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[SHORT COMMUNICATON]

Effects of Vomeronasal Organ Removal on the Sperm Motility in Male Mice

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ABSTRACT—Odors play important roles in the communication of house mice. They release behaviors and prime changes of the physiological conditions of other individuals. In our previous study, we showed that sperm motility was lowered in the subordinate mice comparing with dominant mice. Our hypothesis is that the lowered sperm motility was due to some primer effects by odor substances derived from dominant mice. To test the hypothesis, we destroyed the vomeronasal organ (VNO) of male mice (VNX male) at 5 weeks of age and paired them with intact male mice (Experimental Group). As control group males, intact male mice were kept in pairs (Control Group). At 15 weeks of age, the sperm motility and weights of reproductive organs, and social dominance was analyzed. The subordinate VNX males were found to have high sperm motility comparable to the dominant males. It was suggested that there is male-to-male primer effects, mediated by VNO, that suppress sperm motility of the subordinate mice.

Kew words: primer effect, social dominance, vomeronasalectomy

INTRODUCTION

Pheromone plays important roles in mammalian communications. It releases behavior and primes the physiological changes of other conspecific individuals (Vandenbergh, 1994). Studies on primer effects in male mice have been mainly investigated the hormonal changes of males triggered by female odors but only a few studies have been reported on the influence on sperm so far. For examples of the primer effects on hormones, it is known that female odors stimulate the release of LH (Maruniak and Bronson, 1976) and increase the level of plasma testosterone concentration (Barnard *et al*., 1997) in male mice. As for the primer effects on sperm, stimuli by female odors have been found to increase sperm density (Koyama and Kamimura, 2000) and the odors of adult males given to premature males are known to cause morphological abnormalities of spermatozoa (Aref'ev *et al*., 1986).

In a recent study of ours, we showed that the sperm motility of the dominant male mice is higher than the subordinates (Koyama and Kamimura, 1999). To explain the phenomenon, the following three hypotheses can be postulated: that is, inborn differences, stress effects on subordinates, or primer effect due to the pheromone by dominants. In our previous papers, we showed that male mice isolated after weaning had significantly higher sperm motility than subordinates (Koyama and Kamimura, 1999), and the variance of sperm motility among individual mice was small in puberty and became larger in full adulthood (Koyama and Kamimura, 2003). These studies suggested that the difference of sperm motility was not due to their inborn characteristics, but some environmental factors would have worked after puberty. Since plasma corticosterone concentration did not differ between the dominants and subordinates (Koyama and Kamimura, 2000), stress given to subordinates would be small. To test the remaining hypothesis of the primer effect, we removed the vomeronasal organ (VNX treatment) and kept these VNX male mice with intact males in the present study. Sperm motility was investigated in the pairs where clear social dominance was observed and VNX males became subordinate.

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MATERIALS AND METHODS

Animals and housing conditions

ddY male mice were kept in male-male pairs since 4 weeks of

age. Throughout the study, mice were kept on 12:12 hr L:D cycle (lights were on from 08:00 till 20:00). Food (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water were provided *ad libitum*. A special type of bedding (Petline, Co., Tokyo, Japan) made of paper, which has high capacity to soak up water, was used to avoid exchanging the bedding throughout the experiment in order to keep the odor environment stable. Room temperature was ranged from 15 to 28°C. These housing conditions were within the institutional guideline on keeping laboratory animals.

Vomeronasalectomy treatment

At 5 weeks of age, one of the males in half of the pairs (Experimental Group) went through vomeronasalectomy treatment (VNX males) (M. Ichikawa, personal communication). The left males of these Experimental Group pairs were anaesthetized without any other treatment. The left half pairs were designated as Control Group. Half of these males was sham operated and half was anaesthetized without any other treatment. As there were no differences between the results of these sham operated males and intact males in the Control Group, they were treated as the intact males. As the successful VNX males or transgenic males deficient in vomeronasal organ (VNO) function are reported to become non-aggressive (Bean, 1982; Clancy *et al*., 1984; Stowers *et al*., 2002), we used the VNX males that did not show aggression in the final test on sperm in the present study. In order to investigate the influence of VNX treatment on the health condition, body weights were periodically measured.

Investigation of dominant-subordinate relationships in the paired-males

All the pairs went through resident-intruder tests from 8 to 15 weeks of age in order to determine the dominant-subordinate relationships (Koyama and Kamimura, 1999, 2000). Details of the procedure of the resident-intruder tests are written elsewhere (Koyama and Kamimura, 1999, 2000). Briefly, an intruder mouse was put into the home-cages of each pair until aggression occurred or after 10 min had passed without any aggression. The pairs in which one determined mouse showed aggression at these test were determined to have clear social dominance. And the pairs with clear social dominance were used at the final test on sperm. In the Experimental Group, the pairs with clear social dominance and the pairs in which the VNX males became subordinates were used at the final test on sperm.

Observation of sperm motility and measuring weights of reproductive organs

At 15 weeks of age, sperm motility was investigated. Details of the method to determine sperm motility are described elsewhere (Koyama and Kamimura, 1999, 2000). Briefly, after measuring body weights the mice were anaesthetized using overdose of sodium pentobarbital (Nembutal). Then spermatozoa collected from right cauda epididymis, diluted in Biggers, Whitten, and Whittingham (BWW) medium (Biggers *et al*., 1971; Koyama and Kamimura, 1999), were immediately observed under a phase-contrast microscope (Nikon Optiphoto with Olympus objective SPlan x 20NH). The microscope images observed using a CCD camera (DXC-151, Sony, Tokyo, Japan) were recorded on video tapes (NV-SXSOW Panasonic, National, Tokyo). Thermo Plate (Model MATS-SS, Tokai Hit, Shizuoka, Japan) was used to keep the specimen at 37°C under the microscope. The percentage motility of spermatozoa was determined from the recorded images. Weights of left testes were measured to obtain the parameter to show the level of reproductive activity. The weight of preputial glands that is shown to be smaller in the subordinates (Bronson, 1973) was also determined.

Statistical analyses

ANOVA was used as statistical analysis and Fisher's LSD was used as post-hoc test.

RESULTS AND DISCUSSION

Sperm motility showed clear difference according to the groups and social dominance (ANOVA, Fgroup (1,110) =11.728, P=0.001, Fdominance (1,110)=7.462, P=0.007). In Experimental Group, the sperm motility of VNX subordinate males was as high as that of intact dominant male mice (Fig. 1, right 2 bars). The sperm motility of the intact subordinates of Control Group was significantly low comparing with that of dominants (Fig. 1, left 2 bars), which was consistent with our previous studies (Koyama and Kamimura, 1999, 2000).

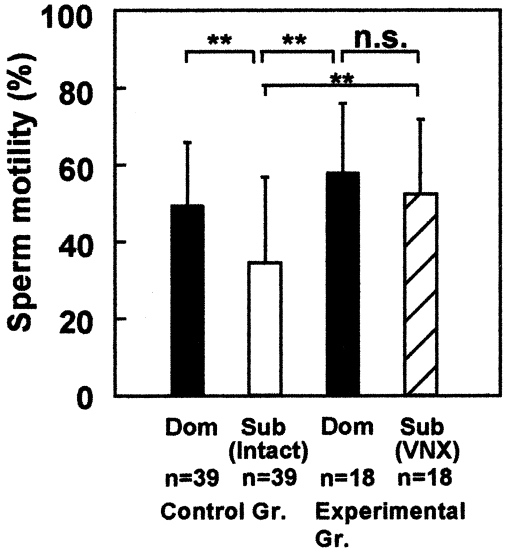

Fig. 1. Sperm motility of intact control pairs and VNX experimental pairs. Dom and Sub mean dominants and subordinates, respectively. Averages±sd of the dominants and subordinates of control and experimental pairs were 50±16, 35±22, 58±18, and 52±19, respectively. **: P<0.01, n.s.: not significant.

It is known that mice become non-aggressive when their VNO are destroyed (Bean, 1982; Clancy *et al*., 1984; Stowers *et al*., 2002). Most of the VNX mice were revealed to be non-aggressive by our resident-intruder tests (85%). They behaved as subordinates in the present study. Under the present experimental condition, it is not clear whether the dominant-subordinate relationships between VNX subordinates and dominants were the same as those between intact mice. However, one of the characteristic features of subordinate mice (Bronson, 1973) was clearly observed in these VNX mice, i.e. they had smaller preputial glands than dominants (Fig. 2a, Fdominance(1,85)=17.175, P<0.001). We could not find out any differences in the behavior of VNX comparing intact subordinates. Therefore, the high sperm motility we found was suggested to be a specific feature of VNX subordinates, i.e. VNX treatment masks the suppressing effects on subordinates, which was probably due to

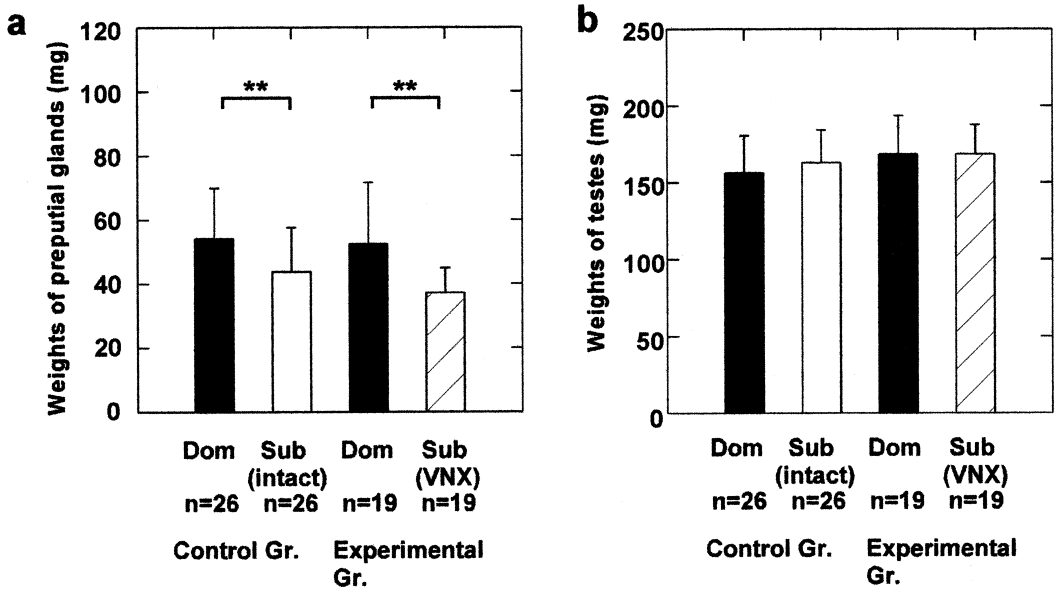

Fig. 2. The weights of preputial glands (a) and testes (b) in the intact control pairs and VNX experimental pairs. Dom and Sub mean dominants and subordinates, respectively. Averages ± sd of the weights of (a) preputial glands and (b) testes of the dominants and subordinates of control and experimental pairs were: (a) 54±15, 44±14, 53±19, and 37±8, respectively, (b) 157±24, 163±21, 169±24, and 169±19, respectively. *: $P<0.05$

pheromones derived from dominants. The VNX subordinate mice would have shown sperm motility as high as the dominants because they were free from the influence of pheromone from the dominants. On the contrary, the lowered weights of preputial glands of subordinates seem to be independent of pheromonal influences. Weights of testes did not show any differences between the groups nor between the social statuses (Fig. 2b), which was consistent with the results of our previous studies (Koyama and Kamimura, 1999, 2000). Such lack of differences in the weights of testes suggests that functional differences in epididymides rather than testes would be related in causing differences in sperm motility between dominants and subordinates. We found no other characteristics that showed difference between dominants and subordinates to be observed.

These characteristics, i.e. the high sperm motility and the lowered weights of preputial glands, of the VNX subordinates were different from the characteristics of isolated males (Koyama and Kamimura, 1999). In our previous study, the isolated males showed higher sperm motility and heavier weights of preputial glands than the subordinates. Sperm motility of the isolated males did not statistically differ from the dominants in that study, but the average value (37±9%) had the tendency to be lower than that of the VNX subordinates in the present study. It is possible to say that the VNX subordinates are not similar to isolated males, although both of these males are free from the odors of other males in higher social status.

Dominant male mice are known to show urine marking more frequently than subordinates (Drickamer, 1995) and the concentrations of volatile compounds in urine, for example α- and β-farnesene, are known to be different according to the social status (Harvey *et al.*, 1989). The odor components that are specifically secreted by dominants would be the first candidates to be investigated whether they are priming physiological changes and depress the sperm motility of

Table 1. Examples of pheromonal controls of the reproductive activities in males and females.

	Odor receiver	
Odor sender	Males	Females
Males	Suppressive effects	Enhancing effects
	ex) suppress sperm motility*	ex) solicit estrus***
	ex) increase abnormal sperm **	
Females	Enhancing effects	Suppressing effects
	ex) increase sperm density****	ex) lengthen estrus cycle***

*: the present study
**: Aref'ev *et al*, 1986
***: Vandenbergh, 1994
****: Koyama and Kamimura, 2000

subordinate males. As known in Bruce effects (Rosser and Keverne, 1985) of female mice, some cognitive processes, of "being in the state of subordinates" in this case, would also have crucial effects. This should be clarified by additional investigations on behavior.

It should be also noted that, there seems to be pheromonal effects that suppress the reproductive activities of the same sex and enhances those of the opposite sex (Table 1). The suppressive influences on the reproductive conditions of others of the same sex are considered to selfishly increase the reproductive success of the odor senders but suppress the reproductive success of the odor receivers. The enhancing effects on the reproductive conditions of the opposite sex would also help to increase the reproductive success of the odor senders. The odors of house mice would be taking important roles in controlling the reproductive success of them.

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