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GENERAL BIOLOGY

Mechanisms of Reproductive Isolation in House Mouse Superspecies Complex Mus musculus s.lato: from Behaviour to Receptors[1]

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The receptor mechanisms of the chemical signals influences underlying species integrity are at the very initial stage of investigation at the present time. In current study test subjects were two sympatric species which do not hybridize under natural conditions: house mouse (*Mus musculus*) and mount—building mouse (*M. spicilegus*) as well as laboratory mice of *M. domesticus* group. The main purposes of the research were: (1) comparative analysis of receptor neurons activation in vomeronasal organ (VNO) of male mice in response to stimulation with receptive con- and heterospecific female odour; (2) comparative analysis of activated pathways from receptor level of VNO to the central nervous system structures in response to stimulation by con-and heterospecific chemical cues. To visualize activated neurons on VNO receptor tissue sections as well as on olfactory bulbs sections in response to stimulation, Fos protein immunohistochemistry was used [1]. Fos protein is a product of *c-fos* known as immediate-early gene which is induced quickly by different stimuli including cell depolarization [2]. Labeling Fos provides a physiological marker of neurons activated in response to specific stimuli.

Half life span of protein Fos is two hours: depending on specific characteristics and neural cell localization optimal exposure time for maximal Fos detection may range from 45 to 90 min [3]. Test subjects were young sexually mature males and females of following species: *M. musculus, M. spicilegus* and *M. domesticus* (laboratory form). To stimulate main and accessory olfactory system males were exposed to receptive con- and heterospecific female bedding for 40 min using half duty cycle (one minute—specific odor, one minute—clean air). To evaluate neural activity at the level of VNO receptor tissue males were exposed to the bedding of receptive con- and heterospecific females for 90 min [4]. Immediately after exposure males were perfused with 3% paraformaldehyde in phosphate buffer. VNO was removed with cartilage capsule as described by C.J. Wysocki [5] and postfixed in paraformaldehyde for two hours. We used standard procedure for fixation of olfactory bulbs, cryoprotection and immunohistochemical staining of VNO and olfactory bulbs sections [6]. We used indirect avidin/biotin method; horseradish peroxidase was used as enzymatic label, diaminobenzidin (DAB) was used as chromogen. Sections were made at 20 μm using cryostat Triangle Biomedical. Immunostaining was made according to standard three day protocol using primary antibodies Santa Cruz Biotechnology (USA): c-fos (4) sc-52, dilution 1: 500. For visualization and counting of Fos positive cells we used Nikon©Eclipse E400 microscope with camera Nikon©Coolpix 990. For picture analyses we used ImageJ (NIH) and Pinnacle studio 8 software.

Male mice were exposed to the bedding containing female chemical signals. Urine and other excretions of receptive female which may contain the bedding are of complex chemical composition including volatile as well as high molecular weight substances. It appears to be that basal VNO sensory neurons mainly activated by higher molecular weight substances and peptides while apical VNO sensory neurons, located closer to the lumen, activated by volatile compounds as well as by higher molecular weight substances (nonvolatile) [7, 8]. In response to exposure of conspecific receptive female bedding to *M. domestius* males ($n = 20$) we recorded Fos-immunoreactivity in both, apical and basal VNO zone, which suggests multicompound nature of chemical signal (Fig. 1). Fos-positive cells were located mainly in rostral part of VNO. Round

[1]The article was translated by the authors.

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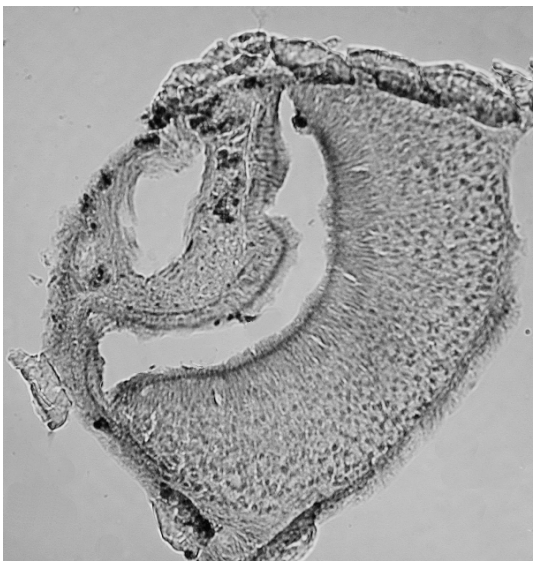

Fig. 1. Neural activity in receptor epithelium of vomeronasal organ in male *Mus musculus domesticus* in response to stimulation with conspecific receptive female chemical signals. Fos-positive neurons are located in both, apical and basal zone of sensory epithelium.

shape and positioning of Fos-positive elements on VNO sections allows identifying them as nuclei of VNO receptor cells. This conclusion is based on literature data on ultra structure of VNO epithelium. Nuclei of receptor, respiratory and supporting cells of VNO differ in shape [9, 10]. In response to exposure of diestral female bedding in house mouse we observed very low level of Fos-immunoreactivity in male VNO epithelium: a very few cells were immunostained. We did not see any Fos-positive cells in VNO epithelium in control animals ($n = 10$) exposed to clean bedding. In response to exposure of receptive *M. spicilegus* female bedding to *M. domesticus* male ($n = 8$) we recorded Fos-immunoreactivity mainly in supporting cells but not in receptor ones (Fig. 2). Specific pattern of activation in sensory epithelium was absent. In response to exposure of receptive *M. spicilegus* female bedding to conspecific male ($n = 8$) we observed Fos-immunoreactivity in receptor VNO epithelium mainly in basal zone (Fig. 3). Thus pattern of VNO receptor cells activation in response to stimulation with receptive female odour was different in males of the two species. Most likely that the lack of specific pattern of activation in VNO receptor tissue in response to stimulation with heterospecific odour may be explained by different chemical composition e.g. by species specificity of pheromonal like substances excreted by receptive female. Exposure of receptive female bedding to conspecific as well as to heterospecific males induced neural activation in the main olfactory bulb (MOB) at the glomeruli level and at the mitral cells level. Moreover neural activation was observed in both: rostral and caudal part of MOB. For the males of all three species, *M. musculus, M. spicilegus, M. domesticus*, in response to stimulation with conspecific receptive female bedding we observed clear pattern of activation in caudal part of accessory olfactory bulb (AOB) which receives projections from basal VNO zone where receptors binding to higher molecular weight substances are expressed [11]. At the same time in response to exposure of receptive M. spicilegus female bedding to *M. musculus* and *M. domesticus* males we did not observe any Fos-immunoreactivity in AOB. Heterospecific receptive female odour did not

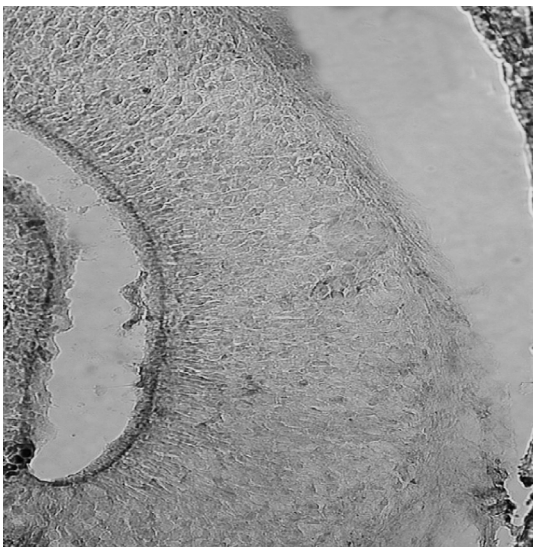

Fig. 2. Lack of activation in receptor epithelium of vomeronasal organ of *M. domesticus* male in response to stimulation with receptive female *M. spicilegus* chemical signals.

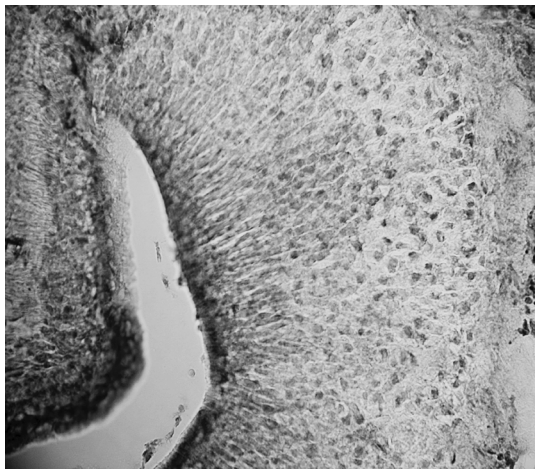

Fig. 3. Neural activity in receptor epithelium of vomeronasal organ in male *M. specilegus* in response to stimulation with conspecific receptive female chemical cues. Fos-positive cells are located mainly in basal zone.

induce neural activation neither at the level of receptor tissue nor at the projecting area of AOB. Thus primary sensory analysis of biological relevance of chemical signal may take part at the level of receptor tissue. It favors the hypothesis about different systems of olfactory communication in two phylogenetic groups of mice (commensal and noncommensal). Based on the results of our previous research and the results of current study the following conclusions could be made [12–14]. In sympatric species of house mouse which do not hybridize under natural conditions, *M. musculus* and *M. spicilegus*, precopulatory reproductive isolation is provided by multiple mechanisms at different levels of organization: from differences in behavioral patterns to differences in receptor signal coding. Precopulatory isolation mechanisms may work at the receptor level as well as through different behavioral responses of individuals to olfactory cues upon interactions of potential sexual partners; taken together these provide reliable reproductive isolation for sympatric species under natural conditions.

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REFERENCES

1. Flavell, S.W. and Greenberg, M.E., *Annu. Rev. Neurosci.,* 2008, vol. 31, pp. 563–590.
2. Sheng, M. and Greenberg, M.E., *Neuron,* 1990, vol. 4, pp. 477–485.
3. Morgan, J.I. and Curran, T., *Annu. Rev. Neurosci.*, 1991, vol. 14, pp. 421–451.
4. Ambaryan, A.V., Voznesenskaia, A.E., Kotenkova, E.V., and Voznessenskaya, V.V., *Chem. Senses*, 2009, vol. 34, no. 3.
5. Wysocki, C.J., Wysocki, L.M., in *Experimental Cell Biology of Taste and Olfaction: Current Techniques and Protocols*, Boca Raton: CRC, 1995, pp. 49–57.
6. DellaCorte, C., in *Experimental Cell Biology of Taste and Olfaction: Current Techniques and Protocols*, Boca Raton: CRC, 1995, pp. 145–142.
7. Kimoto, H., Haga, S., Sato, K., and Touhara, K., *Nature,* 2005, vol. 437, pp. 898–901.
8. Leinders-Zufall, T., Brennan, P., Widmayer, P.S., et al., *Science*, 2004, vol. 306, pp. 1033–1037.
9. Vaccarezza, O.L., Sepich, L.N., and Tramezzani, J.H., *J. Anat.,* 1981, vol. 132, pp. 167–185.
10. Adams, D.R., *Microsc. Res. Tech.*, 1992, vol. 23, pp. 86–97.
11. Rodriguez, I., Feinstein, P., and Mombaerts, P., *Cell*, 1999, vol. 97, pp. 199–208.
12. Sokolov, B.A., Korchagina, Yu.I., Mirzoev, D.A., et al., *Neftegazoobrazovanie i neftegazonakoplenie v Vostochnom Predkavkaz'e* (Oil and Gas Generation and Accumulation in the Eastern Ciscaucasia), Moscow: Nauka, 1990.
13. Ambaryan, A.V. and Kotenkova, E.V., *Usp. Sovrem. Biol.*, 2008, vol. 128, no. 5, pp. 199–214.
14. Voznesenskaia, A.E., Ambaryan, A.V., Kotenkova, E.V., and Voznessenskaya, V.V., *Chem. Senses*, 2005, vol. 30.