Strange Females Increase Plasma Testosterone Levels in Male Mice

Abstract. *Male house mice paired with a normal female for 1 week do not have higher plasma testosterone levels than do males that remain in all-male groups, but paired males have markedly elevated testosterone levels 30 to 60 minutes after the resident female is replaced by another female. Elevation of testosterone levels in these males is similar to that in isolated males paired with a female, does not depend on copulation with the strange female, occurs under housing conditions that permit continuous exposure to the odors of other females and males, and does not occur when the resident female is replaced by another male for 30 to 60 minutes. The elevation thus appears to be a specific endocrine response to an encounter with a strange female. These results, along with previous findings suggesting that strange males affect endocrine function in females, indicate that bisexual encounters are likely to produce endocrine changes in members of both sexes.*

Exteroceptive stimuli from males can alter endocrine function in females (*1*). Exposure to the odor of males accelerates the onset of estrus in female mice, and can block pregnancy (implantation of fertilized eggs) in mated females. Pregnancy block does not occur when females continue to be exposed to the odor of their individual studs, and therefore the block has been described as an endocrine response to a strange male. Pregnancy block by exposure to strange males also has been reported in the nonmurid deermouse and the vole. Recent studies indicate that sex-related stimuli can affect endocrine function in males as well as females. Exposure to females or copulation (or both) have been reported to elevate plasma testosterone (T) levels in rats, rabbits, hamsters, rams, bulls, monkeys, and men (*2*). In the studies with rats, rabbits, hamsters, and bulls, copulation is not required for a rapid increase (within 30 to 60 minutes) in plasma T levels. In male hamsters, rapid increases in plasma T following exposure to vaginal odor can be comparable in magnitude to those following physical pairing with females. Thus the odors of the opposite sex can be adequate stimuli for altering endocrine activities in both males and females.

In this study we determined the short-term effects of an encounter with a strange female on plasma T levels in male mice under conditions intended to minimize such possible general effects of female odor on the male hypophysiogonadal axis. We report that male mice paired for 1 week with a female, and permitted continuous exposure to the odors of other normal females and males in neighboring cages, exhibit high T levels 30 to 60 minutes after the resident female is replaced by another female. The rapid T elevation does not depend on copulation with the strange female, and does not occur if the resident female is replaced by a male.

Subjects were random-bred house mice, *Mus musculus*, more than 55 days old. They were housed in stainless steel cages (22 by 22 by 13 cm) with wire tops, in a common room with a lighting schedule of 14 hours light, 10 hours dark. Plasma T levels were determined by radioimmunoassay (*3*). The assay has a sensitivity of approximately 50 pg of T, and an intra-assay coefficient of variation of 8 percent. Blood samples (one per subject) were collected approximately at the middle of the light period by cardiac puncture without anesthesia. The housing conditions permitted common exposure of subjects to odors in the room, and only the number and sexes of subjects in individual cages were varied. Average T levels were determined for males that (i) remained caged in all-male groups of three to five since weaning, (ii) were removed from all-male groups and paired with a normal female for 1 week, and (iii) were isolated in a separate cage for 1 week. For some of the paired subjects, the resident female was removed 30 to 60 minutes before blood collections, and either another female was introduced or male-male pairs were formed. For some of the isolated males, single females also were introduced into the cages 30 to 60 minutes before collections. All females were caged in groups of four to five prior to pairing with males. Neither males nor females had previous sexual experience. The females that were presented to the isolated males or replaced the resident females of the paired males were not receptive during the 30- to 60-minute exposures and did not copulate. None of these females had vaginal plugs after their short exposure to males.

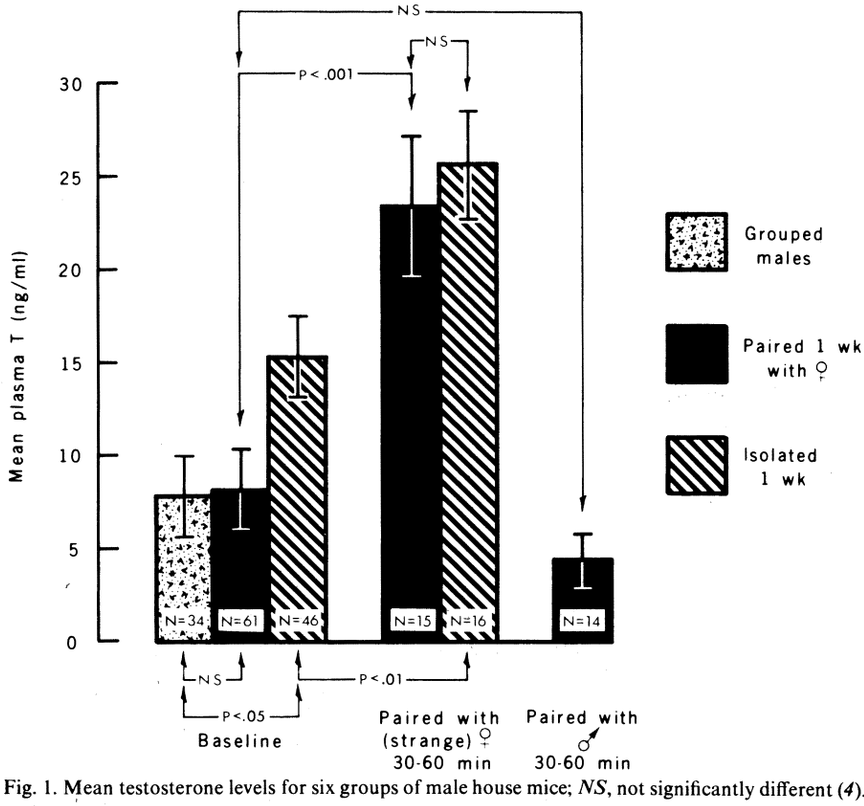

Fig. 1. Mean testosterone levels for six groups of male house mice; *NS*, not significantly different (*4*).

Mean plasma T levels and standard errors for the six conditions are illustrated in Fig. 1. Subjects that remained in all-male groups had an average T level of 7.8 ± 2.2 ng/ml. The mean T level after pairing with a female for 1 week was not different from that of grouped males ($P > .05$), whereas males that were isolated for 1 week did have a higher mean T level ($P < .05$). As expected from findings in other species (*2*), isolated males exposed to a female for 30 to 60 minutes had a higher mean T level than isolated males that were not presented with a female ($P < .01$). Paired males similarly had elevated T levels 30 to 60 minutes after the resident female was re-

placed with another female ($P < .001$), and the mean T level for this group did not differ from that for isolated males that received a female ($P > .05$). In contrast, paired males that were separated from females and paired with males for 30 to 60 minutes did not have elevated T levels. The encounters with strange males did influence T levels in that the variance for the latter group was significantly less than that for any other group (F test, $P < .01$; variances for other groups were homogeneous, $P > .05$), but the trend was toward a depression of T level. This trend was not significant ($P > .05$) when the heterogeneity of variance was taken into account (*4*). The rapid T elevations following encounters with strange females thus appear to be sex-related, and cannot be explained as more general responses to separation from a previous cage mate and pairing with another conspecific. Furthermore, since the males always were exposed to odors of females and other males in the colony room, the elevations in plasma T appear to be specific responses to encounters with strange females and not more general responses to the odors of other mice.

We cannot exclude the possibility that increases in local concentration of odor from the strange females played some role. The males clearly behaved toward these females as potential sex partners. They typically pursued the females from the rear and repeatedly attempted to mount, but were rebuffed. The males also seemed to distinguish females from other males, possibly on the basis of olfactory cues. When male-male pairs were formed, the subjects characteristically approached each other from the front and assumed jousting postures. The trend toward a depression of T levels following encounters with a strange male suggests that these encounters were stressful (*5*).

The large T elevations after exposure to strange females may not have been entirely due to sexual arousal, but also to other social factors such as a rapid establishment of dominant-submissive relationships (*6*). However, the encounters of male mice with strange females were sexually arousing (elicited mounting attempts) and resulted in rapid elevations of plasma T, despite the facts that (i) the males were sexually naive (those in the isolated group paired with females) or had been residing with another female, (ii) the exposure of males to the odors of conspecifics was not restricted prior to the encounters, and (iii) the strange females were unreceptive so that copulation was prevented. These findings raise questions about the interpretation of pregnancy block in female mice as a response to a strange male.

Whitten (*7*) suggested that both the acceleration of estrus in unmated females and the blocking of implantation in mated females could result from exposure to a male pheromone that promotes secretion of follicle-stimulating hormone and consequently causes inhibition of prolactin secretion in females. The male odor also may influence secretion of luteinizing hormone (*8*). Evidence exists that the estrus-accelerating odor is excreted in male urine under androgen control (*1*). The reason for doubt that pregnancy block is solely a response to a male pheromone is that the odor of the stud does not prevent implantation, which suggests that the mated female also must discriminate between the odors of her stud and other animals. Mice can discriminate individuals by their odors (*9*). In many studies of pregnancy block, however, the inseminated females have been exposed to the (strange) males under conditions in which the males in turn could experience an increase in the intensity of female-related stimuli but could not mate. For example, males have been placed in wire chambers and inserted into the females' cages. The present findings suggest that under such conditions the males would develop elevated T levels. Bliss *et al.* (*10*) reported that male rats that copulate to satiation show depressed, rather than elevated, T levels. If similar changes occur in mice (*11*), an even greater difference in T levels for strange males versus studs would be predicted. Analogous considerations would apply for studies in which females are exposed to male odors by being placed in cages previously inhabited by a male, since we found that isolated males have higher baseline T levels than grouped or paired males. Thus Whitten may have been correct in his speculation that both estrus acceleration and pregnancy block result from an endocrine response to an odor common to males. The ability of odors from strange males to block pregnancy might be due to higher concentrations of the testosterone-dependent pheromone in their urine, and might not require individual discriminations by females. Moreover, decreases in male T levels after successful copulation might be required to prevent pregnancy block. Strange males are not effective in blocking pregnancy when studies are conducted with certain inbred strains of mice (*12*). It has been suggested that males of these strains have more similar body odors due to inbreeding and are less easily discriminated by the females. In view of our findings with random-bred mice, however, it would be of interest to determine whether inbreeding has affected the ability of the males to undergo changes in T levels under different housing conditions and after brief exposure to females.

Vandenbergh (*13*) reported that odors on bedding from cages of normal, but not of castrated, males also accelerate sexual maturation in young female mice. He found that the odor from normal males is more effective in accelerating the onset of vaginal opening and first estrus if males are "activated" by placing adult females next to the males, and he speculated that such activation may involve elevations in T levels and higher quantities of pheromone excreted into the bedding. Our results strengthen his speculation. The incidence of urine marking by male mice depends on social variables (*14*). During encounters with a strange female, both the incidence of urine marking and the concentration of pheromone in urine might increase to produce a potent olfactory stimulus for the female. In general, our findings indicate that endocrine changes after bisexual encounters normally occur in members of both sexes, and that more attention should be directed toward these mutual interactions in studies of social influences on endocrine function. Bisexual encounters in adult mice might be conceptualized as involving an exteroceptive feedback loop. Exposure to females promotes behavioral and endocrine activities in males generating stimuli which, in turn, affect behavioral and endocrine activities in females.

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References and Notes

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Dystrophic Spinal Cord Transplants Induce Abnormal Thymidine Kinase Activity in Normal Muscles

Abstract. *The role of the neural tube in the pathogenesis of muscular dystrophy was tested directly. Neural tubes from chicken embryos with hereditary muscular dystrophy and from genetically normal embryos were transplanted into normal recipient embryos. Dystrophic neural tissue induced in muscles of normal hosts high thymidine kinase activity characteristic of dystrophic muscle; normal neural tubes did not. We propose an early inductive effect of the neural tube on the presumptive myoblasts that sets their subsequent course of development, either normal or dystrophic.*

Hereditary muscular dystrophies in man and animals are characterized by progressive weakness accompanied by physiological, biochemical, and morphological changes in the affected muscles. Evidence from both human and animal studies has led to the suggestion that the primary lesion in the diseases is expressed in the nervous system, and that changes in the muscles are secondary to an abnormal neurotrophic influence (*1–4*). A general membrane defect has also been postulated (*5*). In most of the investigations on animals, genetically normal muscle has been innervated with genetically dystrophic nerves, and vice versa, the experiments involving either transplantation of muscle, (*2, 6*), parabiosis (*7*), or culture of muscle and nerve tissue (*3, 8*). Not all the results have agreed on the significance of a peripheral nerve influence (*2–4, 6–8*). We have found that neural tubes from dystrophic chicken embryos transplanted into normal chicken embryos induce a high level of thymidine kinase activity in the genetically normal host muscles. This high activity is characteristic of dystrophic muscle at that stage of development. Transplantation of normal neural tubes had no such effect. We propose that these results may be due to an early irreversible inductive effect of the neural tube on the developing musculature.

Experiments with muscle transplantation designed to test extramuscular influences on the progress of dystrophy have shown that, when muscle is transplanted from dystrophic embryos as young as 12 days in ovo to normal chicks, the phenotypic expression of dystrophy in the donor muscle is unaltered (*9*). However, these experiments have not taken into account the possibility that the defective gene might act extramuscularly at a much earlier state of embryonic development than 12 days in ovo. The direct test of the role of the nervous system in the pathogenesis of hereditary muscular dystrophy is to transplant the nervous system between dystrophic and normal animals before the muscles have been influenced by nerves of their own genotype. To this end we transplanted a neural tube from dystrophic to normal chickens at a time during ontogeny before the first known inductive neuronal influences are expressed on the development of the somites (*10*), and preceding muscle differentiation and innervation (*11*) [stage 13 in (*12*)]. We determined the effect of the transplanted neural tubes on an enzyme activity in the embryonic muscles that is characteristically higher in dystrophic than in normal muscles during development.

Muscular dystrophy in chickens is inherited as an autosomal codominant. The disease affects fast twitch glycolytic muscle fibers and particularly the superficial pectoral muscles that are innervated by the brachial plexus (*13*). The spinal cord region destined to give rise to the brachial plexus was located by reference to the adjacent somites and was transplanted isotopically to the host embryo (Fig. 1). The operation was performed as early as was anatomically possible: that is, as soon as the 21st somite was clearly demarcated from the unsegmented somitic tissue. At this stage of development the differentiation of muscle from the host somites has not occurred and innervation has not been established.

Initial experiments were done to confirm the integrity and location of this type of graft, by transplanting quail neural tubes into chicken embryos. Quail cells can readily be distinguished from chicken cells by their large conspicuous nucleoli (*14*). The brachial motor neurons in the resultant embryos (quail–White Leghorn) were clearly of quail origin. In both quail-chicken and chicken-chicken chimeras no macroscopic evidence of discontinuity between host and donor was observed, and histological examination of the spinal cord at the junction of the transplant and host tissue showed that the graft was anatomically continuous with the host tissue.

Of 65 chicken-chicken transplants, 14 embryos survived to 18 days in ovo. Of these eight had received a neural tube from normal embryos (N → N) and six from dystrophic embryos (am → N). Most embryonic deaths were attributed to surgical trauma and occurred within 3 days of the operation. A second peak in embryonic mortality occurred at 12 to 13 days in ovo and may have resulted from the mechanical stresses on the embryos (*15*).

Thymidine kinase activity in the pectoral muscles of dystrophic chicken embryos at 18 days in ovo is significantly higher than in the pectoral muscles of normal em-

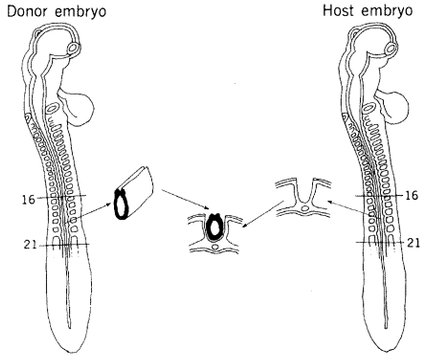

Fig. 1. Diagram of procedure used for transplanting neural tubes. The donor embryo was removed from the egg and washed in saline; the number of somites was then determined. The spinal cord between somites 16 to 21 was removed, washed in 0.25 percent trypsin to remove adherent mesenchyme, and subsequently washed in soybean trypsin inhibitor. It was then stored in Eagle's minimal essential medium buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (25 m*M*) while the host animal was being prepared. The host embryo was exposed by drilling a small window in the shell directly over the embryo, the vitelline membrane above the brachial region was removed, and the spinal cord cut out with a fine steel knife. Care was taken not injure the somites or the underlying notochord. The donor cord was dropped in place from a Pasteur pipette and was oriented by means of a fine tungsten needle under visual control both anteroposteriorly and dorsoventrally. The egg was sealed with sterile Parafilm and incubated with the window side up for a further 16 days.