

## Augmentation of Sensitivity to Urinary Pheromone and Excreting of Urinary Pheromone by Sexual Experiences

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### Introduction

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 Meredith, 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. 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. Immunohistological methods have been used to visualize Fos as a means of identifying neurons that are activated by stimulation. The urinary pheromone-induced increases in Fos-immunoreactivities were eliminated by the removal of the vomeronasal organ in the AOB of rats, indicating that pheromonal information is transmitted to neurons at the AOB (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 AOB 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 AOB.

### Chemical characterization of rat urinary pheromones

Pheromones have been found to be proteins and low mol. wt molecules. The activity of the component in male urine to induce expression of Fos-immunoreactivity in the caudal region of the AOB 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 mol. wts of components with the ability to induce Fos-ir cells in the rat AOB are >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 mol. wt 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 mol. wts <5000 Da carry the activity to induce Fos-ir cells in the rat AOB (Tsujikawa and Kashiwayanagi, 1999). The high mol. wt fraction (>5000 Da) alone loses its ability to stimulate expression because it does not contain low mol. wt substance(s). Major urinary proteins, however, may have a high mol. wt with ability to induce expression of Fos-ir cell in the rat AOB. It is also possible that other protease-sensitive substance(s) with mol. wts ranging from 500 to 5000 Da also induce Fos-ir cells in conjunction with low mol. wt substances. Similar results were obtained in mouse. The application of urine-derived compounds of low mol. wt such as 2,3-dehydro-*exo*-brevicomin induces only hyperpolarizing responses, that is, inhibitory responses, in the vomeronasal sensory neuron of mouse (Moss *et al.*, 1997) and does not induce c-fos mRNA expression in the AOB (Guo *et al.*, 1997).

### Augmentation of pheromonal activities in male urine after sexual experiences

Exposure to urine preparation excreted from 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 inexperienced males of 12 weeks old induced much Fos-immunoreactivity but not significant. Exposure to urine from sexually experienced males (12 weeks old), however, did induce remarkable Fos-ir cells. These results suggest that pheromonal

activities in male urine were augmented by sexual experiences. As described above, a combination of low and high mol. wt 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 mol. wt substances in urine increase after sexual experiences.

## References

- Chateau, D., Roos, J., Plas-Roser, S., Roos, M. and Aron, C.** (1976) *Hormonal mechanisms involved in the control of oestrous cycle duration by the odour of urine in the rat.* Acta Endocrinol., 82, 426–435.
- Guo, J., Zhou, A. and Moss, R.L.** (1997) *Urine and urine-derived compounds induce c-fos mRNA expression in accessory olfactory bulb.* Neuroreport, 8, 1679–1683.
- Halpern, M.** (1987) *The organization and function of the vomeronasal system.* Annu. Rev. Neurosci., 10, 325–362.
- Inamura, K. and Kashiwayanagi, M.** (2000) *Inward current responses to urinary substances in rat vomeronasal sensory neurons.* Eur. J. Neurosci., 12, 3529–3536.
- Inamura, K., Kashiwayanagi, M. and Kurihara, K.** (1997) *Blockage of urinary responses by inhibitors for IP<sub>3</sub>-mediated pathway in rat vomeronasal sensory neurons.* Neurosci. Lett., 233, 129–132.
- Inamura, K., Matsumoto, Y., Kashiwayanagi, M. and Kurihara, K.** (1999a) *Laminar distribution of pheromone-receptive neurons in rat vomeronasal epithelium.* J. Physiol. (Lond.), 517, 731–739.
- Inamura, K., Kashiwayanagi, M. and Kurihara, K.** (1999b) *Regionalization of Fos immunostaining in rat accessory olfactory bulb when the vomeronasal organ was exposed to urine.* Eur. J. Neurosci., 11, 2254–2260.
- Johns, M.A., Feder, H.H., Komisaruk, B.R. and Mayer, A.D.** (1978) *Urine-induced reflex ovulation in anovulatory rats may be a vomeronasal effect.* Nature, 272, 446–468.
- Lydell, K. and Doty, R.L.** (1972) *Male rat odor preferences for female urine as a function of sexual experience, urine age, and urine source.* Horm. Behav., 3, 205–212.
- McClintock, M.K.** (1978) *Estrous synchrony and its mediation by airborne chemical communication (Rattus norvegicus).* Horm. Behav., 10, 264–276.
- Moss, R.L., Flynn, R.E., Shen, X.M., Dudley, C., Shi, J.M. and Novotny, M.** (1997) *Urine-derived compound evokes membrane responses in mouse vomeronasal receptor neurons.* J. Neurophysiol., 77, 2856–2862.
- Pfaff, D. and Pfaffmann, C.** (1969) *Behavioral and electrophysiological responses of male rats to female rat urine odors.* In Pfaffmann, C. (ed.), Olfaction and Taste III. Rockefeller University Press, New York, pp. 258–267.
- Singer, A.G.** (1991) *A chemistry of mammalian pheromones.* J. Steroid Biochem. Mol. Biol., 39, 627–632.
- Tsujikawa, K. and Kashiwayanagi, M.** (1999) *Protease-sensitive urinary pheromones induce region-specific fos-expression in rat accessory olfactory bulb.* Biochem. Biophys. Res. Commun., 260, 222–224.
- Wysocki, C.J. and Meredith, M.** (1987) *The vomeronasal system.* In Finger, T.E. and Silver, W.L. (eds), Neurobiology of Taste and Smell. John Wiley, New York, pp. 125–150.
- Yamaguchi, T., Inamura, K. and Kashiwayanagi, M.** (2000) *Increases in Fos-immunoreactivity after exposure to a combination of two male urinary components in the accessory olfactory bulb of the female rat.* Brain Res., 876, 211–214.