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Research report

Different subregions of the medial preoptic area are separately involved in the regulation of copulation and sexual incentive motivation in male rats: A behavioral and morphological study

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ABSTRACT

The purpose of this study was to investigate whether sexual incentive motivation and copulatory performance are regulated by different subregions of the medial preoptic area (MPOA). Sexual incentive motivation was measured by means of a partner preference test. Both copulatory behavior and sexual incentive motivation were tested in male rats treated with 50 mg/kg of either EGb 761 or a vehicle (distilled water) by gavage for 14 days. Administration of EGb 761 increased the number of intromissions, but had no effect on the number of mounts, mount latency, intromission latency, ejaculation latency, or post-ejaculatory interval. In the partner preference test, the total duration of visits to estrous female rats in both of the groups was significantly different from the total duration of visits to sexually active males. EGb 761 treatment increased the number of ejaculations compared both to vehicle-treated controls on day 14 and the same group on day 0. In comparison with the controls, the EGb 761-treated group showed a significant increase in the number of tyrosine hydroxylase-expressing cells in the dorsal, but not the ventral, subregion of the MPOA, and significantly high dopamine levels in the MPOA. These results indicate that EGb 761 does not affect sexual incentive motivation, but facilitates copulatory performance in male rats, suggesting that the mechanisms responsible for sexual incentive motivation and copulatory performance may be associated with differential functions of MPOA subregions.

1. Introduction

Sexual motivation and copulatory performance are the two main components of male sexual behavior. Sexual motivation is comprised of all the anticipatory actions leading up to sexual activity, such as search for and approach to potential mates. Copulatory performance in animals is assessed by mount frequency, intromission frequency, and ejaculation frequency, while measures of sexual motivation can rest on the behavioral parameters of mount latency (ML), intromission latency (IL), and post-ejaculatory interval (PEI) [9]. In addition, several methods have been used to assess a male's sexual motivation, e.g., pressing a level to gain access to a female, the time spent with a stimulus animal, or pursuit of a receptive female [17]. However, many of these procedures are based on measures of response speed or rate, making them very susceptible to manipulations affecting motor functions, or use different kinds of learned responses complicating further interpretation. For the partner preference test, experimental subjects are placed in a three-chambered apparatus with a sexual incentive female tethered to one outer chamber and a sexual incentive male to the other. The subjects can see, hear, and smell the incentive animals, but physical contact is prevented. Thus, this test is a more ideal method to evaluate sexual incentive motivation in rodents [2,32].

The medial preoptic area (MPOA) plays an important role in the regulation of both sexual motivation and copulatory performance in males. Lesions of the MPOA severely impair copulation in all male vertebrate species [13] and decrease the partner preference for an estrous female in male rats [8,19,28]. Some MPOA neurons in male rats increase firing only during precopulatory female-approaching behavior, while others increase firing only during copulation [36]. Lesions of the dorsal MPOA in rats result in a reduction in copulatory performance, such as a decrease in the frequency of, intromission, and ejaculation, while lesions of the ventral or anterio-dorsal MPOA have no effect on the above-mentioned behavioral parameters [3]. These results, therefore, suggest a functional difference in the mediation of sexual behavior within the MPOA.

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Androgens, particularly testosterone (T), play an important role in the neuromodulation of male sexual motivation and copulatory performance [23]. Lack of T after castration eliminates penile erection, abolishes sexual behavior, and reduces partner preference for an estrous female, and these effects are reversed by T replacement [17]. Research has demonstrated that copulatory effects of androgens are mediated via androgen receptors (ARs) in the MPOA [12,21,22]. Microinjection of hydroxyflutamide, an anti-androgen drug, into the antero-ventral MPOA decreases copulatory behavior in castrated rats receiving T replacement, but has no effect on sexual motivation. In contrast, microinjection of the same drug into the postero-dorsal MPOA does not influence copulatory performance, but decreases sexual motivation [21]. In addition, a decrease in AR density in the medial part of the MPOA, but not in the whole MPOA, has been reported in rats 48 h after copulation to satiety [34]. These data suggest that activation of ARs in different regions of the MPOA may be specifically responsible for sexual motivation or copulatory performance.

Dopamine (DA) has long been known to facilitate sexual function [17]. Microinjection of a DA agonist into the MPOA increases sexual behavior in male rats [14,15], while DA antagonists decrease copulatory behavior, genital reflexes, and the choice of the female's chamber in an X-maze [25,41]. Thus, DA in the MPOA is positively implicated in both copulatory performance and sexual motivation. However, it has recently been shown that dopaminergic neurotransmission is not important for sexual motivation [27]. Agmo has demonstrated that DA has no effect on sexual incentive motivation in male rats [1].

From what has been mentioned above [3,21,34,36], it is reasonable to postulate that sexual performance and sexual motivation are regulated in different subregions of the MPOA. However, there is still no research on whether sexual incentive motivation and copulatory performance are associated with dopaminergic activity in different subregions of the MPOA. Since the treatment of Ginkgo biloba extract (EGb 761) enhances copulation without affecting sexual motivation in male rats [42], we measured sexual incentive motivation in terms of sexual partner preference, copulatory behavior, tissue levels of DA in the MPOA, and stained DA neurons in the MPOA for tyrosine hydroxylase (TH) in EGb 761-treated male rats to reveal the relationship between sexual incentive motivation/copulatory performance and the DA activity in different MPOA subregions.

2. Methods

2.1. Subjects

Long-Evans rats (8 weeks old) were purchased from the Animal Center of the National Science Council, Taipei, Taiwan. The animals were kept in groups of four in a cage (30 cm × 30 cm × 20 cm) in a temperature (22 ± 1 °C)- and humidity-controlled (55 ± 10%) room on a 12-h light–dark cycle (lights off at 17:00 h) with food and water available ad libitum. The experimental protocols were approved by the Animal Care and Use Committee, College of Medicine, National Taiwan University, and all experimental procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Stimulus females

Stimulus females of the same strain (8 weeks old) were ovariectomized under sodium pentobarbital (40 mg/kg, i.p.) anesthesia and implanted subcutaneously with a 5-mm Silastic capsule (1.98 mm ID and 3.18 mm OD) filled with 17 β-estradiol (Sigma) to bring into estrus [39,40,42]. Approximately 1 week after surgery, the females were used as sexual partners for behavioral tests.

2.3. Copulation screening of males

The copulation screening test was performed during the dark phase of the cycle when the rats were 10 weeks old. Each male rat was placed in a circular Plexiglas chamber (45 cm diameter), and a stimulus female was introduced 3 min later; then the number and latency of mounts, intromissions, and ejaculations were recorded over a period of 15 min. The male rats were tested three times at intervals of 5–6 days. Animals that had not ejaculated twice after three testing sessions were not used in the sexual behavior study.

2.4. Treatment

EGb 761 was purchased from Dr. Willmar Schwabe Pharmaceuticals (Karlsruhe, Germany). Male rats (12 weeks old) were randomly divided into two groups, which were treated with 50 mg/kg (1.25 ml/kg) per day of EGb 761 (the optimal dosage for observing male rat copulatory behavior according to our previous studies) [42] or of a vehicle (distilled water) between 07:00 and 09:00 h by gavage for 14 consecutive days.

2.5. Behavioral studies

2.5.1. Sexual incentive motivation

Twenty male rats were used in the behavioral study. The tests were performed between 19:00 and 21:00 h. The testing apparatus consisted of an open-field arena (Plexiglas, 90 cm × 30 cm × 30 cm) with two goal boxes (12 cm × 30 cm × 30 cm) on opposite sides of, and outside, the arena. The passages between the open-field arena and the goal boxes were separated by two acrylic templates with apertures, allowing the rats to see, hear, and smell each other. An estrous female and a sexually active male rat were randomly placed in the goal boxes. At the beginning of the experiment, the subjects were habituated to the testing environment for 3 min, and were then tested for 10 min. After each test, the arena was cleaned before the next animal was introduced. Placing of the forelimbs of the experimental rat within the area (10 cm × 30 cm) in front of the incentive compartments was counted as one visit and the time between the placing and removal of the forelimbs in this area was taken as the duration of each visit. Both the time spent in the incentive zones and the number of visits to each of the incentive animals were recorded. The preference score was calculated by dividing the amount of time spent with the estrous female by the amount of time spent with the estrous female plus the amount of time spent with the active male. A score of 0.5 indicated no preference, while a score higher than 0.5 showed a preference for the estrous female and one lower than 0.5 a preference for the sexually active male [35].

2.5.2. Copulatory behavior testing

After the sexual incentive motivation testing, each male rat was placed in a circular Plexiglas chamber 3 min before the introduction of a sexually receptive female, and was then allowed to copulate for 30 min. The behavioral parameters recorded during the test period were NM (number of mounts), NI (number of intromissions), NE (number of ejaculations), ML (latency from the introduction of the female to the first mount), IL (latency from the introduction of the female to the first intromission), ejaculation latency (EL, latency from the first intromission to ejaculation), and PEI (latency from the first ejaculation to the first intromission of the second copulatory series).

2.6. Radioimmunoassay for serum testosterone levels

Twenty male rats were used for radioimmunoassay and measurement of T levels. Male rats were sacrificed by decapitation approximately 14 h after the last behavioral test and trunk blood was collected in test tubes, kept at room temperature for 30 min, and then centrifuged at 900 × *g* for 30 min at 2 °C, and the serum was collected and stored at −80 °C until tested by radioimmunoassay for T levels. Briefly, after incubation of the serum sample with anti-T antiserum and ^{3}H-T (Amersham) at 4 °C for 24 h, charcoal (Sigma) and dextran-T70 (Pharmacia) were added and the sample was incubated for 15 min in an ice bath, then centrifuged at 1000 × *g* for 30 min to spin down free ^{3}H-T bound to charcoal. The supernatant was poured into 3 ml of Ecoscint A (National Diagnostics), and the samples were counted in a beta-counter. The assay sensitivity was 2 pg per assay tube, and the intra- and inter-assay coefficients of variation were 4.5% (n = 8) and 5.2% (n = 8), respectively.

2.7. Measurement of DA and noradrenaline (NA)

After the last behavioral test, the brain was rapidly removed and immediately frozen in −20 °C isopentane. Serial 180 μm-thick coronal sections were prepared using a cryostat. The MPOA was microdissected bilaterally, homogenized in 0.1 N perchloric acid, and centrifuged at 4 °C at 7800 × *g* for 10 min; then the supernatant was assayed for DA and NA by high pressure liquid chromatography with electrochemical detection [39]. Briefly, 20 μl of supernatant was applied to a C18 reverse phase analytical column filled with ODS-3 (3 μm) (Bioanalytic System, USA) and eluted with a mobile phase of 8.65 mM heptanesulfonic acid (Sigma), 0.26 mM EDTA (Sigma), 6.25% acetonitrile (Merck), 0.35% triethylamine (Merck), and 0.4% orthophosphoric acid (Merck), pH 2.7–3.1, at a flow rate of 0.5 ml/min. The sensitivity of the LC-4C amperometric detector (Bioanalytic System, USA) was 50 nA full scale, and the potential of the working electrode was 0.75 V with respect to an Ag/AgCl reference electrode. The pellets from the centrifugation were solubilized in 0.5 N NaOH and assayed for protein according to the Lowry method [20].