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Chemosensory and Steroid-Responsive Regions of the Medial Amygdala Regulate Distinct Aspects of Opposite-Sex Odor Preference in Male Syrian Hamsters (Mesocricetus Auratus)

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CHEMOSENSORY AND STEROID-RESPONISVE REGIONS OF THE MEDIAL AMYGDALA REGULATE DISTINCT ASPECTS OF OPPOSITE-SEX ODOR PREFERENCE IN MALE SYRIAN HAMSTERS (*MESOCRICETUS AURATUS)*

by

PAMELA M. MARAS

Under the direction of Aras Petrulis

ABSTRACT

In Syrian hamsters, sexual preference requires integration of chemosensory and steroid cues. Although data suggest that separate pathways within the brain process these two signals, the functional significance of this separation is not well understood. Within the medial amygdala, the anterior region (MEa) receives input from the olfactory bulbs, whereas the posterodorsal region (MEpd) is sensitive to steroid hormones. Lesions of either the MEa or MEpd eliminated preference to investigate female over male odors. Importantly, males with MEpd lesions displayed decreased attraction toward female odors, suggesting a decrease in sexual motivation. In contrast, males with MEa lesions displayed high levels of investigation of both female and male odors, suggesting an inability to categorize the relevance of the odor stimuli. These results suggest that both the MEa and MEpd are critical for the expression of opposite-sex odor preference, although they appear to mediate distinct aspects of this behavior.

INDEX WORDS: Chemosensory processing, Reproductive behavior, Odor preference, Sex preference, Y-maze, Habituation-dishabituation

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List of Abbreviations

ACo	anterior cortical nucleus of the amygdala
AHi	amygdalohippocampal area
AOB	accessory olfactory bulb
AOS	accessory olfactory system
BLP	basolateral nucleus of the amygdala
BMA	basomedial nucleus of the amygdala
BNST	bed nucleus of the stria terminalis
BNSTpi	bed nucleus of the stria terminalis, posterior intermediate zone
BNSTpm	bed nucleus of the stria terminalis, posterior mediate zone
CEa	central nucleus of the amygdala
cMEpd	posterodorsal medial amygdala, caudal
Endo	endopiriform nucleus
I	intercalated nucleus of the amygdala
IACUC	Institutional Animal Care and Use Committee
LOT	nucleus of the lateral olfactory tract
ME	medial amygdala nucleus
MEa	anterior medial amygdala
MEad	anterior medial amygdala, anterodorsal
MEa-SHAM	sham surgery group, anterior medial amygdala
MEav	anterior medial amygdala, anteroventral
MEaX	lesion group, anterior medial amygdala

MEpd	posterodorsal medial amygdala
MEpd-SHAM	sham surgery group, posterodorsal medial amygdala
MEpdX	lesion group, posterodorsal medial amygdala
MOB	main olfactory bulb
MOS	main olfactory system
MPOA	medial preoptic area of the hypothalamus
nAOT	nucleus of the accessory olfactory tract
OT	optic tract
PBS	phosphate-buffered saline
PIR	piriform cortex
PLCo	posterolateral cortical nucleus of the amygdala
PMCo	posteromedial cortical nucleus of the amygdala
RIA	radioimmunoassay
SI	substantia innominata
Uni-MEaX	lesion group, unilateral damage to the anterior medial amygdala
Uni-MEpdX	lesion group, unilateral damage to the posterodorsal medial amygdala
VNO	vomeronasal organ

Introduction

In many rodent species, such as the Syrian hamster, the expression of male reproductive behavior relies heavily on the perception of social odors from opposite-sex conspecifics. Two separate olfactory systems, the main olfactory system (MOS) and the accessory olfactory system (AOS), process social odors involved in reproductive behavior (Meredith, 1991; Restrepo, Arellano, Oliva, Schaefer, & Lin, 2004). In the MOS, primary sensory neurons are located in the main olfactory epithelium and respond to general volatile chemicals (Breer, 2003). In contrast, sensory neurons of the AOS are located in a specialized sensory epithelium, called the vomeronasal organ (VNO), and primarily respond to large, non-volatile chemicals (Halpern & Martinez-Marcos, 2003). Sensory neurons of the MOS and the AOS differentially project to the main (MOB) and accessory olfactory bulbs (AOB) in the brain, respectively (Menini, Lagostena, & Boccaccio, 2004). Together, these separate olfactory systems comprise the rodent "chemosensory" system, which is required for the expression of reproductive behaviors.

Male rodent reproductive behavior consists of two phases, the appetitive phase and the consummatory phase. The appetitive phase is characterized by the expression of approach and investigative behaviors that precede mating, whereas the consummatory phase is characterized by the expression of a stereotyped sequence of copulatory behaviors, including mounts, intromissions and ejaculations (Meisel & Sachs, 1994). Both phases of reproductive behavior require chemosensory processing. Removal of the olfactory bulbs in rats (Edwards, Griffis, & Tardivel, 1990; Larsson, 1975) or in mice (Rowe & Edwards, 1972) reduces or eliminates the expression of copulatory behavior. In

Syrian hamsters, either olfactory bulbectomy, (Murphy & Schneider, 1970) or simultaneous deafferentation of the main and accessory olfactory systems (Powers & Winans, 1975), eliminates copulation.

Similar to consummatory behavior, appetitive reproductive behavior also requires chemosensory processing. In male rats, chemosensory deafferentation eliminates preference for receptive females over non-receptive females (Edwards et al., 1990) and reduces non-contact penile erections in the presence of a female, a measure of sexual arousal toward a remote female stimulus (Edwards & Davis, 1997). In male Syrian hamsters, chemosensory processing is required for the extensive anogenital investigation of a receptive female (Devor & Murphy, 1973; Murphy & Schneider, 1970; Powers & Winans, 1975) that initiates the male copulatory sequence. Chemosensory disruption also reduces investigation of female hamster vaginal secretion (Powers, Fields, & Winans, 1979), a potent sexual chemosignal (Murphy, 1973). Thus, chemosensory processing is required not only for the execution of copulatory behavior, but also for the pre-copulatory recognition of, and attraction to, opposite-sex stimuli in the environment.

In addition to chemosensory processing, gonadal hormones regulate male reproductive behavior in many rodent species (Hull, Meisel, & Sachs, 2002). In Syrian hamsters, a reduction of circulating testosterone not only eliminates copulation (Morin & Zucker, 1978; Powers, Bergondy, & Matochik, 1985), but also affects appetitive aspects of male reproductive behavior. Specifically, castrated males show reduced anogenital investigation of a receptive female (Powers et al., 1985) and decreased attraction to female vaginal secretion (Petrulis & Johnston, 1995; Powers & Bergondy, 1983)

compared to intact males. These effects can be reversed by administration of testosterone or its primary metabolites, estradiol and 5 alpha-dihydrotestosterone (Petrulis & Johnston, 1995; Powers & Bergondy, 1983; Powers et al., 1985).

Importantly, the expression of reproductive behavior in male Syrian hamsters requires the integration of chemosensory and hormonal processing (Wood, 1998), and this integration likely occurs within the limbic circuitry that regulates mating behavior (Wood & Coolen, 1997; Wood & Newman, 1995a). Indeed, brain regions involved in reproductive behavior, including the medial amygdala (ME), the medial preoptic area (MPOA) and the bed nucleus of the stria terminalis (BNST), process chemosensory information (Coolen & Wood, 1998; Kevetter & Winans, 1981a, 1981b) and contain steroid receptors (Wood, Brabec, Swann, & Newman, 1992; Wood & Newman, 1993). Unilateral testosterone implants into either the MPOA/BNST or the ME partially restore copulatory behavior in castrated male hamsters (Wood & Newman, 1995b). Unilateral bulbectomy ipsilateral to the steroid implant, however, prevents the associated facilitation of copulatory behavior (Wood & Coolen, 1997; Wood & Newman, 1995a). These data suggest that the limbic mating circuit integrates chemosensory and hormonal cues, and that this integration is required for the normal expression of male reproductive behavior.

The ME has been suggested as one candidate region for the integration of chemosensory and hormonal cues, as it is a target for both types of information. First, the ME receives direct projections from both the main and accessory olfactory bulbs, as well as indirect projections from other cortical chemosensory structures (Coolen & Wood, 1998; Lehman & Winans, 1982). Second, the ME contains dense populations of androgen

and estrogen receptor-containing neurons (Gentry, Wade, & Blaustein, 1977; Li, Blaustein, De Vries, & Wade, 1993; Wood et al., 1992; Wood & Newman, 1993, 1999). This distribution of steroid receptors is found in both the male and female brain (Wood & Newman, 1999). Finally, neurons within the ME show increases in c-fos expression, a measure of neuronal activity, following either copulation (Fernandez-Fewell & Meredith, 1994; Wood & Newman, 1993) or chemoinvestigation of female vaginal secretion (Fernandez-Fewell & Meredith, 1994; Fiber, Adames, & Swann, 1993; Swann, Rahaman, Bijak, & Fiber, 2001; Wood & Newman, 1993).

Functionally, the ME has been shown to play a critical role in odor-mediated appetitive aspects of reproductive behavior (Newman, 1999). Specifically, males with lesions of the ME show reduced anogenital investigation of a receptive female compared to control males (Lehman, Winans, & Powers, 1980). Similarly, lesions of the ME in females eliminates opposite-sex odor preferences and flank marking behavior, and decreases vaginal marking to sexual odors (Petrulis & Johnston, 1999). Finally, in castrated males, steroid implants into the ME can restore anogenital investigation of a receptive female (Wood, 1996; Wood & Newman, 1995b).

Although the ME has been shown to process both chemosensory and hormonal information, detailed anatomical evidence suggests that the two signals are processed in separate, parallel pathways within the ME. Specifically, these studies suggest that the anterior medial amygdala (MEa) processes chemosensory information, whereas the posterodorsal medial amygdala (MEpd) processes signals of hormonal state.

First, the MEa and the MEpd are differentially connected with chemosensory neural circuitry. The MEa has broad connections with both main and accessory olfactory circuits. Specifically, the MEa receives direct projections from both the MOB and the AOB and sends efferents back to the mitral and granule cell layers of the AOB (Coolen & Wood, 1998; Lehman & Winans, 1982). In addition, extensive bidirectional connections link the MEa with cortical chemosensory regions including the nucleus of the accessory olfactory tract (nAOT), endopiriform nucleus (Endo), piriform nucleus (PIR), and the anterior cortical (ACo), posterolateral cortical (PLCo), and posteromedial cortical nucleus (PMCo) of the amygdala (Coolen & Wood, 1998; Gomez & Newman, 1992).

In contrast to the MEa, the MEpd has less extensive connections with chemosensory circuitry. The MEpd does not receive direct projections from the MOB and only sparse, unidirectional projections from the AOB (Coolen & Wood, 1998; Lehman & Winans, 1982). Although the MEpd has moderate connections with the PMCo, part of the accessory olfactory circuit, its connections with nuclei in the main olfactory circuit, including the nAOT, Endo, PIR and ACo, are all substantially less dense than those of the MEa (Coolen & Wood, 1998; Gomez & Newman, 1992).

Second, the MEa and the MEpd contain different concentrations of steroid receptor-containing neurons. The MEpd contains dense concentrations of androgen and estrogen receptor-labeled neurons, whereas the MEa contains considerably fewer steroid-receptor labeled neurons compared to the MEpd (Doherty & Sheridan, 1981; Wood et al., 1992; Wood & Newman, 1993). Steroid implant studies support the role of the MEpd in the hormonal modulation of reproductive behavior. Testosterone (Wood & Newman,

1995b) or estradiol (Wood, 1996) implants facilitate appetitive and some consummatory aspects of reproductive behavior when directed at the MEpd, but not when directed at the MEa.

Lastly, this anatomical separation continues throughout the connected circuit that regulates reproductive behavior. As is the case within the ME, chemosensory and steroid cues may be processed by specific subdivisions of the BNST and MPOA. The posterior medial zone of the BNST (BNSTpm) and the medial subdivisions of the MPOA contain high concentrations of steroid receptor-containing neurons (Li et al., 1993; Wood & Newman, 1993) and are strongly linked to the MEpd (Coolen & Wood, 1998; Gomez & Newman, 1992). The posterior intermediate zone of the BNST (BNSTpi) and the lateral subdivisions of the MPOA contain relatively fewer steroid receptor-containing neurons (Li et al., 1993; Wood & Newman, 1993) and are preferentially connected with the MEa (Coolen & Wood, 1998; Gomez & Newman, 1992). MEpd efferents pass mainly through the stria terminalis, whereas MEa efferents pass through both the stria terminalis and the ventral amygdalofugal pathway (Coolen & Wood, 1998). Importantly, reciprocal fibers connect the chemosensory and steroid-responsive regions of the ME, BNST and MPOA (Coolen & Wood, 1998; Gomez & Newman, 1992), providing a substrate for the integration of chemosensory and hormonal information at each level.

The functional significance of this parallel processing within the ME in mediating reproductive behavior is unclear. Lesion experiments have demonstrated that the MEa, but not the MEpd, is essential for copulation. Lesions of the MEa consistently abolish the expression of all copulatory behavior (Lehman et al., 1980), whereas lesions that

primarily include the MEpd only affect the temporal pattern of these behaviors (Lehman, Powers, & Winans, 1983). In these separate experiments, both MEa and MEpd lesions decrease anogenital investigation, although MEa lesions cause a more robust deficit. Consistent with MEpd's primarily strial projections, lesions of either the MEpd or the stria terminalis itself result in similar behavioral deficits (Lehman et al., 1983).

In contrast to the lesion data, c-fos studies suggest that the MEpd is more involved in the expression of reproductive behavior compared to the MEa. Copulation (Kollack & Newman, 1992) or exposure to female hamster vaginal secretion (Swann et al., 2001; J. M. Swann, 1997) results in higher levels of c-fos expression in neurons in the MEpd than in the MEa. Furthermore, c-fos expression in the MEpd, but not in the MEa, is increased specifically following mating rather than agonistic encounters (Kollack-Walker & Newman, 1995). Finally, clusters of c-fos expression within the MEpd may signal the onset of sexual satiety following multiple ejaculations (Parfitt & Newman, 1998).

Although chemosensory and hormonal regulation of reproductive behavior is likely mediated through distinct circuits within the ME, no studies have directly tested the role of these pathways in regulating odor-guided approach and investigative pre-copulatory behavior. Thus, the current set of experiments tested the function of the MEa and the MEpd in mediating opposite-sex odor preference in male hamsters. Importantly, the expression of these odor preferences requires both an ability to identify the source of each social odor as well as motivation to investigate the sexually relevant female odor. We hypothesized that the chemosensory MEa primarily functions to categorize the

relevance of social odors, whereas the steroid-responsive MEpd mediates the permissive effects of sex steroids on motivation to investigate female odors. If so, then males with lesions of the MEa should show equal investigation of the female odors and male odors, but display normal levels of attraction to the social odors. In contrast, males with lesions of the MEpd may still prefer to investigate female odors compared to male odors, but should show decreased levels of sexual attraction to investigate the female odors.

Experiment 1

The goal of Experiment 1 was to test the role of the MEa and the MEpd in generating preference for opposite-sex odors in male hamsters. Subjects were tested for their preference for female odors over male odors when presented simultaneously in a Y-maze apparatus. One problem with interpreting results in these preference tests, however, is that an observed preference for one stimulus may actually reflect an active avoidance of the other stimulus. Thus, attraction tests, during which each sexual odor was presented opposite a clean odor, was used to measure attraction to each sexual odor alone.

Methods

Subjects

Subjects were sexually naïve male golden hamsters (*Mesocricetus auratus*), purchased from Charles River Laboratory (Wilmington, MA, USA) at 3 weeks of age and were between 3-6 months of age at the time of behavioral testing. All subjects were gonadectomized and implanted subcutaneously with testosterone Silastic capsules at least one week prior to lesion surgery (see below). A separate group of male and female golden hamsters, 3-12 months old, served as odor donors. These hamsters were either

bred in our colony or purchased from Charles River Laboratory. Subjects were unrelated to, and had no previous contact with, these odor donors. All animals were singly housed in solid-bottom Plexiglas cages (36 cm X 30 cm X 16 cm) and maintained on a reversed 14-h light/ 10-h dark photoperiod. Food and water were available *ad libitum*. The Georgia State University Institutional Animal Care and Use Committee (IACUC) approved all animal procedures.

Surgery

Castration and testosterone implant. In male hamsters, exposure to female odors causes an increase in serum testosterone levels (Macrides, Bartke, Fernandez, & D'Angelo, 1974; Pfeiffer & Johnston, 1992), and it is possible that lesions of the ME may interfere with this testosterone surge. Thus, in order to clamp steroid hormone levels across experimental groups, subjects were gonadectomized and given exogenous testosterone.

Subjects were gonadectomized under 1-2% isolfurane anesthesia (100% oxygen) at least one week prior to lesion surgery. Following a midline abdominal incision, the testicles were removed bilaterally via cauterization of the ductus deferens and blood vessels. Vicryl suture (size 4-0, Ethicon, Somerville, NJ) and wound clips were used to close the smooth muscle and the skin incision, respectively. Silastic capsules (i.d. 1.57 mm, o.d. 2.41 mm, Dow Corning, Midland, MI) packed with 20 mm length of crystalline testosterone (Sigma Chemical Co., St. Louis, Mo) were implanted subcutaneously immediately following gonadectomy.

Electrolytic lesions. Subjects were randomly assigned to one of four lesion groups: MEa lesion (MEaX, n = 41); MEpd lesion (MEpdX, n = 24); MEa sham surgery (MEa-SHAM, n = 13); and MEpd sham surgery (MEpd-SHAM, n = 7).

Under 1-2% isolfurane anesthesia (7:3 oxygen: nitrous oxide mix), subjects were positioned in stereotaxic ear bars so that the skull was flat. The temporal muscles were retracted from the skull and small holes were drilled to expose the dura. Bilateral electrolytic lesions were made by lowering a platinum/iridium electrode (0.25 mm diameter, 0.45 mm uninsulated tip, Frederick Haer & Co., Bowdoinham, ME) under stereotaxic control and passing anodal current from a lesion making device (Ugo Basile, Comerio, VA, Italy).

Lesions of the MEa were made by passing 10-12 seconds of 1mA of current at two bilateral sites (four penetrations total). The two stereotaxic coordinates for the MEa lesions were: 0.65 mm posterior to bregma, ± 2.70 mm from the midline, and 7.45 mm below dura; 0.00 mm posterior to bregma, ± 2.70 mm from the midline, and 7.45 mm below dura. Lesions of the MEpd were made by passing 5-6 seconds of 1mA of current at three bilateral sites (six penetrations total). The three stereotaxic coordinates for the MEpd lesions were: 0.50 mm posterior to bregma, ± 2.80 mm from the midline, and 7.20 mm below dura; 0.62 mm posterior to bregma, ± 2.80 mm from the midline, and 7.30 mm below dura; 0.92 mm posterior to bregma, ± 2.70 mm from the midline, and 7.20 mm below dura. Sham surgeries were identical to lesion surgeries except that the electrode was lowered 1.5 mm above the target coordinate and no current was passed.

Gel foam (Pharmacia & Upjohn Co., Kalamazoo, MI) was used to pack the holes and the incision was closed with suture or wound clips.

Odor Stimuli

All odor stimuli were collected from cages that had housed a single odor donor and had not been changed for 10-14 days. When collecting odor samples, clean latex gloves were worn to prevent odor transfer. Odor stimuli consisted of 12 g of soiled cotton bedding (4 Nestlets, ANCARE, Bellmore, NY); 50 ml of soiled corn-cobb litter; one damp cotton gauze pad that was used to wipe the inner walls of the odor donor cage; and another damp gauze pad that was used to wipe the odor donor's bilateral flank and anogenital region ten times each. For female odor stimuli, vaginal secretion was collected onto an additional gauze pad by inducing a female in behavioral estrus into lordosis and gently palpating the vaginal area with a disposable probe. Clean odor stimuli consisted of unsoiled components identical to those of the sexual odor stimuli. All odor stimuli were stored in plastic bags at 4°C until twenty minutes before use. Odor samples older than three months were discarded, and care was taken to ensure that subjects were not tested with the same individual's odor more than once.

Behavioral Testing

To measure preferences for, and attraction to, male and female odors, subjects were tested in an enclosed, Plexiglas Y-maze (Petrulis & Johnston, 1999). The Y-maze consisted of a stem arm (61 cm long) and two side arms (68 cm long). All arms of the maze were 10 cm wide, with walls 10 cm high. The side arms angled off from the stem at 120° and at half of their length, bent back inward 120°. Each side arm had a stimulus

chamber (20 cm long) at its distal end. During testing, a single odor stimulus (see *Odor Collection*) was placed inside each stimulus chamber. Stimulus chambers had perforated doors that allowed air flow, but prevented contact with the odor stimuli. Thus, for all Y-maze tests, subjects were exposed to only the volatile odorants of the stimulus. A start chamber (20 cm long), with a removable, perforated door, was located at the distal end of the stem arm. An electric fan behind the start chamber pulled air from the stimulus chambers through the entire length of the Y-maze. The top of the Y-maze was secured with a clear Plexiglas top to allow for overhead video recording of animal behavior.

Each subject was tested twice in each of four types of Y-maze tests (Clean, Preference, Attraction to female, and Attraction to male), for a total of eight tests (Figure 1). First, to habituate the subjects to the Y-maze and obtain baseline data, subjects were exposed to clean odor stimuli in each stimulus chamber (Clean Test). Next, to test for opposite-sex odor preferences, subjects were exposed to male odors and female odors in opposite stimulus chambers (Preference Test). Last, to test for overall levels of attraction to social odors, subjects were exposed to each sex odor stimulus (males and female) against a clean odor stimulus (Attraction Test). For preference and attraction tests, the stimulus sides were reversed across repeated testing days. For attraction tests, the order of which sexual odor was tested first was counterbalanced across lesion groups.

All testing was done in the first six hours of the dark phase and under dim light conditions. Subjects were placed in the start chamber for one minute, after which, the door was removed and the subjects were allowed nine minutes to explore the maze. Following testing, surfaces of the Y-maze were thoroughly cleaned with 70% alcohol and

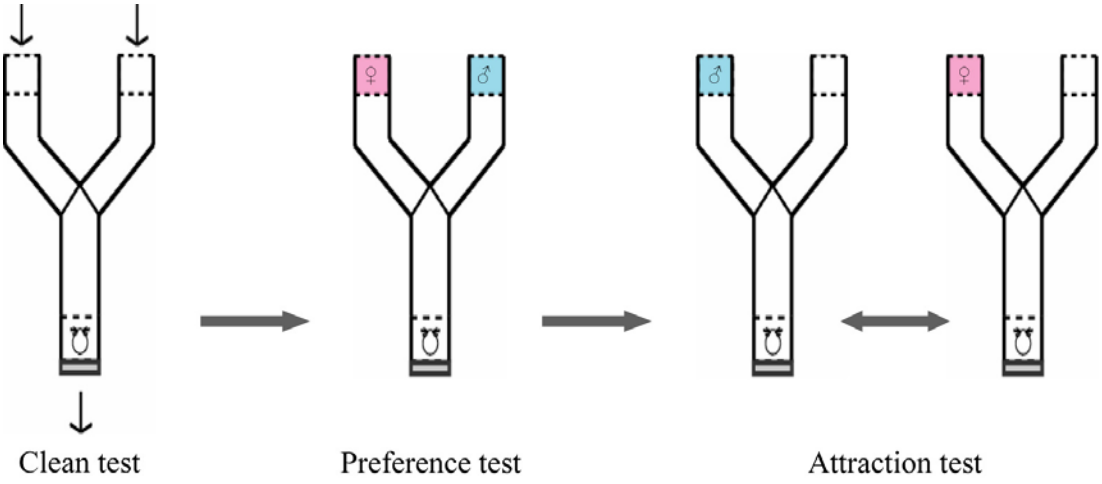

Figure 1. Illustration of Y-maze behavioral testing sequence in Experiment 1. Subjects were tested twice in each type of Y-maze test (eight total tests). Clean tests had clean odor stimuli in both stimulus chambers; Preference tests had male and female odors in opposite stimulus chambers; and Attraction tests had male or female odors opposite clean odors. For Preference and Attraction tests, stimulus sides were reversed across repeated test days. For Attraction tests, which sexual odor was tested first was counterbalanced across lesion groups.

allowed to dry between subjects. To conserve stimulus odors, each odor was used for two consecutive Y-maze tests. The order of subject testing was reversed across repeated testing days to ensure that each subject was tested one time with fresh odor stimuli and one time with re-used odor stimuli.

Video recordings of all Y-maze tests were digitized onto a computer and scored using the Observer for Windows, version 5.0 (Noldus Information Technology B.V., Wageningen, The Netherlands). All observers were blind to the condition of the subject and different observers reached at least an 85% inter-observer reliability score prior to coding behavior. Investigation of the stimulus chamber was coded when the subject made contact with, or directed its nose within 1 cm from, the odor door. The number of entries into each arm and the total duration of investigation of each odor stimulus were calculated for each test.

Histology and Lesion Verification

Following the last behavioral test, subjects were injected with an overdose of Nembutal (100 mg/kg) and transcardially perfused with 200 ml of 0.1M phosphate-buffered saline (PBS, pH 7.4) followed by 200 ml of phosphate-buffered formalin. Brains were post-fixed in phosphate-buffered formalin overnight and then cryoprotected for 48-72 hours in 30% sucrose in PBS solution. Brains were coronally sectioned at 40-µm on a cryostat (-20°C) and stored in PBS until mounting. Every third section was mounted onto glass slides using a 1% gelatin mounting solution and stained with cresyl violet.

Sections were examined under a light microscope for the location and extent of lesion damage compared with published hamster neuroanatomical plates (Morin &

Wood, 2001). Brain sections from subjects with minimum- and maximum-sized lesions were captured at 5X magnification by a Zeiss Axiocam using Axiovision 4.0 software (2002). These lesions were traced onto stereotaxic figures using Adobe Ilustrator CS 11.0 software (2003).

Blood Collection and Radioimmunoassay

Blood samples were collected from the inferior vena cava immediately prior to perfusion and stored in vacutainer collection tubes (VWR, West Chester, PA., 4 ml draw, red/gray) on ice until centrifuging. Samples were centrifuged at 3200 rpms, at 4°C for 20 minutes. Serum was stored in 200µl aliquots at -20°C until the testosterone assay. Testosterone levels were measured by radioimmunoassasy (RIA) kits from Diagnostics System Laboratories (DSL 4000 Testosterone), with a sensitivity range of 0.05-22.92 ng/nl and an inter-assay reliability of 6%, previously validated for hamster serum (Cooper, Clancy, Karom, Moore, & Albers, 2000).

Statistical analysis

For each type of test, data were averaged across both test days. To determine preferences for one odor stimulus over the other, separate mixed-design ANOVAs, with stimulus as the within-subjects factor and lesion group as the between-subjects factor, were performed for each Y-maze test (Clean, Preference, Attraction to female and Attraction to male). Interactions were explicated using simple effects analyses. To compare stimulus investigation times across lesion groups, separate one-way ANOVAs were performed for each stimulus in each Y-maze test. As a measure of general activity level, a one-way ANOVA was used to compare the total number of arm entries made

during the clean tests across lesion groups. Finally, a one-way ANOVA was used to compare testosterone levels across lesion groups.

Results

Lesion Reconstruction

Males were included in the MEaX lesion group (n = 13) or the MEpdX lesion group (n = 12) only if they had extensive bilateral damage to the MEa or the MEpd, respectively. Specifically, males were included in either lesion group only if they had at least 60% bilateral damage of the MEa or the MEpd in at least two stereotaxic planes of section (Morin & Wood, 2001; Figure 2). Data from males were excluded from the analyses if there was either substantial sparing of either region (MEa, n = 14; MEpd, n = 4) or if there was extensive lesion damage to both subnuclei (more than 15% bilateral damage in at least one stereotaxic plane of section; n = 3).

For additional comparison groups, males that had substantial unilateral damage of either the MEa or the MEpd were assigned to the Uni-MEaX lesion group (n = 14) or the Uni-MEpdX lesion group (n = 5), respectively. Males with unilateral MEa lesions typically had partial damage to the anterior cortical nucleus of the amygdala (ACo) and the anterior basomedial nucleus of the amygdala (BMA) contralateral to the MEa lesion. Males with unilateral MEpd lesions typically had partial damage of the intercalated nucleus (I), and the posterior basolateral nucleus of the amygdala (BLP) contralateral to the MEpd lesion.

All males in the MEaX group had lesion damage primarily restricted to the MEa. Seven males had bilateral lesion damage of both the dorsal (MEad) and ventral (MEav)

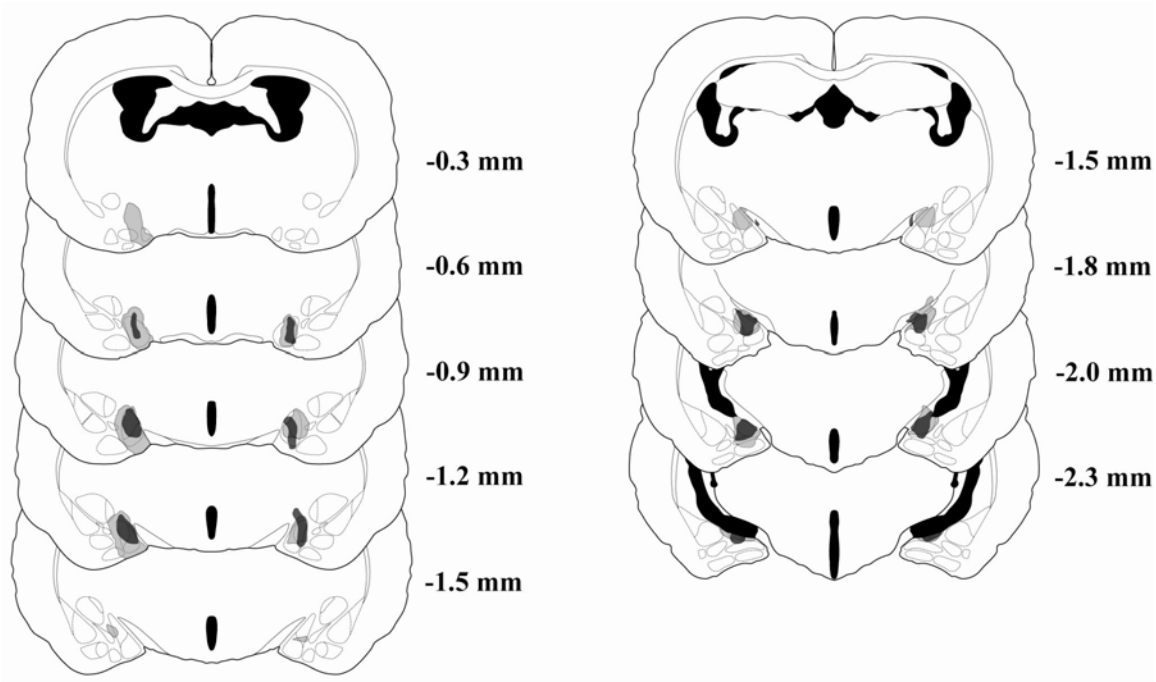

Figure 2. Reconstruction of coronal sections of the largest (light gray) and smallest (dark gray) electrolytic lesions in MEaX males (left panel) and MEpdX males (right panel) in Experiment 1. Sections proceed from anterior (top) to posterior (bottom) levels with the numbers representing the distance posterior from bregma.

regions of the MEa, three males had unilateral damage of both the MEad and MEav, and three males had damage primarily restricted to the MEad. Lesion damage spread to the ventral surface of the brain unilaterally in two males and bilaterally in one male. These males were not different in their behavior compared to males without ventral spread. In addition to damage of the MEa, a subset of MEaX males also had slight, partial damage (less than 15% at only one plane of section) to adjacent nuclei. Eleven males had damage to the most rostral section of the MEpd. This damage was unilateral in six males and bilateral in five males. Males with bilateral, unilateral, or no MEpd spread did not differ in their behavior in the Y-maze.

Other brain areas that had unilateral, partial damage included: central nucleus of the amygdala (CeA, n = 6); optic tract (OT, n = 2); ACo (n = 2); nucleus of the lateral olfactory tract (LOT, n = 1); substantia innominata (SI, n = 1); and I (n = 3). One male had substantial bilateral damage of the CeA and was therefore removed from the MEaX group.

In the MEpdX group, all males had lesion damage primarily restricted to the MEpd, including bilateral (n = 11) or unilateral (n = 1) damage to the caudal region of the MEpd (cMEpd). In addition to damage of the MEpd, some males also had slight, partial (less than 15% at only one plane of section) damage to adjacent nuclei. One male had unilateral damage to the caudal section of the MEa. Other brain areas that were partially damaged in a subset of MEpdX males included: OT (unilateral, n = 2); I (unilateral, n = 3; bilateral, n = 2); amygdalohippocampal area (AHi, unilateral, n = 9); and posteromedial cortical amygdaloid nucleus (PMCo, unilateral, n = 1).

Only electrode tracts were visible in most SHAM males; two MEa-SHAM males and one MEpd-SHAM male also had unilateral cortical damage. These males did not differ in behavior from males without cortical damage and were kept in the analysis. One MEpd-SHAM male died prior to the end of behavioral testing.

Behavioral measures

There was no difference in odor investigation times between MEa-SHAM and MEpd-SHAM groups for any of the Y-maze tests, all $p > .05$; these groups were subsequently combined into one overall SHAM group (n = 19).

Clean tests. All experimental groups investigated the left and right sides of the Y-maze equally, $F(1, 41) = 0.778$, $p > .05$, suggesting that there was no bias towards investigating one stimulus side over the other. Furthermore, when the investigation times were summed for the left and right arms, experimental groups did not differ in their total duration of investigation, $F(2, 41) = 1.818$, $p > .05$, suggesting equivalent levels of general investigation of the stimulus chambers. Levels of activity, as measured by the total number arm entries made in the clean tests, were also not different across experimental groups, $F(2, 41) = 0.887$, $p > .05$. Table 1 summarizes behavioral measures from the clean tests.

Preference tests. There was a significant interaction between experimental group and preference for investigating female over male odors, $F(2, 41) = 5.580$, $p < .01$. SHAM males preferred female odors, indicated by their longer investigation of female odors compared to male odors, $F(1,18) = 9.338$, $p < .01$, but neither the MEaX males,

Table 1. Mean behavioral measures (± SEM) from Clean Y-maze tests in Experiment 1. Investigation times are in seconds. All groups investigated the left and right stimulus sides equally, and there were no differences in general activity or total investigation levels across lesion groups.

	Total number of arm entries	Left stimulus investigation time	Right stimulus investigation time
SHAM	17.368 ± 1.729	55.718 ± 14.448	58. 410 ± 8.907
MEpdX	19.250 ± 1.393	51.512 ± 7.957	65.693 ± 9.581
MEaX	20.962 ± 1.614	75.156 ± 10.687	63.841 ± 9.008

$F(1,12) = 3.624$, $p > .05$, nor the MEpdX males, $F(1,11) = 0.474$, $p > .05$, showed a preference for either odor and investigated the two sexual odors equally (Figure 3a). In fact, the MEaX males showed a trend to prefer the male odors over the female odors, $p = .081$.

When the investigation times of the female and male odors were summed, there was a difference in total duration of odor investigation across experimental groups, $F(2,41) = 19.603$, $p < .001$. Tukey's post-hoc tests revealed that the MEaX males had higher levels of total odor investigation compared to both the MEpdX and SHAM males. This higher level of total odor investigation by the MEaX males is explained by their longer investigation times of both the female odors, $F(2,41) = 3.754$, $p < .05$, and the male odors, $F(2,41) = 24.288$, $p < .001$.

Attraction tests. A significant interaction between experimental group and attraction to the female odors was observed, $F(2, 41) = 4.532$, $p < .05$. Although both the MEaX males, $F(1,12) = 11.596$, $p < .01$, and SHAM males, $F(1,18) = 7.691$, $p < .05$, investigated female odors longer than clean odors, the MEpdX males showed no such attraction and investigated the female and clean odors equally, $F(1,11) = 1.022$, $p > .05$ (Figure 3b). In addition, MEaX males investigated the female odors longer than both the MEpdX and SHAM males, $F(2,41) = 8.940$, $p < .01$. There was no difference, however, in the investigation times of the clean odors across experimental groups, $F(2,41) = 1.754$, $p > .05$.

An interaction between experimental group and attraction to the male odors was also observed, $F(2, 41) = 3.978$, $p < .05$. Only MEaX males, but not MEaX or SHAM

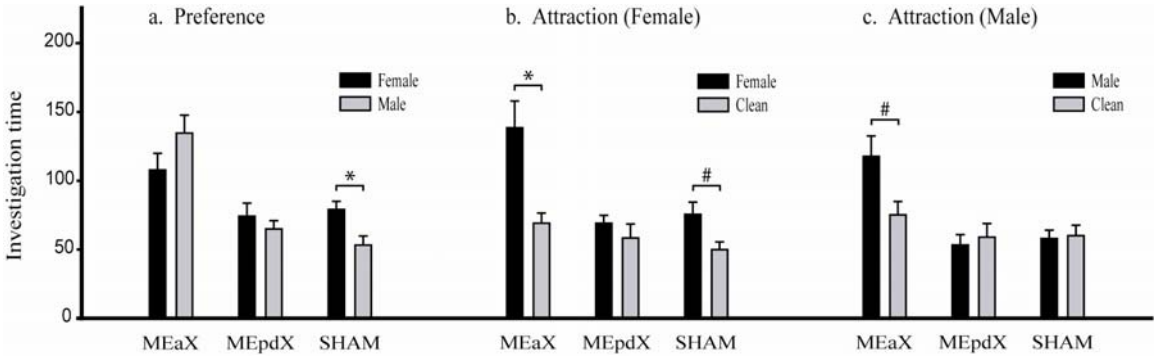

Figure 3. Mean (± SEM) odor investigation times (seconds) from each Y-maze test in Experiment 1. (a) SHAM males, but not MEaX or MEpdX males, preferred to investigate female odors over male odors (b) SHAM males and MEaX males, but not MEpdX males, were attracted to female odors over clean odors and (c) Only MEaX males were attracted to male odors over clean odors. * $p < .01$; # $p < .05$.

males, showed an attraction to the male odors; MEaX males, $F(1,12) = 5.157$, $p < .05$; MEpdX males; SHAM males, (Figure 3c). Furthermore, MEaX males investigated the male odors longer than both the SHAM and MEpdX males, $F(2,41) = 13.125$ $p < .05$. There was no difference, however, in the investigation times of the clean odors across experimental groups, $F(2,41) = 0.956$, $p > .05$.

Effects of unilateral damage of the MEa or the MEpd. Males with unilateral damage of the MEa were indistinguishable from SHAM males in their investigation behavior in each test. Indeed, Uni-MEaX males preferred female odors over male odors, $F(1,13) = 22.181$, $p < .05$, and were attracted to female odors over clean odors, $F(1,13) = 18.005$, $p < .05$, but were not attracted to male odors over clean odors, $F(1,13) = 0.599$, $p > .05$. Although Uni-MEpdX males showed patterns of preference and attraction behavior similar to those of SHAM males, odor investigation times were not significantly different due to the small number of males in this group (n = 5). Table 2 summarizes behavioral measures in the Preference and Attraction tests for Uni-MEaX and Uni-MEpdX males.

Testosterone assay

There was no difference in testosterone levels (ng/nl) between MEa-SHAM and MEpd-SHAM groups, $F(1,17) = .581$, $p > .05$; therefore, these groups were collapsed into one SHAM group for testosterone comparisons. Subsequent analysis showed no difference in testosterone levels across experimental groups, $F(2,41) = .766$, $p > .05$, (MEaX $M = 4.296$, $SD = 2.591$; MEpdX $M = 5.468$, $SD = 2.869$; SHAM $M = 5.120$, $SD = 2.082$).

Table 2. Mean odor investigation times (± SEM) in each Y-maze test from males with unilateral damage of either the MEa or the MEpd in Experiment 1. Uni-MEaX males preferred female odors over male odors and were attracted to female odors over clean odors. Uni-MEpdX males showed a similar pattern of preference and attraction, but odor investigation times were not significantly different. * $p < .01$, compared to investigation time of opposite-odor stimulus in test.

		Uni-MEaX	Uni-MEpdX
Preference	Female odor	128.219 ± 12.352*	125.052 ± 30.141
	Male odor	86.583 ± 9.375	50.228 ± 8.844
Attraction (female)	Female odor	130.637 ± 18.459*	114.342 ± 18.766
	Clean odor	29.541 ± 7.573	68.784 ± 11.944
Attraction (male)	Male odor	95.656 ± 13.591	63.982 ± 8.690
	Clean odor	82.677 ± 11.936	82.102 ± 10.961

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Summary

The results from this experiment demonstrate that both the MEa and the MEpd mediate opposite-sex odor preference in male hamsters. Indeed, neither MEaX nor MEpdX males showed a preference to investigate female odors over male odors. MEaX and MEpdX males were different, however, in their attraction toward social odors. Specifically, SHAM and MEaX males, but not MEpdX males, investigated the female odors over the clean odors. Furthermore, MEaX males were unique in that they were highly attracted to investigate the male odors. MEaX males also displayed notably high levels of social odor investigation, regardless of the sex of the odor stimulus; MEaX males investigated both female and male odors longer than did SHAM and MEpdX males. Importantly, MEaX males did not have increased investigation of the clean odor stimulus, suggesting that their high level of investigation behavior was specific to social odors.

Experiment 2

Experiment 1 demonstrated that males with either MEa or MEpd lesions do not show a preference to investigate female odors over male odors. This lack of preference, however, may reflect either a decrease in sexual motivation to investigate the female odor or a sensory deficit in the ability to discriminate between the two odors. Thus, in Experiment 2, a habituation-dishabituation model was used to explicitly test the ability for subjects to discriminate between male and female odors. In addition, to demonstrate that any deficits observed in this test were specific to social odor discrimination, a subset

of subjects was also tested for their ability to discriminate between two complex, non-social odorants.

Methods

A separate group of male subjects were gonadectomized, implanted with a testosterone capsule and randomly assigned to an experimental group, MEaX (n = 35), MEpdX (n = 29), MEa-SHAM (n = 8) and MEpd-SHAM (n = 10). The procedures in Experiment 2 were identical to those in Experiment 1, except where noted.

Behavioral Testing

A habituation-dishabituation model was used to test discrimination between two odor sources presented sequentially. This approach involves repeated presentations of the same odor source followed by a test presentation of a novel odor source. A decrease in investigation during the repeated presentations indicates a perception of the odors as being the same or familiar. An increase in investigation of the novel odor compared to the last presentation of the habituated odor indicates an ability to discriminate between the two odors (Baum & Keverne, 2002; Johnston, 1993).

The testing sequence consisted of four, 3-minute presentations of repeated odors (habituation) followed by a fifth, 3-minute presentation of a novel odor (test). Five-minute inter-trial-intervals separated each odor trial. Odor stimuli were presented in modified 50 ml polypropylene collection tubes, with ½ cm holes 1 cm apart along the surface of the tube. Wire mesh lined the inner surface of the odor container to prevent contact with the odor stimulus. Thus, for all habituation-dishabituation tests, subjects were exposed to only the volatile components of the stimulus.

Odor containers were placed in the center of the subject's home cage and investigation was scored when the subject's nose contacted or came within 1cm of the odor container. The total investigation times were measured using a stopwatch. Odor containers were cleaned with 70% alcohol and allowed to air dry for 24 hours prior to re-use.

Odor stimuli. Social odor containers consisted of samples of male or female odor stimuli similar to those used in Experiment 1. Non-social odor containers consisted of 0.10g (3 beads) of an artificial odorant, either baby powder or strawberry (International Flavors & Fragrances, Inc., NY), mixed with clean odor stimuli. Care was taken to ensure that odor containers for a particular odor source were not contaminated with odor samples from a different source.

Discrimination of male and female odors. To determine if MEaX and/or MEpdX males can discriminate between sexual odors, subjects were tested with a habituation-dishabituation model using male and female odors as the stimuli. All subjects were given testing sequences with both male and female odors as the habituation stimuli. When tested with female odors as the habituation stimuli, however, both SHAM and lesioned males failed to show an increase in investigation of the test male odor compared to the last presentation of the female odor. Thus, only data from testing sequences using male odors as the habituation stimuli and female odors as the test stimulus are presented (Figure 4a).

Subjects were given repeated presentations of different individual male odors. Thus, subjects were habituated to the sexual identity of the repeated odor, rather than the

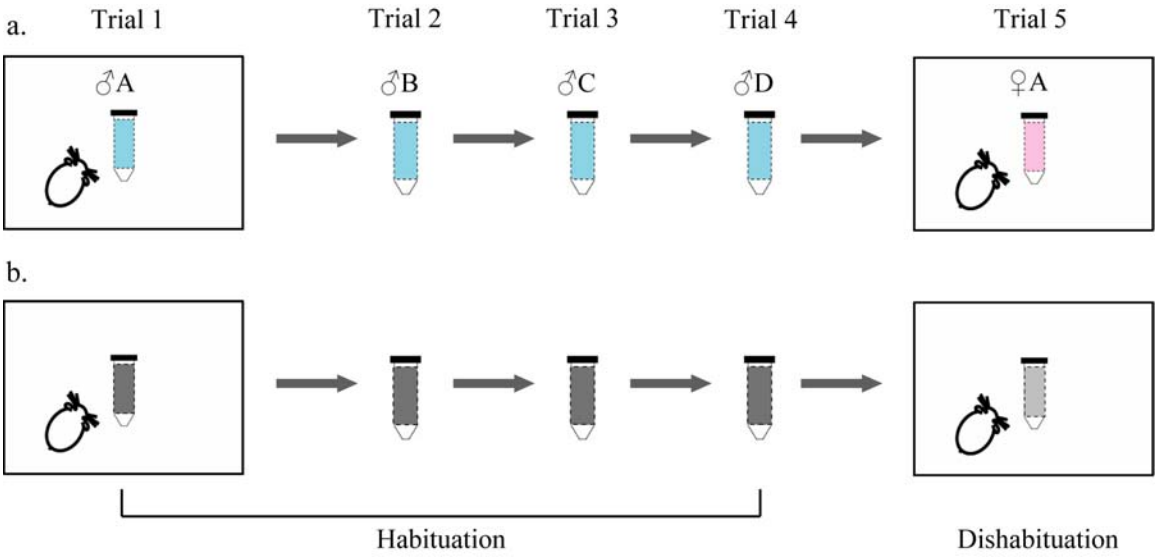

Figure 4. Illustration of habituation-dishabituation testing sequence used in Experiment 2. All tests consisted of four habituation trials followed by one test trial. (a) Sexual odor discrimination tests involved repeated presentations of different individual male odors followed by a test presentation of a female odor (b) Non-social odor discrimination tests involved repeated presentations of a single non-social odor (baby powder or strawberry) followed by a test presentation of the opposite non-social odor.

individual identity of an odor donor. As in experiment 1, each odor stimulus was used for two consecutive trials in order to conserve odor stimuli. Subjects were tested in pairs; one subject was given an individual odor stimulus first, and that odor stimulus was then transferred to a clean odor container to be used as the odor stimulus for the other subject in the pair. In this way, stimulus odors were always presented in a clean odor container in order to avoid transfer of subject odors across trials. The subject pair order was counterbalanced across lesion groups.

Discrimination of non-social odors. In addition to being tested for the ability to discriminate between sexual odors, a sub-set of subjects in experiment 2 (MEaX, n =7 ; MEpdX, n = 8; MEa-SHAM, n = 4; MEpd-SHAM, n = 4) was also tested for their ability to discriminate between two complex, non-social odors. Subjects were tested using one of the non-social odors as the habituation stimulus and the opposite non-social odor as the test stimulus (Figure 4b). Which odor was used for the habituation stimulus was counterbalanced across lesion groups.

Results

Lesion Verification

Subjects were assigned to MEaX (n = 9) or MEpdX (n = 15) lesion groups according to criteria outlined in Experiment 1. Data from males were excluded from the analyses if there was either substantial sparing (MEa, n = 16; MEpd, n = 8) or only unilateral damage (MEa, n = 10; MEpd, n = 3) of either region, or if there was extensive lesion damage to both the MEa and MEpd (n = 3).

All males in the MEaX group had lesion damage primarily restricted to the MEa. Eight males had bilateral damage of both the dorsal (MEad) and ventral (MEav) regions of the MEa, whereas one male had damage primarily restricted to the MEad. Lesion damage spread to the ventral surface of the brain bilaterally in five males. In addition to damage of the MEa, a subset of MEaX males also had partial (less than 15% at only one plane of section) and unilateral damage to adjacent nuclei, including: the most rostral region of the MEpd (n = 2); CeA (n = 1); OT (n = 1); ACo (n = 2); LOT (n = 1); nucleus of the accessory olfactory tract (AOT, n = 1); SI (n = 2); and I (n = 3).

In the MEpdX group, all males had lesion damage primarily restricted of the MEpd, including bilateral damage of the cMEpd. In addition, some males had partial partial (less than 15% at only one plane of section) and unilateral damage to adjacent nuclei, including: the most caudal section of the MEa (n = 1); OT (unilateral, n = 3; bilateral, n = 1); I (unilateral, n = 8); and AHi (unilateral, n = 8).

Only electrode tracts were visible in most SHAM males; one MEa-SHAM male had bilateral cortical damage. This male did not differ in behavior from males without cortical damage and was kept in the analysis.

Behavioral Measures

Discrimination of male and female odors. All lesion groups habituated to the repeated presentations of different male odors, as indicated by a decreased investigation of the male odor on the fourth trial compared to the first trial; MEaX males, $t(8) = 5.768$, $p < .001$; MEpdX males, $t(14) = 8.653$, $p < .001$; and SHAM males, $t(17) = 5.153$, $p < .001$ (Figure 5a). More importantly, all lesion groups discriminated between the male

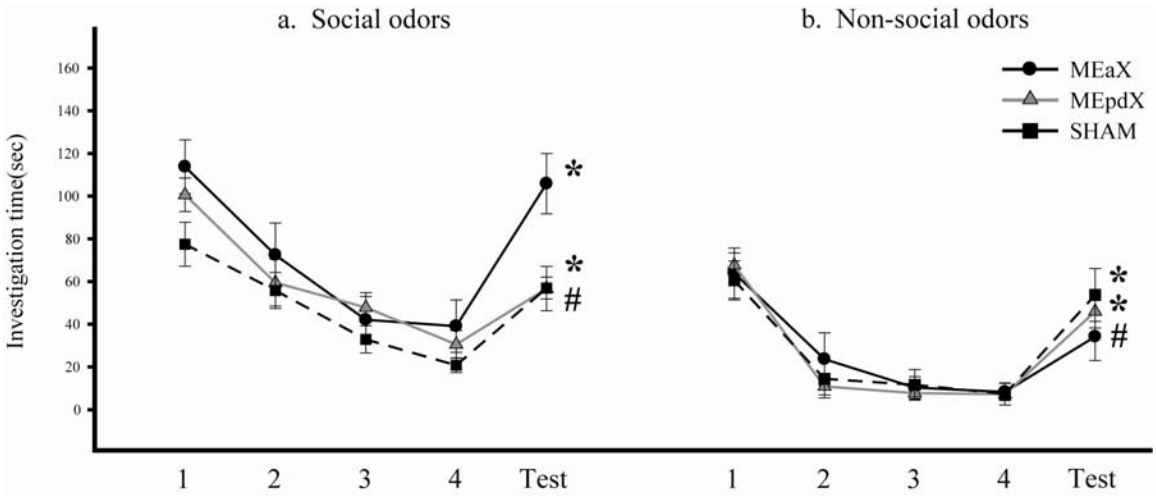

Figure 5. Mean odor investigation times (± SEM) during the habituation-dishabituation tests in Experiment 2. Habituation trials (1-4) were followed by the test trial with the novel odor. (a) In the social odor tests, all groups showed increased investigation of the female odor compared to the fourth presentation of the male odor, * $p < .01$ in MEaX and SHAM males; # $p < .05$ in MEpdX males. (b) In the non-social odor tests, all groups showed increased investigation of the novel odor compared to the fourth presentation of the habituated odor, * $p < .01$ in MEpdX and SHAM males, # $p < .05$ in MEaX males.

odor and female odor, as indicated by an increased investigation of the test female odor compared to the last presentation of the habituated male odor; MEaX males, $t(8) = 3.776$, $p < .001$; MEpdX males, $t(14) = 2.825$, $p < .05$; and SHAM males, $t(17) = 6.686$, $p < .001$. These results show that males in all lesion groups were able to both recognize the repeated male odors as being similar and also to discriminate between the male and female odors.

When the overall levels of investigation during the first presentation of each social odor were compared across lesion groups, MEaX males investigated the female odors longer than both other lesion groups, $F(2,39) = 7.356$, $p < .01$, and there was a trend for MEaX males to investigate the male odors longer than the other groups, $F(2,39) = 3.121$, $p = .055$.

Discrimination of non-social odors. All lesion groups habituated to the repeated presentations of the non-social odor; MEaX males, $t(6) = 5.290$, $p < .01$; MEpdX males, $t(7) = 9.558$, $p < .001$; and SHAM males, $t(7) = 6.879$, $p < .001$ (Figure 5b). Importantly, all lesion groups discriminated between the habituated odor and the other non-social odor; MEaX males, $t(6) = 3.047$, $p<.05$; MEpdX males, $t(7) = 4.739$, $p<.01$; and SHAM males, $t(7) = 4.236$, $p<.01$. These results indicate that lesions of the MEa or the MEpd do not impair discrimination of non-social odors or the ability to perform the habituation-dishabituation task. Furthermore, there was no difference in the levels of investigation of either non-social odor stimulus during the first presentation across lesion groups; baby powder, $F(2,20) = 2.122$, $p > .05$; strawberry, $F(2,20) = .455$, $p > .05$.

Testosterone assay

There was no difference in testosterone levels (ng/nl) between MEa-SHAM and MEpd-SHAM groups, $F(1,15) = 3.716$, $p > .05$; therefore, these groups were collapsed into one SHAM group for testosterone comparisons. Subsequent analysis showed no difference in testosterone levels across lesion groups, $F(2,38) = 2.755$, $p > .05$, (MEaX $M = 7.823$, $SD = 2.925$; MEpdX $M = 5.161$, $SD = 3.160$; SHAM $M = 5.287$, $SD = 2.727$).

Summary

The results from Experiment 2 suggest that the deficits in opposite-sex odor preference observed in MEaX and MEpdX males in Experiment 1 were not due to an inability to discriminate between the male and female odors; both MEaX and MEpdX males were able to discriminate between the social odors when presented sequentially. Furthermore, MEaX or MEpdX males discriminated between two non-social odors. These data confirm that MEaX and MEpdX males did not have general chemosensory deficits and were able to form olfactory memories for the repeated odors in the habituation-dishabituation test.

Consistent with MEaX males in Experiment 1, MEaX males in Experiment 2 also showed elevated investigation of the social odors compared to SHAM and MEpdX males. Indeed, MEaX males investigated the female odor longer, and showed a trend to investigate the male odor on the first trial longer, than both other groups. Importantly, MEaX males investigated the non-social odors at similar levels as the other groups, suggesting that their increased odor investigation is specific to social odors.

General Discussion

Taken together, the results from Experiments 1 and 2 show that both the MEa and the MEpd are critical for the expression of opposite-sex odor preference in male Syrian hamsters, although they appear to mediate distinct aspects of these behaviors. Indeed, MEaX and MEpdX males showed divergent patterns of attraction toward social odors; MEaX males were highly attracted to both male and female odors, whereas MEpdX males did not investigate either social odor more than clean odors. These differences in attraction between the MEaX and the MEpdX males suggest that different mechanisms underlie the opposite-sex odor preference deficits observed in each group. Importantly, the deficits observed in MEaX and MEpdX males did not reflect an inability to discriminate between male and female odors, as both groups distinguished between the social odors when presented sequentially in the habituation-dishabituation test.

The role of the MEpd in opposite-sex odor preference

The opposite-sex odor preference deficits observed in MEpdX males appear to be a result of their decreased attraction to female odors. Thus, the MEpd may be critical for generating the motivation to approach sexually relevant stimuli in the environment. One mechanism by which the MEpd may generate attraction to female odors is activation of the steroid receptors that are located on neurons within this nucleus. Indeed, the MEpd is characterized by dense populations of androgen and estrogen receptor-containing neurons in many rodent species, including rats (Greco, Edwards, Michael, & Clancy, 1998; Simerly, Chang, Muramatsu, & Swanson, 1990), mice (Apostolinas, Rajendren, Dobrjansky, & Gibson, 1999) and hamsters (Doherty & Sheridan, 1981; Wood et al.,

1992). Several converging lines of evidence suggest that the decreases in attraction to female odors observed in MEpdX may be due to the disruption of steroid hormone processing within this MEpd.

First, previous lesion studies demonstrate that the MEpd is important for motivational aspects of sexual behavior. For example, male hamsters with lesions of the MEpd display increased latencies to first mount and ejaculate, as well as decreased anogenital investigation of a receptive female (Lehman et al., 1983). Similarly, in rats, MEpd lesions eliminate the expression of non-contact penile erections in response to a remote female stimulus, as well as the preference for estrous over anestrous females (Kondo & Sachs, 2002; Kondo, Sachs, & Sakuma, 1998). Second, c-fos studies suggest that neurons within the MEpd are specifically activated by sexually arousing stimuli (J. Swann & Fiber, 1997; Veening & Coolen, 1998). In male hamsters, neurons within the MEpd show increases in c-fos expression following mating or exposure to female odor stimuli (Kollack & Newman, 1992; Kollack-Walker & Newman, 1995; Swann et al., 2001). Moreover, this mating induced c-fos expression is colocalized with neurons that contain steroid receptors (Wood & Newman, 1993).

Finally, steroid hormones are required for male attraction to female chemosignals in a variety of rodent species (Bean, Nyby, Kerchner, & Dahinden, 1986; Carr, Loeb, & Wylie, 1966; Ferkin & Gorman, 1992; Hull et al., 2002). In male hamsters, gonadectomy reduces investigation of female odors, and these behaviors can be reinstated either by systemic steroid replacement (Petrulis & Johnston, 1995; Powers & Bergondy, 1983; Powers et al., 1985) or by testosterone or estradiol implants targeted directly into the

MEpd (Wood, 1996; Wood & Newman, 1995b). Similar results have been observed in rats (Baum, Tobet, Starr, & Bradshaw, 1982; Bialy & Sachs, 2002; Huddleston, Michael, Zumpe, & Clancy, 2003). Similar to these behavioral effects, the morphology of MEpd neurons is sensitive to changes in circulating steroids in hamsters (Gomez & Newman, 1991; Romeo & Sisk, 2001) and in rats (Cooke, 2006). Together, these data suggest that the MEpd is steroid-responsive and that steroid processing within the MEpd may be critical for generating sexual motivation.

The role of the MEa in opposite-sex odor preference

In contrast to MEpdX males, deficits in preference of MEaX males appear not to be due to decreased motivation to investigate social odors. In fact, MEaX males displayed high levels of investigation of female odors and inappropriately high levels of investigation of male odors, suggesting an over-generalization of their investigatory response. One interpretation of these data is that the MEa functions as a chemosensory filter to identify or categorize the sexual or social relevance of odors in the environment. Indeed, a failure to categorize an odor source may also explain the notably high levels of odor investigation in the MEaX males. Specifically, disruption of processing within the MEa may generate an error signal that leads to increased olfactory sampling behavior (sniffing) in an attempt to identify the odor stimulus. Importantly, these high levels of investigation were not observed in response to non-social odors (Experiment 2), which suggests that other chemosensory areas, such as the ACo or piriform cortex (Scalia & Winans, 1975), can function to categorize odor stimuli as social or non-social in origin.

If the MEa functions to categorize the relevance of biological odors, then it should process many classes of chemosensory cues. Consistent with this interpretation, in male hamsters, MEa neurons show increases in c-fos expression following exposure to many types of social odors, including female hamster vaginal secretion, male and female hamster flank gland secretions, male and female mouse urine, and male cat urine (Meredith & Westberry, 2004). Furthermore, neurons within the MEa produce similar increases in c-fos expression following either sexual or agonistic encounters (Kollack-Walker & Newman, 1995). In rats, neurons in the MEa, but not in the MEpd, show increases in c-fos expression following exposure to either conspecific alarm pheromones (Kiyokawa, Kikusui, Takeuchi, & Mori, 2005) or components of fox predator odors (Day, Masini, & Campeau, 2004). Together, these data suggest that the MEa may be important for the categorization of biologically relevant odors.

In hamsters, lesions of the MEa not only eliminate copulation, but also eliminate anogenital investigation of a receptive female (Lehman et al., 1980). These findings contrast with those in the current study in which MEaX males displayed increased levels of investigation of social odors. One possible explanation for these differences may be that the subjects in the two experiments were exposed to different types of sensory stimuli. Specifically, subjects in the Y-maze test (present study) were only exposed to the volatile components of the odors, whereas subjects in the mating test (Lehman et al., 1980) were exposed to both volatile and non-volatile chemosignals, as well as auditory, visual and tactile cues from the female. Furthermore, the different behavioral measures used in the two studies measure odor-guided reproductive behaviors in distinct contexts.

Specifically, odor preference in the Y-maze reflects attraction and approach behaviors towards distant odors outside the context of mating, whereas anogenital investigations of a receptive female are specifically expressed as part of the stereotyped sequence of male copulatory behaviors. Thus, these different modes of sensory processing and contextual expression of behaviors may engage distinct neural mechanisms.

In contrast to the results found here and by others (Lehman et al., 1980), male rats with lesions restricted to the MEa show normal copulatory behavior and partner preference for estrous over anestrous females (Kondo & Sachs, 2002). Differences in sexual experience, however, may explain these conflicting results; in both the Lehman et al. and the current study, subjects were sexually naïve, whereas in the Kondo & Sachs study, subjects were given sexual experience prior to lesions of the MEa. In hamsters, sexual experience can modulate the chemosensory processing required for the expression of copulatory behavior. Specifically, removing the VNO abolishes mating behavior in sexually naïve, but not in sexually experienced, males (Meredith, 1986). Furthermore, exposure to female vaginal secretion produces more c-fos expression in areas of the ME, BNST and MPOA in sexually experienced compared to sexually naïve males (Fewell & Meredith, 2002). Thus, previous sexual experience may increase chemosensory processing in areas outside of the MEa such that these areas may subsequently be able to compensate for odor classification if the MEa is damaged.

Interactions between the MEa and MEpd

The current findings provide strong evidence for the distinct functions of the chemosensory and steroid-responsive sub-regions of the ME in generating sex-specific

responses to social odors. However, these sub-regions are strongly interconnected (Coolen & Wood, 1998) and thus likely work in concert to regulate reproductive behavior. Based on our findings and work by others (Wood, 1997), we propose that the nature of this interaction is bidirectional, and that the MEpd functions to increase motivation or arousal towards social stimuli in the environment, probably via its population of steroid-responsive neurons, and that the MEa filters or directs this arousal towards socially and sexually relevant targets. This interpretation predicts that MEaX males would also show high levels of investigation toward heterospecific odors. This proposal also predicts that blockade of androgen and/or estrogen receptors within the MEpd would lead to similar attraction deficits as were observed in MEpdX males.

Parallel processing of chemosensory and steroid hormone cues throughout the mating circuit

The ME is part of an extended network of forebrain nuclei, including the BNST and MPOA, that regulates many aspects of social behavior (Newman, 1999). Importantly, anatomical evidence suggests that the separation of chemosensory and steroid hormone processing observed within the ME is maintained throughout this extended network (Canteras, Simerly, & Swanson, 1995; Coolen & Wood, 1998; Dong, Petrovich, & Swanson, 2001). Furthermore, lesion studies suggest that the expression of sexual preference may require intact processing at all levels of this circuit. For example, similar to MEaX males in the current study, who showed a trend to prefer male odors over female odors, male rats (Paredes, Tzschentke, & Nakach, 1998) and ferrets (Paredes & Baum, 1995) with lesions of the MPOA and anterior hypothalamus also display reversed

partner preference, although these lesion studies did not distinguish between chemosensory and steroid-responsive sub-regions of the MPOA. In the context of mating behavior, male hamsters with lesions restricted to the BNSTpm, a steroid-responsive nucleus, display increases in ejaculation latency and decreases in investigatory behavior similar to those observed in males with MEpd lesions (Powers, Newman, & Bergondy, 1987). If parallel processing is a fundamental principal maintained throughout the mating circuit, then lesions of the chemosensory and steroid-responsive regions of the BNST or MPOA would result in similar odor preference deficits as were observed following MEa and MEpd lesions, respectively. However, if chemosensory and steroid pathways become functionally integrated at higher levels of processing, then lesions of these sub-regions would result in identical deficits in sexual odor preference and attraction.

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