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Male-Induced Puberty in Female Mice: Evidence for a Synergistic Action of Social Cues

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ABSTRACT

It has been established previously that cohabitation with an adult male induces precocial puberty in female mice and, furthermore, that a factor in the male's urine is only in part responsible for this phenomenon. The present four sets of experiments had as their objective a more precise determination of the male's relevant cues. Exposure of immature females of a particular body weight to intact male urine for 54 h yielded a small but consistent level of uterine growth. Cohabitation with a castrated male for the same length of time resulted in no uterine growth. Simultaneous exposure to intact male urine and the presence of a castrated male, on the other hand, yielded uterine growth of a magnitude similar to that obtained by cohabitation with an intact male. Further experiments using wire mesh barriers, opaquing the eyes and/or occluding the ear canals verified that the second type of cue was tactile and, by extension, that castrated males showed the necessary tactile responses. Thus the relevant cues of the male are pheromonal and tactile and these cues act synergistically to induce precocial puberty in young females.

INTRODUCTION

The rate at which female mice mature sexually is influenced markedly by their social environment, in particular by the presence or absence of adult males. Young females housed in male-free environments show delayed, disorganized, and often anovulatory pubertal cycles (Stiff et al., 1974). Cohabitation with an adult male, on the other hand, yields a decidedly well-organized and accelerated puberty (Vandenbergh, 1967; Castro, 1967). While the hormonal bases for the male's actions seem reasonably well understood (Bronson and Desjardins, 1974; Bronson, 1975a), the precise nature of his relevant cues has remained somewhat in doubt. Several studies have concluded that urinary pheromones are, at least in part, responsible for the male's ability to induce precocial puberty (Vandenburgh, 1969; Vandenbergh et al., 1972; Cowley and Wise, 1972). Recently, Drickamer (1974) demonstrated the importance of non-pheromonal cues emanating from the male but his experimental design did not allow a separation of the various non-pheromonal modalities. The present experiments were designed to determine more precisely the nature of the male's active cues and the female's mediating sensory modalities. The results demonstrate unequivocally that two types of stimuli, one a urinary factor and the other of a tactile nature, act synergistically to promote puberty.

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MATERIALS AND METHODS

Four experiments are reported. The first evaluated three techniques for exposing immature females to the odors of male urine. The second and third experiments used different approaches to examine the relative importance of several sensory modalities. The final experiment specifically examined a possible role for visual and high frequency auditory cues. In all cases, female CF-1 mice were purchased from Carworth Farms at 10–10.9 g and immediately housed 6 per cage in a male-free room. They were weighed daily at 0800 h until they reached 15–15.9 g; experimental treatments began at 0900 h of that day and lasted for 54 h until 1500 h of day 3. Uterine weight was used in all cases to assess the relative efficacy of the various social treatments. As presented in detail in the discussion section of this paper, previous research provided the basis for the 54 h experimental period, the critical body weight, and the use of uterine growth as a meaningful index of pubertal change (Bronson and Desjardins, 1974; Bronson, 1975a, b).

Each of the four experiments was conducted as a unit and involved a randomized block design. Because of the probability that air-borne pheromones were involved in this phenomenon, experimental treatments were always separated by room. Five animal rooms were available and all were of similar size, ambient temperature (23 ± 1 C), lighting schedule (14:10 L:D, lights on at 0500 h), and had 12 air exchanges per h. Since one experiment involved 7 treatments, it was conducted in 4 "runs" over a two-week period, with randomization of both the treatments and the delegation of animals to each treatment. The other three experiments were each performed in one week with single shipments of animals. Caging and food, unless

otherwise noted, was 18 × 29 × 13 cm polyethylene and Wayne Breeder Blox. Statistical probabilities were obtained by Duncan's Multiple Range Test following Analysis of Variance. Additional specific methodology will be given with each set of experimental results.

RESULTS

Exposure to Intact Male Urine

This preliminary experiment evaluated three techniques for exposing immature females to the odors of intact male urine. CF-1 females (15–15.9 g) were either isolated, cohabited singly with an adult male, or isolated and exposed to the urine of other intact adult males via one of three procedures: (a) topical exposure by rubbing the general region of the nares and philtrum with a urine-soaked Q-tip three times a day (0.3 ml of urine per female per day); (b) the cage and female were both sprayed with urine from a 1 ml tuberculin syringe (27 gauge needle) three times a day (0.3 ml per female per day); or (c) a timer-controlled infusion pump delivered an aliquot of urine-saline mixture every hour to the bedding of the female's cage through a combination of polyethylene tubing and a pasteur pipette (1 ml of urine and 9 ml of saline per female per day). Urine was collected and pooled from a donor group of 200 isolated adult males and stored at −70 C for not more than a day before use. All experimental treatments were begun with clean cages except male-exposure; in the latter case cohabitation took place in the male's home cage which had not been cleaned for one week.

Mean (± S.E.) uterine weights after 54 h of treatment were as follows: isolated controls, 27 ± 2 mg (N = 12); topical application of urine, 26 ± 2 mg (N = 12); sprayed urine, 43 ± 10 mg (N = 11); timed drip application, 43 ± 6 mg (N = 10); and male-exposed controls, 99 ± 10 mg (N = 12). Thus two techniques, the syringe-spray method and the timed drip method, both induced significant uterine growth ($P<.05$ and $<.01$, respectively) but neither technique produced uterine growth of the magnitude induced by cohabitation with a male. A series of unreported experiments tested the efficacy of greater amounts of urine with no appreciable changes in the results.

Urine Exposure in Combination with Several Social Situations

Immature females were either (a) isolated; (b) cohabitated singly with either an intact or a castrated adult male; or (c) urine-exposed via the syringe-spray method while either isolated or cohabitating singly with either an adult ovariectomized female or an adult castrated male. A final group of isolated females was urine-exposed and vigorously chased around the cage by the gloved hand of an experimenter four times a day, 30 sec each time. As expected, 54 h of cohabitation with an adult male resulted in marked uterine growth (Fig. 1). Urine-exposure resulted in a significant elevation of uterine weight ($P<.05$) but again was relatively ineffective in inducing uterine growth when compared with exposure to an intact male ($P<.001$). Exposure to a castrated male was totally without effect on uterine weight unless combined with exposure to intact male urine, a combination which approached the effectiveness of cohabitation with an intact male ($P>.10$). Chasing with a glove and cohabitation with an ovariectomized female, both when combined with urine-exposure, seemed to result in intermediate effects on uterine growth but in neither case were these effects significantly greater than that resulting from urine exposure alone.

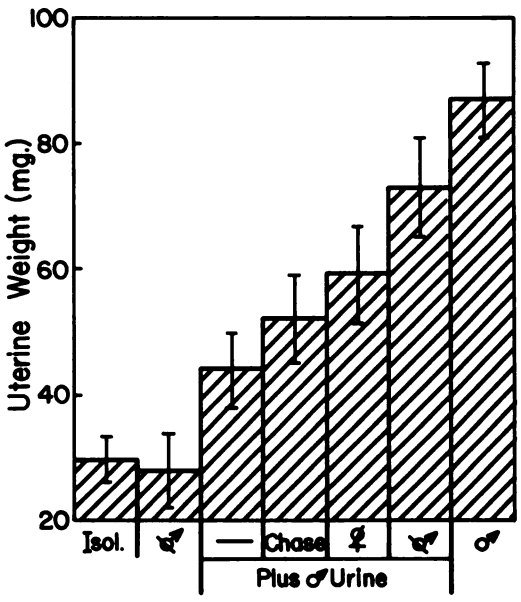

FIG. 1. Mean (± S.E.) uterine weight after 54 h of exposure to isolation, cohabitation with intact or castrate males, or exposure to intact male urine while either isolated or cohabitating with ovariectomized females or castrated males. "Chase" refers to isolated females exposed to intact male urine and periodically chased around their home cages by the gloved hand of an experimenter (see text). N = 14 or 15 females in each treatment.

Separation of the Male and Female by Wire Mesh Barriers

This third study utilized special 36 x 30 x 15 cm cages which were divided into two equal sized compartments by 0.2 cm wire barriers. Single immature females were placed either alone in these cages, housed with a male in the same compartment, housed with a male across the barrier, or they were urine-exposed via the syringe-spray method with or without a male in the other compartment. The stimulus males were housed in their experimental cages for two weeks prior to the experiment. Cohabitation with an adult male in the same compartment yielded a 200 percent increase in uterine weight when compared to that characteristic of isolated controls (Fig. 2). The other three treatments, exposure to urine either with or without a male on the other side of the barrier and no urine exposure with a male in the other compartment, all resulted in intermediate and similar levels of uterine growth ($P<.01$ less than that induced by cohabitation and $P<.05$ greater than the isolated condition in all cases). This experiment establishes that separation of the male and female by a wire mesh barrier (which should inhibit only tactile cues) decreases the effectiveness of the male to a level no greater than that attributable solely to exposure to his urine.

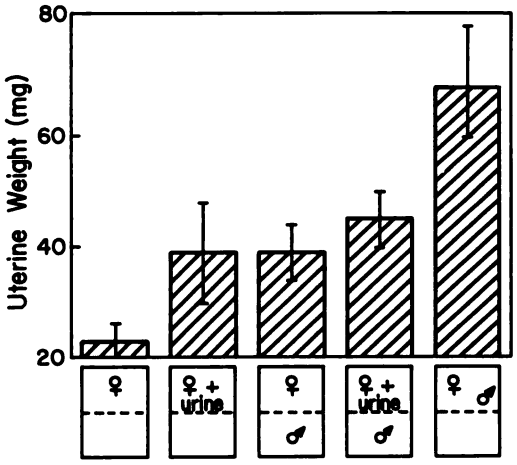

FIG. 2. Mean (± S.E.) uterine weight of females housed in one compartment of a cage divided in two parts by a wire mesh barrier. Females were treated or not with male urine and/or to the presence of an intact male in various combinations depending upon which compartment housed the male (as shown below each histogram). N = 12 females in each treatment.

Form Vision and High Frequency Vocalizations

A final experiment evaluated two treatments, opaquing the cornea and occluding the ear canal, with regard to their potential interference with male-induced uterine growth. Corneal opaquing, to destroy form vision, was accomplished by light acid treatment followed immediately by water rinsing. Ear canals were completely occluded with dental cement to block reception of any high frequency calls from the male. Females having opaque corneas and females having occluded ear canals were either isolated or male-exposed as previously described. Mean (± S.E.) uterine weights at 54 h were as follows: isolated females having occluded ears, 28 ± 3 mg (N = 15); ear occluded and male-exposed, 82 ± 7 mg (N = 13); isolates with opaque corneas, 24 ± 3 (N = 12); and opaqued eyes and male-exposed, 74 ± 10 mg (N = 11). When compared to the effectiveness of male-exposure as revealed in Figures 1 and 2, then, there was certainly no evidence that either corneal opaquing or occluding the ear canals interfered in any way with the uterine response to an adult male.

DISCUSSION

Exposing immature female mice to the presence of an adult male may result in an immediate sub-ovulating release of LH, a marked surge in the level of circulating estradiol, and after a period of time, normal, adult-like, peri-ovulatory changes in LH, FSH, and progesterone (Bronson and Desjardins, 1974). The timing of these reactions to the male may be made reasonably predictable by using females of particular one gram ranges in body weight. CF-1 females weighing 17.5 to 18.5 g, for example, typically complete the entire pubertal cycle in three days, the elevation in LH being observed in 1–3 h, estradiol concentrations in the serum rising 15–20-fold in 6–12 h, and ovulation occurring during the third night (Bronson and Desjardins, 1974). The key role for estrogen in this phenomenon has been verified by successfully replacing the male with an injection of estradiol on either of the first two days of the standard three-day induction period described above (Bronson, 1975a). There is a period in the development of a female mouse during which she exhibits a mature capacity to show the LH-estrogen sequence of release in response to a male and, hence, a maximum uterine response, but also

during which the positive feedback action of estrogen on LH release is not yet mature (Bronson, 1975b). The 15–15.9 g females used in the present studies were at that stage in their development and thus they would not have ovulated "on schedule." The heavier weight class of female was not used in the present experiments because a few control (isolated) females of this weight class would already have been experiencing the prepubertal spurt in uterine growth. Thus initiating our experimental treatments at 15–15.9 g and measuring uterine weight at 54 h (3 pm of day 3, the counterpart of proestrus in our standard pubertal induction period) yielded a mature uterine response to the male at a weight class when no control females would be showing any degree of prepubertal uterine change.

When all experiments are considered together, it becomes obvious that the male is providing two types of cues, pheromonal and tactile. Furthermore, it is obvious that these cues act synergistically in effecting precocial puberty. In those experiments where direct comparisons are possible, cohabitation with an adult male for 54 h yielded 267 percent, 215 percent, and 200 percent increases in uterine weight while exposure to intact male urine by the syringe-spray method yielded increases of only 57 percent, 59 percent, and 70 percent. Thus, as previously suggested by Drickamer (1974; see also Vandenbergh, 1973), urinary factors are not the only type of cue operating in the puberty-induction phenomenon. That the relevant non-urinary cues are tactile would seem to be fully verified by the final two experiments. Opaquing the eyes and occluding the ear canals had no effect on male-induced uterine growth. More importantly, separating the immature females from the adult males by use of a wire mesh barrier yielded a rate of uterine growth no greater than that induced by urine exposure alone (Fig. 2). The experiment with occluded ear canals was suggested by recent findings that male mice emit vocalizations in the ultrasonic range (most often at about 70 kHz) during courting and/or aggressive activities (Sales, 1972; Whitney et al., 1973). Such ultrasounds may be evoked solely by female odors and their production by males is androgen-dependent (Whitney et al., 1974). It is known that sounds in the 70 kHz range can be effectively blocked by even a thin sheet of paper.

A castrated male is apparently capable of approximating the relevant tactile cues of an intact male because simultaneous cohabitation with a castrated male and exposure to intact male urine yielded uterine growth which closely approached that induced by cohabitation with an intact male (Fig. 1). Thus there seems little doubt about the two different types of cues operating in this phenomenon: a urinary priming pheromone which yields a small but consistent effect by itself and tactile cues which are inactive alone but combine synergistically with the urinary factor to yield the full effect. The precise nature of the tactile cues is not revealed by these experiments but it should be noted that the "stair-step" alignment of the data in Figure 1 could suggest a mixture of both specific (to the behavior of normal adult males) and non-specific arousal actions (e.g., fighting and chasing as was observed by intact males as well as by castrated males and ovariectomized females). Even though aggression is typically considered as an androgen-dependent phenomenon in mice, castrated males and ovariectomized females will routinely attack and chase animals that are of considerably smaller size than themselves (Edwards, 1969).

The occurrence of both additive and antagonistic interactions in the regulation of mammalian reproduction by specific ambient stimuli and/or via specific sensory modalities has been documented previously. Thus neither blinding nor surgical anosmia alone alter uterine weight in female rats but the combination of both operations severely depress the uterus (Reiter et al., 1970). Hoffman et al. (1965) report an additive interaction between temperature and photoperiod on testis growth in hamsters, and Eleftheriou et al. (1973) report both facilitory and antagonistic interactions between stressful cues, pheromonal action, and PMS-induced ovulation in immature mice depending upon genetic strain. The present results regarding the relative roles of tactile and pheromonal cues in immature female mice would seem to represent a case of true synergism. In all experiments, urine gave a small but consistent response, exposure to the tactile cues of a castrated male yielded no response, but the combination of these cues yielded almost the full response. There is thus a marked parallel between the type of interaction shown by these external cues and the internal interaction between estrogen and progesterone in inducing ovulation in rats, a classic case of hormonal synergism (Turner, 1966).

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RECOMMENDED REVIEWS

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