Neuroscience 199 (2011) 225–234

BOTH OLFACTORY EPITHELIAL AND VOMERONASAL INPUTS ARE ESSENTIAL FOR ACTIVATION OF THE MEDIAL AMYGDALA AND PREOPTIC NEURONS OF MALE RATS

S. DHUNGEL,[a] M. MASAOKA,[b] D. RAI,[a] Y. KONDO[a,c]* AND Y. SAKUMA[a]

[a]*Department of Physiology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo, Tokyo 113-8602, Japan*

[b]*Department of Life Sciences, the University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan*

[c]*Department of Animal Sciences, Teikyo University of Science, 2-2-1 Senju-Sakuragi, Adachi, Tokyo 120-0045, Japan*

Abstract—Chemosensory inputs signaling volatile and nonvolatile molecules play a pivotal role in sexual and social behavior in rodents. We have demonstrated that olfactory preference in male rats, that is, attraction to receptive female odors, is regulated by the medial amygdala (MeA), the cortical amygdala (CoA), and the preoptic area (POA). In this paper, we investigated the involvement of two chemosensory organs, the olfactory epithelium (OE) and the vomeronasal organ (VNO), in olfactory preference and copulatory behavior in male rats. We found that olfactory preferences were impaired by zinc sulfate lesion of the OE but not surgical removal of the VNO. Copulatory behaviors, especially intromission frequency and ejaculation, were also suppressed by zinc sulfate treatment. Neuronal activation in the accessory olfactory bulb (AOB), the MeA, the CoA, and the POA was analyzed after stimulation by airborne odors or soiled bedding of estrous females using cFos immunohistochemistry. Although the OE and VNO belong to different neural systems, the main and accessory olfactory systems, respectively, both OE lesion and VNO removal almost equally suppressed the number of cFos-immunoreactive cells in those areas that regulate olfactory preference. These results suggest that signals received by the OE and VNO interact and converge in the early stage of olfactory processing, in the AOB and its targets, although they have distinct roles in the regulation of social behaviors.

Key words: olfactory preference, olfactory epithelium, vomeronasal organ, amygdala, preoptic area.

In rodents, olfactory signatures play a key role in social recognition. Chemical signals derived from their body or genital secretions activate the neuroendocrine pathway involved in various social behaviors including aggression, sexual maternal behaviors, and approaching and escaping responses. A considerable body of evidence has shown that the olfactory epithelium (OE) and the vomeronasal organ (VNO) are highly sensitive to such social information. In general, it has been believed that general olfaction involves the OE and the pheromones are detected in the VNO. However, that dichotomy is denied in recent studies, both the OE and VNO receive pheromonal signals in social context (Keller et al., 2009). It is also suggested that those two systems receive different pheromones, for example, in the female mouse, signal of dominant male urine are detected in the VNO, while that of subordinate urine are perceived in the OE (Veyrac et al., 2011).

Surgical ablation of the OE and VNO suggests that they also have different behavioral functions. Removal of the VNO temporarily impairs social recognition in male rats (Bluthé and Dantzer, 1993). Some studies have reported that VNO dysfunction affects sex recognition and expression of sexual behavior in mice (Kimchi et al., 2007; Stowers et al., 2002), although other reports showed that vomeronasal-organ removal (VNOx) disrupted lordosis in female mice whereas no disruptive effect of VNOx has been reported in male mice that were studied under optimal conditions (Kelliher et al., 1999; Pankevich et al., 2004, 2006). OE destruction in male rats suppressed noncontact penile erection but removal of VNO had no effect on this response (Kondo et al., 1999). In female hamsters, removal of the VNO did not impair preference for male odors over female odors (Petrulis et al., 1999).

Projections from the OE and VNO are distinct, the so-called main and accessory olfactory systems (MOB and AOB), respectively. Because the accessory olfactory system and a part of the main olfactory system converge on certain common areas (Pro-Sistiaga et al., 2007), it is hard to segregate the functions of one from those of the other. Indeed, receptors specifically sensing social cues were found in the rat olfactory epithelium (Liberles and Buck, 2006; Lin et al., 2004; Mandiyan et al., 2005; Wang et al., 2006). VNO and OE inputs may complement each other (Brennan and Keverne, 2004; Brennan and Zufall, 2006). Activation of both the MOB and AOB by volatile urinary odors (Martel and Baum, 2007; Muroi et al., 2006) and pheromone stimulation (Xu et al., 2005) has been reported.

In this study, we investigated the effect of sensory deprivation from the OE or VNO on preference behavior for conspecific odors and neuronal activation in the AOB, the medial and cortical nuclei of the amygdala (MeA and CoA),

*Corresponding author. Tel: +81-3-6910-3804; fax: +81-3-6910-3800.
E-mail address: ykondo@ntu.ac.jp (Y. Kondo).

Abbreviations: AOB, accessory olfactory bulb; cFos-ir, cFos-immunoreactive; CoA, cortical amygdale; FITC-SBA, fluorescein isothiocyanate conjugate soybean agglutinin; GRL, granular cell layer; MeA, medial amygdale; MOB, main olfactory systems; MTL, mitral cell layer; OE, olfactory epithelium; OEx, olfactory epithelium lesions; PBS, phosphate-buffered saline; POA, preoptic area; VNO, vomeronasal organ; VNOx, vomeronasal-organ removal.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.09.051

and the preoptic area (POA) in response to odors of estrous females in male rats.

EXPERIMENTAL PROCEDURES

Animals

Male and female Long-Evans rats aged 8 weeks were purchased from the Institute for Animal Reproduction, Ibaraki, Japan and maintained under controlled temperature (23±2 °C) and reversed light/dark photoperiod (lights off from 11:00–23:00) conditions with free access to food and water. The experimental protocols were approved and carried out under the guidelines for the care and use of laboratory animals of Nippon Medical School.

Preparation of animals

Prior to the experiment, all females were ovariectomized under ether anesthesia. Some were brought into estrus by a s.c. injection of 5 μg of estradiol benzoate (dissolved in 0.1 ml of sesame oil, Sigma-Aldrich, St. Louis, MO, USA) at 48 h, and 500 μg of progesterone (dissolved in 0.1 ml of sesame oil, Sigma-Aldrich, St. Louis, MO, USA) at 3–7 h prior to each behavioral test. Following a 1-week acclimation to our laboratory, all males were subjected to three mating sessions with estrous females. Those who failed to ejaculate in the third session were excluded. After the sexual experience, olfactory preference tests (described below) followed by copulation tests were carried out twice weekly as pre-surgery baseline tests.

Surgery

After the baseline behavioral tests, 15 male rats were subjected to olfactory epithelium lesions (OEx) induced by intranasal infusion of 10% zinc sulfate ($ZnSO_4 \cdot 7H_2O$ in 0.5% NaCl solution) under ketamine HCl (25 mg/kg) and sodium pentobarbital (25 mg/kg) anesthesia (Alberts, 1974; Margolis et al., 1974). During infusion, animals were placed in the prone position on an inclined surface while one end of a polyethylene tube was inserted to deliver 100 μl per nostril (at a rate of 0.02 ml/min) and another end of the tube was fitted to a Hamilton syringe attached to a microinfusion pump. Sham-OEx rats were made in the same way but infused with saline. All animals were allowed 4–5 days to recover before post-surgery experiments. To exclude the possible involvement of regenerated OE sensory neurons, all behavioral tests were completed within 6 days of the zinc sulfate treatment.

Another subset of 15 males was subjected to VNOx following the method previously described (Saito and Moltz, 1986). Briefly, under ketamine and sodium pentobarbital anesthesia, each male was held in the supine position on a homemade operating board. Their jaws and tongues were restrained by wire hooks used to keep their mouths open. The vomer bone, which encapsulates the VNO, was exposed by a midline incision of the soft palate and drilled at two points (approximately 5–6 mm apart) to excise the VNO. The incised palate was stitched using an absorbable suture. Corresponding Sham-VNOx males received the same operation without vomer bone removal.

Because sham-OEx (n=7) and sham-VNOx (n=6) were statistically indiscernible in all behavioral tests, they were combined as a single sham group (n=13) for subsequent analyses to compare with OEx and VNOx rats.

Behavioral testing

Hidden food finding test. To examine the abilities of experimental males to smell, hidden food finding tests were carried out after 48 h of food deprivation. In each test, a pellet of laboratory chow was located in a transparent observation cage (50 cm long×30 cm wide×40 cm high) and covered with 5-cm-thick wooden bedding. Each male was placed in the center of the cage and allowed to seek the buried food for 5 min. Time holding the pellet with the forepaws was recorded as latency. Tests were carried out twice, 1 week before and 3 days after the zinc sulfate treatment.

Olfactory preference test. Olfactory preference was assessed using an alternate choice paradigm. The details of the preference chamber have been described previously (Xiao et al., 2004). The apparatus was a three-chambered acrylic observation box (110 cm long×12 cm wide×30 cm high). Each experimental male was placed in the middle compartment and two stimulus rats were placed in the side compartments. Three opaque plates with holes of 3-cm diameter at different levels were assembled into a partition to create divisions between the compartments. A blower connected to the ceiling of the middle compartment through a corrugated flexible tube and maintaining negative pressure in the middle compartment allowed airflow (approximately 0.2 m^3/min) from the two side compartments into the middle compartment. A 2-cm-deep transparent tube at 2 cm from the floor was attached to the holes in both side partition plates facing the middle compartment.

On the day of the olfactory preference test, 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 ovariectomized female; and (3) an intact male and a castrated male. The test order and the positions of the stimulus pairs were counterbalanced, and the intervals between tests were more than 1 h. Before each test, the apparatus was cleaned with 70% ethanol (v/v) and bedded with fresh paper chips (Alpha-dri, Shepherd Speciality Papers; Kalamazoo, MI, USA). During a period of 5 min for acclimation to the apparatus, downstream airflow was made from the middle to the side compartments. Behavioral observations were made for 5 min and recorded by a video camera fixed in front of the preference chamber. Time spent nose-poking in each of the left and right inlets was calculated by an event recorder on a personal computer. Preference scores in each stimulus pair were calculated by determining percent time spent nose-poking toward receptive females (Pairs 1 and 2) or castrated males (Pair 3) relative to total time spent nose-poking toward both stimulus animals.

All males were subjected to twice weekly repetitive olfactory preference tests before surgery. As a post-surgery test, olfactory preference was tested within 1 week after OEx surgery to exclude the influence of regenerated olfactory neurons, and within 2 weeks after VNOx surgery to allow for sufficient recovery (see timeline scheme of behavioral testing shown in Table 1).

Table 1. Timeline schema of behavioral tests

Week	First session	Second session
1	Sexual behavior test	
2	Sexual behavior test	
3	Olfactory preference test	Sexual behavior test
4	Olfactory preference test surgery of OEx and VNOx[a]	
5	Olfactory preference test[b]	Sexual behavior test[b]
6	Olfactory preference test[c]	Sexual behavior test[c]
7	Stimulation with estrous odor	Sacrifice after 2 h

Tests appeared in each line were carried out within the same day in order with an interval more than 2 h.

[a] Surgery was conducted within 1 or 2 d after olfactory preference test.

[b] Recovery period was 1 wk in OEx males and 2 wk in VNOx males (see Method).

[c] In OEx males, the second test of post-surgery was omitted in order to exclude an influence of possible neuronal regeneration.