Behavioural Brain Research 214 (2010) 225–230

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Behavioral characterization of non-copulating male rats with high spontaneous yawning frequency rate

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ARTICLE INFO

Article history:
Received 1 March 2010
Received in revised form 13 May 2010
Accepted 18 May 2010
Available online 25 May 2010

Keywords:
Non-copulating males
Yawning
Male sexual behavior
Sexual motivation
Olfaction
Penile erection

ABSTRACT

An important number of Sprague-Dawley males selected by strict inbreeding process for their high spontaneous yawing frequency (HY) fail to copulate after repeated exposure to receptive females. These HY males that fail to mate are called non-copulators (HYNC). The causes of this behavioral deficit are still unknown. The aim of the present study was to make a detailed behavioral characterization of these animals by evaluating: their partner preference between a sexually receptive female as opposed to a sexually active male; their ability to detect food related odors and their preference for sexually relevant chemosensory cues between bedding from estrous females, bedding from sexually active males and clean bedding. We also evaluated whether these males had alterations in motor function using a rotarod or in their general reward system mediated by opioids by injecting them with 1 mg/kg of morphine to evaluate if they develop conditioned place preference (CPP). At the end of these behavioral tests, we measured their plasmatic levels of testosterone (T). Together, these results will contribute to elucidate the causes of their deficient copulatory performance. Both HYNC and HY copulators (HYC) males showed a clear preference for receptive females as opposed to sexually active males. As well, both groups of animals had a similar ability to detect food related odors. HYC males had a clear preference for estrous female odors as opposed to male or clean bedding, but HYNC males spend the same amount of time sniffing estrous, anestrous, male and clean bedding. In both, HYC and HYNC, morphine induced CPP suggesting that in these males the reward system is functional. No differences were found in motor coordination or in T levels between HY and HYNC males. The behavioral deficit in HYNC male rats cannot be explained by an alteration in: partner preference, food related odor recognition, motor coordination, general reward system, or differences in plasmatic levels of T. However, HYNC males present clear deficits in recognizing sexually relevant odors. These results could, at least in part, explain the deficient execution of copulatory pattern in HYNC males.

1. Introduction

Yawning is a stereotypical behavioral pattern that is commonly associated with other behaviors such as grooming, penile erection, stretching, and arousal [47]. Several research groups have demonstrated an association between yawning and sexual excitement [29]. For example, systemic administration of dopamine agonists elicits yawning and penile erections in rats, mice, monkeys, and humans (review in [18]). In high-yawning (HY) rat subline, a strong correlation between spontaneous yawning and penile erection has been demonstrated [22]. Penile erections increase from 0.4 erections/h in the non-yawning rats to about 2 erections/h in the HY males that exhibit 26–30 yawns/h. The dopamine (DA) agonists; apomorphine and bromocriptine also increase yawning as well as penile erection in low-yawning (LY) and HY sublines [22]. The DA induced yawning and penile erection is blocked by D_2 or D_3 antagonists suggesting that this effect is mediated by D_2/D_3 receptors' family [10–12]. Opiate receptors appear also to be involved in the control of yawning, morphine injected systemically or in the paraventricular nucleus (PVN) of the hypothalamus blocks apomorphine, oxytocin [27] and N-methyl-D-aspartic acid (NMDA) [28] induced penile erection and yawning.

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doi:10.1016/j.bbr.2010.05.028

Yawning is sexually dimorphic, adult males yawn more frequently than females [13,30], suggesting that hormones might play a role in the incidence of this behavior. Castration disrupts sexual behavior, penile erections, and yawning [13,20,35]. The administration of testosterone restores yawning and penile erection frequency to pre surgery levels [34,35]. Cessation of testosterone treatment or administration of testosterone simultaneously with the non-steroidal antiandrogen hydroxyflutamide decreases yawning frequency [13]. In female monkeys, treatment with dihydrotestosterone (a nonaromatizable androgen) induces high levels of yawning from a baseline level of 0.3–4.7 yawns per 30 min after treatment. Female monkeys treated simultaneously with dihydrotestosterone and with the androgen receptor antagonist flutamide show a reduced yawn frequency of just 1.9 yawns per 30 min [19]. Taken together, these observations suggest that androgens are important modulators of yawning and penile erections.

Sprague-Dawley rats that differed in their spontaneous yawning frequency [46] were selectively bred. The High-Yawning (HY) subline has a mean of 20 spontaneous yawns per hour, and the Low-Yawning (LY) one has only an average of 2 yawns/h [17,46]. We have observed that a high proportion of HY males fail to mate after repeated exposure to sexually active females, and we refer to them as HY non-copulators (HYNC). The cause of this behavioral deficit is unknown. Studies in our research group previously demonstrated that Wistar non-copulating male rats (NC) show a reduced sexual motivation for a receptive female when given the opportunity to physically interact with them or when they can only see, smell, and hear her [37,38]. We also demonstrated that NC rats showed a reduced preference for chemosensory relevant olfactory cues from estrous females [37,38].

The aim of the present study was to make a detailed behavioral characterization of HYNC and evaluate if they do not mate due to alterations in: their partner or olfactory preference for sexually receptive females; in their motor coordination or in their plasmatic levels of testosterone. Several lines of evidence indicate that opioids are release during sexual behavior mediating the rewarding properties of mating in males and females rodents as evaluated by conditioned place preference (CPP). For example, systemic administration of the opioid antagonist naloxone blocks the reward state induced by ejaculations in males and females [32]. Moreover, it is well established that the opiate agonist morphine (1 mg/kg, ip) reliably induces CPP. Therefore, we evaluated if this treatment can induce CPP in HYNC to determine if the general reward system mediated by opioids is functional in these males.

2. Materials and methods

We used Sprague-Dawley HY males from F50 generation selectively bred to establish a high incidence of yawning (HY) [46] at the Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla. When males reached adulthood (3–4 months) they were shipped and maintained in the animal facility at the Instituto de Neurobiología at the Universidad Nacional Autónoma de Mexico. Male sexual behavior was registered in four 30-min tests, one per week, with receptive females. Females (2–3 months) were of the same strain and were ovariectomized and brought into estrus by hormone treatment with estradiol benzoate (25 μg/rat, 48 h) and progesterone (1 mg/rat, 6 h) before mating tests. Those HY males that ejaculated in each of the 4 screening tests were included in the group of HY copulating rats (HYC). HY males that did not show any sexual behavior during the 4 tests formed the HY non-copulating group (HYNC). We screen 30 HY males, 8 of them were classified as HYNC and 22 as HYC; we randomly used 8 of the HYC for the present experiments. The same subjects were evaluated in different tests to minimize the use of animals and to have a profile of each subject in the behavioral battery. Thus, they received the olfactory capacity, olfactory preference and partner preference test. For the conditioned place preference (CPP) test 5 HYC and 5 HYNC subjects received systemic morphine administration and 3 males of each group saline. For the motor coordination test we used 8 HYC and 6 HYNC males and for the measurement of testosterone levels 7 HYC and 4 HYNC.

All the experiments were carried out in accordance with the "Reglamento de la Ley General de Salud en Materia de Investigación para la Salud" of the Mexican Health Ministry which follows NIH guidelines and they were approved by the local animal care committee.

2.1. Olfactory capacity

Each rat was placed in an acrylic cage (25 cm × 50 cm × 15 cm) with fresh bedding covering the floor. For each test a piece of Oreo© cookie (5 mm^3) was randomly placed at one of the corners, hidden under the bedding. The time the male took to find the cookie was recorded. If the subject took more than 5 min, the test was ended. Each rat was evaluated twice, with a 1-week interval between tests.

2.2. Olfactory preference

Olfactory preference tests were performed in a chamber (40 cm × 60 cm × 40 cm) containing three bowls (12 cm × 11 cm × 5 cm). Each bowl had either clean, estrous, or male bedding. The clean bedding was clean fresh sawdust. Four sexually experienced adult males were housed together in cages containing fresh bedding, which was collected after 6 h (male bedding). Estrous bedding was obtained from cages containing 4 ovariectomized female rats treated with EB and P as described above. Bedding was collected between 6 and 8 h after P injection. In the odor preference test we and other researchers evaluate the total time that the animals spend sniffing each bowl with the stimulus odors [4,8,37,38]. The average of the 2 tests for each olfactory condition was calculated. The interval between the first and second test was 1 week.

2.3. Partner preference test

The partner preference test was carried out in a 3-compartment box (32 cm × 106 cm × 34 cm). The middle compartment communicates with the lateral compartments through a door. A sexually active male was placed in one of the lateral compartments and a sexually receptive female in the opposite lateral compartment. The stimulus animals wore a harness to prevent crossing into another compartment. Each experimental animal was placed in the middle compartment and was able to physically interact with both stimulus animals. The time spent with each of the stimulus animals was recorded. Two independent tests, separated by a 1-week interval, were done. Each test lasted 10 min.

2.4. Conditioned place preference

For the conditioned place preference test we used a box with 3 different compartments. The 2 lateral compartments are identical in size (23 cm × 37 cm × 32 cm). One lateral compartment is painted white and the floor is covered with sawdust. The other lateral compartment is painted black and moistened with a 2% acetic acid solution. The middle gray-painted compartment (22 cm × 24 cm × 32 cm) communicates with the lateral compartments through sliding doors (10 cm × 10 cm). Briefly, the procedure consisted of a pretest, 6 conditioning sessions, and the test. In the pretest (day 1) the subjects were placed in the middle compartment; after 1 min of habituation, the doors to the lateral compartments were opened, and the animals were allowed to access all 3 compartments for 10 min. The time spent in each of the compartments was recorded. During conditioning, the animals were injected subcutaneously (sc) with vehicle (1 mL/kg, of 0.9% saline) and placed in the preferred saline-paired compartment (non-rewarding) for 30 min (days 2, 4, and 6). On alternate sessions (days 3, 5, and 7), subjects were treated with morphine (1 mg/kg) and 30 min later, placed in the originally non preferred drug-paired compartment (rewarding) for 30 min. Several groups, including ours, have demonstrated that a subcutaneous injection of 1 mg/kg of morphine reliably induces CPP with no motor effects [3,7,23,39]. On day 8 the animals were tested in exactly the same way as the pretest. A group of HYC and HYNC males treated with saline during all sessions was included as a control (saline) for the morphine injection. In order to consider an induction of CPP two criteria were used, the time in the drug-paired side and the preference score (time in the drug-paired side divided by time in drug-paired side plus the time in the saline-paired side) should increase after conditioning.

2.5. Motor coordination

Motor coordination and balance were tested using an accelerating rotarod (AccuScan Instruments Inc). Rats were placed on a rod located 15 cm above a padded platform. The rod was connected to a motor that allows the selection of speed (revolutions per min, rpm) and acceleration rate. Each rat received 9 training trials. Each trial lasted 50 s with the speed increasing from 5 to 15 rpm during the first 30 s. In the last 20 s, the speed was decreased to 5 rpm. Subjects were considered trained when 90% of the males did not fall during the trial. Rats were tested using a longer and more difficult program; that is, the test lasted 135 s, and during that procedure the first 60 s the speed was increased from 5 to 15 rpm. In the remainder of the test, the speed was decreased again but with the rotarod rotating in the direction opposite to that during training. The time the subject stayed on the rotarod and the speed when the rat fell off were recorded.