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10. W. H. Thorpe [*Ibis* **93**, 1 (1951)] noted that behavior patterns may mature and then deteriorate if unused; he called this "instinctive regression." M. Jacobson [*Science* **163**, 543 (1969)] noted that appropriate stimulation during a sensitive period ("functional validation") prevents instinctive regression and is an essential stage in the maturation of certain neural connections. E. B. Hale and A. H. Schulman (in preparation) suggest functional validation as a stage in visual imprinting of turkey poults.
11. Interpretation of the data and preparation of the manuscript were aided by grants from the Frank M. Chapman Memorial Fund and by contract AT (38-1)-310 between the University of Georgia and the Atomic Energy Commission. This is paper No. 3896 in the journal series of the Pennsylvania Agricultural Experiment Station.

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Social Rank in House Mice: Differentiation Revealed by Ultraviolet Visualization of Urinary Marking Patterns

Abstract. *Ultraviolet light has been used to examine urine marks deposited by adult male house mice on filter paper on the floors of their cages during overnight tests. Both the urination frequency and the pattern in which urine was deposited on the filter paper depended upon social rank. Dominant males vigorously marked their entire cage floor, whereas subordinate males typically voided urine in only two to four pools in the corners of their cages.*

In many mammalian species excretory products such as urine, feces, and glandular exudates constitute an important source of chemical communicants (pheromones). Such cues often serve to modify specific behavioral and physiological functions among recipient members of the same population (*1*). Many investigators have documented the importance of urinary pheromones and olfactory reception in both aggressive and sexual behavior in mice (*2*). Physiologically, urinary priming pheromones produced by adult mice have been implicated in both the enhancement and suppression of ovulation in immature and mature females and even in the prevention of implantation in recently inseminated females (*3*).

In the course of an investigation of the relationships between social rank and testicular function in house mice, we noticed that the bladders of subordinate males consistently contained more urine than those of their dominant counterparts killed at the same time of day. This differential accumulation of urine prompted a series of studies. The results that we report here document urinary marking behavior in adult male house mice and demonstrate that the frequency and pattern of urinary marking is strongly dependent upon dominance status. Moreover, our studies illustrate the utility of ultraviolet light as a way of evaluating urinary marking patterns, an approach that will undoubtedly prove useful in elucidating some of the behavioral and physiological consequences of sociality in this and other species.

Laboratory-raised descendants of a wild stock of house mice (*Mus musculus*) were reared in a room maintained at 23°C with a light:dark cycle of 14:10 (lights on at 5 a.m.). Excess food (Wayne mouse breeder diet) and water were provided at all times. Mice were weaned at 21 to 23 days of age and then each animal was isolated in a cage (30 by 30 by 15 cm) where it remained until reaching maturity at 55 to 60 days of age, at which time it was used in one of three experiments.

In the first experiment the relationship between social rank and the volume of bladder urine was examined after 10 hours of pairing (7 p.m. to 5 a.m.). Eleven pairs of dominant (unwounded) and subordinate (wounds on rump or tail) mice were killed by cervical dislocation. We took care to minimize spontaneous urination by killing the animals within 15 seconds after first handling the cage. Urine that was voided during handling and killing was pooled with urine that was aspirated from the bladder with a syringe. The total volume of urine in the syringe was considered as bladder urine.

Table 1. Percentage of total radioactivity recovered from urine deposited on filter paper after dominant and subordinate male mice received a single intravenous injection of [^{3}H]inulin. Each value represents the mean ± the standard error of the mean of the results for three mice.

Hours after injection	Percentage of total radioactivity recovered	
	Subordinate	Dominant
1	30.0 ± 5.4	64.8 ± 1.1
2	6.8 ± 4.3	12.4 ± 2.4
4	32.4 ± 6.8	8.4 ± 1.2
8	18.2 ± 3.1	6.8 ± 0.7
18	9.6 ± 3.7	3.8 ± 0.5
Total	97.0 ± 1.3	96.2 ± 1.2

No urine could be detected in the bladders of eight out of the 11 dominant males. The volume of urine in the bladders of the other three dominant males ranged from 0.06 to 0.14 ml and averaged 0.03 ± 0.01 ml for all 11 dominant males. Urine volume among the subordinate males averaged 0.63 ± 0.08 ml and ranged from undetectable in one animal to 1.02 ml at the extreme. Thus the bladders of the subordinate males typically contained, on the average, at least 20 times as much urine as those of the dominant males.

In the second experiment we used ultraviolet light to examine the frequency and pattern of urinary marking as a function of social status. We obtained urinary marking patterns of ten isolated adult males by placing each male in one compartment of a cage (30 by 30 by 15 cm) that was divided in half by a 0.2-cm wire partition. The bottom of the cage was covered with wood shavings during the day and with Whatman No. 3 MM filter paper between 8 p.m. and 8 a.m. Urinary marks on the filter paper were made visible with the aid of an ultraviolet lamp equipped with a 15-watt tube emitting light at 3666 Å (*4*). Urinary marking patterns of the same males were obtained on the following night after they had been placed in similar cages (cleaned beforehand) as pairs but separated from each other by the wire partition. After urinary marking patterns had been obtained from pairs of mice in each other's presence, the partions were removed for 30 minutes per day for 5 days to permit the establishment of dominance-subordination relationships. Urinary marking patterns were obtained on five consecutive nights. Spontaneous fighting occurred among all pairs immediately after the wire barrier was raised (*5*). A clear dominance-subordination relationship was established in four of the five pairs after the first 30-minute encounter.

Urinary marking patterns presented in Fig. 1 illustrate a typical set of results obtained in these studies. Isolated males deposited urine in 30 to 80 pools or drops over the filter paper as revealed under ultraviolet light (Fig. 1, A and B). A striking change was noted

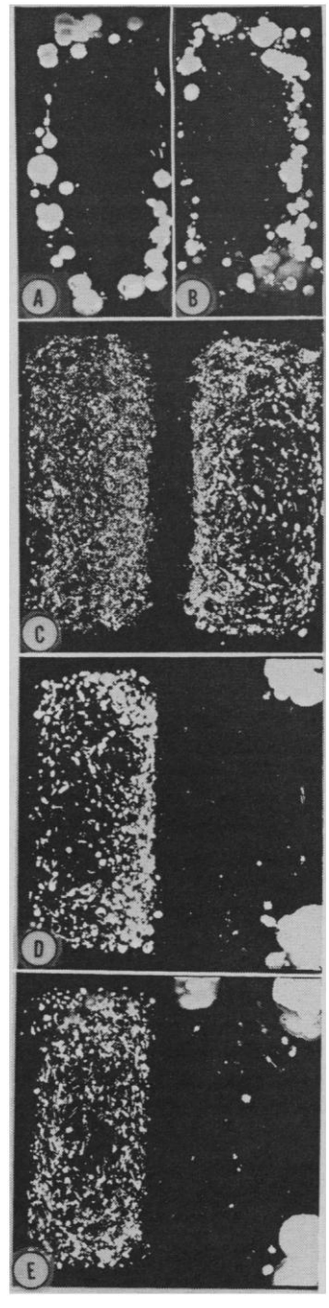

Fig. 1. Urinary marking patterns of adult male house mice revealed by ultraviolet visualization of filter paper placed on the cage floors during overnight tests (8 p.m. to 8 a.m.). (A) and (B) Marking patterns of individually isolated males, each animal occupying one-half of a test cage (30 by 30 by 15 cm) partitioned with a wire-mesh barrier. (C) Marking pattern obtained after two previously isolated males [those used in (A) and (B)] were placed in the same cage but separated by the wire-mesh barrier. (D) Same animals and test situation as in (C) except that this photograph was taken after the firs aggressive encounter (30 minutes) durin which dominance was established by the animal in the left half of the cage. (E) Same animals and test situation as in (C) and (D) except that this photograph was taken after five aggressive encounters (each 30 minutes per day), demonstrating that the dominant male (left half of the cage) continues to deposit drops of urine over the entire cage floor whereas its subordinate (right half of the cage) voids urine in a few pools in the corners of the cage.

in the amount and pattern in which urine was deposited when two males were placed in the same cage but separated by the wire partition (Fig. 1C). Both mice assiduously marked (1000 to 2000 marks) their cage, as evidenced by the striking increase in the number of fluorescent dots instead of pools. Urinary marking patterns obtained on the evening of the first aggressive encounter differed markedly from those obtained on the previous night when the males were physically separated by the wire partition (Fig. 1D). Typically, dominant males continued to mark the entire surface of the cage floor at a high rate ($>$ 1000 marks), whereas subordinate males generally urinated in only two to four pools, usually in the corners of the cages. These patterns were stable at least up to 5 days (Fig. 1E). One pair failed to establish a stable hierarchy during the 5-day period with both animals consistently marking at a high rate. This pair was allowed to fight over a 24-hour period on day 6. Dominance was then firmly established, and the typical differential marking pattern emerged and continued to be maintained.

In a third experiment we examined [^{3}H]inulin excretion after pairs of previously isolated adult males had been allowed to establish social rank during one 30-minute exposure per day for 7 days in a neutral cage. The [^{3}H]inulin (2.5 μc per mouse in 25 μliter of 0.85 percent sodium chloride to give a concentration of 275 mc/mmole) was injected via the tail vein without anesthesia at 4 p.m. Mice were returned to the double-chamber test cages and allowed to urinate on filter paper between 4 p.m. and 10 a.m. Fresh filter paper was placed in the cage at 1, 2, 4, 8, and 18 hours after the injection. After the filter paper had been air-dried, the radioactivity was extracted and counted (*6*). The radioactivity, determined by liquid scintillation counting, was expressed as a percentage of the total radioactivity injected. The rate of deposition of radioactivity after [^{3}H]inulin administration differed markedly in dominant and subordinate males (Table 1). Dominant males deposited 77 percent of the total radioactivity injected within 2 hours after the injection, whereas subordinate males deposited only 37 percent during this same interval. Importantly, the excretion rates declined in a stepwise manner in the dominant males but varied widely in the subordinate males. Despite differences in the temporal pattern of [^{3}H]-inulin deposition, both dominant and subordinate males deposited over 96 percent of the total radioactivity injected within an 18-hour period.

A host of ecological studies have suggested that many mammalian species utilize excretory products to mark objects or animals in their environments (*1*). Our findings extend this concept to mice under laboratory conditions and establish, for the first time, that urination in this species is strongly regulated, in part, by intermale stimuli. All three experiments reveal the same trend. A previously isolated male mouse profusely marks its environment upon being housed in the presence of another male. This pattern is maintained in socially dominant males but completely suppressed in subordinate males housed in the presence of their dominant partners. Under these conditions dominant males usually have empty bladders and eliminate radioactive inulin rapidly, whereas subordinate males eliminate inulin erratically and often have partially full to very full bladders at any point in time. The fact that a 30-minute period of active fighting suppresses urine marking in subordinate males is particularly dramatic and suggests that this behavioral pattern is extremely sensitive to cues originating from a dominant male.

We have since examined over 50 pairs of adult male mice (wild and inbred stocks of *Mus musculus*), and in every instance the same differential type of urinary marking pattern emerges immediately after the establishment of a stable social hierarchy. In all cases vigorous marking occurred when pairs of previously isolated animals were placed in each other's presence; the frequency of marking by isolated males, however, although always lower, was somewhat more variable. The ease with which urinary marking patterns can be studied with ultraviolet light strongly suggests its use for attacking a wide variety of other behavioral and physiological problems related to social rank in this and other species. Although the full behavioral and physiological significance of our

findings remains to be established, it is obvious from the results presented here that dominant male mice have a distinct social advantage over subordinate male mice since they assiduously label their environment with an excretory product that has a demonstrated capacity to elicit profound behavioral and physiological effects on the reproductive processes of females (*2, 3*).

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4. Under the conditions described, mouse urine fluoresces a bright blue. Preputialectomized males show the same pattern of blue-fluorescing marks. In addition, we found that mice receiving a single intraperitoneal injection of 0.25 ml of a 1 percent solution of methylene blue, Sudan black, or basic fuchsin produced urinary marking patterns that were indicative of the dye injected and similar to those that we observed under ultraviolet light.
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6. The filter paper containing radioactivity associated with [^{3}H]inulin was cut into 1-cm squares and extracted five times, each time with 100 ml of water at 70°C for 15 minutes, with constant mixing. Successive extracts were pooled, and an aliquot (1 ml) was removed and placed in a scintillation vial with 10 ml of Insta-Gel (Packard Instrument Company). The scintillation vials were dark-adapted and cooled to 10°C before counting. In all cases sufficient radioactivity was allowed to accumulate to give a probable error of 2 percent or less. We corrected all radioactivity determinations to 100 percent efficiency (expressed in disintegrations per minute) by counting each sample against a ^{226}Ra external standard and comparing the value obtained to a standard correlation curve (count rate of the external standard versus the percent efficiency of samples of known radioactivity containing varying amounts of a quenching additive). Preliminary experiments demonstrated that the extraction procedure described above was adequate to recover 98 ± 1.5 percent of the radioactivity associated with chromatographically pure [^{3}H]inulin.
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Errorless Discrimination, Autoshaping, and Conditioned Inhibition

Abstract. *Pigeons were exposed to a discriminated autoshaping procedure in which brief presentation of a green light on a key was always followed by food whereas presentation of a vertical white line on the key was never followed by food. Pigeons acquired an errorless discrimination by pecking reliably in the presence of the green light but never in the presence of the line. The line inhibited pecking in later tests: when the white line was paired with food, key peck acquisition was retarded; and when the white line was superimposed on the green background, responding was suppressed.*

Discrimination learning is a process through which the environment comes to control an organism's behavior. In a discrimination, an organism's behavior is reinforced in the presence of one stimulus (S+) but is not reinforced in the presence of another stimulus (S−). As a result, the organism comes to respond to S+ but not to S−. Of central importance to the understanding of discrimination learning is an analysis of the origin of nonresponding to S−. Terrace (*1*) has argued that there are two fundamentally different types of discrimination learning, which are distinguished primarily by how nonresponding to S− comes about. In the first type, the organism responds to S− (that is, he makes "errors") during discrimination learning, and responding to S− is eventually inhibited. An organism that learns with errors presumably learns two things: to respond to S+ and not to respond to S−. In the second type, the organism learns without errors, and S− does not come to inhibit responding. Supposedly, an organism that learns without errors learns only to respond to S+. Thus, Terrace contends that nonresponding to S− results from inhibition by S− only if errors occur during learning. The purpose of this report is to determine whether an errorless S− may become inhibitory.

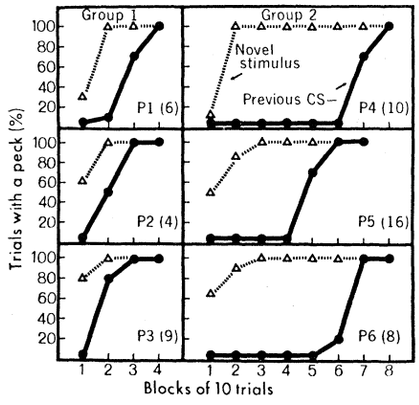

Fig. 1. Mean percentage of trials during which at least one peck occurred for successive blocks of ten trials in the retardation test. Each graph shows data for one bird (identified by *P* plus number), and the total number of errors made by each is shown in parentheses.

Before environmental stimuli acquire control over a skeletal response such as the pecking response of a pigeon, the pigeon often makes many errors (*1*). Terrace demonstrated, however, that the occurrence of errors is not necessary for discrimination learning. He trained pigeons to discriminate without errors between red and green lights. Having begun discrimination training soon after conditioning of the key peck, he initially made S− very different from S+. At first, S− was less bright, of shorter duration, and of different hue than S+. During training, he progressively increased the brightness and duration of S− so that S+ and S− differed only in hue. This method for producing errorless learning is called a fading procedure.

Whether discrimination learning occurs with or without errors, the organism finally responds to S+ but not to S−. However, there are important differences in the behaviors observed during acquisition of a discrimination with and without errors. For example, Terrace argues that when learning with errors occurs, the pigeon emits emotional responses to S−, the S− becomes aversive, and peak shift, behavioral contrast, and inhibitory stimulus control occur (*1*). These features are called "by-products" of discrimination learning (*1*). These by-products are absent if the learning was errorless.

Terrace (*1*) contends that the absence of by-products after errorless learning indicates that an errorless S− is not inhibitory. But testing for the presence of discrimination by-products is not the most direct method of determining whether S− is inhibitory (*2*). For example, if S− is a conditioned inhibitor, then, as a result of learning, it