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Pheromonal signals provide specific information concerning the identity, gender, endocrine, and social status of different members of the population in a variety of mammals (Halpern, 1987; Wysocki and Meredeth, 1987). Pheromones in urine excreted from male and female rats induce various changes in gonadal functions such as reflex ovulation in the absence of coitus and mounting (Johns *et al.*, 1978), a reduction in the oestrous cycle of female rats from 5 to 4 days (Chateau *et al.*, 1976), and oestrous synchrony among female rats living together (McClintock, 1978).

The vomeronasal organ is the peripheral chemoreceptor organ of the vomeronasal system. Regulation of gonadal functions by urinary pheromones has been well established in the rodent vomeronasal organ. Vomeronasal sensory neurons project information to the accessory olfactory bulb (AOB) located on the dorso-caudal surface of the main olfactory bulb. Immunohistological methods have been used to visualize Fos as a means of identifying neurons that are activated by stimulation. The induction of Fos has been widely used as an assay for studying the excitability of populations of neurons within many

different regions of the brain. The urinary pheromone-induced increase in Fos-immunoreactivities were eliminated by the removal of the vomeronasal organ in the AOB of rats (Inamura *et al*., 1999a).

Augmentation of sensitivity of male rats to female urinary pheromone after sexual experiences

Sexually experienced Long-Evans male rats prefer oestrous to dioestrous urine odor, and dioestrous urine odor to distilled water odor (Pfaff and Pfaffmann, 1969; Lydell and Doty, 1972). Sexually inexperienced males do not exhibit these preferences, indicating that there may exist a temporally discrete information source for sexually experienced male rats that may accurately indicate a given female's state of sexual receptivity. Information regarding the females' endocrine state is transmitted to males by means of urinary pheromones. The expression of Fos-ir cells in the accessory olfactory bulb of sexually experienced male rats was compared with that from sexually inexperienced male rats following exposure to oestrous urine (Sakamoto *et al*., submitted for publication). In the localized region (lateral and rostral regions) of the periglomerular cell layer, many more Fos-ir cells were expressed in the sexually experienced rats than in the inexperienced rats, which suggests that sexual experience promotes the formation of a memory of a pheromone found in oestrous urine at the periglomerular cell layer of the accessory olfactory bulb.

Chemical characterization of rat urinary pheromones

Pheromones have been found to be proteins and low molecular weight molecules. The activity of the component in male urine to induce expression of Fos-immunoreactivity in the caudal region of the accessory olfactory bulb of female rats was abolished by papain treatment, while that in the rostral region was not (Tsujikawa and Kashiwayanagi, 1999). The pronase treatment of male urine abolished the expression of immunoreactivity in the rostral region as well as in the caudal region, suggesting that at least two urinary peptides (papain-sensitive and -insensitive ones) with the ability to stimulate the vomeronasal organ of female rats are contained in male Wistar rat urine.

Exposure to the substances remaining after dialysis (> 100 Da) induced Fos-ir cells in the AOB of female Wistar rats, while the dialyzed urine preparation (< 100 Da) did not induce a remarkable number of Fos-immunoreactive (Fos-ir) cells (Yamaguchi et al., 2000). These results suggest that the molecular weights of components with the ability to induce Fos-ir cells in the rat AOB are over 100 Da. Exposure of the female rat vomeronasal organ to either the dialyzed urine preparation (< 500 Da) or the remaining substances (> 500 Da) of male rats did not induce expression of Fos-ir cells in the AOB, whereas exposure to a mixture of these preparations did induce expression (Yamaguchi *et al*., 2000). In rats, the application of urine preparations without dialysis induces inward currents in vomeronasal sensory neurons under the voltage-clamp condition (Inamura and Kashiwayanagi, 2000) and increases in impulse frequency (Inamura et al., 1997;1999b), which in turn lead to the expression of Fos-ir cells in the AOB (Inamura et al., 1999a). These results suggest that the combination of high and low molecular weight substances is

responsible for depolarization, increases in impulse frequency, and the expression of Fos-immunoreactivity in the AOB. Exposure to crude urine and an ultrafiltrated urine preparation (< 5000 Da) induces significant Fos expression in the mitral/tufted cell layer of the AOB, while exposure to either the substances remaining after ultrafiltration (> 5000 Da) or a control salt solution did not, suggesting that components with molecular weights below 5000 Da carry the activity to induce Fos-ir cells in the rat AOB (Tsujikawa and Kashiwayanagi, 1998). The high molecular weight fraction (>5000 Da) alone loses its ability to stimulate expression because it does not contain low molecular weight substance(s). Major urinary proteins, however, may have a high molecular weight with ability to induce expression of Fos-ir cell in the rat AOB. It is also possible that other protease-sensitive substance(s) with molecular weights ranging from 500 to 5000 Da also induce Fos-ir cells in conjunction with low molecular weight substances.

In mouse, the application of urine-derived compounds of low molecular weight such as 2,3-dehydro-*exo*-brevicomin induces only hyperpolarizing responses, that is, inhibitory responses, in the vomeronasal sensory neuron (Moss *et al*., 1997) and does not induce c-fos mRNA expression in the AOB (Guo *et al*., 1997).

Augmentation of pehromonal activities in male urine after sexual experiences

Exposure to urine preparation excreted form young male (10 weeks old) rats without a sexual experience did not induce remarkable expression of Fos-ir cells in the mitral/tufted cell layer of AOB (Tomioka et al., submitted for publication). Urine preparations excreted from sexually experienced males of 12 weeks old induced much Fos-ire cells but not

significant. Exposure to urine from sexually inexperienced males (12 weeks old) did induce remarkable Fos-ir cells. These results suggest that pheromonal activities in male urine were augment by sexual experiences. As described above, a combination of low and high molecular weight substances is necessary for the increases in Fos-immunoreactivity in the AOB of rats. Exposure to a mixture of the dialyzed urine preparation (< 500 Da) of sexually experienced males and the remaining substances (> 500 Da) of sexually inexperienced males did not induce expression of Fos-ir cells in the AOB. However, exposure to a mixture of the dialyzed urine preparation (< 500 Da) of sexually inexperienced males and the remaining substances (> 500 Da) of sexually experienced males did induce remarkable expression. These results suggest that pheromonal activities of high molecular weight substances in urine increase after sexually experiences.

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