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Electrical microstimulation of the nucleus incertus induces forward locomotion and rotation in rats

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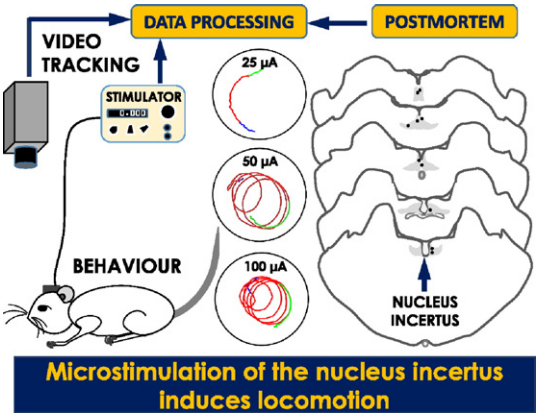
HIGHLIGHTS

- Nucleus incertus modulates hippocampal theta which is associated with locomotion.
- Microstimulation of nucleus incertus in rats was sufficient to induce locomotion.
- Nucleus incertus plays a role in behavioral activation and locomotion.

GRAPHICAL ABSTRACT



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ABSTRACT

Locomotion is essential for goal-oriented behavior. Theta frequency oscillations in the hippocampus have been associated with behavioral activation and initiation of movement. Recently, the nucleus incertus, a brainstem nucleus with widespread cortical and subcortical projections, has been reported to modulate the septo-hippocampal axis triggering theta activity in the hippocampus. This suggests that activation of the nucleus incertus would induce movement. In this study, we investigated the effects of electrical microstimulation of the nucleus incertus on locomotion in conscious rats. Rats chronically implanted with microelectrodes targeting the nucleus incertus were electrically stimulated while their behavior was tracked. High frequency electrical microstimulation of the nucleus incertus was sufficient to induce forward locomotion and rotation. The latencies of evoked locomotion were consistent with a role of the nucleus incertus in modulating premotor areas, possibly the septo-hippocampal axis. Electrical microstimulation of the nucleus incertus increased velocity, mobility and rotations during stimulation and post-stimulation. These results suggest that the nucleus incertus plays a role in behavioral activation and locomotion.

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1. Introduction

The nucleus incertus (NI) has long been hypothesized to be involved in behavioral activation [1]. Locomotion to execute behaviors is an inevitable consequence of behavioral activation. Locomotion and hippocampal theta oscillations are very tightly coupled in an awake animal [2–5]. Although the role of the NI in generation of hippocampal theta oscillations is well-established [6,7], evidence for a causal role for the NI in locomotion is lacking.

While the motor cortex and associated areas are directly involved in the initiation and maintenance of locomotion, many other regions such as the septo-hippocampal circuitry have been implicated in the premotor control of locomotion and the transition of the brain into a state appropriate for processing inputs during locomotion. For instance, theta oscillations are observed in the hippocampus of rodents hundreds of milliseconds before the onset of locomotion and throughout the movement period representing a brain state specialized for encoding spatially-related sensory input [1–4]. Optogenetic stimulation (at theta frequencies) of the glumatergic neurons in the medial septum and diagonal band of Broca (MSDB), the theta pacemaker, has been shown to evoke locomotion [5–6]. Similarly, high frequency (50–100 Hz) stimulation of certain hypothalamic nuclei, supramammillary nucleus, rostral pontine oralis nucleus (RPO) and raphe nuclei have been shown to evoke or inhibit hippocampal theta oscillations and, respectively, initiate or reduce locomotion [7–12].

The NI has dense projections to and from the MSDB. In addition, its projections span many of the other regions involved in modulation of hippocampal theta oscillations including the midbrain raphe nuclei, lateral habenula, RPO, certain hypothalamic nuclei such as the supramamillary nucleus, and the hippocampus itself. It also receives inputs from some of these regions [1,8,9]. These initial anatomical findings suggest that the NI is in a position to modulate hippocampal theta oscillations and pre-motor control of locomotion. Subsequently, a study demonstrated that high-frequency stimulation of the NI, evokes theta oscillations in the hippocampus [10]. Similar to the RPO and DRN, the NI also generates theta which is coupled with hippocampal theta activity [7]. In addition, the NI acts as a mediator between the RPO and medial septum-hippocampus system. Acute inactivation of the NI prevented RPO stimulation-induced hippocampal theta in the anesthetized rat [10]. The NI is a chief source of the highly conserved neuropeptide, relaxin-3, in the brain, which has been shown to be involved in modulating theta via the medial septum [11]. Relaxin-3 expressing neurons of the NI spontaneously fire in a phase-locked manner to the initial ascending phase of hippocampal theta [6]. Relaxin-3 signaling in the medial septum and hippocampus modulates theta activity and promotes spatial memory measured by the spontaneous alternation task [11]. Supporting the functional relevance of the NI in spatial memory, the reversible inactivation of the NI with lidocaine delayed the acquisition and retrieval phase in the Morris water maze [12]. Additionally, a host of studies has shown that neurons in the NI exhibit hippocampal theta-locked firing and theta-rhythmicity [6,10,13]. Therefore, as hippocampal theta is associated with locomotion, and the role of NI in modulating hippocampal theta is well-established, in the present study we hypothesized that activation of the NI would trigger locomotion and tested this hypothesis by direct microstimulation of the NI in freely-moving rats.

2. Materials and methods

Adult male Sprague–Dawley rats (300–350 g), obtained from InVivos Pte Ltd., Singapore, were utilized in this investigation. The procedures were conducted under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore and in compliance with the guidelines of the National Institutes of Health Guide for Care and Use of Animals. Rats were housed in pairs in individually ventilated cages that were maintained in a temperature controlled room (22 °C–24 °C) with a 12 h light–dark cycle. The animals were given ad libitum access to food and water and were acclimatized to the housing conditions for at least 5 days. All behavioral assessments were conducted during the light phase of the 12 h light-dark cycle.

2.1. Surgery

Each rat was anaesthetized with an intra-peritoneal injection of a ketamine (75 mg/kg body weight) and xylazine (10 mg/kg body weight) cocktail. Subsequently, it was mounted on a stereotaxic frame and body temperature was maintained at 37 ± 0.5 °C with a homoeothermic heating blanket. A trephine hole was drilled above the NI (AP: −9.7 mm; ML = 0.1 mm from the Bregma) [14]. A twisted bipolar nickel chromium stimulation electrode (custom-made in the laboratory from 125 μm diameter 80% nickel, 20% chromium wire) was tested for connectivity before implantation into the NI. The electrode was held in place with dental cement and anchoring screws fitted to the skull. The rats were then allowed a rehabilitation period of 1 week, with analgesic (carprofen) and antibiotic (enrofloxacin) treatments injected subcutaneously for the first 5 days.

2.2. Drugs

Ketamine (Parnell Manufacturing Pty Ltd.; Alexandria, NSW, Australia), xylazine (Ilium Xylazil, Troy Laboratories Pty Ltd.; Glendenning, NSW, Australia), enrofloxacin (Baytril 5%, Bayer Health Care; Seoul, Korea) and carprofen (Carprieve, Norbrook Laboratories (GB), Ltd.; Carlisle, UK) were freshly prepared in sterile isotonic saline (B. Braun, Germany) before use. Pentobarbital (Valabarb) was purchased from Jurox Pty Ltd., Australia.

2.3. Behavior

On complete recovery of the rat, it was exposed to a circular open arena (diameter: 120 cm), and connected to a tethered stimulation head mount. A dual output square pulse stimulator (S88X, GRASS Technologies, U.S.A.) and a photoelectric stimulus isolation unit (PSIU6X, GRASS Technologies, U.S.A.) were used for electrical stimulation. The stimulation protocols employed included a (1) 100 Hz stimulation train (with pulse width: 0.25 or 0.5 ms) lasting for 4 or 10 s with an inter-train interval of 10 s or a (2) 1 pulse stimulation given every 10 s in a subset of animals. The current intensity was progressively increased from 25 μA in 25–50 μA steps. No further increases in current intensity were used when a maximal response (locomotion) indicated that a saturation current was reached, as judged by 3 observers, or 700 μA was reached. The stimulation at each current intensity was repeated a minimum of 6 times (and not > 10 times). A minimum of 3 progressively increasing current intensity values were used for each animal. These stimulation protocols have been previously successfully employed in our laboratory and have been found to produce maximal NI-induced effects in target regions [15].

The behavior of the rat was recorded with Ethovision XT10 (Noldus, Netherlands). Raw position information of the animal (center point) was acquired using a 25 Hz sampling rate. Using this information, linear velocity of the animal, number of rotations and mobility were calculated. Linear velocity of the animal was defined as the composite differences between x and y coordinates of the center point of the animal between samples. The number of rotations were counted by measuring the direction of motion of an animal about external points. The animal had to rotate 180° to complete a rotation. A threshold of 50° was set to count rotations. That is, if the animal initially while rotating one way, rotated for 50° in the opposite way, resulted in the initial rotation reading being discarded and a rotation in the opposite direction being initiated. Once the cumulative number of rotations were counted, using the aforementioned methodology, for various epochs, the results