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ROLE OF THE VOMERONASAL SYSTEM IN INTERSEXUAL ATTRACTION IN FEMALE MICE

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Abstract—Although it is generally accepted that rodents' sociosexual behavior relies mainly on chemosignals, the specific roles played by the vomeronasal and olfactory systems in detecting these signals are presently unclear. This work reports the results of three experiments aimed at clarifying the role of the vomeronasal system on gender recognition and intersexual attraction, by analyzing the effects of lesions of the accessory olfactory bulbs (AOB) in chemically naïve female mice. The first experiment demonstrates that lesions of the AOB abolish the preference that females show for male-soiled bedding in tests in which the females can contact the bedding, thus having access to both volatile and involatile male chemosignals. The second experiment shows that airborne male-derived chemosignals are not attractive to intact, chemically naïve females but tend to be preferentially explored by females whose AOB has been lesioned. However, repeated exposure to male-soiled bedding has opposite effects in sham-operated and AOB-lesioned female mice. Whereas after this experience sham-operated females show an (acquired) attraction toward male airborne chemosignals, in AOB-lesioned females the same experience makes male-derived volatiles aversive. Finally, in the third experiment we have confirmed that our AOB-lesioned females are able to detect urine-borne male odorants, as well as to discriminate them from the synthetic terpene geraniol. These findings strongly suggest that in mice, the involatile male sexual pheromone that is intrinsically attractive is detected by the vomeronasal system of the females. In addition, the repeated experience of females with male-soiled bedding would probably allow the association of this pheromone, acting as unconditioned stimulus, with olfactory stimuli (odorants) that therefore would become conditioned attractors to the females.

Key words: olfactory system, pheromones, reward, emotional learning, sexual behavior.

Rodents' socio-sexual behavior is largely dependent on chemosignals. Males and females scent-mark using urine (Hurst and Beynon, 2004), vaginal fluids or other secretions (Petrulis and Johnston, 1997). Scent marks contain different molecules that, together, act as efficient chemosignals. These include lipocalins (Cavaggioni and Mucignat-Caretta, 2000), such as the major urinary proteins of mice (Cheetham et al., 2007; Stopka et al., 2007), the alpha 2u globulins of rats (Beynon and Hurst, 2004) or the aphrodisin of female hamsters (Briand et al., 2004), other large peptides secreted by exocrine glands (e.g. lachrymal glands; Kimoto et al., 2005), small peptides such as those related to the Major Histocompatibility Complex (MHC) type I (Boehm and Zufall, 2006) and small volatiles that probably are tightly bound to lipocalins (Sharrow et al., 2002). This combination of highly volatile, moderately volatile and involatile molecules can be detected by the olfactory epithelium and/or vomeronasal organ (VNO), which consequently are fundamental for elaborating adaptive responses to conspecifics' chemical cues.

In this context, several studies have explored the role of both sensory organs on sexual behavior in mice. The results of these works are, however, partially inconsistent. Some of them indicate that the VNO is needed for detection of possible mates, so that its removal impairs sexual behavior in both females (Keller et al., 2006a) and males (Clancy et al., 1984), as well as several precopulatory behaviors such male-to-female ultrasonic vocalizations (Wysocki et al., 1982). In contrast, both male (Leypold et al., 2002; Stowers et al., 2002) and female mice (Kimchi et al., 2007) deficient in the TRPC2 channel, involved in sensory transduction by vomeronasal cells, show increased mounting toward conspecifics, irrespective of their gender, as well as decreased aggressions toward males. These findings suggest that the VNO mediates gender discrimination thus promoting gender-specific behaviors. However, why lesions of the VNO decrease, whereas 'genomic lesions' of the organ increase sexual behavior, remains difficult to explain. In this sense, it has been suggested (Kimchi et al., 2007) that surgical removal of the VNO often results in occlusion of the nasal cavity, and therefore the behavioral deficits observed in VNO-lesioned animals would actually be the consequence of additional olfactory impairment.

In mice and other rodents, pheromone-mediated intersexual attraction is very likely a key step in mate detection leading to sexual encounter. However, although there is no doubt that males prefer female- to male-derived chemosignals and vice versa, whether attractive sexual pheromones are olfactory and/or vomeronasal stimuli is presently unclear. Some authors report that lesions of the VNO result in a loss of attraction toward non-volatile chemosig-

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Abbreviations: AOB, accessory olfactory bulb; CM/CM, castrated male-soiled bedding test; CM/M, intact vs. castrated male-soiled bedding two-choice test; Ger, geraniol; MHC, major histocompatibility complex; MTMT, (methylthio)methanethiol; PBS, phosphate-buffered saline; VNO, vomeronasal organ.

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nals (the detection of which requires contact with the scent source) of conspecifics of the other gender (Pankevich et al., 2004; Keller et al., 2006a). This suggests that pheromone-mediated intersexual attraction depends on vomeronasal-dependent chemosignals, the olfactory system playing a minor role, since when contact with the scent source occurs, olfactory stimuli are surely detected. In agreement with this, using two-choice chemoinvestigation tests we have shown that chemically naïve female mice, deprived from any experience with male chemosignals, show a clear preference to explore male-soiled bedding vs. female- (Moncho-Bogani et al., 2002) or castrated male–soiled bedding (Martínez-Ricós et al., 2007). However, this attraction (which is independent of the levels of sexual steroid hormones, Moncho-Bogani et al., 2004) is not observed when the females explore the bedding through a perforated methacrylate platform, thus having access only to male-derived volatiles. This suggests that volatiles detected by the olfactory epithelium (Moncho-Bogani et al., 2005) are not attractive to chemically naïve females, although they can become secondarily attractive after learning induced by repeated exposure to male-soiled bedding (allowing contact with the bedding; Moncho-Bogani et al., 2002, 2005). A similar situation is found in male mice. Thus, males are attracted by female urine (Pankevich et al., 2004) and lesions of the VNO abolish this attraction irrespective of whether contact with the urine is allowed or not, thus suggesting that involatile, VNO-detected urine-borne pheromones mediate attraction of males toward females.

However, several pieces of evidence support two different alternative views on this issue. Fist, Keller et al. (2006a) have shown that both intact and VNO-lesioned female mice show attraction toward male-derived volatiles, whereas destruction of the olfactory epithelium suppresses attraction for both volatile and involatile male chemosignals (Keller et al., 2006b), thus suggesting that volatiles detected by the olfactory epithelium might be fundamental for intersexual attraction. On the other hand, several volatiles present in male urine that are known to stimulate vomeronasal neurons (Leinders-Zufall et al., 2000) have been shown to be attractive for female mice (see Dulac and Torello, 2003, for a review), thus suggesting that volatiles detected by the VNO mediate female attraction for male chemosignals.

To try to clarify this issue we have performed three experiments using chemically naïve female mice. In the first experiment, we have analyzed the effects of bilateral lesions of the accessory olfactory bulb (AOB) on the preference of chemically naïve females for intact male- vs. castrated male-soiled bedding, in tests in which the females had access to the bedding. By lesioning the AOB instead of the VNO, we can be sure that the effects of the lesions cannot be attributed to occlusion of the nasal cavity. In the second experiment, we assess the effects of AOB lesions on the attraction of females for male-derived volatiles, before and after repeated experience with male chemosignals.

This experiment was designed to re-examine the role of the olfactory epithelium in the detection of innately attractive sexual chemosignals, as well as analyzing whether the vomeronasal system is involved in the acquisition of attraction to male volatiles, e.g. if non-volatile, vomeronasally-detected male pheromones can act as unconditioned stimuli for the acquisition of conditioned attraction to male odorants. Finally, in the third experiment we use the habituation–dishabituation procedure to assess whether AOB-lesioned females are able to detect male odorants and to discriminate them from other odorants.

EXPERIMENTAL PROCEDURES

Experiment 1: Effects of AOB lesions on innate attraction of females for male chemosignals

In this experiment we tested whether innately attractive non-volatile male pheromones contained in male-soiled bedding are detected through the vomeronasal system. To do so, we carried out electrolytic lesions of the AOB in a group of females (Lesion group) and sham surgery in another group (Sham group). Then, we compared the preference of Sham and Lesion groups toward male chemosignals by means of two-choice tests between bedding soiled by intact and castrated males.

Animals and experimental design. Experimental animals (n=41) were adult (more than 9 weeks of age) CD-1 female that had never contacted adult males nor male-derived secretions or excretions. Animals were treated throughout according to the EEC guidelines for European Communities Council Directives of 24 November 1986 86/609/EEC using procedures approved by the Committee of Ethics on Animal Experimentation of the University of València. Specifically, we asked for veterinarian assessment to avoid animal suffering and we tried to minimize the number of animals by using a within-subject experimental design when possible.

Surgery and post-surgical care. Animals were randomly assigned to the control (sham surgery) or the AOB-lesion group and received the corresponding surgery. For all surgical procedures, animals were anesthetized with sodium pentobarbital (Sigma, St Louis, MO, USA; 60 mg/kg i.p.) and atropine (Sigma; 0.4 mg/kg) was injected intraperitoneally to reduce cardio-respiratory depression. Then, animals were placed in a stereotaxic frame (David Kopf Instruments 963-A, Tujunga, CA, USA) and lesions were aimed at the AOB at the following coordinates: +3.5 anterior to bregma, 1 mm from the midline and 1.3 mm deep from the brain surface (adapted to the CD-1 strain from Franklin and Paxinos, 2001). To avoid damaging the prefrontal cortex covering the AOB and the inferior cerebral vein, with the consequent risk of a serious hemorrhage, we approached the AOB by inserting the electrode at more rostral levels (antero-posterior coordinate +3.95 mm) with an angle of 48°. Electrolytic lesions were made by passing a constant (negative) current of 0.8 mA (using a current generator; Ugo Basile, Comerio, VA, Italy) for 15 s through a stainless steel electrode of 250 μm diameter, insulated except for 500 μm of the tip (Ugo Basile). Sham surgery consisted in introducing the electrode using a similar approach without passing any current.

Behavioral tests. Tests were performed in rectangular methacrylate cages (25 cm wide, 50 cm long and 30 cm high) with two glass dishes (6 cm diameter and 5.5 cm high), containing 13 g of soiled bedding, located in opposite sides of the cage.

After at least 1 week of recovery after surgery, both Sham (n=21) and Lesion females (n=20) were habituated to the experimenter and to the test cage for 10 min daily during four consec-