Hormones and Behavior 63 (2013) 606–614

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

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

The medial preoptic area is necessary for sexual odor preference, but not sexual solicitation, in female Syrian hamsters

Luis A. Martinez *, Aras Petrulis

Georgia State University, Neuroscience Institute, 100 Piedmont Ave SE, Atlanta, GA, 30303, USA

ARTICLE INFO

Article history:
Received 27 August 2012
Revised 28 January 2013
Accepted 1 February 2013
Available online 12 February 2013

Keywords:
Olfaction
Chemosensory
Proceptive
Sexual motivation
Appetitive

ABSTRACT

Precopulatory behaviors that are preferentially directed towards opposite-sex conspecifics are critical for successful reproduction, particularly in species wherein the sexes live in isolation, such as Syrian hamsters (*Mesocricetus auratus*). In females, these behaviors include sexual odor preference and vaginal scent marking. The neural regulation of precopulatory behaviors is thought to involve a network of forebrain areas that includes the medial amygdala (MA), the bed nucleus of the stria terminalis (BNST), and the medial preoptic area (MPOA). Although MA and BNST are necessary for sexual odor preference and preferential vaginal marking to male odors, respectively, the role of MPOA in odor-guided female precopulatory behaviors is not well understood. To address this issue, female Syrian hamsters with bilateral, excitotoxic lesions of MPOA (MPOA-X) or sham lesions (SHAM) were tested for sexual odor investigation, scent marking, and lordosis. MPOA-X females did not investigate male odors more than female odors in an odor preference test, indicating that MPOA may be necessary for normal sexual odor preference in female hamsters. This loss of preference cannot be attributed to a sensory deficit, since MPOA-X females successfully discriminated male odors from female odors during an odor discrimination test. Surprisingly, no deficits in vaginal scent marking were observed in MPOA-X females, although these females did exhibit decreased overall levels of flank marking compared to SHAM females. Finally, all MPOA-X females exhibited lordosis appropriately. These results suggest that MPOA plays a critical role in the neural regulation of certain aspects of odor-guided precopulatory behaviors in female Syrian hamsters.

Introduction

Precopulatory behaviors that aid in the identification and localization of potential mating partners are an important component of reproductive behavior for most mammals (Beach, 1976). For species typified by non-cohabitating sexes such as Syrian hamsters (*Mesocricetus auratus*), these behaviors are essential for successful mating (Gattermann et al., 2001; Pfaff et al., 2008). Female Syrian hamsters engage in a number of different precopulatory or solicitational behaviors, including vaginal marking (a stereotyped scent marking behavior resulting in the deposition of vaginal secretion) and preferential approach towards, and investigation of, opposite-sex odors (Petrulis, 2009). Although both vaginal marking and opposite-sex odor preference are behavioral responses that are preferentially directed towards male compared to female odors (Johnston, 1977; Martinez and Petrulis, 2011; Petrulis and Johnston, 1999; Petrulis et al., 1999), odor preference is more clearly linked to the identification and localization of potential mating partners, whereas vaginal marking plays a key role in attracting mates. Indeed, the deposited secretion is highly attractive to male hamsters (Johnston, 1974; Johnston and Schmidt, 1979; Kwan and Johnston, 1980), and females deposit these marks in such a way as to direct the male to her nesting area (Lisk et al., 1983).

The expression of both sexual odor preference and vaginal marking depends critically on an interconnected set of brain areas that are more broadly involved in processing conspecific odor information (Petrulis, 2009). These areas include the medial amygdala (MA), the bed nucleus of the stria terminalis (BNST), and the medial preoptic area (MPOA) (Wood, 1998). Odor information detected by the main and accessory olfactory systems is initially processed by MA and relayed to MPOA, either directly or via BNST (Coolen and Wood, 1998; Wood and Swann, 2005). Not surprisingly, neurons in these brain areas exhibit selective activation to opposite- vs. same-sex odors in both male and female hamsters (DelBarco-Trillo et al., 2009; Maras and Petrulis, 2010). These areas also appear to play specific, functional roles in female precopulatory behaviors. For example, lesions of MA eliminate preferential investigation of male vs. female odors and reduce overall levels of vaginal marking by female hamsters, but do not eliminate preferential vaginal marking in response to male odors (Petrulis and Johnston, 1999). In contrast, females with lesions of BNST do not vaginal mark preferentially to male odors, but do display a normal preference to investigate male odors more than female odors (Martinez and Petrulis, 2011). These data suggest that although BNST may be a critical component of the neural circuit regulating vaginal marking responses to sexual odors, it is not

* Corresponding author. Fax: +1 404 413 5471.
E-mail address: lmartinez10@student.gsu.edu (L.A. Martinez).

0018-506X/$ – see front matter
http://dx.doi.org/10.1016/j.yhbeh.2013.02.003

necessary for the expression of sexual odor preference; therefore, odor information relevant for sexual odor preference processed by MA likely bypasses BNST and continues to other limbic/hypothalamic areas connected to MA, such as MPOA.

Although there is substantial evidence suggesting that MPOA is broadly involved in regulating sexual behavior in rodents (Hull and Dominguez, 2007; Sakuma, 2008), its specific role in odor-guided female precopulatory behaviors is not clear. In rats, radiofrequency lesions of MPOA decrease the amount of solicitational behaviors towards, and time spent with, a sexually-experienced male, and disrupt females' preference for intact compared to castrated male rat odors (Xiao et al., 2005). Furthermore, excitotoxic lesions of MPOA decrease the preference of female rats to spend time with intact males compared to estrous females (Guarraci and Clark, 2006). However, it should be noted that other researchers have found no effects of electrolytic lesions of MPOA on sexual odor/partner preference in female rats (Paredes et al., 1998) or ferrets (Robarts and Baum, 2007). Although comparable data for the role of MPOA in sexual odor preference in female hamsters is not available, this area does appear to be involved in other precopulatory behaviors that can be induced by opposite-sex odors. Indeed, large electrolytic lesions of MPOA that also damaged BNST decrease vaginal marking during interactions with males (Malsbury et al., 1977), and radiofrequency lesions of MPOA decrease ultrasonic vocalizations by females following exposure to male hamsters (Floody, 1989).

A significant limitation of previous studies examining the role of MPOA in odor-guided precopulatory behaviors is the lack of specificity in disrupting MPOA vs. nearby areas such as BNST. This is a critical issue given that these areas are highly interconnected and share similar patterns of connectivity with other brain areas that regulate precopulatory behaviors (Coolen and Wood, 1998; Simerly and Swanson, 1986, 1988; Wood and Swann, 2005). As mentioned above, we have recently utilized discrete, excitotoxic lesions in order to determine the role of BNST in preferential vaginal marking and sexual odor investigation (Martinez and Petrulis, 2011); however, it is not known if MPOA plays either a comparable or dissociable role from that of BNST in the regulation of these behaviors. In order to address this issue, we administered excitotoxic lesions of MPOA to female Syrian hamsters and measured the effects of lesions on sexual odor investigation and scent-marking responses. Given that specific lesions of MPOA disrupt sexual odor preference in male hamsters (Been and Petrulis, 2010), we hypothesized that MPOA would be necessary for the normal expression of preferential investigation of male odors by females. Furthermore, given the previously observed effects of MPOA/BNST disruption (Malsbury et al., 1977), and considering that implants of estradiol specifically into MPOA increase the expression of vaginal marking (Takahashi and Lisk, 1987; Takahashi et al., 1985), we hypothesized that MPOA would also be necessary for maintaining the overall levels of vaginal marking.

Materials and methods

Overview of design

Subjects were initially screened for adequate levels of vaginal marking to male odors (>5 marks/10 min), and then received either bilateral, excitotoxic lesions of MPOA or sham surgeries. Following recovery, subjects underwent a series of behavioral tests. First, subjects were tested for their investigatory responses to male and female odors (odor investigation tests). This consisted of an initial test to familiarize subjects with the testing apparatus (Clean test), followed by a volatile odor preference test utilizing conspecific odor stimuli (Odor preference test). Second, subjects were tested for their ability to discriminate the sexual identity of odor stimuli using a habituation–discrimination task (Odor discrimination test). Subjects were then tested for scent-marking responses to male or female stimuli on two days of the estrous cycle, diestrous day 2 and proestrus. Finally, to verify that MPOA lesions had not disrupted the ability of females to display copulatory behavior, subjects were tested during behavioral estrus for receptive sexual responses to a sexually experienced male.

Subjects

Adult female Syrian hamsters (*M. auratus*) were purchased from Harlan Laboratories (Indianapolis, IL, USA) at approximately 7–9 weeks of age. In addition to experimental subjects, a separate group of unrelated adult male and female Syrian hamsters was purchased from Harlan Laboratories to serve as stimulus animals. Animals were either individually housed (experimental subjects) or group housed (3–4 same-sex animals per cage; stimulus animals) in solid-bottom polycarbonate cages containing corncob bedding and cotton nesting material (Nestlets; Ancare, Bellmore, NY). Subjects and stimulus animals were maintained on a reversed light cycle (14:10 light:dark; lights out at 10 am), with all behavior testing occurring during the first 4 h of the dark portion of the light cycle. Food and water were available ad libitum. Animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23; revised 1996) and approved by the Georgia State University Institutional Animal Care and Use Committee. It should be noted that none of the survivable manipulations utilized in the present study resulted in animal mortality; furthermore, that all efforts were made to minimize the total number of animals used and their suffering.

Estrous cycle monitoring

Prior to screening for sufficient vaginal marking levels, subjects were examined daily for eight consecutive days in order to determine their stage of the estrous cycle. Subjects were gently restrained while vaginal secretion was manually extruded using a disposable probe, and the consistency of the secretion was examined for stringy consistency indicative of behavioral estrus (Orsini, 1961). Once the day of behavioral estrus was identified, the two cycle days prior to estrus were defined as diestrous day 2 and proestrus, respectively (Johnston, 1977). Estrous cycles were also monitored for eight days following surgery to ensure that this procedure had not disrupted cyclicity. Finally, in order to verify that estrous cycle stage had been properly inferred from cyclic changes in vaginal secretion consistency, females were tested for sexual receptivity in response to a male prior to the conclusion of behavioral testing (see Lordosis test below). In all cases, 'day' refers to the dark phase of the light cycle.

Surgery

At two to three months of age, subjects were assigned to either a MPOA lesion group (MPOA-X) or a sham lesion group (SHAM). A matched random assignment procedure was used, such that initial levels of vaginal marking in response to male odors on proestrus were equivalent across subjects assigned to the MPOA-X and SHAM groups (see below). Subjects were first anesthetized with 2–3% isoflurane gas (Baxter, Deerfield, IL) in an oxygen (70%) and nitrous oxide (30%) mixture, and then placed within a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) with ear- and incisor-bars positioned such that the top of the skull was level. Following a midline scalp incision, the skin and underlying temporal muscles were retracted to expose the skull. A hand-operated drill was then used to make holes in the skull in order to expose dura. For MPOA-X subjects, the excitotoxin *N*-methyl-D-aspartic acid (20 mg/ml, 25 nl per injection site; Sigma, St. Louis, MO) was injected bilaterally via a Hamilton microinjection syringe (701R 10 μl syringe; Hamilton, Reno, NV) under stereotaxic control. A single injection of excitotoxin was made per hemisphere, using the following coordinates: Anterior–posterior, +1.7 mm (relative to bregma); medial-lateral, ±0.6 mm