Behavioural Brain Research 205 (2009) 219–225

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

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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ARTICLE INFO

Article history:
Received 16 April 2009
Received in revised form 6 June 2009
Accepted 15 June 2009
Available online 21 June 2009

Keywords:
Medial preoptic area
Sexual motivation
Copulatory performance
Partner preference
Dopamine
EGb 761

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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doi:10.1016/j.bbr.2009.06.025

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].

2.8. Immunohistochemistry

2.8.1. Perfusion and tissue preparation

Eight male rats were used in the immunohistochemistry. Approximately 14 h after the last behavior, the animals were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused through the aorta with 300 ml of 0.9% saline containing 0.1% sodium citrate, followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brain was removed, immersed in the same fixative for 2 h at 4 °C, and preserved in 30% sucrose in 0.1 M phosphate-buffered saline, pH 7.4, at 4 °C. These tissues were then embedded in OCT compound (Sakura) and stored at −80 °C. Serial 30 μm-thick coronal sections were cut on a cryostat, collected in 0.05 M Tris-buffered saline (TBS), pH 7.4, and stored at 4 °C until processed for immunohistochemistry.

2.8.2. TH immunohistochemistry

This procedure was performed on every third consecutive free-floating section. The sections were washed for 3× 10 min in TBS, and then incubated for 1 h at room temperature in 1% hydrogen peroxide and 10% methanol in TBS to block endogenous peroxidase activity. To prevent nonspecific binding of the primary antibody, the sections were incubated for 1 h in TBS containing 10% normal goat serum (Vector Laboratory) and 0.1% Triton X-100. They were then incubated overnight at room temperature with rabbit anti-TH antibody (1:500; Chemicon) diluted in blocking buffer (2% normal goat serum and 0.02% Triton X-100 in TBS). After washing, the sections were incubated for 1 h at room temperature with biotinylated goat anti-rabbit IgG antibody (1:300; Vector Laboratory) diluted in blocking buffer, then the sections were incubated for 1 h at room temperature with peroxidase-conjugated streptavidin (DAKO) diluted in 1:300 in TBS. After three rinses in TBS, the sections were incubated with 3,3′-diaminobenzidine tetrahydrochloride solution, rinsed in TBS, and wet-mounted on gelatin-coated slides. After drying, the slides were dehydrated and coverslipped with Permount (Fisher Scientific). Labeled neurons with a clearly visible nucleus were counted in each section.

2.8.3. Quantification of TH immunohistochemistry

Cells showing TH immunoreactivity (TH-ir) were counted in bilateral 0.6 mm square areas of the MPOA (0.3 mm posterior from the bregma, 0.6 mm lateral to the border of the third ventricle, 0.6 mm inferior to the median preoptic nucleus) on the basis of the rat brain atlas [29]. To examine the relationship between different MPOA subregions and male copulatory behavior, cells showing TH-ir in this brain area were counted in the dorsal and ventral subregions (Fig. 1). We captured and quantified images of the sample area by using a light microscope (Zeiss Axio Imager. A1, Germany) equipped with an AxioCamICc3 and by using an image analysis system (Image Pro Plus 4.5, Media Cybernetics).

2.9. Statistical analysis

Quantitative data were analyzed for statistical significance by means of Statistic 6.0 (StatSoft). Sexual performance and sexual incentive motivation were analyzed by two-way repeated ANOVA followed by Fisher's post hoc test to establish the significance of differences between mean values. Differences in levels of DA, NA, and serum T and in the number of neurons showing TH-ir were evaluated by Student's *t* test. A paired *t*-test was used for analyzing the number of TH-ir cells in different subregions in the same group. *P* values less than 0.05 were considered statistically significant. All quantitative data are reported as the mean ± SEM.

3. Results

3.1. Effects of EGb 761 on male copulatory behavior

As shown in Table 1, an increase in the NI was seen in male rats treated for 14 days with EGb 761 compared to the same group on day 0. ANOVA revealed a significant effect of day ($F(1,26) = 8.85$, $P < 0.01$) on the NI. Post hoc analysis resting on Fisher's method showed that the EGb 761-treated rats displayed a higher NI than the same group on day 0 ($P < 0.05$). For the NE, ANOVA revealed a main effect of treatment ($F(1,26) = 8.73$, $P < 0.01$) and a significant effect of day ($F(1,26) = 9.75$, $P < 0.01$). Post hoc comparison showed that EGb 761 treatment caused a significant increase in the NE compared to the vehicle-treated group on day 14 ($P < 0.001$) or the EGb 761-treated group on day 0 ($P < 0.001$). For EL, ANOVA detected a significant main effect of treatment ($F(1,26) = 4.74$, $P < 0.05$), and a subsequent Fisher's post hoc analysis showed that a significant decrease in the EL had taken place in EGb 761-treated animals when compared to the vehicle-treated group on day 14 ($P < 0.05$) (Table 1). No significant difference in ML, NM, IL, or PEI was seen between the EGb 761-treated group and controls.

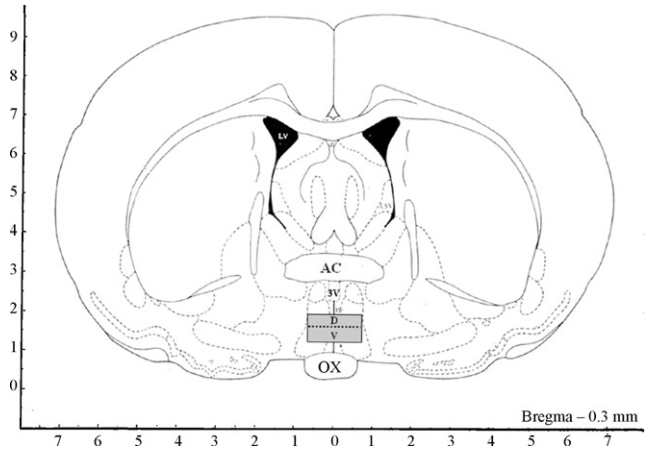

Fig. 1. Schematic drawing illustrating the MPOA and the area where TH-ir neurons were counted. The depicted coronal section is 0.3 mm posterior from bregma according to Paxinos and Watson's atlas [29]. 3 V, third ventricle; AC, anterior commissure; OX, optic chiasm; D, dorsal subregion of MPOA; V, ventral subregion of MPOA.

3.2. Effects of EGb 761 on male sexual incentive motivation

Both control and EGb 761-treated rats showed a preference for the estrous female in the partner preference test, with no significant difference between the two groups (Fig. 2A). Repeated two-way ANOVA showed no treatment effect in the total duration of visits to estrous females ($F(1,18) = 2.11$, $P = 0.16$) or to sexually active males ($F(1,18) = 0.01$, $P = 0.91$) in either of the two groups (Fig. 2B). Similarly, EGb 761 treatment had no effect on the total number of visits to either estrous females ($F(1,18) = 3.27$, $P = 0.09$) or sexually active males ($F(1,18) = 0.27$, $P = 0.61$) compared to the controls (Fig. 2C). In addition, no significant difference in the mean duration of visits to estrous females ($F(1,18) = 0.01$, $P = 0.91$) or sexually active males

Table 1
Effect of EGb 761 on male rat copulatory performance.

Group	Day	*N*	Mount latency (s)	Number of mounts	Intromission latency (s)	Number of intromissions	Ejaculation latency (s)	Number of ejaculations	Post-ejaculatory intervals (s)
Vehicle	0	14	152.8 ± 50.6	5.7 ± 1.4	185.0 ± 49.4	30.1 ± 2.5	677.9 ± 59.1	1.7 ± 0.1	372.9 ± 16.3
EGb 761	0	14	186.0 ± 102.9	6.1 ± 1.7	216.1 ± 100.3	33.4 ± 3.2	574.0 ± 79.6	1.8 ± 0.2	360.1 ± 13.2
Vehicle	14	14	142.9 ± 35.8	6.9 ± 1.9	178.4 ± 42.1	36.2 ± 4.4	620.1 ± 66.5	1.7 ± 0.1	387.7 ± 17.8
EGb 761	14	14	66.4 ± 25.7	5.6 ± 1.2	68.1 ± 26.0	42.7 ± 2.8[a,*]	407.6 ± 49.5[b,*]	2.6 ± 0.2[a,b,***]	336.4 ± 10.5

The data are presented as the mean ± SEM.
[a] Compared to the same group on day 0.
[b] Compared to the vehicle-treated group on the same day.
[*] $P < 0.05$.
[***] $P < 0.001$.

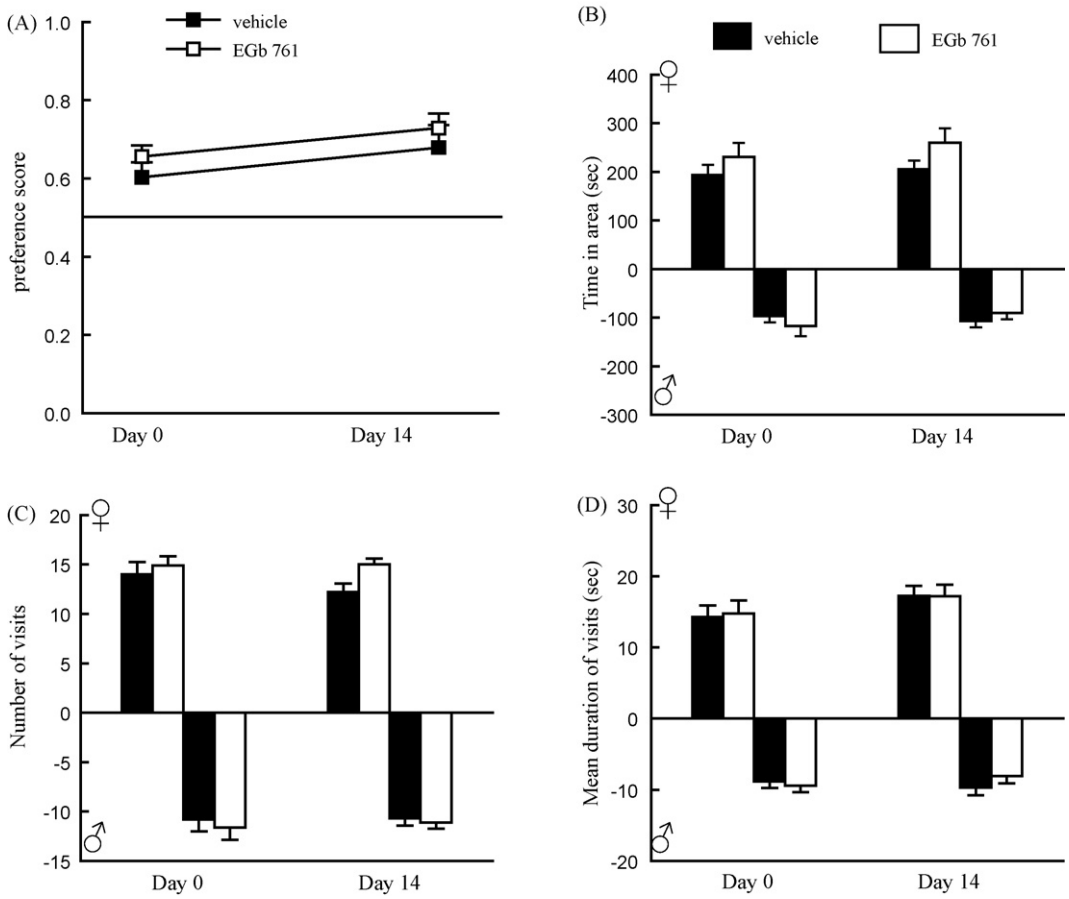

Fig. 2. Effects of EGb 761 treatment on sexual motivation in male rats. (A) Preference score in the partner preference test for male rats treated with vehicle or EGb 761. (B) Time spent by male rats in the vicinity of estrous females or sexually active males. (C) Number of visits paid to the stimulus rats. (D) Mean duration of visits spent in the vicinity of estrous females or sexually active males. The data are the mean ± SEM for days 0 and 14.

($F(1,34) = 0.19$, $P = 0.67$) was seen between the EGb 761-treated and control groups (Fig. 2D).

3.3. Serum T levels

Serum T levels in rats treated for 14 days with EGb 761 were not different from those in controls (df = 16, $t = -3.36$, $P = 0.73$) (Fig. 3).

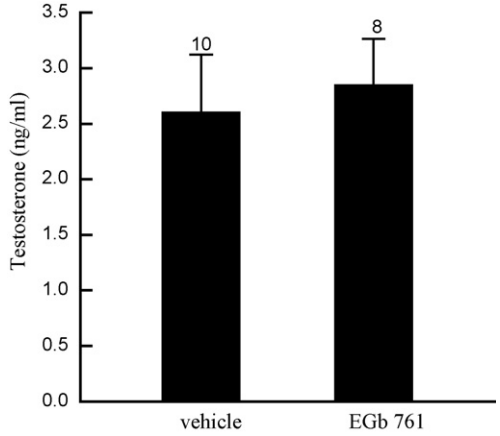

Fig. 3. Serum T levels in the two groups on day 14. Each bar represents the mean ± SEM for the number of rats indicated above each bar.

3.4. Concentrations of DA and NA

As shown in Fig. 4A, EGb 761 treatment caused a significant increase in the DA contents in the MPOA (df = 18, $t = -2.37$, $P < 0.05$). No significant difference was seen in NA levels in the MPOA (df = 18, $t = 0.51$, $P = 0.61$) (Fig. 4B).

3.5. TH-ir positive neurons in the MPOA

Fig. 5 shows the results from an immunohistochemical study concerning the effects that EGb 761 could have on the number of TH-expressing neurons in the MPOA (Fig. 5A and B). A significant increase was seen in the number of TH-expressing neurons in the MPOA (df = 6, $t = -3.22$, $P < 0.05$) (Fig. 6A) in EGb 761-treated rats compared to controls.

Fig. 6B shows a significant increase in the number of TH-ir neurons in the dorsal subregion of the MPOA after EGb 761 administration compared to the same region in the vehicle-treated group (df = 6, $t = -7.10$, $P < 0.001$). There was a tendency to exhibit a higher number of TH-ir neurons in the dorsal subregion of the MPOA than in the ventral MPOA subregion after EGb 761 treatment (df = 3, $t = 2.85$, $P = 0.06$).

4. Discussion

In the present study, EGb 761 treatment significantly enhanced male copulatory performance in rats in terms of increased NI and NE, but had no effect on the ML, IL, or PEI (Table 1), which are

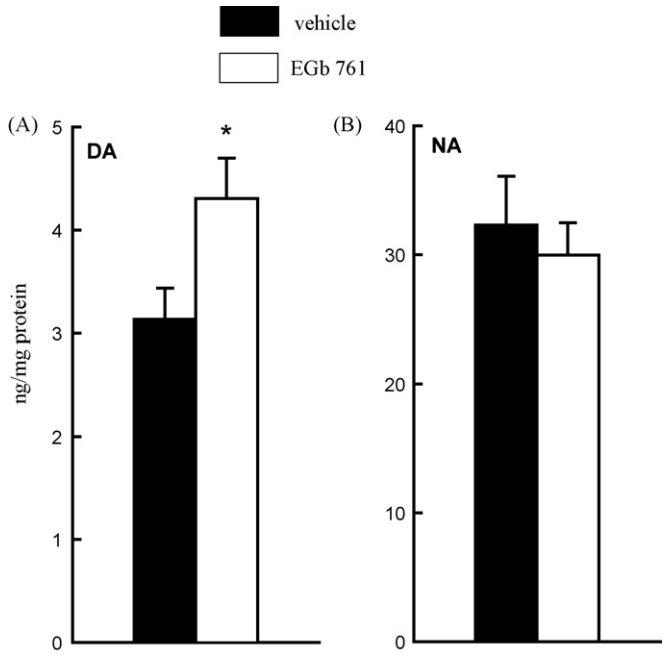

Fig. 4. DA and NA levels on day 14 in the MPOA. The data are the mean ± SEM for $n = 10$. $^{*}P < 0.05$ compared to the vehicle-treated controls.

commonly considered indicators of sexual motivation in rodents. Both controls and EGb 761-treated animals spent more time with estrous females than with sexually active males in the partner preference test (Fig. 2A and B); however, there was no significant difference in the duration (Fig. 2B) or number of visits (Fig. 2C) to estrous females between these two groups. These data clearly indicate that EGb 761 treatment does not influence sexual motivation in male rats evaluated in reference to ML, IL, PEI, or sexual incentive motivation when measured by means of a partner preference test, but significantly increases copulatory performance.

Administration of herbal extracts, such as *Panax ginseng*, *Panax quinquefolium*, and *Tribulus terrestris*, has been reported to improve male sexual behavior in rodents. These herbal extracts increase sexual motivation and/or copulatory performance. Treatment with *P. ginseng* increases ejaculation frequency and decreases EL and PEI, but has no effect on mount frequency, ML, intromission frequency, or IL in male rats compared to controls [18]. While treatment of rats with *P. quinquefolium* for 1 day decreases EL without affecting ML or IL, long-term treatment with the same herbal extract results in a significant decrease in ML and IL [26]. In addition, long-term

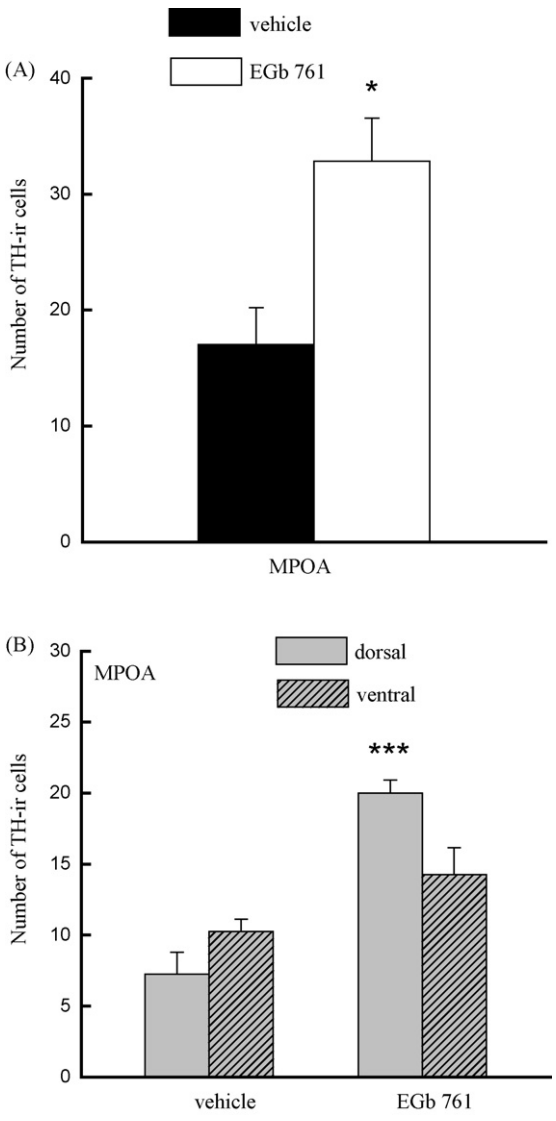

Fig. 6. Quantification of the number of TH-immunoreactive neurons in the MPOA (A) and in the subregions of the MPOA (B) on day 14. The data are the mean ± SEM for $n = 4$. $^{*}P < 0.05$, compared to the vehicle-treated controls; $^{***}P < 0.001$, compared to the same subregion in the vehicle-treated controls.

treatment with *T. terrestris* increases mount frequency and intromission frequency and decreases ML, IL, EL, and PEI [10]. Together, these results show that some herbs can affect both sexual motivation and copulatory performance, but that other herbs influence

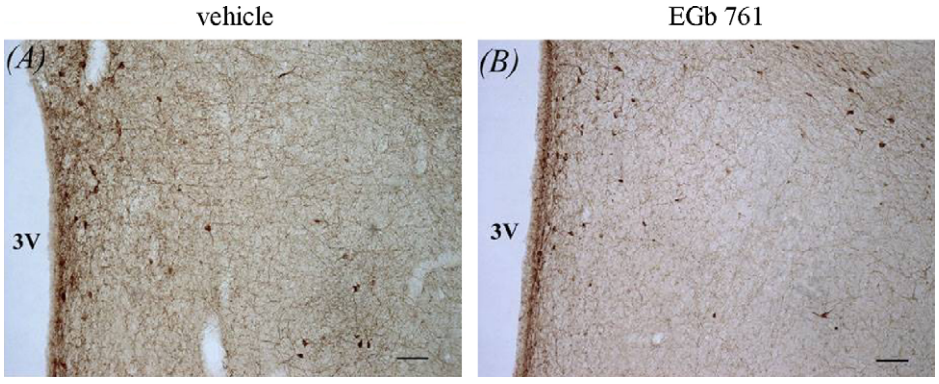

Fig. 5. Photomicrographs showing TH-immunoreactivity in the MPOA in vehicle- and EGb 761-treated male rats on day 14. The scale bars represent 100 μm and the magnification is 10×. 3V, third ventricle.

only one. These results suggest that different mechanisms mediate sexual motivation and copulatory performance.

In general, DA plays a key role in different aspects of the male sexual responses, including sexual motivation, sexual arousal, penile erection, and ejaculation [17]. However, it has been reported that DA does not influence sexual motivation in rats. Male rats with microinjection of DA receptor agonist (apomorphine) into the ventral tegmental area have no influence on sexual motivation expressed as proportion of choices the receptive female [16]. In addition, treatment with dopaminergic compounds (apomorphine or amphetamine) has no effect on sexual incentive motivation in male rats [1]. In the present study, treatment with EGb 761 significantly increased MPOA DA contents (Fig. 4A), but had no effect on sexual incentive motivation (Fig. 2). Our results support the viewpoint that DA is ineffective in sexual incentive motivation in male rats [1]. Also, our findings showed that EGb 761 treatment significantly increased copulatory performance (Table 1); these results seem to imply that DA in the MPOA is important for copulatory performance, but not for sexual incentive motivation. Furthermore, our findings are consistent with the notion that preoptic DA is not significantly involved in sexual motivation [27].

Several studies have demonstrated that the subregion of the MPOA is associated with sexual motivation and copulatory performance [3,21,36]. Furthermore, it has been reported that dorsal MPOA may be more important than other MPOA subregions for copulatory performance [3]. Our results showed that EGb 761 treatment significantly increased the number of TH-ir neurons in the dorsal subregion, but not in the ventral subregion, of the MPOA compared to the same subregions in controls (Fig. 6B). The behavioral data showed that EGb 761 treatment did not influence sexual incentive motivation, but increased copulatory performance in the present study. These data suggest that DA neuron activity in the dorsal subregion of the MPOA may be involved in the regulation of copulatory performance, and that the ventral subregion of the MPOA may be associated with sexual incentive motivation.

Both sexual motivation and copulatory performance are controlled by T. Castration eliminates partner preference in rats [2,11] and hamsters [4], and its effects are overcome by T replacement. T propionate treatment restores copulatory performance to pre-castration levels in copulating castrated rats, but results in a significant increase in preference for estrous females in comparison with intact subjects or the same rats after castration [31]. Further, Portillo et al. [30] reported that copulating male rats showed a significantly higher percentage of ejaculators than sexually sluggish animals, which take a longer time to ejaculate when tested on repeated occasions with receptive females, and both of the groups showed a significant preference for estrous females, with no difference between the two groups regarding either preference for estrous females or serum T levels. Similar to these findings, our present findings showed that EGb 761 treatment significantly increased copulatory performance (Table 1) without affecting sexual motivation (ML, IL, and PEI) (Table 1), sexual incentive motivation (Fig. 2), or serum T levels (Fig. 3) compared to controls. These results indicate that T is essential for male sexual behavior, but that the serum T threshold to induce sexual incentive motivation or copulatory performance may be different.

Our previous study [5], which was on middle-aged (18–19-month-old) rats, showed that higher DA levels are seen in the MPOA in rats displaying ejaculations than in the MPOA in (1) rats displaying mounts and intromissions but displaying no ejaculation and (2) noncopulating rats; furthermore, there was no significant difference in MPOA DA levels between the last two groups. Our present results showing that the EGb 761-treated group displayed a greater number of ejaculations (Table 1) and had higher DA levels in the MPOA (Fig. 4) than the controls are in agreement with the notion that MPOA DA levels are closely associated with regulation of ejaculation in rats.

Since kaempferol, a major ingredient of Ginkgo biloba, is a potent monoamine oxidase B (MAO-B) inhibitor [38] and prevents DA degradation and increases its availability, the EGb 761-induced increase in DA content in the MPOA (Fig. 4A) seen in the present study might be due to the MAO-B inhibitory activity of kaempferol. TH catalyses the rate-limiting step in DA biosynthesis and therefore is commonly used as a marker for dopaminergic neurons. TH is present in neuronal cell bodies in the MPOA [33,37]. In the present study, EGb 761 treatment increased the number of TH-positive cells in the MPOA compared to controls (Fig. 6A), this increase being specific to the dorsal subregion (Fig. 6B), suggesting that EGb 761 treatment increases DA neuron activity in the dorsal MPOA, thus increasing DA synthesis and, in turn, facilitating copulatory performance.

The MPOA has NA terminals; it receives the noradrenergic projections from the brain stem [7,24]. Our previous study found no differences in NE tissue levels in the MPOA between middle-aged and young-adult rats, although the young-adult rats displayed better copulatory behavior than the middle-aged ones [5]. In the present study, treatment with EGb 761 significantly increased TH activity in the MPOA, but did not influence MPOA NE tissue levels compared to controls (Fig. 3B). Taken together, these data suggest that NA tissue levels in the MPOA are not related to male sexual behavior in rats.

Results based on behavioral observations from other laboratories [30,31] and our own [6,40] show that copulatory performance seems dissociable from sexual motivation, and the mechanisms involved are not yet understood. A few limitations of the present study should be considered. First, since we measured the DA content of the MPOA as a whole instead of in the dorsal or ventral subregion separately, we cannot say whether the DA content in these subregions correlates with the numbers of TH-ir neurons in these subregions. Another limitation is that, although we measured serum T concentrations, we did not examine AR expression in the MPOA. Further research on the role of ARs in different MPOA subregions in male sexual behavior would help in understanding the dissociation of sexual motivation and copulatory performance. Finally, only one dose (50 mg/kg) of EGb 761 and one treatment duration (14 days) were used in the present study, so we do not know whether different results would have been obtained if higher or lower doses of EGb 761 or a longer or shorter duration of treatment had been used. For example, it is possible that a lower dose of EGb 761 might facilitate sexual motivation, but not sexual performance, while a higher dose might affect both. However, this does not preclude the fact that the behaviors can be separated.

In summary, we conclude that the ventral subregion of the MPOA may regulate sexual incentive motivation, while the dorsal MPOA subregion may contribute to the control of copulatory performance.

Acknowledgement

This study was supported by a grant from the National Science Council (NSC 95-2413-H002-026), ROC.

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