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Social Inhibition of Sexual Maturation in Female and Male Bank Voles (*Clethrionomys glareolus*)

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Kruczek M., Marchlewska-Koj A. & Drickamer L. C., 1989: Social inhibition of sexual maturation in female and male bank voles *(Clethrionomys glareolus)*. Acta theriol., 34, 33: 479—485 [With 2 Tables]

Puberty of female bank voles, *(Clethrionomys glareolus)* (Schreber, 1780) was estimated by uterine weight, and the number of Graafian follicles. In male bank voles puberty was assessed by testes weight, accessory gland weight, and index of spermatogenesis. Sexual maturation of males was inhibited by the presence of intact or castrated-testosterone treated males. Adult males did not affect the age of puberty of females. Urine of grouped intact or ovariectomized females strongly inhibited puberty of juvenile females. Males reared in the presence of female urine had less advanced spermatogenesis than non-exposed males. These findings indicate that in *Clethrionomys glareolus* the social interaction between conspecifics is manifested mainly by inhibition of the onset of puberty.

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1. INTRODUCTION

Delayed sexual maturation of both sexes may be one of the most important factors limiting reproductive activity in high density populations of rodents. Several studies have shown that the onset of puberty in house mice, deermice, and voles is inhibited or stimulated by chemosignals released by mature conspecificis (see reviews: Babb & Terman, 1982; Vandenbergh, 1983; Drickamer, 1986).

Our previous observations (Kruczek & Marchlewska-Koj, 1986) indicate that bank vole females (*Clethrionomys glareolus*) reared in a high density populations show delayed sexual maturation in comparison to females bred in isolation. A number of different stimuli — tactile, auditory, visual and olfactory — may have operated in these experiments (Bronson, 1979).

The purpose of the present study was to identify the social cues mediating the influence of adult male or female bank voles on maturation of conspecifics.

2. MATERIAL AND METHODS

The bank voles used in the experiments came from an outbred stock colony reared in our laboratory. The animals were maintained in metal cages (40×20×25 cm) at 18—20°C,

on photoperiod schedule of 14 h light : 10 h dark; humidity was not controlled. Food (oats, wheat, carrots and red beets) and water were provided in excess once daily. Wood shavings were used as bedding material and were changed once each week.

The experiments were conducted in spring and summer to avoid the influence of the season on the rate of sexual maturation.

The animals were reared in sexually mixed groups until 21 days of age. After weaning, males and females with body weights 8.5 to 9.5 g were used for experiments. Juvenile males and females were separated and housed in unisexual groups of 2-3 animals per cage during the next 3 weeks. Depending on the experimental protocol they were either kept isolated, or in the presence of an adult intact male, castrated male, castrated-testosterone treated male, or they were exposed to male urine.

When juvenile males and females were reared with adult females they were frequently attacked and wounded by adult females. In view of these findings, only the influence of female urine on puberty was investigated. The juvenile animals were exposed to the urine of females reared alone (isolated female), grouped females or ovariectomized grouped females.

A cage with juvenile males or females was connected by glass tubing with a metabolic cage placed above in which one male or one female was maintained for 24 h. Only urine reached the cage below and all faeces and food were diverted to a special container.

Urine donors were housed singly or animals per cage (grouped females). The animals were transferred to a metabolic cage each day for 24 hours; after that period they returned to a home cage and were left undisturbed for one weeks.

Females were ovariectomized and males were castrated at least 4 weeks before use. Castrated males were injected s.c. with 500 µg testosterone propionate in paraffin oil (Polfa, Jelenia Góra, Poland) every day starting from 3 days before use.

Young males and females were weighed and killed when 42 days old. In males, both testes, seminal vesicles and coagulating glands were dissected out and weighed (the accessory glands were weighed jointly). The left testis was fixed in Carnoy's fluid, sectioned at 7 µm and stained with haematoxylin and eosin. The functional state was classified using the spermatogenic index (SI) (Grocock & Clake, 1974). The spermatogenic index (values from 5 to 0) gives a measure of seminiferous epithelium activity, 5 representing complete spermatogenesis with abundant sperm production and 0 the presence only of Sertoli cells and spermatogonia. The spermatogenic index was determined for each left testis on 10 seminiferous tubules situated in the center o testicular cross-section.

In females the uterus was weighed. Both ovaries dissected out and fixed in Bouin's fluid, serially sectioned at 7 µm and stained with haematoxylin and eosin. The Graafian follicles were classified according to diameter and to the number of granulosa cells in the largest cross-section of each follicle (Pedersen & Peters, 1968).

Data were analysed by using ANOVA and Duncan's New Multiple Range Test.

3. RESULTS

There were no significant differences between body weights of 42 day-old females and males across all experimental groups. Also the presence of intact males or their urine did not affect the uterine weights and number of Graafian follicles of young females (Table 1). On the other hand, juvenile males reared in the presence of intact adult males or castrated-testosterone treated males had significantly smaller testes than males from other experimental groups. This correlated with low-

Table 1

Body and uterine weights and number of Graafian follicles in females and body, testicular and accessory glands (seminal vesicles and coagulation glands) weigts and spermatogenic index (SI) in males of bank voles exposed to males or their urine. Mean ∓ SEM not followed by the same letter are significantly different (Duncan's New Multiple Range Test) at $p<0.05$.

	N of	Weight of		N of	N of	Weights of			SI
	fe-males	body (g)	uterus (mg)	Graafian follicles	males	body (g)	testes (mg)	seminal ves. +coag. glands (mg)	
Control	10	14.5±1.0	12.9±1.5	2.2±0.9[1]	9	16.6±0.9	316.0±32.4[a]	36.9±9.4[a]	3.1±0.06[a]
Urine of intact male	6	12.1±0.5	10.5±1.4	2.5±0.4	8	15.1±0.9	297.0±23.7[a]	14.6±2.5[ab]	3.1±0.06[a]
Intact male	7	13.1±0.9	10.1±1.6	3.0±0.5	8	15.5±0.6	148.4±30.5[b]	5.6±0.9[b]	1.7±0.07[b]
Castrated male	—	—	—	—	8	16.5±0.9	254.8±53.6[a]	31.7±13.8[a]	2.8±0.08[a]
Castrated+test. male	—	—	—	—	6	14.9±0.1	83.9±8.0[b]	3.8±0.3[b]	1.8±0.10[b]

[1] For histological procedure ovaries of 6 randomly chosen females were used.

Table 2

Body and uterine weights and number of Graafian follicles in females and body, testicular and accessory glands (seminal vesicles and coagulation glands) weigts and spermatogenic index (SI) in males of bank voles exposed to female urine. Mean ± SEM not followed by the same letter are significantly different (Duncan's New Multiple Range Test) at $p<0.05$.

	N of	Weight of		N of	N of	Weights of			SI
	fe-males	body (g)	uterus (mg)	Graafian follicles	males	body (g)	testes (mg)	seminal ves. +coag. glands (mg)	
Control	10	14.5±1.0	12.9±1.5[a]	2.2±0.9[a,1]	9	16.6±0.9	316.0±32.4	36.9±9.4	3.1±0.06[a]
Isolated female	7	14.5±0.6	15.0±1.5[a]	0.7±0.2[ab]	7	17.6±0.7	287.1±38.6	21.3±8.1	2.8±0.09[b]
Grouped females	8	13.9±0.6	9.4±1.4[b]	0	6	15.2±0.9	244.2±44.2	21.6±6.3	2.3±0.13[c]
Ovx, grouped females	6	11.9±0.9	6.2±1.1[b]	0.8±0.4[ab]	—	—	—	—	—

[1] For histological procedure ovaries of 6 randomly chosen females were used.

er index of spermatogenesis. In seminiferous tubules only round spermatids were found whereas elongated spermatids and a few spermatozoa were present in testes of control males, reared with castrated males or exposed to male urine. Males kept with intact males or castrated-testosterone treated males also had smaller accessory glands than other experimental groups.

Males reared from day 21 to day 42 in the presence of intact males or castrated-testosterone treated males (Table 1) had testicular weights and accessory glands weights similar to the same organs in 21 day-old males (63.7 ± 5.0 mg and 2.3 ± 0.1 mg respectively; the differences were not significant, $p > 0.05$.)

Urine of grouped intact or ovariectomized female bank voles strongly affected the puberty of juvenile females (Table 2). This appeared as lower uterine weights than in non-exposed or exposed to the urine of isolated females. Also Graafian follicles did not develop in ovaries of young females reared in the presence of grouped female urine.

Juvenile males reared in the presence of urine from singly housed or grouped females had less advanced spermatogenesis than non-exposed males. Males exposed to the urine of singly housed females or grouped females inhibited spermatogenesis at the level of elongated spermatids (Table 2).

4. DISCUSSION

The results of the present experiments clearly show that delay in the onset of puberty in female bank voles is evoked by the urine of grouped adult females. The release of chemosignals is not affected by ovariectomy. In this respect there is a strong similarity between female mice (Drickamer, 1977; Drickamer *et al.*, 1978) and voles. Also in female pine voles (*Pitymys subterraneus)* relaese of chemical signals which delay puberty is not controled by ovarian hormones (Frankiewicz & Marchlewska-Koj, 1986). As indicated by our experiments in bank voles, urine of adult females influences the reproductive system of juvenile males. This appears as inhibition of spermatogenesis. Rissman *et al.*, (1984) showed that *Microtus californicus* males were delayed in attaining puberty when reared on family bedding or bedding of the mother. In our experiments females different from mothers were used as the urine donors but a certain degree of kinship between donors and young males cannot be excluded.

Male stimulation of earlier puberty in females has been documented in number of rodents: *Mus musculus* (Vandenbergh, 1969); *Peromyscus maniculatus* (Teague & Bradey, 1978); *Microtus pennsylvanicus*

(Baddloo & Clulow, 1981); and *Microtus agrestis* (Spears & Clarke, 1986). However, our results show that in bank voles the presence of males or their urine neither increased the uterine weights nor the number of Graafian follicles during 3 weeks of exposure.

The influence of social interaction between males on their hormonal activity is less documented. McKinney & Desjardins (1973) found that exposure of juvenile male mice to adult males impaired spermatogenesis and decreased accessory gland weights. In *Peromyscus maniculatus* the presence of males or their urine strongly inhibited reproductive activity of juvenile males (Lawton & Whitsett, 1979). Our results indicte that juvenile male bank voles reared with intact male or castrated-testosterone treated males showed delayed maturation. As indicated by testicular weights and accessory gland weights the repreductive system did not develop during the 3 weeks of the experiments and remained nearly at the level of 21-day old males. In bank voles, testosterone is required to evoke the inhibitory effect on the reproductive system in young males.

Behavioral observations indicate that adult males did not fight with juvenile males and females. Aggressive behavior and wounding were never noted in these experimental groups. Adult males stayed together with juvenile animals in their common nest. Male behavior was entirely different from adult female habits. As mentioned in the METHODS females were very aggressive toward young animals of both sexes.

We suggest that in bank voles the inhibitory effect of adult males may be brought about by chemosignals released from a source other than urine. In *Microtus agrestis* the presence of adult males decreased the development of seminiferous tubules in juvenile males reared on a short photoperiod (Spears & Clarke, 1986). Also Milligan (1976) found that in this species pregnancy block was evoked only when a female was in direct contact with a strange male.

Our findings indicate that in *Clethrionomys glareolus* the social interaction between conspecifics is manifested mainly by inhibiton of reproductive activity which appears as the delay of the onset of puberty in both sexes. This phenomenon might be involved in the regulation of multi-annual cycles with the peaks every three to five years followed by a pronounced decline in the density of population. On the other hand, in *Mus musculus* where no such fluctuation have been reported, both activatory and inhibitory olfactory signals influence on the reproductive activity in feral population (Bronson, 1979)

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WPŁYW WARUNKÓW SOCJALNYCH NA TEMPO DOJRZEWANIA PŁCIOWEGO SAMIC I SAMCÓW NORNICY RUDEJ (*CLETHRIONOMYS GLAREOLUS*)

Streszczenie

W prezentowanej pracy jako wskaźnik dojrzewania płciowego samic przyjęto ciężary macic oraz liczbę pęcherzyków Graafa, natomiast u samców takimi wskaźnikami były ciężary gonad i gruczołów dodatkowych oraz stopień zaawansowania spermatogenezy. Zahamowanie procesu dojrzewania płciowego młodych samców stwierdzono tylko w przypadku, gdy były one hodowane w obecności dorosłego samca lub samca kastrowanego, któremu podawano testosteron. Nie stwierdzono natomiast wpływu obecności dorosłego samca na tempo dojrzewania płciowego młodych samic. Wyraźne opóźnienie dojrzewania płciowego samic zanotowano pod wpływem działania moczu zgrupowanych i owariektomizowanych samic. Proces spermatogenezy u młodych samców hodowanych w obecności moczu pojedynczej samicy był znacznie mniej zaawansowanny w porównaniu z samcami kontrolnymi.

Na podstawie otrzymanych wyników wysnuto wniosek, iż wzajemne socjalne oddziaływania w obrębie gatunku przejawiają się zahamowaniem procesu dojrzewania płciowego.