A comparison of the aversive and female attractant properties of urine from dominant and subordinate male mice

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The urine of individual dominant and subordinate male mice was tested for male-aversive and female-attractant properties using dominant and subordinate male donors and Ss and estrous and anestrus female Ss in open-field tests. The results indicate (1) the presence of a factor in the urine of dominant males which proves aversive to both dominant and subordinate Ss, (2) the presence of a female attractant in the urine of dominant males, (3) naive females must be in estrus to respond positively to male urine. The results are discussed in terms of preputial and coagulating gland contents, androgen levels, and possible territorial functions.

Olfaction is an important regulator of social behavior in the mouse (Bronson, 1971), and urine, with its strong odoriferous properties, would seem to provide a particularly suitable olfactory signal. Experiments performed in this laboratory clearly demonstrate the presence of an androgen-dependent aversive pheromone in male mouse urine which discourages prolonged investigation, at least by subordinate males, of an area marked with such urine (Jones & Nowell, 1973a).

Krames, Carr, and Bergman (1969) showed that male rats prefer to investigate the odor of submissive males rather than that of dominant males, and we have found that only the urine of dominant male mice causes avoidance by subordinates, the urine of subordinate males being no more effective than water in this respect (Jones & Nowell, 1973b). However, we wanted to know what effect the aversive pheromone would have on dominant males and, for this reason, the following cross-over experiment which investigated the responses of dominant and subordinate Ss to the urine of dominant and subordinate males was performed. Thus, the main object of Experiment I was to determine whether a male mouse, when placed in a small open field, one half of which had been treated with the urine of either dominant or subordinate male mice, would spend more time in one half than in the other.

Female rats can discriminate between the odors emanating from sexually active vs sexually inactive members of the opposite sex (Carr, Loeb, & Dissinger, 1965), and Scott and Pfaff (1970) have shown that female mice prefer urine collected from intact males to that obtained from castrates. Experiment II compared the responses of female mice in estrus and anestrus to the urine of both dominant and subordinate males by an identical method to that used in Experiment I.

As the coagulating glands and preputial glands of male mice are involved, respectively, in the production of aversive (Jones & Nowell, 1973c) and female-attractant

pheromones (Bronson & Caroom, 1971), the weights and secretory contents of these glands were compared in dominant and subordinate animals.

METHOD

Albino mice of the Tuck T. O. strain, bred and maintained by the University of Hull Zoology Department, were used. The 20 urine donors, 10 dominant and 10 subordinate, and the 20 Ss, 10 dominant and 10 subordinate, used in Experiment I were approximately 4-month-old males which had been isolated for 1 month. Dominant/subordinate relationships were established by randomly pairing the isolate males 10 days before the experiment began until one mouse of each pair consistently assumed submissive postures (Grant & Mackintosh, 1963). A wire mesh divider was then put into each cage. This divider was removed at regular 2-day intervals, and the mice were allowed to fight for a short period; in every case, the dominant/subordinate relationship was maintained. The 20 Ss used in Experiment II were sexually naive 4-month-old females housed in groups of six since weaning.

Method of Urine Collection

The urine collection apparatus was a modification of another described elsewhere (Jones, Dilks, & Nowell, 1973), in that each cage was divided into two compartments with a funnel accurately fitting and draining each half. Ten dominant/subordinate pairs chosen as urine donors were placed into the collection apparatus, the dominant being put into one compartment and the subordinate into the other. The mice were allowed 24 h acclimatization before collection began. Urine was collected over 16 h daily, from 1800 to 1000 h the following morning. The urine was then tested for its aversive (Experiment I) and its female-attracting (Experiment II) properties.

EXPERIMENT I

The aversive efficacy of the urine was tested by measuring the investigatory behavior of the 10 dominant and 10 subordinate Ss in an open field, which consisted of a black metal tank measuring 16 x 11 x 9 in. high and containing a sheet of unprinted newspaper, divided into two equal halves by a faint pencil line, as substrate. Each S was put into a small Perspex cage which was then placed on the midline in the test tank. Trials were not

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Table 1

Means and Standard Errors of the Time Spent (in Seconds) by Dominant and Subordinate Male Mice in Each Half of an Open Field, One Half of Which Had Been Treated With the Urine of Either Dominant or Subordinate Males

Test Situations	Time Spent in Clean Half	Time Spent in Treated Half
Subordinate S vs dominant male urine	202.8 ± 4.4	97.2 ± 4.4*
Subordinate S vs subordinate male urine	152.0 ± 3.8	148.0 ± 3.8
Dominant S vs dominant male urine	187.0 ± 6.1	113.0 ± 6.1*
Dominant S vs subordinate male urine	149.7 ± 3.5	150.3 ± 3.5

Note—Probability values derived from analysis by the G statistic.

* $p < .001$

started until the S emerged from the small cage, which was then removed and washed in preparation for the next trial.

Four test situations were used in this experiment, i.e., the responses of dominant and subordinate Ss to the urine of dominant and subordinate males were measured. In all cases, one half of the open-field paper substrate was spotted with nine equidistantly spaced drops of urine. Twenty trials of 5-min duration were performed in each of the four test situations; thus, each mouse was tested with both urine types twice and underwent four trials in all. Trials were randomly rotated between the various categories in an attempt to eliminate habituation to any particular test situation and were always carried out between 1000 and 1400 h under constant white light. Preference for either half of the open field was measured by the accumulated time spent in each half during the trial. The tank was cleaned with a mild solution of disinfectant after each trial and the sheet of paper was changed.

Results

Table 1 shows the means and standard errors of the time spent in each half of the open field under all test conditions. The times spent in each half were compared using the G statistic. Following angular transformation, a two-way analysis of variance was performed, and the percentage of time spent in the clean half was compared between groups by means of the t test. When one half of the open field was treated with the urine of dominant males, both dominant and subordinate Ss showed a significant preference for the clean, untreated half. However, the preference shown by subordinates for the clean half was significantly greater ($p < .001$) than that shown by the dominant Ss. When the urine of subordinate males was used, neither the subordinate nor the dominant Ss showed any significant preference for either half of the open field.

EXPERIMENT II

The female-attracting properties of the urine of dominant and subordinate male mice was tested using 20 sexually naive females as Ss and identical apparatus and methods to those used in Experiment I. Immediately prior to testing, a vaginal smear was taken from each S to determine the stage of the estrous cycle. Those showing a late proestrous to early estrous smear were used in the "estrous" category; those in late estrous, with heavy vaginal cornification, were discarded and all other stages were defined as "anestrous" and tested as such.

Both estrous and anestrous females were tested using the urine of both dominant and subordinate males, and 20 trials of 5-min duration were performed in each of the four test situations. Preference for either half of the open field was measured by the accumulated time spent in each half during the trial.

Results

Table 2 shows the means and standard errors of the time spent in each half of the open field in all the test situations. When the urine of dominant males was used, estrous females showed a significant preference for the urine-treated half, but these females showed no preference for either half when the urine of subordinate males was used. Treatment of one half of the open field with the urine of either dominant or subordinate males induced no significant preference for either half by anestrous female Ss.

Organ Weights

The dominant and subordinate urine donors were sacrificed, and the left adrenal, preputial, and coagulating glands were cleaned and weighed. The wet weights of the preputial and coagulating glands were recorded and, after the expulsion of their secretory contents by pressure from a blunt instrument, their dry weights were also recorded. All gland weights are expressed relative to body weights.

Table 2

Means and Standard Errors of the Time Spent (in Seconds) by Estrous and Anestrous Females in Each Half of an Open Field When One Half Had Been Treated With the Urine of Either Dominant or Subordinate Male Mice

Test Situations	Time Spent in Clean Half	Time Spent in Treated Half
Anestrous female vs subordinate male urine	152.9 ± 4.1	147.1 ± 4.1
Anestrous female vs dominant male urine	152.5 ± 2.0	147.5 ± 2.0
Estrous female vs subordinate male urine	154.7 ± 3.2	145.3 ± 3.2
Estrous female vs dominant male urine	120.5 ± 2.5	179.5 ± 2.5*

Note—Probability values derived from analysis by the G statistic.

* $p < .001$

RESULTS

Table 3 shows the means and standard errors of the organ weights and of the percentage weights of preputial and coagulating gland secretions. The adrenal glands were significantly heavier in the subordinate than in the dominant males. No significant differences were found between the weights of either the preputial or the coagulating glands when comparing dominant and subordinate males. However, in the case of both glands, significantly greater relative weights of secretory contents were found in the subordinate animals.

DISCUSSION

The results of Experiment I support those of previous work carried out in this laboratory (Jones & Nowell, 1973a) which demonstrated the presence of an aversive pheromone in male mouse urine that discourages prolonged investigation, at least by subordinate males, of an area marked with such urine. The present results suggest that we can now extend this statement to include dominant males, since the dominant Ss used in Experiment I showed a marked preference for the clean half of the open field when the other half had been treated with the urine of dominant males. Subordinate males showed a significantly greater preference for the clean half of the open field than did the dominants. It seems reasonable to expect the dominant Ss to possess higher androgen levels than the subordinates, and it is possible that the previous experience of the Ss, together with their endocrine status, may govern their responses to the urine of a strange male. In this way, the urine may represent a greater threat to the less aggressive subordinates with low androgen levels than it does to the aggressive dominant males.

When the urine of subordinate males was used, neither subordinate nor dominant Ss showed a significant preference for either half, thus indicating that the aversive pheromone is not released by the subordinate males. The combination of coagulating gland secretion with urine is essential for expression of the aversive pheromone (Jones & Nowell, 1973c) and, although there is no significant difference between the wet weights of the coagulating glands of the dominant and subordinate urine donors, the glands of the dominants contain significantly less secretion than those of the subordinates. This high weight ratio of secretory epithelium to secretion in the dominant animals is reminiscent of the findings on the preputial gland by Hucklebridge, Nowell, and Wouters (1972) and it is suggested that, in both these glands, this high ratio is indicative of a relatively higher activity than in the subordinate animal.

It is suggested that the absence of secretion of the androgen-dependent pheromone by subordinate animals is a direct result of lower androgen production as a reciprocal of the increase in ACTH release (Bullock &

Table 3
Means and Standard Errors of the Relative Wet Weights of the Adrenal, Preputial, and Coagulating Glands and of the Percentage Weights of Preputial and Coagulating Gland Secretions of Dominant and Subordinate Male Mice

Gland Weights and Percentage Secretions	Dominant Male Mice (mg)	Subordinate Male Mice (mg)
Adrenal gland	6.07 ± 0.14	7.23 ± 0.25†
Preputial gland	159.6 ± 5.9	140.0 ± 6.5
Coagulating gland	63.3 ± 5.6	56.7 ± 2.6
Percent preputial gland secretion	14.8 ± 2.4	33.5 ± 2.8*
Percent coagulating gland secretion	26.0 ± 1.6	44.5 ± 2.1*

Note—Probability values (two-tailed) derived from analysis by the Mann-Whitney U test. **p* < .02 †*p* < .002.

New, 1972) which must follow the high level of social stress to which these mice have been subjected.

The results of Experiment II indicate that only the urine of dominant males possesses any female-attracting properties and that sexually naive females must be in estrous to respond positively to this urine. The females showed no significant preference for either half of the open field when the urine of subordinate males was used. It is known that female mice prefer to investigate the urine odors of intact males to those of castrates (Scott & Pfaff, 1970) and that the male's preputial glands, an androgen-dependent tissue (Mugford & Nowell, 1972), are involved in the production of a female attractant (Bronson & Caroom, 1971). The preputial glands of the dominant males show a significantly lower relative sebum content than do those of the subordinates, which, as previously suggested, indicates that these glands are more active.

Our finding that only estrous females were attracted to the urine of dominant males supports that of previous work carried out on rats (Carr et al, 1965) which showed that naive females had to be receptive before they would show a preference for male urine odor.

The release of aversive and female-attractant pheromones in the wild situation would be beneficial to a male animal in two senses. They would help to maintain the integrity of his territory by discouraging trespass by other males and would also insure the presence of a potential mate.

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