Hormones and Behavior 59 (2011) 193–199

Contents lists available at ScienceDirect

Hormones and Behavior

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

Olfactory preference in the male rat depends on multiple chemosensory inputs converging on the preoptic area

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ARTICLE INFO

Article history:
Received 1 October 2010
Revised 8 November 2010
Accepted 12 November 2010
Available online 20 November 2010

Keywords:
Sexual odor
Preference
Olfactory pathways
Preoptic area
Amygdala

ABSTRACT

Both volatile and nonvolatile molecules are involved in chemosensory communication in rodents. Volatile odors from physically inaccessible estrous females induced increased numbers of c-Fos-positive cells in the preoptic area (POA) and in the cortical nucleus of the amygdala (CoA) of male rats. The numbers of c-Fos-positive cells in the medial nucleus of the amygdala (MeA) increased in response to the nonvolatile odors of bedding soiled with the excreta of estrous females. In an alternate choice paradigm, male rats carrying ibotenic acid lesions in either the MeA or the CoA—or a combination of both—distinguished the odors of estrous females from those of males, although the time spent sniffing the stimuli was diminished. Males with POA lesions showed complete loss of this capability. Males carrying either of the lesions did not detect differences between estrous and anestrous females or between intact and orchidectomized males. Lesions in the POA or MeA severely impaired male sexual behavior, whereas a CoA lesion had no effects. Thus, c-Fos-positive cells in the CoA might be involved in chemosensory transmission relevant to certain social contexts, but not in the execution of male sexual behavior. The POA is indispensable for both olfactory preferences and sexual behavior. The residual olfactory preference in males with MeA or CoA lesions or the combination of both could reflect an additional route for chemosensory transmission from the main olfactory bulb to the POA.

Introduction

Chemosensory signals emitted by conspecific individuals of opposite sexes play key roles in sexual and social behavior in most mammalian species. The signals are received in sensory neurons of the olfactory epithelium (OE) and the vomeronasal organ (VNO) and processed and relayed in the main and accessory olfactory bulbs (MOB and AOB, respectively). They are then integrated in the medial and cortical nuclei of the amygdala (MeA and CoA, respectively) and/or the preoptic area (POA). The MeA and POA include a large number of neurons with sex steroid receptors (Simerly et al., 1990), suggesting an involvement of the MeA and POA in regulating sex-specific and sex steroid-dependent olfactory preference for the odor of conspecific individuals (Xiao et al., 2004). Receptive female rats are attracted by the odor of sexually active males rather than by that of females or orchidectomized males, whereas sexually active male rats prefer the odor of receptive females over that of ovariectomized (ovx) females or of intact males (Xiao et al., 2004). In a social context, male rats usually show a distinct olfactory preference for castrated males over intact males (Xiao et al., 2004). This preference toward same-sex conspecific animals is male specific. Orchidectomy eliminates both of the preferences (Xiao et al., 2004) and hormonal replacement with either testosterone or estradiol restores them (Kondo et al., 2004).

On the other hand, we have little knowledge about the neural circuitry of olfactory preference. Chemosensory stimulation with soiled bedding collected from estrous females markedly increased the number of c-Fos immunoreactive cells in the MeA and POA of male rats (Hosokawa and Chiba, 2005; Hurtazo and Paredes, 2005; Paredes et al., 1998a,b). For female rats, we have reported that destruction of these areas impaired the olfactory preference for sexually active males over inactive males (Xiao et al., 2005). Small lesions in the MeA impaired noncontact penile erection in male rats induced by estrous female odors but were not sufficient to suppress the olfactory preference for estrous females (Kondo and Sachs, 2002).

In this study, we examined the effect of destruction in the POA and its chemosensory pathways—the medial and cortical nucleus of the amygdala—to clarify the neural basis of control of olfactory preferences. Infusions of ibotenic acid produced neuron-specific and axon-sparing lesions in the MeA, the CoA and the POA. In these animals, olfactory preferences were tested for three pairs of stimulus animals: (1) receptive vs. ovx females; (2) receptive females vs. intact males and (3) intact vs. orchidectomized males. The aim was to elucidate the chemosensory pathways essential for olfactory preference.

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doi:10.1016/j.yhbeh.2010.11.011

Materials and methods

Animals

Male and female 8-week-old Long-Evans rats were purchased from the Institute for Animal Reproduction (Ibaraki, Japan). All the animals were housed two or three per cage under controlled temperature (23 ± 2 °C) and reversed light/dark illumination (lights off from 11:00 to 23:00) with free access to food and water. The experiments were performed according to protocols approved by the Nippon Medical School Committee for Animal Experimentation. As stimulus partners for behavioral tests, females were ovariectomized under ether anesthesia and brought into behavioral estrus by subcutaneous injections of 5 μg estradiol benzoate (dissolved in 0.1 ml sesame oil) at 48 h and 500 μg progesterone (dissolved in 0.1 ml sesame oil) at 3–7 h prior to use. Several ovx females and orchidectomized males without hormone treatment were prepared as sexually inactive stimulus animals. Following 1 week of acclimation, males were made familiar with the apparatus and given sexual experience (once a week for 3 weeks). Males that ejaculated in the third session of sexual experience were included in the following experiments. After this sexual experience session, two olfactory preference tests (each followed by sexual behavior tests) were carried out weekly as pre-surgery baseline tests.

Olfactory preference testing

The olfactory preference was determined by an alternate choice paradigm. The preference chamber was as described (Xiao et al., 2004). The apparatus was a three-chambered acrylic observation box (110 cm long × 12 cm wide × 30 cm high). In this, each experimental male was placed in the middle compartment and two stimulus rats were placed separately in the side compartments. Three opaque plates with 3 cm diameter holes at different levels were assembled into a partition for divisions between compartments. A blower connected to the ceiling of the middle compartment through a corrugated flexible tube was used to generate airflow from the two side compartments to the middle one by maintaining a negative pressure (flow rate ~0.2 m^3/min). A 2 cm deep, transparent tube 2 cm above the floor was attached to the hole of both side partition plates facing the middle compartment. Thus, the only stimuli available for experimental males were volatile odors, detected by main olfactory system.

On the day of olfactory preference testing, each experimental male was subjected to three preference tests with different pairs of stimulus animals: (1) a receptive female and an intact male, (2) a receptive female and an ovx female and (3) an intact male and a castrated male. The test order and the position of the stimulus pairs were counterbalanced and the intervals between the tests were greater than 1 h. Before each test, the apparatus was cleaned with 70% ethanol (v/v) and bedded with fresh paper chips (Alpha-dri, Shepherd Specialty Paper, Watertown, TN, USA). During 5 min of acclimation to the apparatus, the downstream airflow was directed from the middle to the side compartment. Five minutes of behavioral observation were recorded by a tripod video camera fixed in front of the preference chamber. Times spent nose-poking in to the left and right inlets were calculated by an event recorder on a personal computer. Preference scores in each stimulus pair were calculated as the time spent nose-poking towards receptive females (Pairs 1 and 2) or castrated males (Pair 3) as a percentage of the total time spent nose-poking towards both stimulus animals.

Male sexual behavior testing

On each behavioral test day, sexual behavior with estrous females was also measured following a series of preference tests. Each male was first placed in a transparent observation cage (50 cm long × 30 cm wide × 40 cm high) bedded with wooden shaving and acclimatized for 5 min. Behavioral tests were started by the introduction of estrous females and terminated after the first ejaculation or when 60 min had elapsed. The numbers and latencies of mounts and intromissions, and the latency of ejaculation were recorded.

Neurotoxic lesioning

After the baseline olfactory preference and sexual behavioral tests, male rats were subjected to bilateral lesions of the POA ($n = 15$), the MeA ($n = 14$), the CoA ($n = 11$) or to a larger extent including both the MeA and CoA ($n = 9$). Sham-lesioned control males were also used corresponding to each of those lesions ($n = 6$ for each). Brain surgery was carried out under ketamine HCl (25 mg/kg, im) and sodium pentobarbital (25 mg/kg, ip) anesthesia. Each male was secured in a stereotaxic frame with an incisor bar placed 3.3 mm below the interaural line. Axon-sparing lesions were induced by microinjection (200 nl/min flow rate) of 1 μl (1.3 μl for MeA + CoA lesions) ibotenic acid solution (5 μg/μl in 0.01 M phosphate buffered saline, PBS, pH 7.4; Sigma-Aldrich, St. Louis, MO, USA) with a 27-gauge injection cannula connected to a 10 μl Hamilton syringe. The cannula was located at the following coordinates: MeA, anteroposterior (AP) −2.8 mm, mediolateral (ML) ± 3.8 mm, dorsoventral (DV) −9.5 mm; POA, AP −0.4 mm, ML ± 0.8 mm, DV −8.6 mm; CoA, AP 2.8 mm, ML ± 4.2 mm, DV −10.0 mm and MeA + CoA, AP −2.8 mm, ML ± 4.0 mm, DV −9.7 mm from the bregma. After infusion, the cannula was left for another 3 min to prevent backflow. Sham lesion groups were generated in the same way but were injected with PBS alone. Although every lesioned group had sham operation controls, these were combined into a single sham group (SHAM) for data analyses.

After a 1-week recovery period, two olfactory preference tests (each followed by sexual behavior tests) were weekly carried out as post-surgery tests.

Histology

After the second post-surgery behavior tests, the animals were euthanized for histological examination. Experimental males were anesthetized deeply with an overdose of sodium pentobarbital (>80 mg/kg, ip) and killed by cardiac perfusion with saline followed by fixation with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brains were removed and kept in the same fixative overnight. After replacing the fixative with 30% sucrose in PB, 40 μm thick frontal sections were cut using a freezing microtome. Alternate sections were processed for NeuN immunohistochemistry (a marker for differentiated neurons) to determine the damaged area of the brain precisely. The remaining sections were stained with cresyl violet to identify the brain cell nuclei. Free-floating sections were incubated in 3% normal goat serum and 0.25% Triton X-100 in 0.01 M PBS followed by anti-NeuN antibody (mouse monoclonal IgG; 1:200, Millipore, Billerica, MA, USA) for 16 h at 4 °C. Then, the sections were processed using the ABC method using ABC Elite kits (Vector Laboratories, Burlingame, CA, USA) and developed with diaminobenzidine (0.25 mg/mL) in 0.01 M PBS containing 0.03% H_2O_2.

c-Fos immunohistochemistry

To identify the brain areas activated by chemosensory signals involved in olfactory preference, another subset of 13 male rats was used for studying c-Fos localization. These rats were subjected to 60 min exposure to one of three different stimuli: clean bedding (CL, $n = 4$); estrous female soiled bedding (SO, $n = 4$) and volatile odors from inaccessible estrous females (ES, $n = 5$). For the CL and SO bedding exposures, the rats were selected randomly to be placed alone in the middle compartment of the preference apparatus (without airflow) containing fresh paper chips as above, or SO bedding respectively. SO bedding (1 week of use by the test animals) was collected from home cages of ovx females that had been

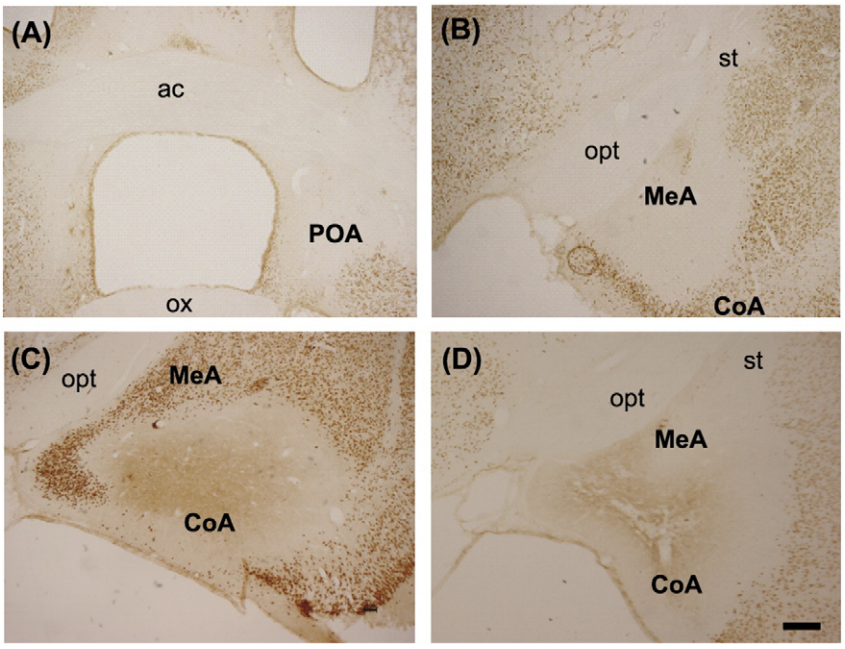

Fig. 1. Photomicrographs of sections including lesions to the preoptic area (POA) (A), medial amygdala (MeA) (B), cortical amygdala (CoA) (C) and MeA + CoA (D). The sections were stained immunohistochemically for NeuN, a marker of differentiated neurons. No immunostained neurons were observed in the destroyed areas. Abbreviations: ac, anterior commissure; opt, optic tract; ox, optic chiasma; st, stria terminalis. Scale bar = 200 μm.

induced to develop estrus as described above. For ES exposure, estrous females were kept in both of the side compartments of the preference apparatus and experimental males were placed in the middle compartment attached to the blower to generate airflow for odor presentation. Immediately after the stimulation, all the males were sacrificed by cardiac perfusion as above; 40-μm-thick frontal sections were obtained using a freezing microtome after removing them from the fixative and resuspending then in 30% sucrose in PB. Free-floating coronal sections were processed for c-Fos immunohistochemistry. The sections were incubated in 0.01 M PBS with 3% normal goat serum and 0.25% Triton X-100 followed by c-Fos antibody (rabbit polyclonal IgG, 1:20,000, Calbiochem, Gibbstown, NJ, USA) for 4 days at 4 °C. Subsequently, the sections were processed by the ABC method as above and developed with diaminobenzidine (0.25 mg/mL) in 0.01 M PBS containing 0.03% hydrogen peroxide. Densely stained cell nuclei with sharp boundaries at × 20 magnification were counted as c-Fos immunoreactive neurons. The three areas counted were as follows: (1) the POA, defined as a paraventricular square (330 × 330 μm), 200 μm below the anterior commissure, (2) the MeA, defined as a square (330 × 330 μm) between the caudal tail of the stria terminalis and the optic tract and (3) the CoA, defined as a square (330 × 330 μm) below the stria terminalis and 200 μm dorsal to the ventral surface of the brain. The MeA and CoA were on the same plane. We determined the mean numbers of c-Fos immunoreactive cells in three serial sections for each area.

Statistical analysis

Differences in time and preference scores for the total nose-poking time among the SHAM, POA, MeA, CoA and MeA + CoA lesioned groups before and after surgery were compared by analysis of variance (ANOVA) followed by Student–Newman–Keuls post hoc tests. When appropriate, all sexual behavior and ejaculation parameters between pre- and post-surgery groups were examined using Mann–Whitney nonparametric *U* tests and or Kruskal–Wallis tests. Mount and intromission numbers were examined by ANOVA followed by Student–Newman–Keuls *post hoc* tests. Differences in c-Fos-positive neurons among rats exposed to CL, SO and ES bedding odors were analyzed by ANOVA followed by Student–Newman–Keuls *post hoc* tests. Times spent nose-poking during olfactory preference tests toward the pairs of stimulus animals were analyzed using paired Student's *t*-tests. The threshold for significant difference was accepted at $P<0.05$.

Results

Neurotoxic lesions

In all the lesioned and SHAM rats, needle tracks were identified in Nissl-stained sections. The tip of each cannula was located within the appropriate brain nucleus of each animal that demonstrated the expected damage (see below). We excluded all the data from males with insufficient or misplaced damaged regions confirmed by NeuN histochemistry in identifying the extent of the excitotoxic lesion. Finally, we had seven males with POA lesions, six with MeA lesions, five with CoA lesions and five with MeA + CoA lesions. In the rats with POA lesions, neuronal loss was observed in the center of the preoptic area and the ventral part of the bed nucleus of the stria terminalis (Fig. 1A). Enlargement of the third ventricle was frequently observed, which might have resulted from the decreased cell mass and the shrunken preoptic tissue. All MeA lesions covered the posterodorsal part of the MeA completely (Fig. 1B). The ventral part of the MeA was found to remain intact in most rats. CoA lesions covered the posteromedial cortical amygdala and posterolateral cortical amygdala (Fig. 1C). The damaged regions of CoA lesions also included the ventral portion of the basomedial amygdaloid nucleus. Destruction of extended lesions in the MeA + CoA group of rats was seen over the medial amygdala including the posterodorsal and the posteroventral parts and covered the cortical amygdala including the posteromedial and posterolateral CoAs (Fig. 1D).

We also had 24 vehicle injected sham-treated males (six in each lesion group). No neuronal loss was observed in any of those animals and behavior patterns were indistinguishable among them, so those data were combined as one SHAM group for further statistical analyses of behavioral tests.

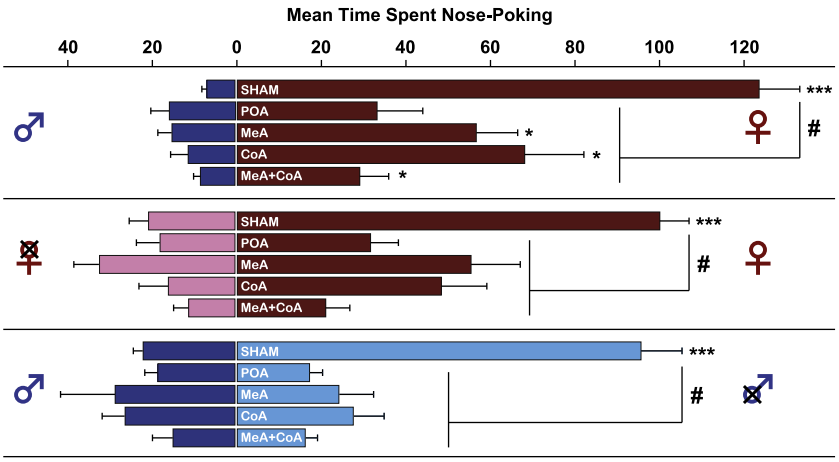

Fig. 2. Mean time spent nose-poking (± SEM) toward each of the stimuli of all groups in the post-surgery test. Gender symbols to the left and right of each panel indicate pairs of stimulus animals (a cross over the male or female symbol means gonadectomy). Hashes indicate a significant decrease in total nose-poking time, compared with that of the (pooled) SHAM control group (ANOVA followed by Student–Newman–Keuls test). Asterisks indicate significant differences in nose-poking time towards the preferred animal side from the opposite side (Student's *t*-test; $^{*}P<0.05$, $^{***}P<0.001$).

Olfactory preferences

Data from the second test of each pre- and post-surgery series were considered for analysis. Nose-poking times to explore each stimulus were determined in rats subjected to POA, MeA, CoA, MeA + CoA lesions and in SHAM rats.

In the pre-surgery test, sexually experienced males of all groups displayed a consistent, male-typical, olfactory preference behavior. They spent significantly longer times in nose-poking toward receptive females (SHAM, $P<0.001$; POA, $P<0.001$; MeA, $P<0.01$; CoA, $P<0.001$ and MeA + CoA, $P<0.01$, by paired *t*-tests) than towards intact males or ovx females in all groups. They also showed significantly longer times poking their noses towards castrated males when presented with intact males as a choice (SHAM, $P<0.001$; POA, $P<0.01$; MeA, $P<0.01$; CoA, $P<0.05$; MeA + CoA, $P<0.05$, by paired *t*-tests).

Effects of lesions

POA lesions significantly suppressed the times spent nose-poking toward preferable conspecific odors. This treatment eliminated the olfactory preferences in all stimulus pairs; that is, the time spent nose-poking toward estrous females (compared with intact males or anestrous females) or castrated males (compared with intact males) were not different (Fig. 2). Further post hoc analyses (ANOVA followed by Student–Newman–Keuls) showed that the POA-lesioned rats spent significantly shorter times in nose-poking toward estrous females versus intact males ($P<0.05$, $F(4, 42)=13.31$, $P<0.001$) or anestrous females ($P<0.05$, $F(4, 42)=14.75$, $P<0.001$) as well as towards castrated males versus intact males ($P<0.05$, $F(4,42)=12.06$, $P<0.001$).

Lesions in the amygdala including the MeA and the CoA, as well as POA lesions, also suppressed the time spent nose-poking (Student–Newman–Keuls, $P<0.05$, see Fig. 2). However, males with MeA or CoA lesions still showed significant preference for the odor of an estrous female when it was paired with the odor of an intact male. Males with lesions in the MeA and CoA spent significantly longer times in nose-poking toward estrous females than towards intact males (MeA, $t=4.610$, $df=4$, $P<0.01$; CoA, $t=3.810$, $df=5$, $P<0.05$). When the stimulus of estrous females was paired with that of ovx females, the decreased rate in nose-poking by these groups yielded no significant preference toward estrous females. The preference for castrated over intact male odors was eliminated by both MeA and CoA lesions.

In this study, we also examined extended lesions covering both the MeA and CoA regions (MeA + CoA group). The effect was not synergetic with those of the individual MeA and CoA lesions. MeA + CoA lesions significantly suppressed the time spent nose-poking for preferable odors (Student–Newman–Keuls, $P<0.05$, see Fig. 2) and the lesioned males showed no preference for estrous females over ovx females or for castrated males over intact males. However, as with the MeA and CoA-lesioned males, they still showed significantly longer times nose-poking towards estrous females than they did towards intact males ($t=3.773$, $df=4$, $P<0.05$).

Copulatory behavior

On each test day, sexual behavior with receptive females was tested following a set of preference tests. Destruction of the POA, MeA and MeA + CoA regions produced prolonged latencies in mounting, intromission and ejaculation times ($P<0.001$ by Kruskal–Wallis test; $\chi^2=20.449$, 36.293 and 36.493, respectively). In contrast, males with lesions in the CoA showed a relatively normal level of sexual behavior, although their latency of intromission was increased significantly (Table 1). Ejaculation was suppressed completely by lesions to the POA, MeA and MeA + CoA regions, whereas all males in the SHAM and CoA lesion groups displayed ejaculation within the 60 min observation time (Table 1).

c-Fos expression after chemosensory stimulation

Another 13 separate males exposed to clean bedding, to bedding collected from estrous females or to direct stimulation with the odor of estrous female rats, also showed preference for estrous female odors over male or anestrous female odors (mean preference scores of the three groups, 87.4 ± 3.01% and 79.5 ± 3.30%, respectively) and for castrated male odors over sexually active male odors (78.4 ± 3.27%). There were no significant differences in olfactory preference behavior for conspecifics. Similarly, none of the recorded parameters of sexual behavior of males were significantly different between groups. Results of counting c-Fos-positive neurons in the POA, MeA and CoA are summarized in Fig. 3A. In the POA, increased c-Fos labeled cells were observed after stimulation by both estrous female odors and estrous soiled bedding odors, compared with those induced by clean bedding odors (Student–Newman–Keuls, $P<0.05$, ANOVA, $F(2, 10)=11.961$, $P<0.01$). There were no significant differences in the numbers of c-Fos-positive neurons counted between rats exposed to estrous soiled bedding odors or to estrous female odors. The same pattern of increase was found for the CoA region. Estrous soiled bedding and estrous female odors exposures significantly augmented the numbers of c-Fos-positive cells (Student–Newman–Keuls, $P<0.05$, $F(2, 10)=11.319$, $P<0.01$) and

Table 1
Sexual behavior in the post-surgery test.

Groups	Number of intromission	Latencies (s) to			% of animals displaying		
		Mount	Intromission	Ejaculation	Mount	Intromission	Ejaculation
SHAM ($n=24$)	11.7 ± 1.4	5	8	229	100	100	100
POA ($n=7$)	0.6 ± 0.4^{ab}	3600^{ab}	3600^{ab}	3600	42.2	28.5	0
MeA ($n=6$)	0.2 ± 0.1^{ab}	79^{a}	3600^{ab}	3600^{ab}	50	16.6	0
CoA ($n=5$)	10.4 ± 3.4	9	68^{a}	1258^{a}	100	100	100
MeA + CoA ($n=5$)	0^{ab}	43^{ab}	3600^{ab}	3600^{ab}	60	0	0

Median values of latencies are expressed in seconds. Significantly different [a] from the SHAM control group and [b] from the CoA lesioned group ($P<0.05$ by Kruskal–Wallis test). Number of subjects is given in the parentheses following group names.

no significant difference between estrous soiled bedding and estrous female odor stimulation was found in the CoA region.

Significant increases in c-Fos labeled cells were found in the MeA region after stimulation with the odors of soiled bedding from estrous females (Student–Newman–Keuls, $P<0.05$, $F(2, 10) = 10.883$, $P<0.01$). However, direct stimulation by the airborne odor of estrous females failed to increase c-Fos-positive cell counts in the MeA.

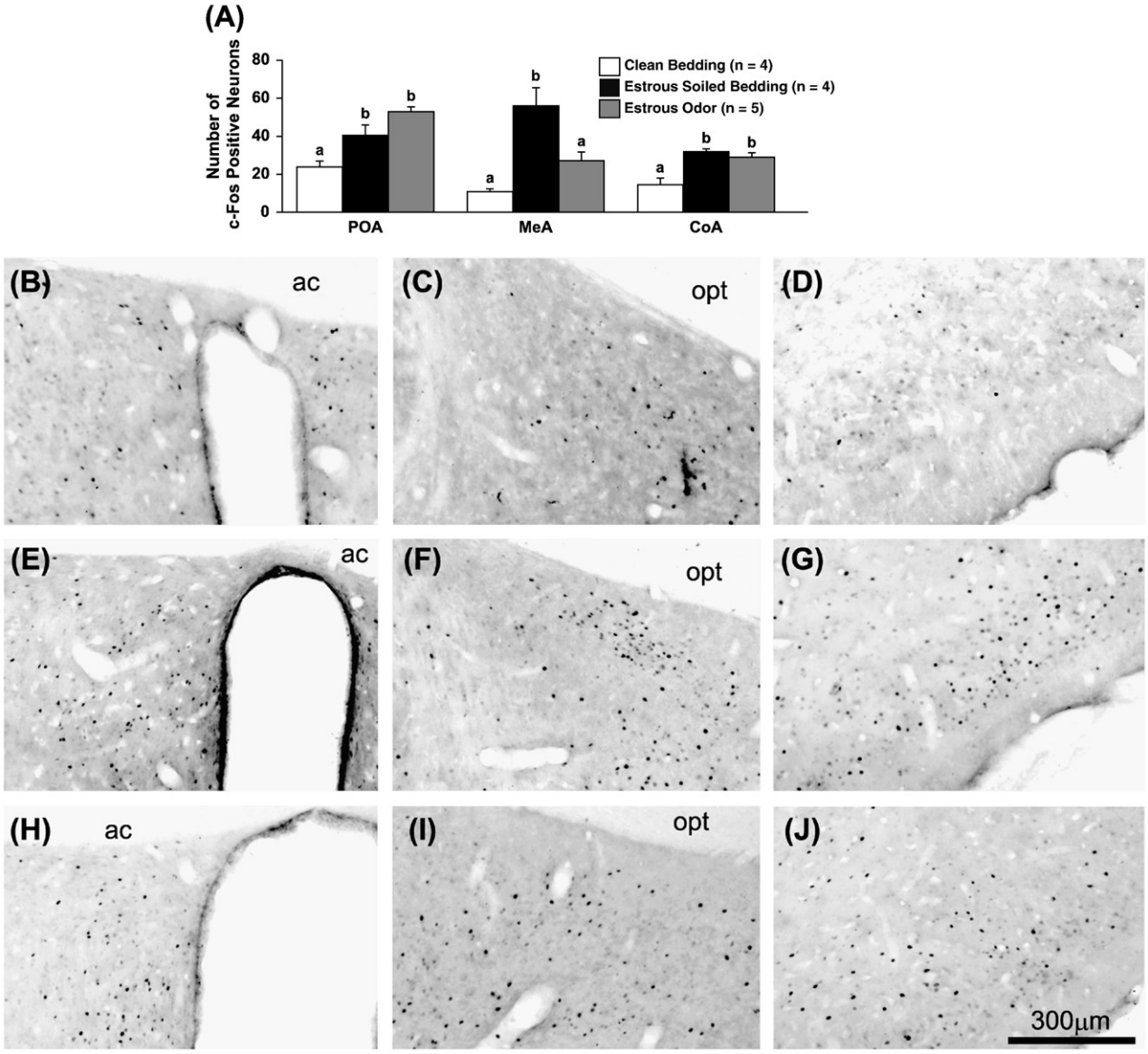

Fig. 3. (A) C-Fos immunostaining in the POA, MeA and CoA regions after exposure to clean bedding, soiled bedding of estrous female cages and airborne estrous female odors. Different letters above the columns in each brain area indicate statistically significant differences from each other (ANOVA followed by Student–Newman–Keuls test). The vertical bar in each column indicates the SEM. (B–J) Corresponding representative c-Fos immunostained sections of the POA (left; B, E, H), the MeA (center; C, F, I) and the CoA (right; D, G, J) after exposure to clean bedding (upper; B–D), soiled bedding (middle; E–G) and airborne estrous female odors (lower; H–J). Abbreviations: ac, anterior commissure; opt, optic tract.

Discussion

Chemosensory signals play a key role in social and sexual behavior in both male and female rats. As mentioned before, the nasal cavity of the rat has two distinctive chemosensory organs: the OE involved in general olfaction and the VNO receiving pheromonal (social) odors. The signals received in the OE travel to the olfactory tubercle and the piriform cortex via the MOB; then they are delivered to various olfactory regions of the brain. In addition, some signals containing biological and innate messages such as social and predator odors (Kobayakawa et al., 2007) can travel to the CoA (Kevetter and Winans, 1981). On the other hand, pheromonal information received in the VNO is sent to the MeA via the AOB. Recently, anatomical studies described a direct projection from the main olfactory bulb to the MeA in rats (Pro-Sistiaga et al., 2007) and mice (Kang et al., 2009), suggesting interactions between the main and vomeronasal systems at a level of the amygdala. Furthermore, these signals travel to the POA and the bed nucleus of the stria terminalis through the stria terminalis (Tsutsui et al., 1994) and the amygdalofugal pathway (Kondo and Yamanouchi, 1995; Masco and Carrer, 1984). This stream of chemosensory information is thought to establish a neural substrate to regulate various social activities such as sexual, maternal and aggressive behaviors.

Chemicals received in the OE are volatile and conveyed via the airflow. In contrast, chemicals detected in the VNO must be non-volatile because of its structural restriction; the detection requires active inhalation by the so-called vomeronasal pump (Ben-Shaul et al., 2010; Meredith et al., 1980). In our present study, estrous soiled bedding was assumed to contain both volatile and nonvolatile stimuli because animals were allowed direct contact with it. Stimulation with the soiled bedding odor induced the production of significantly more c-Fos-positive cells in the MeA and CoA than did clean bedding odor, suggesting that the estrous soiled bedding odor stimulates both the OE and VNO. In contrast, airborne odors of estrous females are assumed to produce only volatile stimulation. Indeed, a significant increase of c-Fos-positive cells in the amygdala was observed only in the CoA but not in the MeA. Those results suggest that the chemosensory processing of the OE and VNO are relatively independent at the level of the amygdala though direct projections from the main olfactory bulb to the MeA (Kang et al., 2009). In addition, we have reported previously that sexual behavior could activate the AOB and MeA even after surgical removal of the VNO (Kondo et al., 2003).

Chemosensory signals processed in the MeA and CoA might converge upon the POA because stimulation with both the estrous soiled bedding odor and the airborne odor significantly increased the numbers of c-Fos-positive cells in the POA. In this study, we clearly demonstrated that destruction of the POA in male rats drastically suppressed their olfactory preference for conspecific odors. Although our current study is the first systematic analysis of the involvement of the POA in olfactory preference, we have some fragmentary knowledge regarding the POA and sexual partner preference. Electrolytic or chemical lesions in the POA decreased the preference for tethered receptive females over anestrous females (Edwards and Einhorn, 1986; Edwards et al., 1996). Suppression of neuronal activities in the POA by local application of lidocaine also reduced the time spent in the vicinity of inaccessible estrous females (Hurtazo et al., 2008). Although there are studies reporting that electrolytic destruction of the POA reversed sexual preference, that is, males with POA lesion preferred male rats rather than females, in male rats (Paredes et al., 1998a,b) ferrets (Alekseyenko et al., 2007), we could not affirm such effects in our experimental conditions. Presumably, the difference is caused by the means by which stimuli are presented: airborne vs. direct contact. The POA lesions in our study instead eliminated olfactory preferences. A considerable body of evidence has reported that the POA is an essential structure for expression of male sexual behavior; lesions in this region reduce sexual motivation in a variety of mammalian species (Hull and Dominguez, 2007; Sakuma, 2008). The male rats with POA lesions also showed severe impairment not only in sexual behavior but also in olfactory preference for estrous females in this study. Furthermore, POA lesions also affect preference for the odor of castrated males, suggesting the involvement of the POA in processing both sexual and nonsexual social signals.

Destruction of the MeA and CoA, the source areas of POA inputs for activation of sexual behavior (Dominguez et al., 2001), both significantly decreased the times spent nose-poking toward preferable stimulus animals. Those eliminated the olfactory preferences for estrous over anestrous females and for castrated over intact males, supporting our previous study of MeA lesions in female rats (Kondo and Sakuma, 2005). The similar effectiveness of MeA and CoA lesions suggests that there is an interaction of OE inputs with the vomeronasal system. Recently, several studies also reported possible interactions between the main and vomeronasal systems (Kondo et al., 2003; Muroi et al., 2006; Slotnick, et al., 2010). In mice, VNO inputs have been suggested to be critical for olfactory preference (Achiraman et al., 2010) and sex discrimination (Stowers et al., 2002), although this is contested (Pankevich et al., 2004). At least, the VNO is involved in preference for involatile social chemicals in mice (Keller et al., 2006a,b; Pankevich et al., 2004, 2006). In this study, we did not examine the effects of disrupting VNO inputs. At the level of the amygdala, the main olfactory and vomeronasal systems are both involved in the regulation of olfactory preference in the rat. In contrast to the similar effects of MeA and CoA lesions on olfactory preferences, these lesions exhibit different effects on copulatory behavior patterns; thus, males with MeA lesions showed significantly delayed initiation of sexual behavior and severely suppressed intromissions and ejaculations (Kondo, 1992), whereas males with CoA lesions showed similar levels of copulatory activities to those of sham males (Giantonio et al., 1970). Neural substrates regulating olfactory preference and sexual behavior might overlap partly but have different compositions.

Although males with MeA or CoA lesions showed a decreased rate of nose-poking toward estrous females when paired with intact males, they still showed significant olfactory preference for estrous females over intact males. At first, we considered these outcomes to be caused by complementary actions of the MeA and CoA after a loss of the other. Then, we made extended lesions including both the MeA and CoA. Males with MeA + CoA lesions showed decreased nose-poking times, similar to those of POA lesioned males but less than those of single MeA or CoA lesions (but not statistically significant). However, males with MeA + CoA lesions still showed a small (residual) but significant preference for estrous females over intact males. One possibility is that the residual effect may be caused by an insufficient extent of MeA + CoA lesions in covering all the areas processing these olfactory signals. Because the MeA and CoA are long structures running rostrocaudally, the penumbra might be involved in such small preferences after the lesions. Another possibility is the involvement of a third pathway: other than the MeA and CoA. Even after such combined lesions of the MeA and CoA, most of the main olfactory system is still working, so the rats are not totally anosmic and can smell food and other substances. Inputs from the OE are delivered to the piriform cortex and subsequent areas such as the anterior olfactory nucleus. The POA finally determines the phenotype of olfactory preference after the convergence of those inputs through the multiple pathways. Further study is required for understanding the chemosensory processing involved in social behavior.

Acknowledgments

This study was partly supported by Grants-in-Aid for Scientific Research to Y.K. (18590225) and Y.S. (S081035) from Japan Society for the Promotion of Science.

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CURRICULUM VITAE

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- Total Teaching experiences for MBBS, MD, BSc Nursing, BMIT, BMLT since Masters in medical Physiology: **11 years** (Including PhD graduation to till date)

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Training / Special School attended

1. A month training in Neurophysiology, Implantation Biology and Sleep laboratory at Physiology Department, all India Institute of Medical Sciences (AIIMS), New Delhi, India.
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3. Second school of Neuroendocrinology (Organized by International Neuroendocrine Federation.
4. International Brain Research Organization-Asia Pacific Regional Committee (IBRO-APRC) associate school, Penang, Malaysia
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6. Summer School of Neuroendocriniology, organized in conjunction with FENS (Federation of European Neuroscience Societies), IBRO (International Brain Research Organization), the European Research Council COST action BM1105 and the University of Monash, Australia, 27 July-2nd August 2013 , Prato, Italy

Achievement/Scholarship/Others

1. Outstanding Performance Excellence Award, 18th Nippon Medical School Research Conference for Foreign Researchers (2007), Tokyo, Japan

2. Best Presentation Award, 2nd school of Neuroendoncrinology (2009), Yufuin, Japan

3. Japan Student Services Organization (JASSO) scholarship (2008-2009) "Honors Scholarship for privately Financed International Students"

4. External Examiner for 1st year MBBS/BDS Annual Examination, 26th July to 9th August 2012, BP Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal.

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6. University External examiner for MBBS (Bachelor of Medicine and Bachelor of Surgery) annual examination, 6th January – 8th January 2013 at Institute of Medicine (IOM, Tribhuvan University) Maharajgunj, Kathmandu, Nepal.

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8. An **Organizing General Secretary** of Historic **"First Annual Conference of Physiology and Applied Sciences" ACPAS-2013,** held on 27-28 September 2013 at Nepalese Army Headquarter Army Officer's Club and Kathmandu University School of Medicine (KUSM), Dhulikhel Hospital, Kavre.

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5. International Neuroendocrine federation (INF) (under process)

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PUBLICATIONS

National Presentation

1. B Upadhaya, D Puri, N Baral, **S dhungel**. Indigenous plant remedies in Nepal. 4th Annual celebrations scientific Programme, 4 Sept 1997, B P Koirala Institute of Health Sciences, Dharan, Nepal.
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5. S Kumar, BH Paudel, **S Dhungel** and R Khadka. Effect of cold induced pain and mental task on Breath Holding Time. 5th Annual celebrations scientific Programme, 7 Sept 2000, B P Koirala Institute of Health Sciences, Dharan, Nepal.
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7. **S Dhungel**, R Sinha, M Sinha, BH Paudel, MB Mandal and N Bhattacharya. Behavioral effects of formalin pain in obese rabbit. 9th Annual celebrations scientific Program, 7 Sept 2002, B P Koirala Institute of Health Sciences, Dharan, Nepal.
8. **S Dhungel.** Neuropeptides and Neuroendocrine Regulation of Social Behavior. First Annual Conference of Physiology and Applied Sciences, ACPAS-2013, Kathmandu, Nepal, 27-28 September 2013.

International Presentation

8. **S Dhungel**, R Sinha, M Sinha, MB Mandal, N Bhattacharya. Nociceptive Response In Diet Induced Obese rabbit. Poster presentation on 4th Prof. Baldev singh Symposium on "Pain: New Dimension", All India Institute of Medical Sciences, **New Delhi, India,** 9th Feb 2002.

9. **Sunil Dhungel**, Susumu Urakawa, Yasuhiko Kondo and Yasuo Sakuma. Differential Roles of the Medial Amygdala and Preoptic Area in the Control of Conspecific Odor Preference in Male Rats. 84th Annual meeting of Physiological Society of Japan, **Osaka, Japan,** March 20th – 22nd March 2007.

10. **Sunil Dhungel**, Susumu Urakawa, Yasuhiko Kondo and Yasuo Sakuma. Medial amygdala and preoptic area regulate conspecific odor preference in male rats. Society for Behavioral Neuroendocrinology (SBN, www.sbn.org) Annual Meeting, **Asilomar, Pacific Grove, California, USA, June 21st - 24th 2007**.

11. **Sunil Dhungel,** Susumu Urakawa, Yasuhiko Kondo and Yasuo Sakuma**.** Functionaldiversity of the medial amygdala and the preoptic area in controlling sexual preference and behavior of male rats**.** 67th Annual meeting of Japanese Society of Animal Psychology (JSAP), Waseda University**, Tokyo,** Japan 7th – 8th October 2007.

12. **Sunil Dhungel**, Yasuhiko Kondo and Yasuo Sakuma. Conspecific olfactory preferences of male rats carrying lesions of the medial amygdala or the preoptic area**.** US/Japan Neurosteroid symposium 2008 Nagaragawa Convention Center, **Gifu, Japan,** September 8th- 11th 2008.

13 **Sunil Dhungel**, Susumu Urakawa, Yasuhiko Kondo and Yasuo Sakuma. Functional differences of medial amygdala and medial preoptic area in the regulation of conspecific odor preferences. Japan/China Symposium 2008. Strategies to Reduce Risks on the Brain Development Contingent to Urbanization, University of Tsukuba, **Tsukuba, Japan** October 23rd - 25th 2008.

14. **S Dhungel**, S Urakawa, Y Kondo and Y Sakuma. Olfactory preference of male rats after destruction of the medial amygdala or the medial preoptic area. XXXVI International Congress of Physiological Sciences (IUPS) 2009 **Kyoto, Japan** July 27th-1st August 2009**.**

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20. **Sunil Dhungel.** Olfactory preference of male rats after destruction of vasopressin cells in anterior olfactory nucleus. Summer School of Neuroendocrinology, **Prato, Italy**, July 26-August 2nd 2013.

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3. Kumar S, Paudel BH, **Dhungel S**, Khadka R. Effect of Cold Induced Pain and Mental Task on Breath Holding Time. ***Journal of Nepal Medical Association,* 2001,41: 262-265.**

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10. **Sunil Dhungel,** Dilip Rai, Yasuhiko Kondo, Katsuhiko Nishimori, Yasuo Sakuma. Oxytocin impairs social behavior in male and female. ***(Journal of neuroscience*** *under preparation****)***