Olfactory control of aggressive and sexual behavior in the mouse (*Mus musculus* L.)

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Olfactory factors in the urine of male and female housemice exerted reciprocal, "mirror-image" effects upon aggression and sexual behavior. Male urine painted on female intruders facilitated aggression but inhibited copulation. Female urine painted on male intruders inhibited aggression but facilitated copulation. These effects were not due to the influence of urinary factors upon either intruder novelty or competing responses of escape and investigation.

Male mice typically display strikingly different behaviors towards male vs female conspecifics. Male intruders are viciously attacked, whereas female intruders are not attacked but, instead, are sexually mounted (Scott & Frederickson, 1951; Mackintosh, 1970). Several different olfactory cues (pheromones) present in the urine of intruders could potentially control these divergent behaviors: either an aggression-facilitating factor in male urine or an aggression-inhibiting factor in female urine could account for the differences in attack behavior. Conversely, either a sexual-attraction factor in female urine or a sexual-repellant factor in male urine could account for the differences in copulatory behavior.

The present study tested whether or not urinary pheromones govern the elicitation and suppression of aggression and copulation in the mouse. Previous studies have shown that the urine of novel males increases fighting and fighting-related activities (Mackintosh & Grant, 1966; Archer, 1968; Ropartz, 1966a, b; Mugford & Nowell, 1960). However, these studies confounded the effects of novelty with that of potential "identifier" pheromones capable of specifically eliciting aggression. In the present study, effects of male urine were tested by comparing the behavior displayed towards females smeared with male urine vs females swabbed with saline. The effects of female urine were tested by comparing the behavior displayed towards males smeared with female urine vs males swabbed with saline. Independent measurements were made of the aggressive, sexual, investigative, emotional, and escape tendencies of the S towards these intruders.

SUBJECTS

Test Ss totaled 96 male mice. Forty-eight of these came from a randomly mated F_3 population derived from 16 pairs of feral *Mus musculus* live-trapped in a local food-manufacturing plant. The remaining 48 came from the C57/BL6 inbred strain supplied by the Jackson Laboratory. In addition, eight male and eight female mice of the A/JAX strain (Jackson Laboratory) were used as standardized intruders. All animals were raised and maintained under standard laboratory conditions and were 90-120 days old at the onset of testing.

APPARATUS

Ss were tested in wire mesh home cages, 12 x 6 x 8 in. The bottoms of the cages were covered with San-o-cel, and the tops were covered with removable Plexiglas plates. Observations were recorded with a remotely controlled Esterline-Angus recorder connected to a portable pushbutton panel. Urine was collected in 16 specially designed metabolism cages which housed the A/JAX intruders.

PROCEDURE

All test Ss were isolated at 28 days of age and housed in wire mesh home cages. The A/JAX intruders were housed in metabolism cages for 10 days prior to testing. These animals were used both as intruders and as urine sources. At the onset of testing, intruders were washed in detergent bottles, rinsed, and then assigned to one of six treatment categories: F-S (female intruders swabbed with saline about their anal-genital region), F-M (female intruders swabbed with male urine), F-F (female intruders swabbed with female urine), M-S (male intruders swabbed with saline), M-F (male intruders swabbed with female urine), and M-M (male intruders swabbed with male urine).

A/JAX intruders were assigned randomly to C57 and wild Ss, resulting in eight Ss from each stock being assigned intruders from each of the six treatment groups. Each test S received an intruder from just one of these treatment groups throughout testing. The behavior of A/JAX intruders is stereotyped, involving boxing defensive postures or fleeing tendencies almost exclusively. Intruders were dropped in the S's home cage for 10-min intervals on 10 successive days, and the following responses of the S were recorded: (1) fighting—any instances of biting or chasing of the intruder, (2) cowering—freezing with all limbs rigid and with the front of the S oriented towards the intruder, or any flight tendencies of the S away from the intruder, (3) investigating—any nosing, licking, or grooming of the intruder, (4) tail rattling—any side-to-side vibrating tail movements made by the S, (5) mounting—any attempts to climb on top of the intruder accompanied by ejaculatory thrusts. The total proportion of time spent fighting, cowering, or investigating was recorded for every S during each of the 10 test sessions. Since instances of mounting and tail rattling were relatively brief and rare, the total number of discrete occurrences per test session was recorded for these responses. All testing was done under dim red illumination 1 h after the start of the night phase of a reversed daylight cycle. Testing was run blind in that the E recording the behavior was different from the E applying saline and urine to the intruders.

RESULTS

The effects of male and female urine are summarized in Table 1. Male urine contained factors which facilitated aggression, but inhibited copulation. Conversely, female urine contained factors which inhibited aggression but facilitated copulation.

The effects of male urine are analyzed in the top half of Table 1. On Trial 1, females smeared with male urine (F-M) are treated just like normal males (M-M): none of the behaviors displayed towards F-M intruders differs significantly from that displayed towards M-M intruders. Both wild and C57 Ss, however, attack F-Ms significantly more than they do females smeared with saline (F-S). This latter difference persisted until Trial 10 for both the wilds ($t = 1.806$, $p < .05$) and the C57s ($t = 1.794$, $p < .05$). The aggression-facilitating effect of male urine cannot be attributed to the suppression of competing responses. Neither wild nor C57 Ss displayed any significant difference in cowering or investigative tendencies towards F-M vs F-S intruders. While C57s did show a suppression of copulation with F-M intruders, the additional time made available for aggressive activity was insignificant. Mounting occupied only .011 of the first-trial interval for the F-S group compared to .003 for the

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Table 1
Effects of Male and Female Urine Factors (Trial 1 Data)

Response of Ss		Treatment of Intruders: vs Wilds F-S	vs Wilds F-M	vs Wilds M-M	vs C57s F-S	vs C57s F-M	vs C57s M-M
Fighting (Proportion of Session)	M	0*	.042	.018	.005*	.032	.138
	SD	0	.071	.032	.0044	.034	.190
Mounting (Instances)	M	0	0	0	1.630†	.125	0
	SD	0	0	0	2.00	.037	0
Tail-Rattling (Instances)	M	.111	.82	0	0*	.83	.70
	SD	.325	1.34	0	0	1.21	1.11
Cowering (Proportion of Session)	M	.079	.055	.072	.109	.091	.014
	SD	.137	.059	.110	.173	.151	.036
Investigating (Proportion of Session)	M	.210	.161	.106	.228	.191	.239
	SD	.151	.135	.056	.173	.151	.036

Response of Ss		vs Wilds M-S	vs Wilds M-F	vs Wilds F-F	vs C57s M-S	vs C57s M-F	vs C57s F-F
Fighting (Proportion of Session)	M	.055*	0	0	.044†	.004	0
	SD	.084	0	0	.037	.009	0
Mounting (Instances)	M	0	0	0	0*	1.25	.60
	SD	0	0	0	0	1.79	.80
Tail-Rattling (Instances)	M	.64	0	0	.222	.375	.200
	SD	1.37	0	0	.416	.992	.40
Cowering (Proportion of Session)	M	.029	.031	.105	.138	.121	.100
	SD	.026	.064	.144	.246	.214	.173
Investigating (Proportion of Session)	M	.128	.069	.070	.263	.261	.334
	SD	.103	.133	.098	.171	.205	.095

*$p < .05$ (one-tailed t test), †$p < .025$ (one-tailed t test)

F-M group. Table 1 also reveals that male urine facilitated tail-rattling of C57 Ss. This effect was also suggested in wild Ss but was not statistically significant ($t = 1.270$, n.s.).

Table 1 indicates that male urine acted as a sexual inhibiter. C57 Ss mounted F-M animals significantly less than they did F-Ss. This effect cannot be attributed to the elicitation of competing aggressive responses. The free time available for copulation (excluding time spent in aggression, cowering, or investigation) was greater for the F-M group ($P = .67$ for the test interval) than for the F-S ($P = .66$). The inhibiting effect of male urine on copulation by C57 Ss was transient, however. By Trial 10 there was no significant difference in the amount of copulation directed towards F-M vs F-S females ($t = .990$, n.s.).

Wild Ss exhibited no copulatory behavior in any of the test groups. Hence, the effects of urinary factors upon copulation could not be tested in this stock.

The effects of female urine are analyzed in the bottom half of Table 1. On Trial 1, the application of female urine on male intruders resulted in their being treated like normal females: none of the behaviors exhibited towards the M-F group differed significantly from the F-F group. However, M-Fs were attacked significantly less by both C57 and wild Ss than were M-Ss. The aggression-inhibiting properties of female urine persisted until Trial 10 for both wild Ss ($t = 1.784$, $p < .05$) and C57 Ss ($t = 3.73$, $p < .01$). The inhibiting effect of female urine upon aggression was not due to the elicitation of competing responses. The amount of cowering or investigative tendencies by both C57 and wild Ss did not differ for M-F vs M-S groups. While C57 Ss mounted intruders from the M-F group more often than they did those from the M-S group, the total proportion of free time available for aggressive activity towards M-Fs ($P = .62$) was greater than for M-Ss ($P = .60$).

Female urine acted initially as a sexual attractant. On the first trial, C57 Ss directed considerably more mounting attempts towards M-F intruders than towards M-S. This difference was not due, in any significant extent, to the suppression of incompatible aggressive responses (proportion of time available for mounting = .55 for M-F vs .61 for M-S). However, the facilitatory effect of female urine upon mounting did not persist until Trial 10 ($t = 1.00$, n.s.).

DISCUSSION

Aggression is facilitated by a factor in the urine of male opponents and suppressed by a factor in female urine for both genetically wild and domesticated C57/6 male mice. These effects are large and persistent enough to account for the typical differences in attack directed towards male vs female intruders. Previous studies have shown that aggression is enhanced by urine from strange males, but these effects may have been produced by a spectrum of unfamiliar odors (novelty effect) rather than a specific sexual identifier (pheromone effect). These studies utilized male urine collected either from different strains (Mackintosh & Grant, 1966; Archer, 1968; Ropartz, 1966a, b) or from different treatment groups (Mugford & Nowell, 1970) than the animals used as intruders. The latter study showed that urine from normal, noncastrated males would enhance aggression toward castrated opponents with whom the test Ss had already become familiar. The authors did not test whether the urine of castrates might enhance aggression towards familiar, noncastrated opponents. In the present study, testing of the aggression facilitating and inhibiting properties of urine was accomplished by comparisons between urine- and saline-treated intruders of the same sex. The only systematic difference between intruders of the same sex was the source of urine or saline applied to them. The urine came from animals identical to the intruders in their experimental treatment, genotype, age, and all characteristics other than sex. Thus, any difference in the effects of male and female urine were due to the sex of the source and not to differences in the source's novelty with respect to the intruder. Nor can aggression towards F-Ms be explained by the "disconcerting" effects upon test Ss of smearing intruders with urine of the opposite sex, since no aggression was directed towards M-Fs.

No previous study has tested whether urinary factors might affect aggression indirectly by acting upon competing responses. Mice respond to intruders not only with aggressive behavior, but also sexual, escape, and investigative tendencies (Clark & Schein, 1966; Banks, 1962; Scott, 1953). In the present study, urinary factors did not affect escape or investigation, and copulation and aggression were sufficiently infrequent

to substantially compete with each other.

Copulation by C57 males was initially facilitated by female urine and inhibited by male urine—a reciprocal "mirror image" of the aggression effects. However, the effects upon copulation were not sufficiently persistent to completely account for the sensory control of sexual behavior. This may have been due either to an incomplete removal of urinary cues by the prewashing procedure or to antagonism by other cues mediating sexual recognition.

Previous work has indicated that male mouse urine contains factors capable both of synchronizing estrous in females (Marsden & Bronson, 1964) and blocking pregnancy (Dominic, 1966). Together with present findings, this suggests the existence of unitary pheromones capable of integrating a multiple of divergent physiological and behavioral responses. The identification of such a pheromone has been assisted by the recent finding that testosterone injections in spayed female mice elicited attack by males (Mugford, 1970).

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