Research report

Disruption of footshock-induced theta rhythms by stimulating median raphe nucleus reduces anxiety in rats

Yi-Tse Hsiao [a], Pei-Lu Yi [a,b,**], Chiung-Hsiang Cheng [a], Fang-Chia Chang [a,c,d,*]

[a] Department of Veterinary Medicine, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan
[b] Department of Sports, Health & Leisure, College of Sports Knowledge, Aletheia University, Tainan Campus, Taiwan
[c] Graduate Institute of Brain & Mind Sciences, College of Medicine, National Taiwan University, Taipei, Taiwan
[d] Graduate Institute of Acupuncture Science, College of Chinese Medicine, China Medical University, Taichung, Taiwan

HIGHLIGHTS

- Inescapable footshock stimulation increases LF theta rhythms and anxiety.
- High frequency (100 Hz) stimulation of MRN desynchronizes LF theta power.
- Administration of bicuculline into MRN decreases LF theta power.
- Desynchronization of LF oscillations reduces footshock-induced anxiety.

ARTICLE INFO

ABSTRACT

Theta rhythms generated in the hippocampus are controlled by the pacemaker in the medial septum-diagonal band of Broca (MS-DBB). The median raphe nucleus (MRN) transmits serotonergic signals to the MS-DBB, which suppresses the septo-hippocampus-produced theta waves, whereas GABAergic interneurons in the MRN facilitate the generation of theta oscillations. Animal studies have indicated that fear increases theta oscillations. Moreover, anxiolytics reduce reticular formation-elicited theta rhythms and theta blockade decreases anxiety. In this study, we hypothesized that the MRN mediates anxiety reduction caused by the theta blockade. Our results demonstrated that inescapable-footshock stimulation significantly increased the power of low-frequency theta oscillations (4–7 Hz) in rats. Both the electrical stimulation of MRN and administration of bicuculline into the MRN successfully desynchronized footshock-induced theta oscillations. Compared to the naïve rats, inescapable-footshock stimulation diminished the entry percentage and time spent in the open arms of the elevated plus maze (EPM), behavioral indicators of anxiety. Rats treated with either MRN stimulation or bicuculline administration to desynchronize theta oscillations reduced anxiety caused by the inescapable-footshock stimulation. Our results demonstrated that the electrical stimulation of MRN or blockade of the GABAergic pathways in the MRN interferes with theta oscillations and reduces anxiety, implicating the role of MRN.

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1. Introduction

Theta waves are sinusoidal oscillations which are generated by the hippocampus and several subcortical nuclei (e.g., medial septum-diagonal band of Broca (MS-DBB), dorsal raphe nucleus, ventral tegmental nucleus of Gudden, and anterior thalamic nuclei) [1]. The frequency ranges of theta rhythms differ among species. In rats, the acceptable spectrum of theta rhythms is between 4 and 10 Hz [1]. Although theta oscillation is commonly observed in the electroencephalogram (EEG) spectra, its function is still unclear. In general, theta waves occur during stress [2,3], memory processing [1,4,5], orienting, exploratory [1,3], or rapid eye movement (REM) sleep [1,6]. In fact, theta rhythms can be divided into two types: type-1 and type-2 theta rhythms [1]. These two types of theta waves are determined by pharmacological manipulations [7–10]. Administration of atropine inhibits type-2 oscillations, but type-1 theta waves are resistant to anticholinergics [7–10]. Under normal condition, frequencies of type-2 theta waves (around 4–9 Hz) are lower than type-1 theta oscillations (around 6–12 Hz) [2,9,11,12].

* Corresponding author at: School of Veterinary Medicine, National Taiwan University, No. 1, Sec. 4., Roosevelt Road, Taipei, Taiwan. Tel.: +886 2 3366 3883.
** Corresponding author at: Department of Sports, Health & Leisure, College of Sports Knowledge, Aletheia University, Tainan Campus, Taiwan.
Tel: +886 6 570 3100x7716.
E-mail addresses: pyi67@hotmail.com (P.-L. Yi), fchang@ntu.edu.tw (F.-C. Chang).

According to behavioral properties, type-1 theta is predominant during waking periods in rats, especially during voluntary movement [8–10]. In contrast, type-2 theta dominates EEGs during REM sleep [6] and arousal immobility in rats [8–10,12]. Type-2 theta is also observed when predators are nearby [2,9]. Frequencies of type-1 and type-2 theta waves may be overlapped, and the predominant frequency may change slightly in different situations. For example, type-2 theta waves are detected at a frequency higher than 9 Hz in the presence of predators [9]. After treatment with atropine to suppress type-2 theta, dominant theta waves with frequencies lower than 7 Hz are still observed when animals elicit low-speed motor behaviors, such as face washing and teeth chattering in rats [10], as well as rearing and hopping in rabbits [9]. Thus, there is no exact frequency range that distinguishes between type-1 and type-2 waves. Particularly with electrically elicited theta, it can be shown that frequency and type are independent of each other – with movement or non-movement being the key determinant of cholinergic sensitivity. In the present study we simply defined theta waves, with frequencies between 4 and 7 Hz defined as low-frequency (LF) theta waves, and frequencies between 7 and 10 Hz as high-frequency (HF) theta waves [13]. The relationship between theta waves and animal behaviors (e.g., learning, memory, fear and anxiety) has been demonstrated. For example, the blockade of theta waves impairs initial learning in water mazes, and the restoration of theta rhythms recovers the ability to learn [14]. Both theta waves and context-dependent fear are disrupted by blocking neuronal gap junctions within the dorsal hippocampus in freely moving rats [15]. Studies have shown that the oscillation of theta waves is correlated to stress and anxiety. Anxiolytic drugs inhibit reticularly-elicited theta powers in rats, therefore the strength of theta waves could be used as a neurophysiological index for evaluating the efficacy of anxiolytics [16,17]. To further investigate the role of theta oscillations in anxiety, our current study was designed to manipulate the generation of theta waves and subsequently try to influence the anxiety level.

Several brain regions contribute to the production of theta waves [1], and these brain structures could be potential candidates for the disruption of theta wave generation. The MS-DBB is the primary structure controlling theta rhythms. Inactivation or lesion of MS-DBB neurons completely abolishes theta oscillations [18]. The median raphe nucleus (MRN) could further modulate theta rhythms. Studies have reported that GABAergic interneurons of MS-DBB, which suppress theta processing, receive serotoninergic afferents from the MRN [19–21]. Jackson et al. have demonstrated that directly applying 100 Hz of electrical stimulation to the MRN desynchronizes hippocampal theta waves [22]. In addition to its role in the regulation of theta waves, the MRN modulates behaviors in response to the stressors. Our previous study revealed that inescapable footshock stimulation increases the power of LF (4–7 Hz) theta oscillations through the activation of GABAergic pathways in the MRN, which blocks the ability of MRN to desynchronize theta waves [13]. Sainsbury et al. have also demonstrated that low-frequency theta waves increase after inescapable footshock stimuli and that the increase is not due to conditioning [12]. The aforementioned evidence suggests that anxiety and theta oscillations may influence each other via MRN activities. In the present study, we hypothesized that high frequency (100 Hz) electrical stimuli of MRN or blockade of the GABAergic pathways in the MRN interferes with theta functions and reduces anxiety. We herein employed inescapable footshock stimulation as an acute stressor in rats to trigger the enhancement of theta waves. A high frequency electrical stimulus (100 Hz) of MRN, or an administration of bicuculline into the MRN, was applied to desynchronize theta waves, and then the responses of the elevated plus maze (EPM) were employed to measure the footshock-induced anxiety levels.

2. Material and methods

Stock solution of bicuculline (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in pyrogen-free saline (PFS). The stock solutions were stored at −20 °C until used. The dose of bicuculline employed in these experiments was 0.025 μg based upon our previous results [13]. The total volume for each injection was 1 μl and the duration of injection was 3–5 min. Our previous study has indicated that microinjection of 1 μl solution into the MRN did not cause the MRN lesion [13].

Male Wistar rats (250–300 g; National Laboratory Animal Breeding and Research Center, Taiwan) were used in the present study. Animals were anesthetized by 50 mg/kg Zoletil® (Tiletamine:Zolazepam = 1:1; Virbac, Carros, France). Three screw EEG electrodes were surgically implanted on the right frontal and parietal lobes and the left occipital lobe as previously described [6]. The occipital electrode was used as a reference. Rats that received electrical stimulation to desynchronize theta oscillations were implanted with a concentric bipolar electrode (model: CBBPE10, 33 gauge, pencil point tip, I.D. 50 μm, FHC, Bowdoinham, ME, USA) into the midline area of both sides of the MRNs (AP, −7.9 mm; ML, 0 mm; DV 7.5 mm relative to bregma). This electrode could stimulate both right and left sides of the MRNs. A microinjection guide cannula (26 gauge, O.D. 0.46 mm, I.D. 0.24 mm, Plastics One, Roanoke, VA, USA) was slowly implanted into the MRN (AP, −7.9 mm; ML, 0 mm; DV 7.5 mm relative to bregma) in rats which received bicuculline administration. Coordinates were adopted from the Paxino and Waton rat atlas [23]. The insulated leads from EEG screw electrodes were routed to a Teflon pedestal (Plastic One). The Teflon pedestal was then cemented to the skull with dental acrylic (Tempron, GC Co., Tokyo, Japan). The incision was treated topically with polysporin (polymixin B sulfate–bacitracin zinc) and the animals were allowed to recover for seven days prior to the initiation of experiments. These rats were housed separately in individual recording cages (home cages) in an isolated room, in which the temperature was maintained at 23 ± 1 °C and the light:dark (L:D) rhythm was controlled in a 12:12 h L:D cycle (40 W × 4 tubes illumination). Food and water were available ad libitum. Ibuprofen was dissolved in drinking water and applied for three days after surgery. Experiments were performed one week after recovery from surgery. All procedures performed in this study were approved by the National Taiwan University Animal Care and Use Committee.

On the second postsurgical day, rats were connected to the recording apparatus via a flexible tether. Experimental protocols were executed after one week of recovery. The location of the microinjection cannula was confirmed by injecting 0.5% trypan blue dye at the end of the experiment. The recording data were included for subsequent analyses only when the injection target had been confirmed inside the rats' MRN. Animals were habituated by daily handling and injections of PFS timed to coincide with scheduled experimental administrations.

Signals from the EEG electrodes were fed into an amplifier (model V75-01, Coulbourn Instruments, Lehigh Valley, PA, USA). The EEG was amplified (factor of 10,000) and analog bandpass was filtered between 0.1 and 40 Hz (frequency response: ±3 dB; filter frequency roll off: 12 dB/octave). The amplified EEG signals were then input to the iWorx system, an analog-digital converter (model: IX-214, iWorx Systems, Inc., Dover. NH, USA). The EEG signals were recorded by LabScribe 2.0 Software (iWorx Systems, Inc.) with a sampling rate of 100 Hz. EEGs were analyzed with LabScribe 2.0 and the open-source Chronux algorithms (http://chronux.org/) run by the Matlab Signal Processing Toolkit for the fast Fourier transform (FFT) and multi-taper time-frequency spectrum. The concentric bipolar electrode in the MRN was connected to a stimulus isolator (model: A360, World Precision Instruments, Sarasota,