Female Urinary Chemosignals Influence Scent-Marking Behavior in Male Mongolian Gerbils *(Meriones unguiculatus)*

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The influence of females on the amount of scent-marking behavior displayed by male Mongolian gerbils was investigated. Males isolated from females scent mark at a low level which increases more than two-fold if females are present in the room for three weeks without direct contact with the males. A similar increase is obtained by application of pooled female urine directly onto the males' noses.

Introduction

Mongolian gerbils *(Meriones unguiculatus)* scent mark their environment by rubbing their ventral scent gland on low objects. The hormonal regulation of this scent-marking behavior has been widely investigated [1, 2]. Field observations showed that marking in gerbils might be directed towards females [3]. In addition, females and female urine both instantly increased the frequency of home-cage marking in males [4]. In contrast to these direct effects of females and female urine, the experiments described here present evidence for long-term influences of female urinary chemosignals on the level of male scent marking behavior.

Material and Methods

Subjects were 17 male Mongolian gerbils *(Meriones unguiculatus)* aged 12 to 15 months with ontogenetic data available from a preceding study [1]. All males were kept in one room separate from the colony and housed singly in opaque macrolon cages (size: $37 \times 20 \times 15$ cm) with wire mesh tops. Food and water were available ad lib. The air conditioned room (22 ± 1 °C, 55% rel. humidity) was maintained on a light cycle of 12:12 hours (lights off from 13.00 to 01.00 hours) with sufficient light present during the dark phase to allow observation of the animals. The room's volume was 50 m^3 and the air was renewed 16 times per hour.

The behavior of the males was observed in an open field apparatus (60×60 cm, transparent walls 25 cm; modified from [5]) marked off into 16 rectangles with a cylindric plastic "marking" peg at each of the 9 line intersections. The animals were observed inside the animal room in random order for 5 minutes 2–4 hours after lights off. The number of marking events directed to one of the pegs (marking activity) was registrated together with the number of fields entered by the animal (locomotor activity).

The behavior of the males was observed for several weeks under unstimulated males-only conditions. Then 17 virgin, sexually mature females (5–8 months of age) were placed in separate cages in the room with the experimental males. No direct contact was possible between males and females. At least one female was positioned next to every male. Urine was collected from 20 other females and kept frozen as a pool until used [6]. There was no attempt to determine the estrous state of the females.

For determination of serum testosterone levels, approximately 100 µl blood were collected from the saphenous vein of every male once a week [1]. After centrifugation, serum was kept frozen at −25 °C until the radioimmunoassays were performed (for details see [7]).

The Wilcoxon matched pairs signed ranks test was used for within-group comparisons. All calculations were corrected for tied observations; differences were considered significant when below the 0.05 level (two tailed). Correlation coefficients (r_s) were calculated using Spearman's rank correlation.

Results and Discussion

During the males-only period, the number of ventral scent gland marks per 5 minutes remained more or less constant for each individual. However, marking activity between different individuals ranged from almost no marking at all to about 20 marks per five minutes [1]. The mean marking activity of the males during males-only conditions was 8.3 ± 1.7 (mean ± SEM, $n = 17$).

When the stimulus females were present in the room, the marking activity of the males increased to an individually different level that was maintained as

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long as the females were present. Three weeks of permanent female stimulation were necessary until no further changes in behavioral activity could be observed; the mean marking activity then was about 130% higher than before stimulation (19.4 ± 2.6; mean ± SEM, $n = 17$, $p < 0.001$).

This increase in marking activity was not mediated by a concurrent increase in testosterone concentrations, since no significant changes could be found in the samples collected weekly (males-only: 1.9 ± 0.1 ng/ml; with females: 2.0 ± 0.1 ng/ml; $n = 17$, N.S.). In addition, locomotor activity was not affected by the different conditions (males-only: 137.9 ± 5.9 fields; with females: 141.1 ± 6.0 fields; $n = 17$, N.S.). The female-stimulated maximum marking activity of the males matched the respective individuals maximum marking activity during puberty almost exactly ($r_s = 0.98$; $p < 0.001$; Fig. 1). This confirms the earlier results on individually fixed scent marking activities in gerbils [1]. However, the high marking activity during puberty was accompanied by almost twice as high testosterone levels as in adults with or without female stimulation (puberty: 3.8 ± 0.4; $n = 8$; significantly different from males-only and female situation with $p < 0.02$).

The female-induced marking represents a reproducible effect; it is not a learned response, since both males and females were sexually naive and did not have direct contact with any other animal throughout the experiments. When the females were removed at the end of the experiment, marking decreased to the lower unstimulated levels, and, with females returned, marking increased again.

Tactile and visual cues were prevented by the experimental conditions since no direct contact between males and females was permitted. Thus auditory or chemical signals might be the reason for the increase in marking activity. To specifically test for chemical influences on marking behavior 10 µl of pooled female urine were applied twice a day to each nasal groove of males kept without females [6, 8]. This treatment induced a significant increase in marking activity in the urine-treated group compared with water treated controls (Fig. 2). Similar to the effect of the presence of females inside the room, it took several days to achieve the constant higher level of marking activity.

These experiments show that chemical signals present in the urine of females cause a specific modification of the males' scent marking response in a

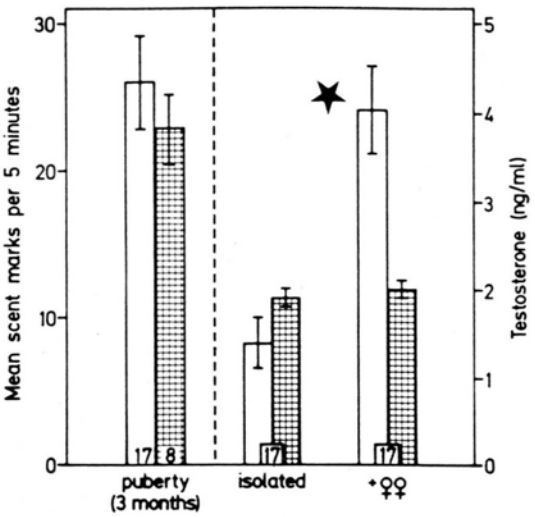

Fig. 1. Scent marking activity (□) and serum testosterone concentration (▦) of male gerbils under males-only conditions and three weeks after introduction of females into the room without direct contact to the males (mean ± SEM, number of males as indicated at the bottom of the bars; *: $p < 0.001$). Almost identical marking activities were obtained during puberty and female-stimulated conditions, however, the mean testosterone concentration was not affected by this treatment as compared to the higher level during puberty [1].

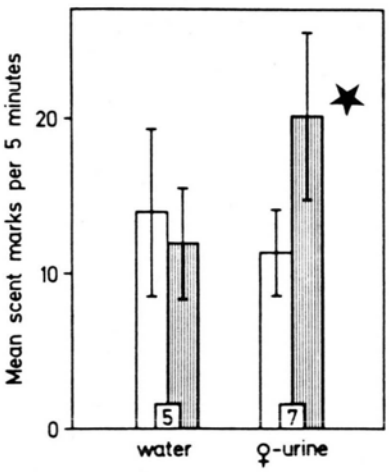

Fig. 2. Scent marking activity of male gerbils before (□) and after (▨) application of 20 µl water and pooled female urine, respectively, twice a day (mean ± SEM, number of males as indicated at the bottom of the bars; *: $p < 0.01$; 5 tests per individual before and 5 days after initiation of treatment).

standardized test situation. Present experiments employ the application of urine fractions to the nostrils of males as a biotest for the chemical identification of the factor stimulating scent marking.

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