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Research paper

A neural connection between the central part of the medial preoptic nucleus and the bed nucleus of the stria terminalis to regulate sexual behavior in male rats

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HIGHLIGHTS

- c-Fos expression in the MPNc was increased following sexual behavior in male rats.
- Some c-Fos-expressing neurons of the MPNc project axons to the BNST.
- MPNc projections to the BNST may control male rat sexual behavior.

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ABSTRACT

The medial preoptic nucleus (MPN) is a regulatory center for male sexual behavior. It consists of sexually dimorphic structures that are male biased, and these structures are found in the central part of the MPN (MPNc). The bed nucleus of the stria terminalis (BNST) also participates in male sexual behavior, and receives efferent neural projections from the MPNc. In this study, we examined if MPNc neurons projecting to the BNST are activated in male rats displaying sexual behavior. Fluoro-Gold (FG; a retrograde neural tracer) was injected into the BNST of male rats, which were separated into two groups: (1) those in contact with estrous female rats and displayed sexual behavior followed by ejaculation and (2) those without contact with estrous female rats. In both groups, protein expression of c-Fos (a neuronal activity marker) and calbindin (a location marker of the MPNc) were detected by fluorescent immunohistochemistry. The number of c-Fos-immunoreactive cells with or without FG labeling in the MPNc was also measured. The number of c-Fos-immunoreactive cells significantly increased following ejaculation. Approximately 10% of FG-labeled cells in ejaculation male rats were immunoreactive for c-Fos, and this percentage value was significantly higher in this group compared with control male rats. Overall, these results suggest that efferent projections from the MPNc to the BNST function to control sexual behavior in male rats.

1. Introduction

Sex differences in the brain underlie the control of sex-biased or sex-specific behaviors through a neural circuitry in which its structures and functions may themselves exhibit sex differences. The medial preoptic nucleus (MPN) is a regulatory center of sexual behavior. In many male species of vertebrates (e.g., killifish, newt, snakes, lizards, quails, cats, dogs, rats, mice, and monkeys), MPN lesions have shown to disrupt male sexual behavior [1]. Therefore, the MPN of male individuals plays a facilitating role in the regulation of sexual behavior. Electrical or pharmacological stimulation of the MPN facilitates sexual behavior in male rats, resulting in a short latency of ejaculation [2–4]. The MPN of rats is composed of the medial, lateral, and central parts. The central part of the MPN (MPNc) contains a cluster of neurons with a higher cell density compared with the other parts. The MPNc is referred to as the sexually dimorphic nucleus of the preoptic area (SDN-POA), which is five- to seven-fold larger and contains more neurons in male than female rats [5,6]. Moreover, homologs of the SDN-POA have been identified in other species, including humans [7–14]. Neurochemical properties of the MPNc are not completely understood. However, many neurons expressing calbindin—a calcium-binding protein—are included in the MPNc. The volume of calbindin-expressing region in the MPNc is two- to four-fold larger in male

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than female rats, and the number of calbindin neurons of male rats is greater than that of female rats [7,15,16]. Previous studies have suggested that the MPNc is involved in sexual orientation and sexual behavior in male rats [17,18]. However, the neural circuitry for exhibiting the physiological function of the MPNc remains undetermined.

A comprehensive neuroanatomical study using a neuronal anterograde tracer has demonstrated unique efferent projections in each part of the MPN in rats [19]. Specifically, the MPNc contains many neurons projecting to the bed nucleus of the stria terminalis (BNST), whereas such projection neurons are relatively small in the lateral and medial parts of the MPN [19]. The BNST as well as the MPN is known as a region relating to male sexual behavior. BNST lesions have been shown to impair sexual behavior in male rats [20]. In the MPN and the BNST, the expression of c-Fos—a marker of neuronal activity—is increased in male rats that copulate with estrous female rats [21,22]. These findings indicate that neurons of both the MPN and BNST in male rats are activated by social interaction with estrous female rats. Neural projections from the MPNc to the BNST may function as a part of neural circuit to regulate male sexual behavior. However, there has been no evidence to show a neural functional role for the regulation of male sexual behavior between the MPNc and other regions. In the current study, we aimed to determine if MPNc neurons projecting to the BNST are activated by sexual behavior in male rats.

2. Materials and methods

2.1. Animals

Male Wistar rats 8–13 weeks old and weighing 300–500 g were purchased from Japan Laboratory Animals, Inc. (Tokyo, Japan). They were housed under a 12-h light-dark cycle at 23 °C, with free access to water and food. Experiments were conducted according to the Guidelines for the Care and Use of Experimental Animals of Saitama University (Saitama, Japan).

2.2. Experimental design

A screening test was given to male rats to select vigorous male rats. The screening test showed intromission at five times ($n = 15$). The neuronal retrograde tracer Fluoro-Gold (FG) was injected into the BNST on the right side, and sexual behavioral tests were carried out in a dark room under a dimmed red light a week later to obtain male rats that ejaculated at one time ($n = 8$). These rats were designated as the ejaculation group. Control male rats ($n = 7$) were placed in a dark room without female rats. Sixty minutes after ejaculation or after they were placed in the dark room, animals were sacrificed for histological analysis on coronal brain sections.

2.3. Injection of FG

Animals were anesthetized with an intraperitoneal injection of medetomidine hydrochloride (0.3 mg/kg of body weight), midazolam (4 mg/kg of body weight), and butorphanol tartrate (5 mg/kg of body weight), and then placed in a stereotactic device for brain surgery (Summit Medical, Tokyo, Japan). A glass capillary (tip outer diameter: 50 μm) filled with 4% FG solution (Fluoro-Chrome, Englewood, CO, USA) was inserted into the BNST on the right side. The tip of the glass capillary was lowered 1.0 mm caudal to the bregma, 1.4 mm right to the midline, and 6.4 mm below the dura with reference to the rat brain atlas [23,24]. FG was then iontophoretically injected under a positive current (5.0 μA, 7-s pulse) for 3 min using a current source (Digital Midgard Precision Current Source, Stoelting Co, IL, USA). After the injection, the glass capillary was gradually pulled out under a negative current of 0.5 μA to prevent leakage of FG.

2.4. Behavioral test

Sexual behavioral tests were performed in a dark room under a dimmed red light. Sexual behavior of each male rat was observed in a breeding cage (440 × 280 × 205 mm) after one female rat was placed in the cage. Female rats used for the behavioral test were ovariectomized and subcutaneously injected with estradiol benzoate (20 μg) and progesterone (500 μg) 48 h and 4 h before use, respectively.

For the selection of vigorous male rats, male sexual behavior was observed for 30 min until male rats showed an intromission at five times. This screening test was carried out in 3- to 4-day intervals. Male rats exhibiting five times intromission in one test session were used for further tests in this study.

Brain surgery was performed on the selected vigorous male rats to inject FG, and sexual behavioral tests were then performed. Sexual behavior of FG-injected vigorous male rats was observed for 60 min in each test session. This test was performed three times at 2- to 3-day intervals, until the first ejaculation was observed. Immediately after ejaculation, male rats were separated from female rats and isolated in a breeding cage for 60 min before they were euthanized and brain samples taken. Control male rats, which were also selected by aforementioned screening test, were placed in the dark room that was used for behavioral tests for 60 min without female contact.

2.5. Tissue preparation and immunohistochemistry

Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (64.8 mg/kg body weight). Heparin (1000 U/kg body weight) was used to avoid blood clotting. Animals were then transcardially perfused with 0.05 M ice-cold phosphate-buffered saline (PBS; pH 7.4), followed by perfusion fixation with ice-cold 4% paraformaldehyde in 0.05 M phosphate buffer (PB; pH 7.4). Brains were postfixed with the same fixative at 4 °C overnight and then immersed in 30% sucrose and 0.05 M PB at 4 °C for 3–4 days. Coronal sections of frozen brains were cut at a thickness of 30 μm using a cryostat (CM1900 UVA; Leica Microsystems, Wetzler, Germany).

Coronal brain sections were rinsed in 0.05 M PBS and treated with 5% normal goat serum (NGS) in 0.05 M PBS containing 1% Triton X-100 (PBST; pH 7.4) for 1 h at room temperature. The sections were incubated with a rabbit anti-c-Fos antibody (1:20,000; Calbiochem, La Jolla, CA, USA) and a mouse anti-calbindin antibody (1:5,000; Sigma–Aldrich, St. Louis, MO, USA) in 5% NGS-PBST at 4 °C overnight. The sections were then incubated with goat anti-rabbit IgG conjugate with Alexa Fluor 488 (1:400; Invitrogen, Carlsbad, CA, USA) and goat anti-mouse IgG conjugate with Alexa Fluor 647 (1:400, Invitrogen) for 2 h at room temperature. Sections were mounted on Matsunami amino silane-coated glass slides and coverslips placed on top with mounting medium (Fluoromount; Diagnostic BioSystems, Pleasanton, CA, USA).

2.6. Image analysis

Brain sections were observed under a fluorescent microscope (BZ-9000; Keyence, Osaka, Japan). To determine the location of the FG injection site, we compared this site with the distribution of calbindin-immunoreactive (ir) cells, as the principal nucleus of the BNST (BNSTp) can be detected by calbindin (Fig. 1). For cell counting in the MPNc, the distribution of calbindin-ir cells was used as a reference. Calbindin-ir cells are localized in the center of the MPNc, and the volume of calbindin-ir region in the male MPNc is larger