40 cm) connected with a 100 cm long corridor). The floor of one of the cage was made of metal grid (rods equally spaced every 1.2 cm) connected to a discontinuous shock source.

Conditioning procedures were performed after one week of recovery and consisted of three phases: I – arena familiarization before conditioning (1–2 days); II – conditioning phase I: when rats were put in the shock box and were exposed to 6–8 pairings (separated by 2-min blocks) of an acoustic stimulus (tone of 70 dB, duration 1 s) and a 1-s electrical foot-shock 7 s later (duration 0.1 s pulse rate 0.50–0.60 mA). The tone was associated with the subsequent aversive stimulus, causing freezing, which lasted from the sound stimulus until the electric shock; III – conditioning phase II: when the gate to the corridor was manually opened by the experimentator 5 s after the tone (but before the foot-shock), and rats were allowed to escape from the aversive stimulus box to the neutral box. This procedure allowed us to repeatedly induce both: attentional-theta rhythm episode during the immobility after the acoustic tone (when the rats were waiting for the gate to be open) and active avoidance response (escape to the neutral box to avoid the foot-shock) (Fig. 1(I)).

2.4. Experimental protocol

Acquisition of hippocampal local field potentials (LFP – through a JFET preamplifier, filtered 0.1–1000 Hz, amplified × 1000, and digitized at 4 kHz), as well as animals' behavior data (video camera recording), was carried out through the whole duration of the experiment.

In baseline conditions, animals spent 20 min in the neutral box, and then were placed in the aversive stimulus box for 1 min, followed by presentation of the acoustic tone (pre-injection control of immobility and avoidance response). The animals which performed (I) clear and stable immobility after the tone presentation, as well as (II) active locomotion after opening of the gate (escape) were then bilaterally infused into the VTA (0.5 μl volume/side, infusion lasted for 1 min) with 20% solution of procaine ($N = 5$), 10 μg amphetamine ($N = 6$) or water (drug vehicle, $N = 6$). After the drug infusion rats were placed in the neutral box. Then every 4 min (during the first 20 min after procaine or water infusion) or 9 min (after amphetamine infusion) till the end of the experiment (60 min after the infusion) the rats were placed in the foot-shock box for 1 min, followed by the presentation of acoustic tone and gate opening (and foot-shock in case of no escape from the shock box) (Fig. 1(I)). Each animal received intracerebral infusion of drugs in a randomised order, with at least 3 days gap between each experiment.

After completion of the experiment, electrolytic small lesions (an anodal current of 100 μA/15 s) were performed through the hippocampal electrodes to mark the tips locations, and an intra-VTA injection of alcian blue dye was made to assess the location of the injection cannulas tips. Brain sections were cut using frozen tissue technique and the positions of the recording electrodes and injection cannulas were verified.

2.5. Statistical analysis

Signal analysis was performed off-line with SPIKE 2 and GraphPad Prism 6 (GraphPad Software, USA) software, on artifact-free fragments of hippocampal LFP.

Fast Fourier transformation (FFT) was calculated for 5-s samples, chosen from the hippocampal LFP recordings obtained during immobility induced by presentation of the acoustic tone in the foot-shock box, in pre- and post-injection conditions. The maximal peak power (FFT peak magnitude, P_{max}) was analyzed in five frequency bands (0.1–3, 3–6, 6–9, 9–12 and 12–15 Hz). To eliminate inter-subject variability, P_{max} was expressed as a percentage of the pre-injection baseline value (from 5 s attentional immobility after the tone presentation) taken as 100%, for each frequency