Pharmacology, Biochemistry and Behavior 91 (2009) 581–589

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Orexin A attenuates unconditioned sexual motivation in male rats

Y.J. Bai [a,b], Y.H. Li [a], X.G. Zheng [a], J. Han [a,b], X.Y. Yang [a], N. Sui [a,*]

[a] Key Laboratory of Mental Heath, Institute of Psychology, Chinese Academy of Sciences, Beijing, China
[b] Graduate School of Chinese Academy of Sciences, Beijing, China

ARTICLE INFO

Article history:
Received 7 December 2007
Received in revised form 12 September 2008
Accepted 25 September 2008
Available online 12 October 2008

Keywords:
Unconditioned sexual motivation
Orexin
Preference score
Intracerebroventricle

ABSTRACT

Orexins are neuropeptides involved in multiple neurophysiological functions such as reward and motivation. However, it is not clear whether orexins are implicated in sexual motivation. This study aims to evaluate the effects of orexin A and the OX_1R antagonist SB334867 on unconditioned sexual motivation. Forty-five male Wistar rats are divided into four groups. The four groups are respectively administered intracerebroventricularly with saline, orexin A (1, 10 μg), 10% DMSO (cyclodextrin) and SB334867 (5, 15 μg) 10–15 min before sexual motivation tests. The preference for a receptive female to a male in an open arena with two tethered animals is designated as unconditioned sexual motivation. The results show that orexin A reduces the female preference (reducing time in the female zone and/or increasing time in the male zone), the number of visits for the female zone and the total distance traveled in sexually high-motivated males. SB334867 has no effect on the female preference, the number of visits and the distance traveled in either sexually high-motivated or low-motivated males. Our experiments reveal that centrally administered orexin A attenuates sexual motivation in high-motivated males although endogenous orexin A might not play an important role in the expression of unconditioned sexual motivation.

1. Introduction

Orexins (hypocretins) (De Lecea et al., 1998; Sakurai et al., 1998) are neuropeptides exclusively expressed in the lateral hypothalamus (LH) and adjacent regions (Siegel, 2004). Orexin peptides take two forms, orexin A and orexin B, which bind to two G-protein-coupled receptors, OX_1R and OX_2R (Sakurai et al., 1998). Initially, orexins were found to facilitate feeding (Sakurai et al., 1998) and to be involved in multiple physiological functions, such as arousal, energy homeostasis and neuroendocrine activities (Martynska et al., 2005; Winsky-Sommerer et al., 2003). Recently, it was reported that orexins play an important role in reward-associated behaviors. For instance, hunger, food presentation and food-associated contextual cues can activate orexin neurons (Harris et al., 2005; Mileykovskiy et al., 2005; Sakurai et al., 1998). Orexins have also been shown to increase motivation for food (Thorpe et al., 2005) and contribute to the expression of drug-seeking behaviors (Harris et al., 2005). So, it is hypothesized that orexin neurons form an interface between the internal physiological state of an organism and motivated behaviors (Scammell and Saper, 2005). It has also been suggested that expectation of a reward may activate orexin neurons (Dayas et al., 2008; Harris et al., 2005).

The question of whether orexins are implicated in sexual behavior, which is considered a reward-related behavior, remains unknown. Two previous studies support a role for orexins in sexual behavior. In one study, analysis of Fos immunoreactivity in orexin neurons reveals that orexin neurons were activated during male copulation while systemic pretreatment with selective OX_1R antagonist SB334867 impaired copulatory behavior (Muschamp et al., 2007). In another study, microinjections of orexin A in the medial preoptic area (MPOA), a critical structure for male sexual behavior, facilitated copulatory behavior in male rats (Gulia et al., 2003). In fact, male sexual behavior comprises two basic phases. The appetitive phase consists of the sexual motivation which is manifested by approaching a mate whereas the consummatory phase involves the act of copulation. Appetitive behaviors are highly variable and presumably determined by the context, while copulatory behaviors are usually highly stereotyped (Paredes and Agmo, 2004). Also, the two components of male sexual behavior are partly independent and controlled by different neural mechanisms (Paredes and Agmo, 2004). As mentioned above, orexin neurons appear to be activated by motivating stimuli and are extensively involved in exploration for the potential reward. Consequently, it is possible that orexin neurons are activated before copulation (i.e. during the appetitive phase) by signals associated with sexual behaviors. In previous studies (Gulia et al., 2003; Muschamp et al., 2007), the two different components of sexual behavior were not considered when trying to determine the involvement of orexin neurons in sexual behavior. In the present study, the preference for a receptive female to a male was used to examine unconditioned sexual motivation in male rats (Agmo, 2003b, 2004). In addition, the effects of orexin A and OX_1R antagonist SB334867 on female preference were evaluated.

* Corresponding author. Tel.: +86 10 64857369; fax: +86 10 64857369, +86 10 64872070.
E-mail address: suin@psych.ac.cn (N. Sui).

0091-3057/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.09.018

It is worth noting that individual differences may exist in male sexual motivation (Agmo, 2003a; Rhen and Crews, 2002). In this work, we intentionally considered the effects of orexin A or OX_1R antagonist SB334867 on female preference in high-motivated and low-motivated males.

2. Methods

2.1. Animals

Wistar rats (Charles River laboratories in Beijing, China) were housed four per cage in a controlled temperature (20–24 °C) colony room with a 12:12 h light–dark (light on at 08:00) cycle. The relative humidity was 40–60%. Forty-five males were sexually naïve rats weighing 330±20 g. Four sexually naïve females weighed 250 g upon arrival to the animal facilities. Food and water were available ad libitum. All tests were performed between 12:00 and 20:00 during the light phase of the cycle. The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the Regulations for the Administration of Affairs Concerning Experimental Animals (China, 1988).

2.2. Surgery

2.2.1. Stereotaxic surgery

Male rats were implanted under anaesthesia (chloral hydrate, 0.4 g/kg) with a unilateral stainless steel cannula (o.d. 0.6 mm, i.d. 0.35 mm, 8 mm in length) directed towards the lateral ventricle (coordinates: AP −0.8 mm, ML ±1.4 mm, DV −3.5 mm from the skull surface, according to Paxinos and Watson, 1997). The guide cannulae were secured with three small screws and dental cement. After at least 1 week of recovery, rats were handled daily (1 min/day) before being tested. Correct placement of cannulae was verified by an intense drinking response (water consumed>5 ml in 30 min) to angiotensin II (25 ng ICV). Two rats were eliminated due to lack of intense drinking response.

2.2.2. Ovariectomy

Females were bilaterally ovariectomized under chloral hydrate (0.4 g/kg) anesthesia at least 2 weeks before use. Artificial estrus was induced by subcutaneous treatment with estradiol benzoate (25 μg/rat) and progesterone (1 mg/rat) about 48–52 h and 4–6 h before tests, respectively.

2.3. Drugs

Orexin A (Tocris, UK) was dissolved in physiological saline. SB334867, selective OX_1R antagonist (Tocris, UK) was dissolved in 10% Dimethyl Sulphoxide (DMSO) and 2% β-cyclodextrin (Sigma, USA). β-estradiol 3-benzoate (EB) and progesterone (P) (Sigma, USA) were dissolved in peanut oil.

2.4. Apparatus

The apparatus for unconditioned sexual motivation was a little modified from earlier studies (Agmo, 1999). A rectangular arena (100×50×45 cm high) had two openings (25×25 cm) diagonally opposed on the long walls (see Fig. 1). Cages (15×25×25 cm high) containing the male or female rats (sexual incentives) could be fitted to these two openings. The front of the cages was made of wire mesh (1-mm wire, mesh size 10×10 mm), which allowed the subjects to see, smell and touch the animals in the cages. The arena and cages were made of Plexiglas with a black and rough surface inside. A virtual area (30×20 cm) adjacent to each cage was defined as the incentive zone. The location and movement of rats were monitored by a video camera

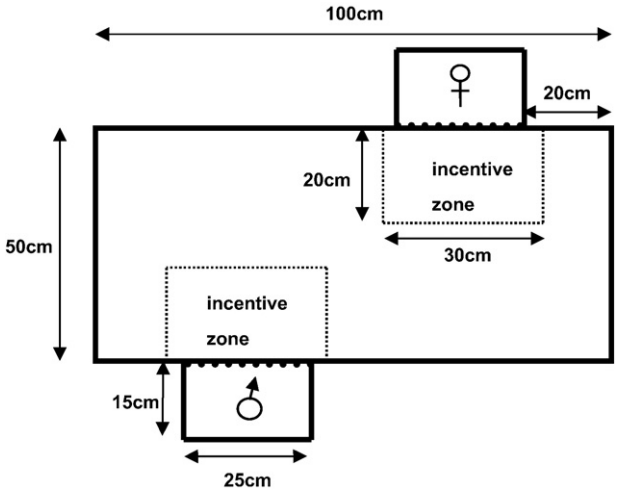

Fig. 1. Apparatus for unconditioned sexual motivation (according to Agmo A, 1999).

suspended on the ceiling (about 2 m above the test arena). The camera was connected to a computer via a signal transmitter. The behavioral parameters were automatically recorded using professional software designed by Taiji Software Company in Beijing of China. The testing apparatus was placed in a room with dim white light provided by three incandescent bulbs (15 W). This light intensity was necessary for adequate functioning of the videotrack system and did not affect male sexual motivation.

2.5. Procedure

The procedure for the sexual motivation test was identical to earlier studies (Agmo, 2003b). First, the subjects were familiarized to the test arena for 3 days, 10 min/day, without incentive rats in cages. Before each test, the arena and the cages were carefully cleaned with 0.1% acetic acid in water. Then, the incentives (one female and one male rat) were placed in their cages. About 5 min later, the first experimental subject was introduced into the middle of the arena and observed for 10 min. Thereafter, the subject was removed and the next subject was introduced. No cleaning was performed between two subjects. The position of the cages was randomly changed during the test. The following indices were recorded: the time spent in incentive zones, the number of visits to the zones and the total distance traveled. Unconditioned sexual motivation was quantified by the preference score (time spent in the female incentive zone/[time spent in the female incentive zone+time spent in the male incentive zone]).

All males were randomly divided into four groups, which were administered i.c.v. with saline, orexin A, 10% DMSO (cyclodextrin) and SB334867 respectively. In order to reduce the number of animals used, each group was repeatedly tested with a vehicle or with a given drug in different doses (Muschamp et al., 2007). Before every experimental test, the subjects were pretested for their sexual motivation baseline without any drug treatment. After the pretest, the test was carried out on the next day. We did find apparent individual differences in male sexual motivation. A number of animals expressed high or mild preference scores while some others showed very low preference with scores of around 0.5. Accordingly, the males in all groups were respectively subdivided into two groups based on their performance during the pretest. The animals with a preference score above the median were placed in a high-motivation subgroup whereas those below the median were placed in a low-motivation subgroup. During the test, all rats were introduced into the arena 10–15 min after i.c.v. infusions of drugs or vehicle. To avoid the disturbing effect of drug doses, orexin A (1, 10 μg) and SB334867 (5, 15 μg) were administered

from the low to high doses. In addition, all subjects were allowed to rest for 3 days (drug washout period) before the second pretest and experimental test. The injection cannulae extended 1 mm beyond the tip of the guide cannulae. Infusions, 5 μl per injection, lasted 90 s with the injector left in position for an additional 60 s to allow for diffusion.

2.6. Statistical analysis

Data from the high-motivation and low-motivation subgroups were analyzed separately. The preference score was evaluated with a two-way ANOVA for repeated measures on one factor. The within-subject factor was "test" ("pretest" versus "test"), and the between-subject factor was "treatment" ("vehicle" versus "drug"). A repeated measures three-way ANOVA was performed on the times spent in incentive zones. The two within-subject factors were "zone" ("male zone" versus "female zone") and "test" ("pretest" versus "test"), and the between-subject factor was "treatment" ("vehicle" versus "drug"). The number of visits was analyzed with a repeated measures two-way ANOVA. The within-subject factor was "zone" ("male zone" versus "female zone"), and the between-subject factor was "treatment" ("vehicle" versus "drug"). In case of significant interaction, analyses of simple effects were performed. The distance moved after treatment with the vehicle and drugs was

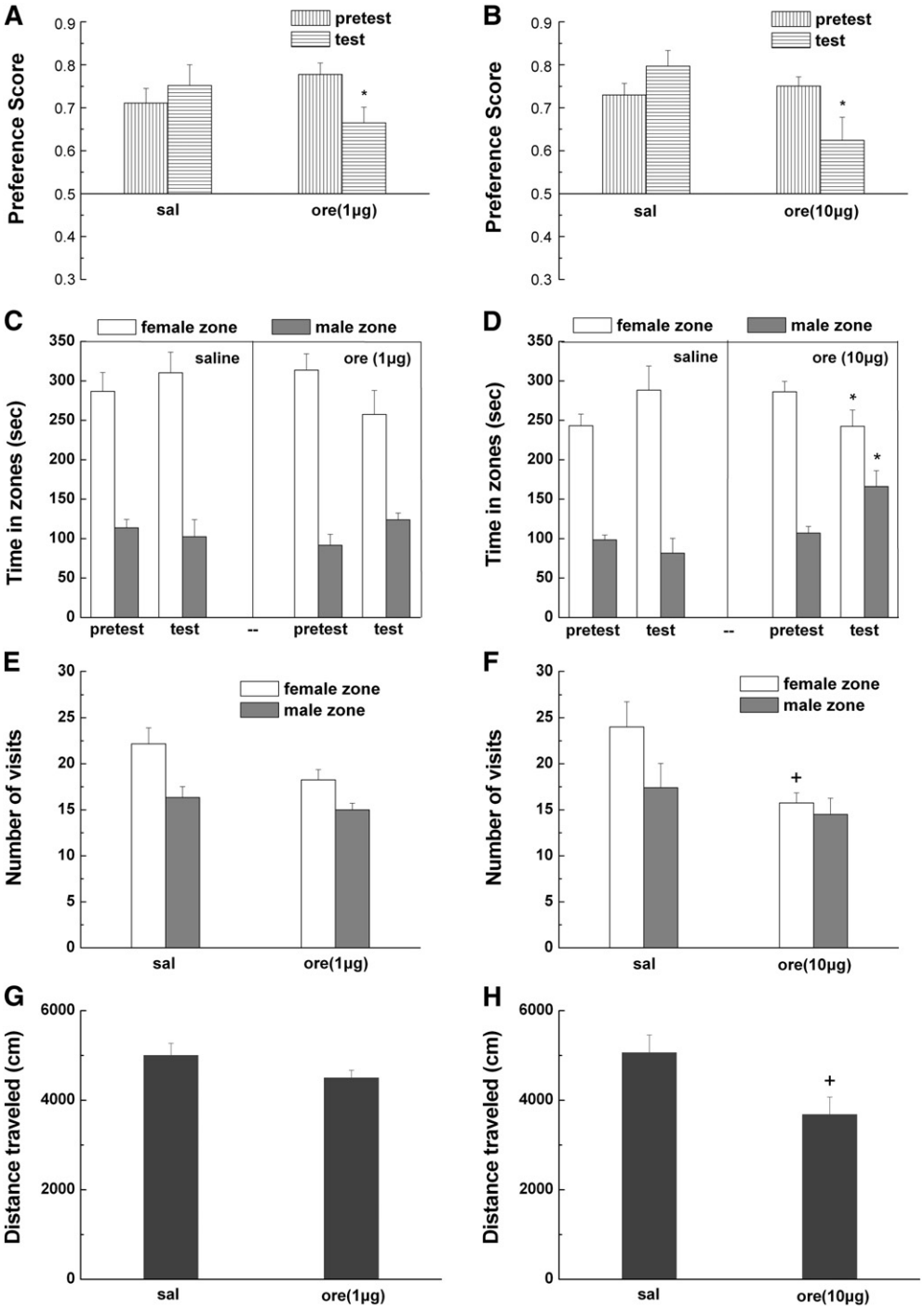

Fig. 2. Preference score in the high-motivation subgroups treated with saline ($n=6$) and 1 μg of orexin A ($n=6$) (A), or with saline ($n=6$) and 10 μg of orexin A ($n=6$) (B). Time spent in incentive zones (C and D). No drug treatment was performed during pretest. Number of visits to incentive zones after treatment with saline or orexin A (E and F). Distance moved in male rats treated with saline or orexin A (G and H). Data presented are mean±S.E.M. * $p<0.05$, compared with pretest. $^{+}$ $p<0.05$, compared with the saline-treated group.

compared by means of a *t* test. Pearson coefficient of correlation was used to reveal the correlation between the preference scores during the two pretests. A two-tailed significance level of .05 was used.

3. Results

3.1. Effect of orexin A on unconditioned sexual motivation in males

The preference for the female in an individual subject is relatively stable across both pretests except for 3 subjects. After 3 males were eliminated (they were eliminated for the entire analyses), a significant Pearson correlation was found between the preference scores during the first pretest and those during the second pretest ($r^2=0.33$, $p<0.01$) in all the subjects (males treated with vehicle or drugs). Moreover, the preference scores in the vehicle-treated groups showed a significant correlation between the first pretest and test ($r^2=0.47$, $p<0.01$) or between the second pretest and test ($r^2=0.48$, $p<0.01$). Therefore, most of the animals were always in the high- or low-motivation subgroups, except that 6 subjects were incorporated into the different subgroups in the two pretests. Since their preference scores were

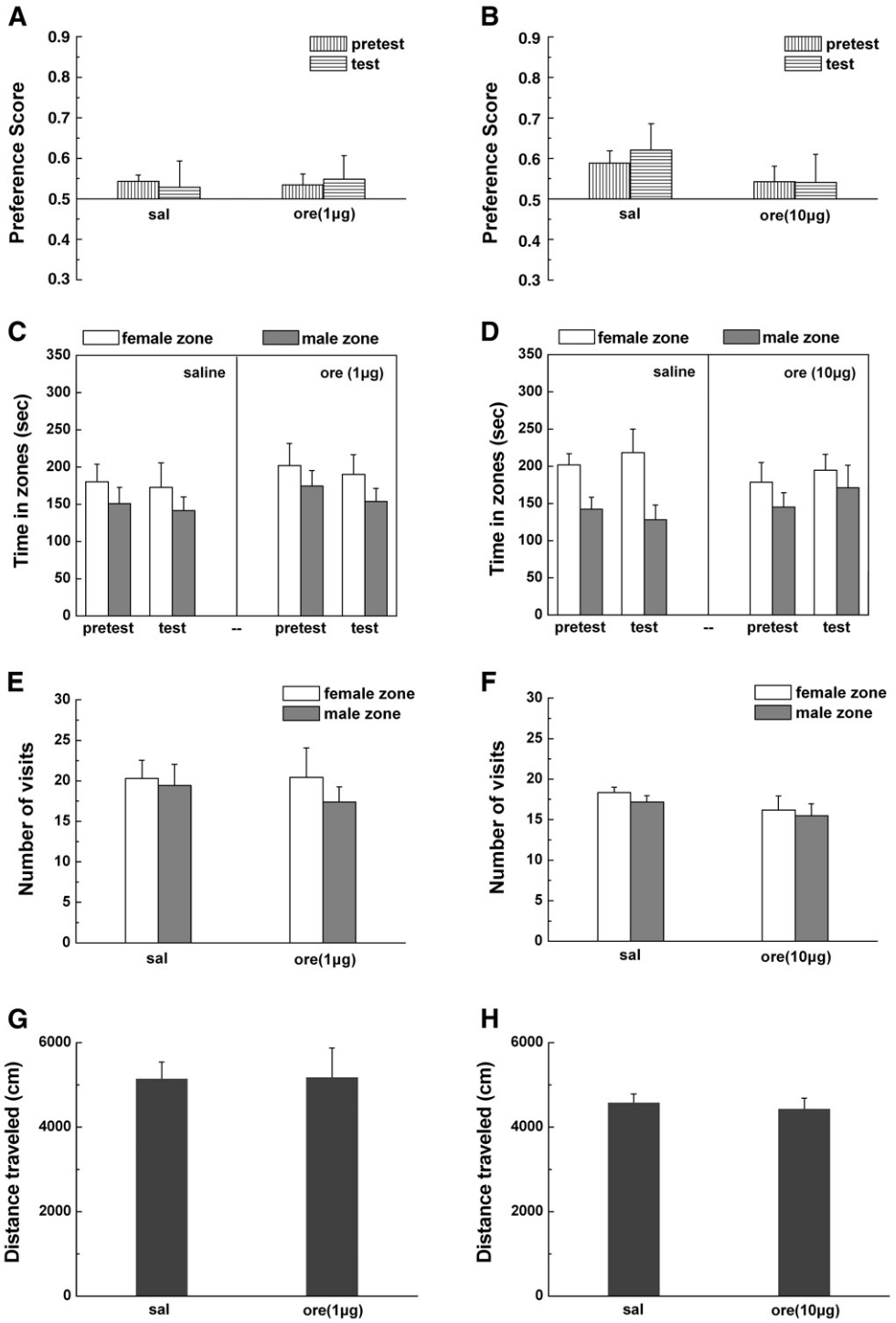

Fig. 3. Preference score in the low-motivation groups treated with saline ($n=7$) and 1 μg of orexin A ($n=5$) (A), or with saline ($n=6$) and 10 μg of orexin A ($n=6$) (B). Time spent in incentive zones (C and D). No drug treatment was performed during pretest. Number of visits to incentive zones after treatment with saline or orexin A (E and F). Distance moved in male rats treated with saline or orexin A (G and H). Data presented are mean±S.E.M.

always near the median (around a score of 0.64) during the two pretests, we thought that the results were not apparently affected.

The drug dose was not treated as a factor since the subjects were repeatedly tested with the vehicle or with a given drug in two doses. Fig. 2A–D shows the effects of orexin A on female preference in high-motivation subgroups. The preference scores were influenced by orexin A. This was demonstrated by significant "treatment"×"test" interactions with orexin A at doses of 1 and 10 μg (Fig. 2A: $F(1, 10)=9.21$, $p<0.05$; Fig. 2B: $F(1, 10)=7.03$, $p<0.05$). The analysis of simple effects showed that the preference scores were decreased by orexin A at doses of 1 μg (Fig. 2A: $F(1, 10)=9.89$, $p<0.05$) and 10 μg (Fig. 2B: $F(1, 10)=6.02$, $p<0.05$) during the test compared with that during the pretest. For the time in incentive zones, when orexin A was given at a dose of 1 μg, there was a significant "zone"×"test"×"treatment" interaction (Fig. 2C: $F(1, 10)=5.43$, $p<0.05$). Further analysis showed a near significant interaction between "test" and "treatment" for

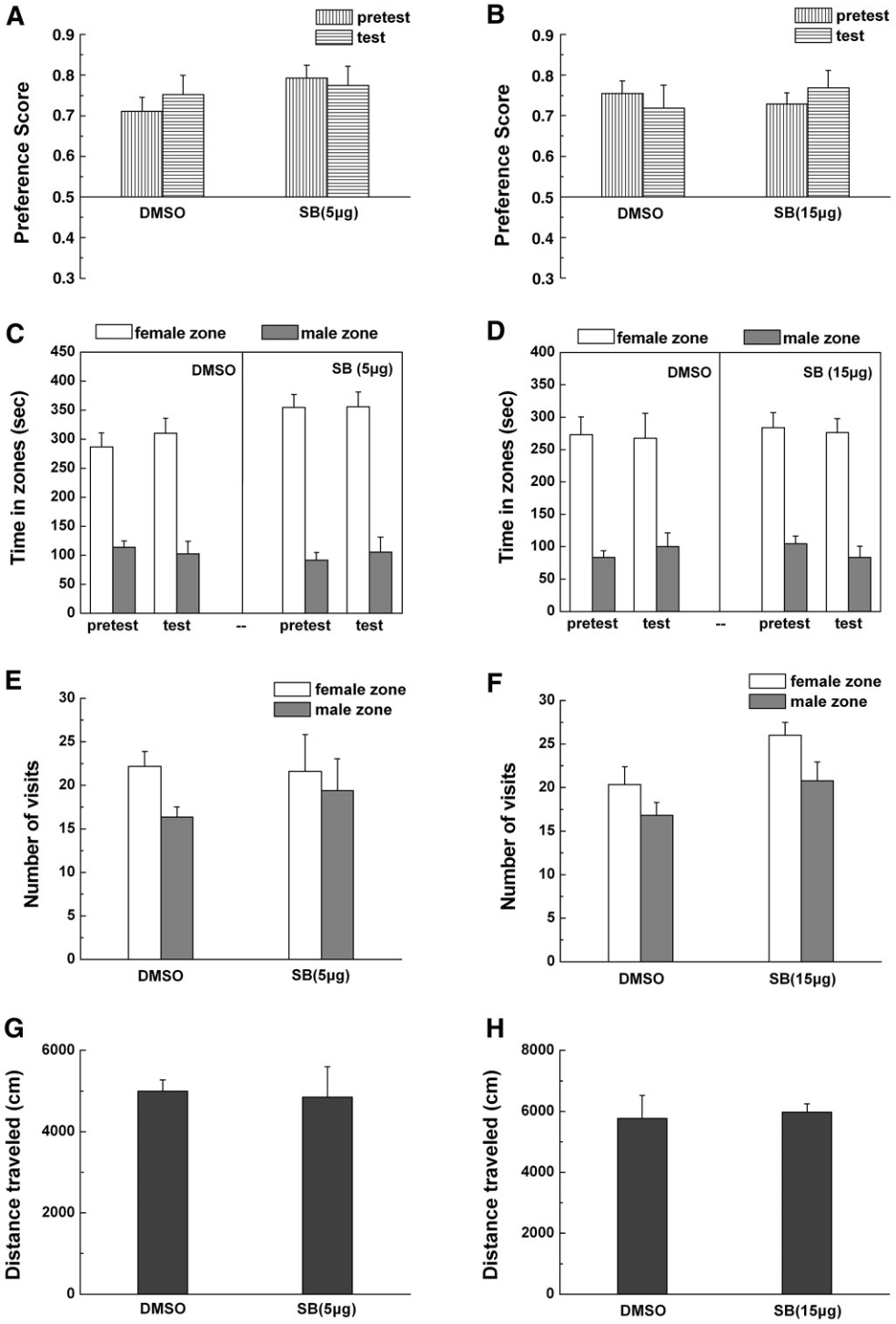

Fig. 4. Preference score in the high-motivation groups treated with DMSO ($n=6$) and 5 μg of SB334867 ($n=6$) (A), or with DMSO ($n=6$) and 15 μg of SB334867 ($n=5$) (B). Time spent in incentive zones (C and D). No drug treatment was performed during pretest. Number of visits to incentive zones after treatment with DMSO or SB334867 (E and F). Distance moved in male rats treated with DMSO or SB334867 (G and H).

the time spent in female zones (F (1, 10)=4.57, p=0.058) and no significant "test"×"treatment" interaction for the time spent in male zones (F (1, 10)=3.08, p=0.11). The analysis of simple effects showed that the time spent in the female zone during the test appeared to be reduced by orexin A compared with that during the pretest despite a near significant statistical difference (F(1, 10)=4.54, p=0.059). After delivering orexin A at a dose of 10 μg, a significant "zone"×"test"×"treatment" interaction (Fig. 2D: F(1, 10)=8.14, p<0.05) was found. Further analyses showed a significant "test"×"treatment" interaction for the time in female zones (F (1, 10)=8.59, p<0.05)

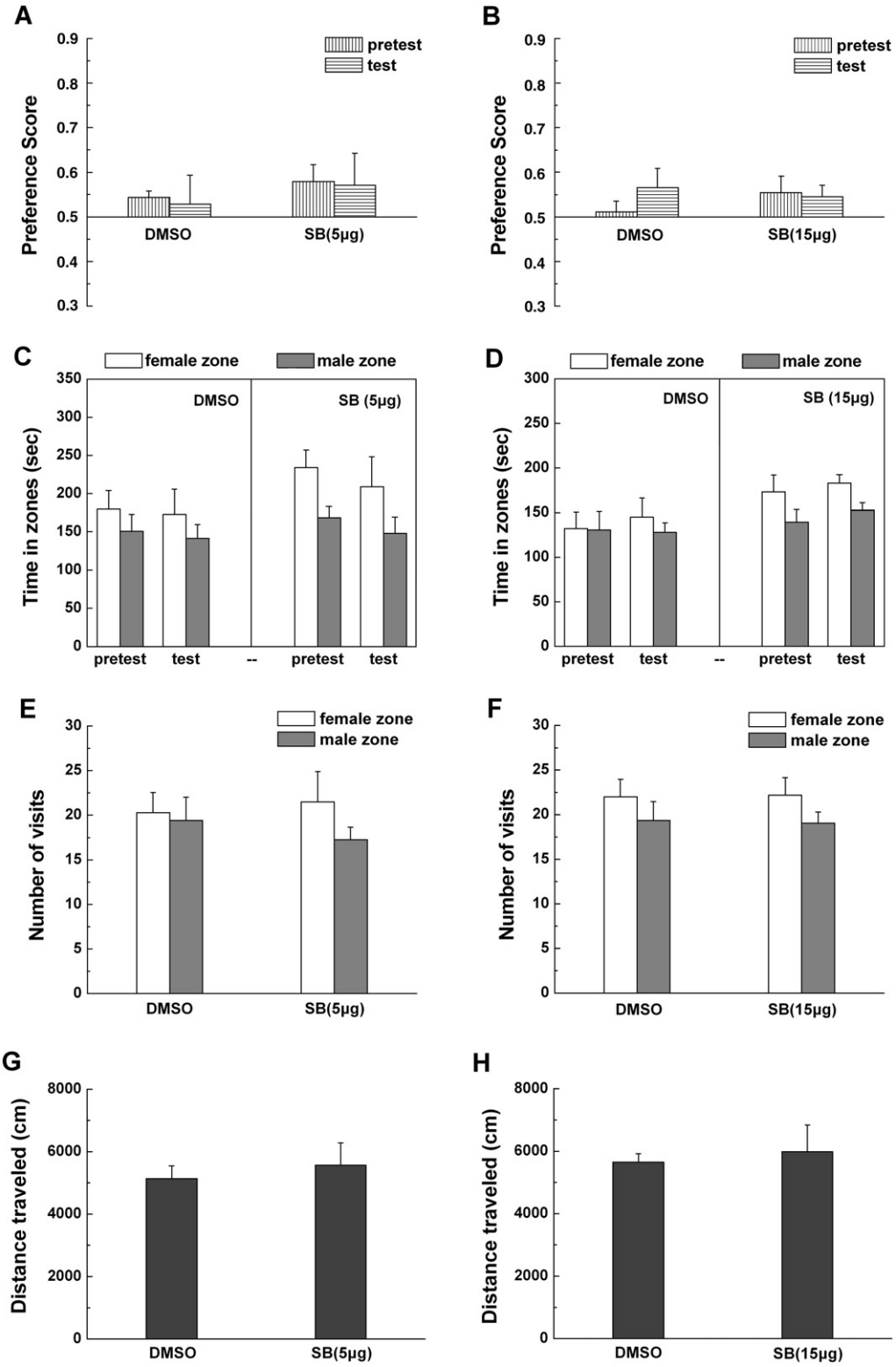

Fig. 5. Preference score in the low-motivation groups treated with DMSO (n=7) and 5 μg of SB334867 (n=5) (A), or with DMSO (n=6) and 15 μg of SB334867 (n=4) (B). Time spent in incentive zones (C and D). No drug treatment was performed during pretest. Number of visits to incentive zones after treatment with DMSO or SB334867 (E and F). Distance moved in male rats treated with DMSO or SB334867 (G and H).

and a significant "test"×"treatment" interaction for the time in male zones ($F(1, 10)=4.78$, $p=0.05$). For the orexin A-treated group, the time spent in the female zone during the test was reduced compared with that during the pretest ($F(1,10)=5.44$, $p<0.05$), and the time spent in the male zone during the test was increased compared with that during the pretest ($F(1,10)=7.53$, $p<0.05$). It was found that female preference was attenuated by orexin A at the two doses because of the reduced time spent in the female zone and/or increased time spent in the male zone. Concerning the number of visits to the incentive zones, there was no significant "treatment"×"zone" interaction after treatment with saline and orexin A at a dose of 1 μg (Fig. 2E: $F(1,10)=1.90$, $p=0.20$). When saline and orexin A at a dose of 10 μg were delivered, a significant interaction between "treatment" and "zone" was found (Fig. 2F: $F(1, 10)=10.23$, $p<0.01$). The analysis of simple effects showed that the number of visits for the receptive female in the orexin A-treated group was significantly reduced compared with that in the saline-treated group ($F(1, 10)=10.59$, $p<0.01$). It was demonstrated that the subjects in the high-motivation subgroup significantly reduced the number of visits for the female zone after delivery of orexin A at a dose of 10 μg. The distance traveled was not affected by orexin A at a dose of 1 μg (Fig. 2G: $t(10)=1.53$, $p=0.16$) and decreased after treatment with orexin A at a dose of 10 μg (Fig. 2H: $t(10)=2.48$, $p<0.05$).

In low-motivation subgroups, the preference scores were not influenced by orexin A. This was demonstrated by no significant "treatment"×"test" interaction with orexin A at doses of 1 and 10 μg (Fig. 3A: $F(1, 10)=0.17$, $p=0.69$; Fig. 3B: $F(1, 10)=0.15$, $p=0.71$). Similarly, for the time in incentive zones, there was no significant "zone"×"test"×"treatment" interaction when two doses of orexin A were given (Fig. 3C: $F(1, 10)=0.01$, $p=0.91$; Fig. 3D: $F(1, 10)=0.40$, $p=0.54$). This shows that female preference in sexually low-motivated subjects was not influenced by orexin A. For the number of visits, there was no significant "treatment"×"zone" interaction after treatment with saline and orexin A at two doses (Fig. 3E: $F(1, 10)=0.34$, $p=0.57$; Fig. 3F: $F(1, 10)=0.05$, $p=0.82$). In contrast to the high-motivation subgroup, the distance traveled was not affected by orexin A at doses of 1 μg (Fig. 3G: $t(10)=0.05$, $p=0.96$) or 10 μg (Fig. 3H: $t(10)=0.44$, $p=0.67$).

The above results suggest that exogenously delivered orexin A exerted an inhibitory effect on sexual motivation in high-motivation subgroups but spared low-motivation subgroups possibly due to the "floor effect". In order to examine the role of endogenous orexin A in sexual motivation, experiments were done to determine the effect on male sexual motivation when OX_1R is blocked by the specific antagonist SB334867.

3.2. Effect of SB334867 on unconditioned sexual motivation in males

Figs. 4 and 5 indicate the effects of SB334867 on high-motivation and low-motivation subgroups. The preference scores in two subgroups could not be regulated by SB334867 because no interaction between "treatment" and "test" in high-motivation subgroups with SB334867 at doses of 5 μg (Fig. 4A: $F(1, 10)=1.92$, $p=0.20$) and 15 μg (Fig. 4B: $F(1, 9)=0.01$, $p=0.92$) or in low-motivation subgroups with SB334867 at doses of 5 μg (Fig. 5A: $F(1, 10)=0.004$, $p=0.95$) and 15 μg (Fig. 5B: $F(1, 8)=0.61$, $p=0.46$) was found. For the time spent in incentive zones, there was no "zone"×"test"×"treatment" interaction when SB334867 was given at 5 μg (Fig. 4C: $F(1, 10)=1.14$, $p=0.31$) or 15 μg (Fig. 4D: $F(1, 9)=0.31$, $p=0.59$) in the high-motivation subgroup. Similarly, in the low-motivation subgroup, there was no "zone"×"test"×"treatment" interaction when SB334867 was given at 5 μg (Fig. 5C: $F(1, 10)=0.01$, $p=0.93$) or 15 μg (Fig. 5D: $F(1, 8)=0.05$, $p=0.83$). This suggests that unconditioned sexual motivation in males was not affected in either the high-motivation or the low-motivation subgroup. The number of visits was not affected by 5 μg (Fig. 4E: $F(1, 10)=2.08$, $p=0.18$) or by 15 μg of SB334867 (Fig. 4F: $F(1, 9)=0.46$, $p=0.52$) in the high-motivation subgroup. In the low-motivation subgroup, the number of visits was not affected by two doses of SB334867 (Fig. 5E: $F(1, 10)=1.45$, $p=0.26$; Fig. 5F: $F(1, 8)=1.36$, $p=0.28$). The distance traveled was not affected by SB334867 at the two doses in the high-motivation subgroup (Fig. 4G: $t(10)=0.18$, $p=0.86$; Fig. 4H: $t(9)=0.23$, $p=0.82$) or in the low-motivation subgroup (Fig. 5G: $t(10)=0.56$, $p=0.59$; Fig. 5H: $t(8)=0.44$, $p=0.67$).

4. Discussion

In the present study, centrally administered OX_1R antagonist SB334867 had no effect on female preference in males, and orexin A attenuated the female preference in sexually high-motivated males. This is in contrast to previous research which reported that systemic pretreatment with SB334867 impairs copulatory behavior (Muschamp et al., 2007) and that applications of orexin A in the MPOA facilitate male copulation (Gulia et al., 2003). However, it may be incorrect to infer intensity of sexual motivation from copulatory performance alone because the neural mechanisms underlying components of sexual motivation and copulation in rats can be dissociated (for a recent review: Paredes and Agmo, 2004). In addition, injections of orexin A in the cerebral ventricles were likely to have different behavioral effects than local injections in the MPOA. In fact, the inhibitory effect of orexin A found in our experiment suggests that i.c.v. injections of orexin A may influence motivation by acting at other regions of the brain that contain orexin fibers and receptors.

A more conceivable explanation for the lack of effect of SB334867 on the preference for the receptive female is that endogenous orexin A is not involved in the expression of unconditioned sexual motivation. Orexin A-expressing neurons may be uniquely activated by events associated with consummatory behaviors. Harris et al. (2005) found that orexin A-expressing neurons in the lateral hypothalamus only responded to food- and drug-paired contexts but not to novel items-paired context where no consummatory behavior had happened. In the present study, sexually naïve rats were used, and no copulation was permitted during the tests. It is possible that only presentation of a receptive female without copulatory behavior (consummatory behavior) does not serve as a powerful stimulus for activation of orexin neurons. Consequently, infusions of SB334867 did not influence the preference for receptive females. As new and specific pharmacological agents are developed, the involvement of the OX_2R in sexual behavior should be examined.

In fact, the previous studies indicate that orexin A may contribute toward the increased sexual arousal because systemic injection of SB334867 significantly increased intromission latency (Muschamp et al., 2007) and infusions of orexin A in the MPOA significantly decreased mount and intromission latencies (Gulia et al., 2003). The mount and intromission latency are indicators of sexual arousal which determines how easily copulation is initiated in the presence of a receptive female (for a recent review: Paredes and Agmo, 2004). The previous study also shows that applications of orexin A in the MPOA increased intromission frequency and decreased ejaculation latency, indicating an improvement of sexual performance (Gulia et al., 2003). In contrast to the roles of orexin A in sexual arousal and performance, the lack of involvement of endogenous orexin A in sexual motivation is revealed in the present study. Therefore, as far as the roles of orexin A in sexual behavior are concerned, there may be a dissociation between appetitive behavior and copulatory behavior.

Unexpectedly, the female preference was attenuated by exogenously delivered orexin A in sexually high-motivated rats in this experiment. Sex, as a potent natural reward, is closely associated with activity of the mesolimbic dopamine (DA) systems that mediate reward and motivation. Microdialysis studies have indicated that dopamine is released into the nucleus accumbens (NAc) upon presentation of an inaccessible receptive female (Damsma et al., 1992) or bedding from receptive females (Louilot et al., 1991). However, exact roles of dopamine in sexual motivation are still unclear. Manipulations of the dopamine

system have no effect on unconditioned sexual motivation (Agmo, 2003a; Ellingsen and Agmo, 2004), and even lesions of the NAc leave unconditioned sexual motivation intact (Kippin et al., 2004). Nevertheless, there also exists the evidence that dopamine antagonism attenuates the unconditioned sexual motivation in males (Lopez and Ettenberg, 2001). Therefore, although orexin neurons have extensive projections to the ventral tegmental area (VTA) and excite the mesolimbic DA system (Fadel and Deutch, 2002; Korotkova et al., 2003; Narita et al., 2006), the effect of orexin A on unconditioned sexual motivation in this study may not be mediated by the DA system.

At this point, we can only speculate about the mechanism of inhibitory effect of orexin A on female preference in males. As mentioned above, orexin receptors are extensively distributed in the brain, and centrally administered orexin A may work through multiple areas. Among of them, orexin neurons play an important role in maintaining arousal and responses to stress. Orexin receptors are expressed in the hypothalamic paraventricular nucleus (PVN) and pituitary, and mediate enhanced production of adrenocorticotropin-releasing factor (CRF), adrenocorticotropin (ACTH) and corticosterone (Spinazzi et al., 2006). Moreover, orexin receptors are highly expressed in the extended amygdala of the basal forebrain including the anxiety-related regions such as the central amygdala and bed nucleus of the stria terminalis (Baldo et al., 2003; Marcus et al., 2001). One study showed that i.c.v. infused orexin A significantly elevates intracranial self-stimulation (ICSS) thresholds in rats, and orexin-induced reinstatement of cocaine seeking is blocked by CRF/noradrenergic antagonism, indicating a stress-like response elicited by orexin A (Boutrel et al., 2005). Another study demonstrates that i.c.v. delivered orexin A has an anxiogenic-like effect similar with that of CRF in the mouse light–dark exploration test and in the mouse elevated plus-maze test (Suzuki et al., 2005). It can be seen that orexin A given centrally may generate a physiologically or psychologically negative state. As is well known, sexual behavior is an instinctive behavior which can be influenced by physiological, psychological and social conditions. For example, corticosterone can rapidly reduce male odor preferences in female mice (Kavaliers and Ossenkopp, 2001). Furthermore, male rats do not show preference for a receptive female when placed in an unfamiliar testing environment (Agmo, 2003b). Therefore, orexin A may have induced a stress-like state that interfered with male preference for a receptive female in this study. It is possible that the neural interactions involving the stress systems and sexual reward/motivation systems may be involved in the mechanism underlying the inhibitory effect of orexin A on sexual motivation observed in the present study.

The dose of orexin A employed in this experiment was similar to other studies examining the roles of orexin A on food intake, arousal and neuroendocrine functions. For instance, orexin A in doses of 1 and/or 10 μg i.c.v. administered to rats stimulates food intake (Edwards et al., 1999; Rodgers et al., 2000). Intracerebroventricular delivery of orexin A in doses of 1 and/or 10 μg increases arousal, locomotor activity (beam breaks), grooming behavior and the levels of prolactin, growth hormone and corticosterone in plasma (Hagan et al., 1999).

It might be argued that the reduction in the amount of approach of sexually receptive females is due to the increased appetitive behavior for food, since the same doses of orexin A stimulate a number of food intake behaviors which may reflect higher food motivation. However, we know that orexin A not only increases food intake in rats upon presentation of food, but also stimulates water intake when water is available (Kunii et al., 1999). Additionally, orexin A reinstates cocaine-seeking behavior when animals are placed in the environment where drug-taking behavior once took place (Boutrel et al., 2005). In fact, orexins have a prominent role in regulating the stability of arousal. A growing body of evidence seemly suggests that the function of the orexin system is to maintain an appropriate level of arousal and motivation, allowing various goal-oriented behaviors (food, thirst, sex or drug) to take place. Therefore, the food intake effect may result from arousal rather than direct feeding pressure (Adamantidis and De Lecea, 2008). In our opinion, the type of behavior elicited by orexin A might depend on the specific events encountered by animals. Based on the above statements, it is reasonable to think that orexin A induced a "hyperarousal" state which is related to stress or an anxiety-like response. In this state, the incentive properties of estrous females were reduced and the rats became more alert to non-sexual stimuli, thus diverting the amount of time dedicated to seeking a mate. Certainly, this needs to be verified in further experiments.

Orexins are always reported to induce hyperactivity in rodents, which may include large movements (ambulation and rearing) as well as grooming behaviors (Hagan et al., 1999; Nakamura et al., 2000). In the present experiment, only ambulatory activity was recorded, and the total distance moved in rats was not influenced by 1 μg orexin A but mildly reduced by 10 μg orexin A. This phenomenon is consistent with the results in another two studies. One reports that orexin A (3 μg) has no effect upon cage transits in rats, but increases the duration of grooming behavior and freezing behavior as well as the number of rears (Jones et al., 2001). The other shows that spontaneous horizontal activities are not increased by orexin A (1 and 10 μg) in mice placed in a novel environment (Suzuki et al., 2005). However, orexin A in a dose of 10 μg significantly increases the time spent in grooming behavior in rats (Hagan et al., 1999). Grooming behavior could be considered to reflect an underlying anxiety state (Lowry and Moore, 2006). In this respect, orexin A seems to resemble CRF. It is reported that CRF dramatically increases non-ambulatory activity and mildly increases ambulatory locomotion in a familiar environment, but decreases locomotor activity (number of line crossings) when rats were tested in an unfamiliar environment (open field) (Lowry and Moore, 2006). In our study, the testing apparatus can constitute a novel environment for males (especially for the high-motivated subjects) as a result of the presentation of the incentive animals. In fact, we observed that the subjects were shuttling frequently between the two incentive zones shortly after the tests began while the numbers of visits for both incentive zones were reduced when orexin A was delivered (Fig. 2F). Obviously, mild decrease of the distance moved in rats cannot account for reduction of the preference score, since the latter occurs only when ambulatory activity is severely impaired (Agmo, 2003a).

In summary, centrally administered orexin A attenuates male preference for the receptive female in high-motivated rats. These results suggest that endogenous orexin A might not play an important role in the expression of male unconditioned sexual motivation since there was a lack of effect on male preference for the receptive female for the OX_1R antagonist SB334867. This paper represents the first report on the effect of orexins on sexual motivation which contributes to the growing understanding of the role of orexins in various motivated behaviors.

Acknowledgements

The study was supported by the National Basic Research Program Grants (2009CB522002 & 2003CB515404 to N. Sui), the CAS Grant (KSCX1-TW-R-68 to N. Sui), the NNSF Grants (30770719,30600184,30470578 to N. Sui, Y.H. Li, X.G. Zheng) and the Grant (07CX081008 to Y.H. Li) from the Institute of Psychology, CAS. We thank Dr. G. Kirouac for his careful proofreading of this manuscript.

References

Adamantidis A, De Lecea L. Physiological arousal: a role for hypothalamic systems. Cell Mol Life Sci 2008;65:1475–88.

Agmo A. Sexual motivation — an inquiry into events determining the occurrence of sexual behavior. Behav Brain Res 1999;105:129–50.

Agmo A. Lack of opioid or dopaminergic effects on unconditioned sexual incentive motivation in male rats. Behav Neurosci 2003a;117:55–68.
Agmo A. Unconditioned sexual incentive motivation in the male Norway rat (*Rattus norvegicus*). J Comp Psychol 2003b;117:3-14.
Agmo A, Turi AL, Ellingsen E, Kaspersen H. Preclinical models of sexual desire: conceptual and behavioral analyses. Pharmacol Biochem Behav 2004;78:379–404.
Baldo BA, Daniel RA, Berridge CW, Kelley AE. Overlapping distributions of orexin/hypocretin- and dopamine-beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. J Comp Neurol 2003;464:220–37.
Boutrel B, Kenny PJ, Specio SE, Martin-Fardon R, Markou A, Koob GF, et al. Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. Proc Natl Acad Sci U S A 2005;102:19168–73.
Damsma G, Wenkstern D, Pfaus JG, Phillips AG, Fibiger HC. Sexual-behavior increases dopamine transmission in the nucleus-accumbens and striatum of male-rats – comparison with novelty and locomotion. Behav Neurosci 1992;106:181–91.
Dayas CV, McGranahan TM, Martin-Fardon R, Weiss F. Stimuli linked to ethanol availability activate hypothalamic CART and orexin neurons in a reinstatement model of relapse. Biol Psychiatry 2008;63:152–7.
De Lecea L, Kilduff TS, Peyron C, Gao XB, Foye PE, Danielson PE, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998;95:322–7.
Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA, Bloom SR. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. J Endocrinol 1999;160:R7–12.
Ellingsen E, Agmo A. Sexual-incentive motivation and paced sexual behavior in female rats after treatment with drugs modifying dopaminergic neurotransmission. Pharmacol. Biochem. Behav. 2004;77:431–45.
Fadel J, Deutch AY. Anatomical substrates of orexin–dopamine interactions: Lateral hypothalamic projections to the ventral tegmental area. Neuroscience 2002;111:379–87.
Gulia KK, Mallick HN, Kumar VM. Orexin a (hypocretin-1) application at the medial preoptic area potentiates male sexual behavior in rats. Neuroscience 2003;116:921–3.
Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, et al. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci U S A 1999;96:10911–6.
Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. Nature 2005;437:556–9.
Jones DN, Gartlon J, Parker F, Taylor SG, Routledge C, Hemmati P, et al. Effects of centrally administered orexin-B and orexin-A: a role for orexin-1 receptors in orexin-B-induced hyperactivity. Psychopharmacology (Berl) 2001;153:210–8.
Kavaliers M, Ossenkopp KP. Corticosterone rapidly reduces male odor preferences in female mice. Neuroreport 2001;12:2999–3002.
Kippin TE, Sotiropoulos V, Badih J, Pfaus JG. Opposing roles of the nucleus accumbens and anterior lateral hypothalamic area in the control of sexual behaviour in the male rat. Eur J Neurosci 2004;19:698–704.
Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci 2003;23:7-11.
Kunii A, Yamanaka A, Nambu T, Matsuzaki I, Goto K, Sakurai T. Orexins/hypocretins regulate drinking behavior. Brain Res 1999;842:256–61.
Lopez HH, Ettenberg A. Dopamine antagonism attenuates the unconditioned incentive value of estrous female cues. Pharmacol Biochem Behav 2001;68:411–6.
Louilot A, Gonzalez-Mora JL, Guadalupe T, Mas M. Sex-related olfactory stimuli induce a selective increase in dopamine release in the nucleus accumbens of male rats. A voltammetric study. Brain Res 1991;553:313–7.
Lowry CA, Moore FL. Regulation of behavioral responses by corticotropin-releasing factor. Gen Comp Endocrinol 2006;146:19–27.
Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, et al. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 2001;435:6-25.
Martynska L, Wolinska-Witort E, Chmielowska M, Bik W, Baranowska B. The physiological role of orexins. Neuro Endocrinol Lett 2005;26:289–92.
Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 2005;46:787–98.
Muschamp JW, Dominguez JM, Sato SM, Shen RY, Hull EM. A role for hypocretin (orexin) in male sexual behavior. J Neurosci 2007;27:2837–45.
Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, et al. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. Brain Res 2000;873:181–7.
Narita M, Nagumo Y, Hashimoto S, Narita M, Khotib J, Miyatake M, et al. Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. J Neurosci 2006;26:398–405.
Paredes RG, Agmo A. Has dopamine a physiological role in the control of sexual behavior? A critical review of the evidence. Prog Neurobiol 2004;73:179–226.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Orlando, FL: Academic; 1997.
Rhen T, Crews D. Variation in reproductive behaviour within a sex: neural systems and endocrine activation. J Neuroendocrinol 2002;14:517–31.
Rodgers RJ, Halford JC, Nunes de Souza RL, Canto de Souza AL, Piper DC, Arch JR, et al. Dose-response effects of orexin-A on food intake and the behavioural satiety sequence in rats. Regul Pept 2000;96:71–84.
Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998;92:573–85.
Scammell TE, Saper CB. Orexin, drugs and motivated behaviors. Nat Neurosci 2005;8:1286–8.
Siegel JM. Hypocretin (orexin): role in normal behavior and neuropathology. Annu Rev Psychol 2004;55:125–48.
Spinazzi R, Andreis PG, Rossi GP, Nussdorfer GG. Orexins in the regulation of the hypothalamic–pituitary–adrenal axis. Pharmacol Rev 2006;58:46–57.
Suzuki M, Beuckmann CT, Shikata K, Ogura H, Sawai T. Orexin-A (hypocretin-1) is possibly involved in generation of anxiety-like behavior. Brain Res 2005;1044:116–21.
Thorpe AJ, Cleary JP, Levine AS, Kotz CM. Centrally administered orexin A increases motivation for sweet pellets in rats. Psychopharmacology 2005;182:75–83.
Winsky-Sommerer R, Boutrel B, de Lecea L. The role of the hyopcretinergic system in the integration of networks that dictate the states of arousal. Drug News Perspect 2003;16:504–12.