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Nucleus incertus inactivation impairs spatial learning and memory in rats

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HIGHLIGHTS

- Nucleus incertus inactivation impairs retrieval of working memory.
- Nucleus incertus inactivation reduces hippocampal c-fos and pCREB levels.
- Nucleus incertus is involved in acquisition and retrieval of spatial reference memory.

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ABSTRACT

Nucleus incertus (NI) is a pontine nucleus which releases mainly GABA and relaxin-3 in rats. Its suggested functions include response to stress, arousal, and modulation of hippocampal theta rhythm. Since the role of NI in learning and memory has not been well characterized, therefore the involvement of this nucleus in spatial learning and memory and the aftermath hippocampal levels of c-fos and pCREB were evaluated. NI was targeted by implanting cannula in male rats. For reference memory, NI was inactivated by lidocaine (0.4 μl, 4%) at three stages of acquisition, consolidation and retrieval in Morris water maze paradigm. For working memory, NI was inactivated in acquisition and retrieval phases. Injection of lidocaine prior to the first training session of reference memory significantly increased the distance moved, suggesting that inactivation of NI delays acquisition in this spatial task. Inactivation also interfered with the retrieval phase of spatial reference memory, as the time in target quadrant for lidocaine group was less, and the escape latency was higher compared to the control group. However, no difference was observed in the consolidation phase. In the working memory task, with inter-trial intervals of 75 min, the escape latency was higher when NI was inactivated in the retrieval phase. In addition, c-fos and pCREB/CREB levels decreased in NI-inhibited rats. This study suggests that nucleus incertus might participate in acquisition of spatial reference, and retrieval of both spatial reference and working memory. Further studies should investigate possible roles of NI in the hippocampal plasticity.

1. Introduction

The hippocampal formation as a major structure in the processes of learning and memory is connected to a variety of higher and lower brain areas and nuclei. Brainstem inputs to the hippocampus have modulatory roles in memory processes, and some are thought to exert their effects by generating or affecting hippocampal theta rhythm which is suggested to be involved in learning and memory [1]. A pontine structure which is recently identified and implicated in modulation of theta rhythm is nucleus incertus (NI) [2–4].

NI which is located ventral and medial to the posterodorsal tegmental nucleus constitutes a medial part, pars compacta (NIc) and the lateral pars dissipata (NId) [5]. NI neurons are heterogeneous and secrete GABA and some neuropeptides as co-transmitter, the most prominent of which is relaxin-3 and is highly abundant in NI in the mammalian brain [6]. On the other hand, glutamatergic projections from NI to septohippocampal system have been recently identified [7]. Many of NI neurons are rich in corticotropin releasing hormone receptor type 1 (CRF1), suggestive of its role in response to stress [8,9]. Indeed studies have reported that behavioral stress [10–12] or direct administration of CRF [13] activates NI neurons. Some other receptors are expressed in NI neurons including RXFP3 itself which is the cognate receptor of the relaxin-3 [6,12,14,15].

NI projects to the hippocampal formation, the medial septal nucleus, nucleus of the diagonal band, the amygdala and many other areas. And its main inputs are from the medial septum and nucleus of the diagonal band, the medial part of the lateral habenula, the median raphé nucleus, and the contralateral nucleus incertus [5,16].

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Diverse projections along with neurochemical, electrophysiological and behavioral investigations have urged investigators to propose different functions for NI such as response to stress [8,10,12,17], feeding behavior [18], arousal [5,16,19–22], and effects on hippocampal theta rhythm [3,4,23,24]. Brain theta rhythm which can be recorded from many areas including hippocampus is involved in functions like active waking behavior and REM sleep, encoding and retrieval of memory, spatial navigation/exploration, and also sensorimotor integration [2, 25,26]. Septal complex, hypothalamus and many brainstem nuclei, like reticularis pontis oralis (RPO), are thought to be principal structures engaged in the generation of theta rhythm [4]. RPO-elicited theta rhythm in hippocampus depends on NI, and RXFP3 antagonist in septal area reduces theta rhythm [3,24]. This functional connectivity was later approved by tracing studies [27]. These observations have led to the notion that NI is a relay point between RPO and the medial septum. Moreover, theta-like oscillations have been shown in NI under experimental conditions which is synchronous with hippocampal theta rhythm [23]. Lately, NI was shown to have relaxin-3 positive and negative neurons, with the former shown to have phase-lock activity with hippocampal theta when stimulated by CRF [28]. The contribution of NI to hippocampal theta rhythm raises the possibility that this nucleus in involved in cognitive tasks such as learning and memory. In a spontaneous alternation task, RXFP3 antagonist reduced alternation score, indicative of disrupted spatial working memory [24]. In another study, electrolytic lesion of NI in rats delayed extinction of the conditioned fear [29]. More recently NI ablation with CRF-saporin conjugate increased freezing behavior in cued fear conditioning [30].

Behavioral studies evaluating the role of NI in learning and memory are limited and long term hippocampus-dependent memory has not been investigated. The present study aimed to identify the role of NI in different phases of spatial reference and working memory formation in rats. This was achieved by using the Morris water maze (MWM) paradigm and injection of lidocaine into the NI. Reversible inhibition by lidocaine has been extensively used in our laboratory and elsewhere to inactivate neurons so that their functions can be eliminated for a limited time period [31–34]. And in the case of NI, it inhibits all types of neurons, leaving no NI-driven response. Since the MWM is a hippocampal dependent task, NI effects on learning and memory were assessed by measuring hippocampal c-fos and pCREB levels.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (230–280 g) were obtained from our own breeding colony. They were housed four per cage in a temperature and humidity controlled animal house facility where a 12:12 h light/dark cycle, with lights on at 7:00 am, was applied. Rats had free access to food and water during the study. They were handled by the experimenter from two weeks before the surgery. The behavioral testing was done during the light phase. Experiments were carried out in accordance with recommendations from the declaration of Helsinki and the internationally accepted principles for the experimental use of rats.

2.2. Surgery

Rats were anesthetized by ketamine and xylazine (100 and 2.5 mg/kg respectively, i.p). Under aseptic conditions, scalp and fascia were removed and the skull was exposed. After drilling the trephine hole, a 15 mm cannula was inserted into the nucleus incertus (AP: −9.8, ML: 0, DV: 7–7.5). To avoid excessive hemorrhage, the cannula was inserted with a 16° angle, almost 2 mm caudal to the vertical insertion point. Two jewelers' screws were inserted into the skull, and dental cement was applied to fix the cannula. A stylet was inserted into the cannula to prevent its blockade. Rats were returned to their cages and after one week behavioral tests were started.

2.3. Microinjection procedure

Before injection, rats were gently restrained by hand and the stylet of cannula was removed. The injection needle (30-gauge) was connected to a Hamilton syringe through a 20-cm piece of polyethylene tubing. The needle was inserted 0.5 mm beyond the tip of the cannula to reach the NI; so the cannula itself was in the vicinity of NI and did not cause damage to it. Then 0.4 μl of sterile saline or 4% lidocaine hydrochloride (Sigma, USA) in saline was injected slowly over a 2-min period. The injection needle was left in the guide cannula for an additional 60 s to facilitate diffusion of the drug, before it was slowly withdrawn.

2.4. Behavioral testing

2.4.1. Morris water maze apparatus

The water maze used in our study is a dark circular pool (150 cm in diameter and 60 cm high) filled with water to a depth of 45 cm. A Plexiglas platform (11 cm diameter) covered with black rubber was located 2 cm below the water surface in the center of one of the arbitrarily designed north-east (NE), south-east (SE), south-west (SW) or north-west (NW) orthogonal quadrants. The platform provided the only escape from the water. Extra-maze cues included racks, curtains, a door, and pictures on the walls around the room where the water maze was housed. The cues were kept fixed in positions during the study so that all the rats could use the same visual cues. Swimming was recorded by a CCD camera (Panasonic Inc., Japan) hanging from the ceiling above the MWM apparatus and locomotion tracking was measured by a video tracking system for automated analyzing of animal's behavior using the Ethovision software (version XT7, Netherlands).

2.4.2. Habituation

To promote animal adaptation with the test environment and reduce the level of stress, one day before starting the hidden-platform training sessions, rats were given a 60 s swim in the tank where there was no platform.

2.4.3. Reference memory

The training sessions consisted of two blocks of 4 trials per day (block interval: 10 min, trial interval: 1 min), for three consecutive days as reported previously in our lab [35]. The platform was located in the center of the third quadrant near the southern labeled direction. Other seven directions, from southwest to southeast, were engaged to release the rats in a quasi-random manner, always facing the wall of the tank. Rats had 60 s to swim and find the platform, where after 2 s of being stationary on it, recording was stopped by the software. If the animal failed to reach the platform within 60 s, it was directed by the experimenter to the platform and left there for 10 s. Twenty-four hours after the third session the probe test was given, during which the platform was removed from the tank and rats swam for the whole 60 s. For the probe test, rats were released opposite to where the platform was placed. For the recorded tracks, escape latency, distance moved, velocity and time spent in target quadrant were calculated for subsequent analyses.

2.4.4. Working memory

The protocol for assessment of working memory started like the reference memory with two blocks of four trials each day, for three days while the platform was located in a same position [31,36]. Then with one day break, rats were given two trials per day (trial interval: 75 min) for four consecutive days with the platform placed in a different position each day. On the fifth day, rats did one training trial, and 75 min later the probe trial was performed in which the platform was removed.