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# Role of the Vomeronasal Organ on the Estral Cycle Reduction by Pheromones in the Rat

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The role of he vomeronasal organ on the estral cycle reduction induced by pheromones is studied in adult female wistar rats. The animals were divided in three groups: I, intact rats; II, vomeronasalectomized rats (VNX); and III, sham operated rats (sham). Each group was submitted to another three distinct conditions from the day they were weaned (21 days old): Isolated female rats; with male odors from two adult males of tested sexual potency, and isolated rats again. The isolated intact rats show mainly 5 day length cycles. The groups I and III (intacts and sham) with male odors, show 4 day length cycles. The VNX animals show 5 day cycles in any one experimental conditions. These results support the idea that the vomeronasal organ is the receptor of the male reducing cycle pheromone in the female rat.

**Key words:** Vomeronasal organ, Estral cycle, Pheromones.

It is a known fact that the estrous cycle of female mice can be influenced by stimuli originating in the male (10, 22, 39). In the rat, several effects have been described that are produced by exteroceptive stimuli. These effects range from a modification of the pattern of behaviour in newborn rats (24, 25) to modifications in the sexual patterns of behaviour of adult rats (11, 23, 37). In relation to modifications in the rat estrous cycle, it has been established that odour of the male urine is capable of reducing the ovarian cycle duration of the female rat from 5 to 4 days (3, 12).

Apparently, stimuli that modify the patterns of the estrous cycle in female mice and rats do so through olfactory structures and nervous endings situated inside the nasal cavities (9). On the other hand it has been proposed that hypothalamic hypophyseal structures are involved in the mechanisms determining pseudopregnancy in female mice in the presence of strange males (10). There is evidence that the estrous cycle-reducing pheromone acts in some way on nervous mechanisms that regulate the secretion of gonadotropins, and it has been suggested that pheromones control the storage and release of FSH by the hypophysis (14). Furthermore, other authors (29) have

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proposed that the estrous cycle-reducing pheromone influences directly the luteal function of the ovary by modifying the ovarian secretion of progesterone.

The apparent evidence of the pheromonal effects on the cycle length in the female mice and rat, does not apply to the elucidation of which structure is the exteroceptive receptor capable of detecting and transmitting the information to the nervous centers. However, the observation that the axons of the sensory cells of the vomeronasal organ (VNO) end in the accessory olfactory bulb (6, 35) and the evidence that some of the pheromonal stimuli can be transmitted through the olfactory accessory bulb (30, 32, 33) suggest that the VNO could be the receptor structure of the phemoronal stimuli.

Nevertheless, the idea that the VNO could be a pheromonal sexual chemoreceptor is not new, for this possibility has been stated in various occasions, with respect to female mice and rats, and also in a range of domestic and non-domestic mammals that live in complete freedom in their own environment (7, 8, 15-20, 41). The participation of accessory olfactory bulb in relation to estrous-reducing stimuli, has also been evidenced (31,32). Likewise, the vomeronasal organ seems to participate in the estrous cycle regulation as it has been reported previously (26).

## Materials and Methods

Ninety seven sexually inexperienced female Wistar rats were used weighing 39.78 ± 6.7 g at the time of weaning on postnatal day 21. They were vomeronasalectomized or sham operated 39 days later (postnatal day 60). Their body weight was then 179 ± 12.43 g.

In order to observe the animals, they were situated in cages of 40×25×14 cm in the number of 4-5 animals in each, were kept at room temperature (24±2°C) and exposed to the natural solar rhythm from September to March. The food supply consisted of a standard laboratory diet and water *ad libitum,* with permanent and direct access to it. The estrous cycle was determined daily by microscopic observations of vaginal smear, reconstructing the phases of the estrous cycle according to NALVANDOV (27).

Bilateral vomeronasalectomy was performed under sodium pentobarbital anaesthesia (3.5 mg/100 g). The animal was fixed in decubito supine position with its head directed towards the operator. Keeping the animal's mouth wide open, the palate was exposed and the operative field was delimitated with the aid of a 10 × stereoscopic microscope. An incision was made in the midline of the palate mucosa. The edges of the wound were separated and the os incisivi was exposed. The os incisivi and the capsule of the VNO were trepanned with a fine electric drill. The VNO was extracted with the aid of a microaspirator and the cavity was checked with a needle. The hole of trepanation was filled with sterile gelatine sponge. The soft tissue was sutured with silk or catgut n.° 000000. Antibiotics were administered in the drinking water for a week after the operation. The survival rate was about 90 %. The other 10 % died during the operation or before recuperation from anaesthesia. The sham operation includes anaesthesia, mucosa incision, os incisive trepanation and exposition of vomeronasal capsule, as soon as the closure of surgical wound. The histological control of the VNO lesion showed a total disappearance of its normal structure in a ramdomized sample of lesioned rats.

The rats were divided into three groups. Their estrous cycle was controlled daily between 9 a.m. and 11 a.m., immediately after the vaginal opening. They were kept away from the males from the moment they were 21 days old.

In a second stage, rats in group I were left intact; those in group II were vomeronasalectomized and those in group III were sham operated. In this stage, cages containing adult males of proved sexual potency were placed on top of the cages that contained female rats. In this way the female rats could smell the odour from male excretions. The estrous cycle was controlled as previously described. Finally all the rats were again isolated and the estrous cycle length determined once more. «Chi» square test to two independent samples were applied for statistical analysis (36).

## Results

The results obtained with respect to the length of the estrous cycle in female rats under different experimental conditions can be seen in Table I. The results are expressed in percentages, considering 100 % the number of cycles observed in each stage of the experiment. In intact and isolated rats there is a predominance of a 5 day length cycle. In the discussion the appearance of a high number of anomalous duration cycles (less than 4 days and more than 5) during this stage of the experiment, will be referred to. When rats are put in contact with the male odours, there is an evident increase in the cycle of 4 days length and a decrease in the 5 day cycle. At the same time, a low percentage of anomalous cycles appears. When these animals are isolated again, a similar situation to that of the first phase has appeared; that is, there is a decrease in the percentage of the 4 day cycle and an increase in the percentage of the 5 day cycle; the percentage of anomalous cycles remaining relatively low.

Relative to group I, the percentage of cycles of 5 day length is significantly higher in female rats isolated from males, whereas in the rats in contact with the male odours, the highest percentage corresponds to a 4 day cycle. In the group, the percentage of cycles of more than 5 days is significantly higher in isolated rat whose cycles control was initiated immediately after the vaginal opening. There are no significant statistical differences between the anomalous cycles of rats in contact with the male odours and the same animals isolated again.

Isolated rats belonging to the second group show a predominance of a 5 day cycle immediately following the vaginal opening. When they were 60 days old, rats belonging to this group were subjected to a bilateral vomeronasalectomy. Under these conditions no variation in the percentage distribution of the length of cycles was found. The distribution was similar to that obtained at the previous stage, except that the percentage of anomalous cycles decreased to the levels found in group I (rats in contact with male odours). When the vomeronasalectomized rats were isolated again, the distribution of the cycles was similar to that obtained in previous stages.

The statistical analysis shows that the duration of the cycles under different experimental conditions are not statistically diferent, except in the case of cycles of 6 days length.

The female rats, sham operated (group III), isolated from males, placed in contact with male odours and isolated again, behave statistically in a similar way to the intact rats of group I.

Global results show that in intact isolated rats a 5 day length cycle predominates, whereas in intact rats placed in contact with the male odours, a 4 day length cycle predominates. This also occurs in rats that have been sham operated. In contrast, bilaterally vomeronasalectomized rats behave as if they were isolated in any one of the experimental conditions.

Table I. *Number of cycles and percentage of the latter length in rats belonging to different experimental groups.*

| | Group | | 3 days or less | | | 4 days | | | 5 days | | | 6 days | | | 7 days or more | | | Total cycles |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | | No. | % | | No. | % | | No. | % | | No. | % | | |
| | IT 1 | | 2 | 1.49 | | 16 | 11.94 | | 62 | 46.27 | | 24 | 17.91 | | 30 | 23.87 | | 134 |
| | | p* | | | N.S. | | | <0.01 | | | <0.01 | | | <0.01 | | | <0.01 | |
| I | IT 2 | | 4 | 2.38 | | 108 | 64.28 | | 45 | 26.78 | | 6 | 3.57 | | 5 | 2.98 | | 168 |
| | | p** | | | N.S. | | | <0.01 | | | <0.01 | | | N.S. | | | N.S. | |
| | IT 3 | | 7 | 5.11 | | 43 | 31.38 | | 71 | 51.82 | | 10 | 7.30 | | 6 | 4.38 | | 137 |
| | IT 1 | | 8 | 5.19 | | 25 | 16.23 | | 89 | 57.79 | | 16 | 10.39 | | 16 | 10.39 | | 154 |
| | | p* | | | N.S. | | | N.S. | | | N.S. | | | N.S. | | | N.S. | |
| II | VX 2 | | 7 | 5.69 | | 29 | 23.58 | | 78 | 63.41 | | 5 | 4.07 | | 4 | 3.25 | | 123 |
| | | p** | | | N.S. | | | N.S. | | | N.S. | | | <0.05 | | | N.S. | |
| | VX 3 | | 6 | 7.89 | | 11 | 14.47 | | 43 | 56.58 | | 10 | 13.16 | | 6 | 7.89 | | 76 |
| | IT 1 | | 1 | 0.72 | | 13 | 9.42 | | 84 | 60.87 | | 18 | 13.04 | | 22 | 15.94 | | 138 |
| | | p* | | | <0.05 | | | <0.01 | | | <0.01 | | | N.S. | | | <0.01 | |
| III | SH 2 | | 9 | 7.03 | | 62 | 48.44 | | 45 | 35.16 | | 8 | 6.25 | | 4 | 3.12 | | 128 |
| | | p** | | | <0.05 | | | <0.01 | | | <0.01 | | | | | | N.S. | |
| | SH 3 | | 1 | 0.75 | | 32 | 24.06 | | 93 | 69.92 | | 2 | 1.50 | N.S. | 5 | 3.76 | | 133 |

IT: Intact rats; VX: Vomeronasalectomized rats; SH: Sham operated rats. 1: Isolated rats. 2: With male odours. 3: Isolated again. * «Chi» square test 1 v. 2. ** «Chi» square test 2 v. 3. N.S.: Non significant.

## Discussion

The high percentage of irregular cycles in rats isolated 21 days after their birth, has an antecedent in experiments performed in female mice (38), showing that vaginal and uterine cycles in female mice isolated when they were 21 days old are prolonged in time and are disorganized.

These results agree with those described above in the female rat, as the highest percentage of anomalous cycles are of prolonged duration (more than 5 days, Table I). These cycles were those that follow immediately the vaginal opening, with diestrous phases generally prolonged.

It is a known fact that intact female rats in contact with adult male urine odours reduce the length of the cycle from 5 to 4 days (2, 3, 12, 13). However, vomeronasalectomized rats present a predominance of a 5 day cycle under any experimental condition, that is, vomeronasalectomized rats are not capable of reducing the duration of the cycle from 5 to 4 days when they are put in contact with odours from male rats. Keeping in mind that sham operated rats present a cyclic behavior that is statistically identical to the one that intact rats present, it can be supposed that the VNO of the female rat acts as receptor of the pheromones that, emitted by the adult male, are capable of reducing the cycle length from 5 to 4 days. These results are in accordance to those of SÁNCHEZ-CRIADO (31), who has achieved similar data in rats with stereotaxic electrocoagulation of the accessory olfactory bulb, the second step of vomeronasal system pathway.

The fact that, in the past, the receptions of these external influences have been attributed to the olfactory sense (3-5, 28) probably is due to a false interpretation of the olfactory deficits. The technique designed to ablate the vomeronasal organ is very specific. To get to the vomeronasal organ through the palate, the nervous structures of nasal cavities (olfactory receptors, organ of Masera, trigeminal endings and terminal nerve endings) remain intact. Such technique practically eliminates the obstacles to interpret the discussed results. These obstacles have been pointed out extensively (1, 40).

The pheromonal stimulation of the VNO acts, probably, on the hypothalamus-hypophyseal-gonadal axis through the sensorial accessory olfactory bulb-cerebral amygdala (vomeronasal system) pathway according to SCALIA's dual concept of the Olfactory System (34, 35). This concept is valid not only from a morphological point of view. Physiologically it has been shown that the ablation of some portion of the vomeronasal system may report clear changes in the reproductive neuroendocrinology in rats, since such ablation does not seem to influence the sexual behavior of the female rat (21).


## Resumen

Se estudia en ratas hembras adultas, destetadas a los 21 días de edad, el papel del órgano vomeronasal sobre la reducción del ciclo estral inducida por feromonas. Se disponen tres grupos experimentales: I, ratas intactas; II, ratas vomeronasalectomizadas (VNX); y III, falsamente operadas (sham). Cada grupo, a su vez, fue sometido a otras tres distintas situaciones: Ratas aisladas de machos; con olores de machos procedentes de dos machos adultos con potencia sexual comprobada, y ratas aisladas de nuevo. Las ratas intactas aisladas de olores de machos muestran en los tres grupos ciclos de 5 días de duración predominantemente. Las de los grupos II y III (intactas y sham) con olores de machos, presentan ciclos de 4 días. Los animales VNX, en cualquier condición experimental, tienen ciclos de 5 días. Estos resultados apoyan la idea de que el órgano vomeronasal en la rata hembra es el receptor de la feromona del macho que reduce la duración del ciclo estral.

## References


1. ALBERTS, J. R.: *Physiol. Behav.*, **12**, 657-670, 1974.
2. ARON, C. L.: *Archs. Anat. Mist. Embryol. Norm. Exper.*, **56**, 209-216, 1973.
3. ARON, C. L.: *Physiol. Rev.*, **5**, 229-283, 1979.
4. ARON, C. L., ROOS, J. and ROOS, M.: *Neuroendocrinology*, **6**, 109-117, 1970.
5. ARON, C. L., ROOS, J. and ROOS, M.: *J. Interdiscipl. Cycle Res.*, **2**, 239-246, 1971.
6. BARBER, P. C. and RAISMAN, G.: *Brain Res.*, **81**, 21-30, 1974.
7. BARBER, P. C. and RAISMAN, G.: *Proc. Intern. Union Physiol. Sci.*, **12**, 698, 1977.
8. BELLRINGER, J. F., PRATT, H. P. M. and KEVERNE, E. B.: *J. Reprod. Fert.*, **59**, 223-228, 1980.
9. BRONSON, F. M.: Pheromones (A. Neuberger and E. L. Tatum, eds.). North Holland Pub. Co. London, 1974, pp. 344-365.
10. BRUCE, H. M.: *Nature*, **184**, 105-106, 1959.
11. CARR, W. J., LOEB, L. S. and WYLIE, N. R.: *J. Comp. Physiol. Psychol.*, **62**, 336-338, 1966.
12. CHATEAU, D., ROOS, J. and ARON, C. L.: *C.r. Sèanc. Soc. Biol.*, **166**, 1110-1113, 1972.
13. CHATEAU, D., ROOS, J. and ARON, C. L.: *C.r. hebd. Sèanc. Acad. Sci. Paris*, **276**, 2823-2826, 1973.
14. CHATEAU, D., ROOS, J., ROOS, M. and ARON, C. L.: *C.r. Sèanc. Soc. Biol.*, **168**, 1422-1427, 1974.
15. ESTES, R. D.: *Mammalia*, **36**, 315-341, 1972.
16. GALLEGO, A., SÁNCHEZ-CRIADO, J. E. and MORA, O.: In «Determination of behavior by chemical stimuli» (Steiner J. E. and Granchow, J. R., eds.). IRL Press. London, 1982, pp. 57-64.
17. GURTOVOI, N. N. and NIKOL'SKILL, V. S.: *Biol. Nauki*, **16**, 28-32, 1973.
18. JOHNS, M. A., FEDER, M. M., KOMISARUK, B. R. and MAYER, A. D.: *Nature*, **272**, 446-447, 1978.
19. JONHSTON, R. E. and RASMUSSEN, K.: *Physiol. Behav.*, **33**, 95-104, 1984.
20. KANEKO, N., DEBSKI, F. A., WILSON, M. C. and WHITTEN, W. K.: *Biol. Reprod.*, **22**, 873-878, 1980.
21. KELCHE, C. and ARON, C. L.: *Physiol. Behav.*, **33**, 45-48, 1984.
22. LEE, S. VAN DER and BOOT, L. M.: *Acta Physiol. Pharmac. Neerl.*, **4**, 442-443, 1955.
23. LEMAGNEN, J.: *Arch. Sci. Physiol.*, **6**, 295-332, 1952.
24. LEON, M. and MOLTZ, H.: *Physiol. Behav.*, **8**, 683-686, 1972.
25. MOLTZ, H. and LEON, M.: *Physiol. Behav.*, **10**, 69-71, 1973.
26. MORA, O. A. and GALLEGO, A.: *Proc. Intern. Union Physiol. Sci.*, **13**, 525, 1977.
27. NALVANDOV, A. V.: In «Reproductive physiology: Comparative reproductive physiology of domestic animals, laboratory animals and man» (Salisbury, G. W. and Crampton, E. W., eds.). W. H. Freeman, Co. San Francisco, 1958, pp. 98-124.
28. PARKES, A. S. and BRUCE, H. M.: *Science*, **134**, 1049-1054, 1961.
29. ROSER, S. and CHATEAU, D.: *C.r. Sèanc. Soc. Biol.*, **168**, 829-834, 1974.
30. SÁNCHEZ-CRIADO, J. E.: *Endocrinologia*, **26**, 16-19, 1979.
31. SÁNCHEZ-CRIADO, J. E.: *Rev. esp. Fisiol.*, **35**, 137-142, 1979.
32. SÁNCHEZ-CRIADO, J. E.: In «Olfaction and endocrine regulation» (Breipohl, W., ed.). IRL Press. London, 1982, pp. 209-221.
33. SÁNCHEZ-CRIADO, J. E. and GALLEGO, A.: *Acta Endocrinol.*, Suppl. **225**, 255, 1979.
34. SCALIA, F. and WINANS, S. S.: *J. Comp. Neurol.*, **161**, 31-55, 1975.
35. SCALIA, F. and WINANS, S. S.: In «Mammalian olfaction, reproductive processes and behavior» (Doty, R. C., ed.). Academic Press, 1976, pp. 7-28.
36. SIEGEL, S.: Estadística no paramétrica. Trillas, México, 1975.
37. STERN, J. J.: *Physiol. Behav.*, **5**, 519-524, 1970.
38. STIFF, M. E., BRONSON, F. M. and STETSON, M. H.: *Endocrinology*, **94**, 492-496, 1974.
39. WHITTEN, W. K.: *J. Endocr.*, **13**, 399-404, 1956.
40. WYSOCKY, C. J.: *Neurosci. Behav. Rev.*, **3**, 301-341, 1979.
41. WYSOCKY, C. J., KATZ, Y. and BERNHARD, R.: *Biol. Reprod.*, **28**, 917-922, 1983.