Variation in Length of the Estrous Cycle in Mice Due to
Former Intrauterine Proximity to Male Fetuses 1

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ABSTRACT

Female mouse fetuses occupying an intrauterine position between male fetuses exhibit longer estrous cycles in adulthood than do females normally rearing in utero next to other female fetuses. This difference in adult cycle length was observed consistently when the two types of females were maintained under a variety of social conditions. Thus, these data suggest a fundamental biasing of the intrinsic timing of the estrous cycle by former intrauterine proximity to males in this and possibly other species in which more than one fetus is present in the uterus.

INTRODUCTION

The mammalian estrous cycle encompasses a complex series of changes in the brain, pituitary and ovary, all synchronized by hormonal and environmental cues to promote ovulation and sexual receptivity. The length of this cycle varies considerably between species and, in most cases, variation among conspecifics is also observed (Allaria et al., 1971; Nequin et al., 1979; Schwartz, 1969). The present report documents one source of such individual variation in mice, namely, that there is a direct effect of intrauterine proximity to males on the length of a female’s estrous cycle during later adult life.

In the present experiments, female mouse fetuses located in utero between two male fetuses (designated here as 2M females) and female fetuses adjacent to other female fetuses (0M females) were collected by cesarean delivery and fostered to dams that had just given birth naturally. The length of the estrous cycles of these two types of females was compared in adulthood while they were either (1) housed individually or grouped in the presence of a male or (2) housed individually or grouped in an environment free of males. This spectrum of housing conditions was deemed an experimental necessity because of the sensitivity of the mouse’s estrous cycle to regulation by social (pheromonal and tactile) cues. It is well established now, for example, that female mice emit cues that prolong the estrous cycles of other females, while stimuli emanating from adult male accelerates cycling (Bronson, 1979; Vandenbergh, 1974; Whitten and Chalmers, 1973). Thus, among females that are grouped together in the absence of males, prolonged and irregular cycles are usually observed, while adult females that are either grouped with males present or housed individually exhibit shorter and more regular cycles. The present experiments were designed to determine whether possible effects of intrauterine position on estrous cycle length would relate to differential sensitivity to extrinsic (social) cues or reflect a more fundamental influence on the intrinsic timing of the adult estrous cycle.

MATERIALS AND METHODS

One hundred and fifty adult CF-1 females were paired with males each morning between 0800–1000 h, transimplanted female-housing vaginal plug (15–15 each day) were individually housed (Day 9 of pregnancy). On Day 19 of pregnancy at 0900 h, the females that had not yet begun to deliver (about 75% of the pregnant females) were killed by cervical dislocation and the fetuses were removed from the uterus. The sex of the pups was determined by observing the size of the scrotal sac and the 0M and 2M females were tabbed. The pups were removed from the mothers that had just delivered naturally and a experimental pups from the same intrauterine position were

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fostered to each dam. The 0M and 2M females were weaned when 27 days old and kept in foster-litter groups until being assigned to an experiment. Animal rooms were maintained at 23 ± 1°C on a 14L:10D cycle with lights on at 0600 h. Separate rooms were available for the separation of males and females when necessary.

RESULTS

Our initial experiment was conducted with 60-day-old 0M and 2M females that had been housed since birth in a room containing males. A total of 20 females from each intrastene position were placed in 30 × 30 × 15 cm cages that were divided in half by a wire-mesh partition. Five females from the same foster-intrastene position were housed on one side of the screen and an adult male was housed on the other side to accelerate the females’ cycles. One complete cycle was measured for each animal by daily vaginal lavage (1300–1400 h). A complete estrous cycle was considered to be the number of days between fully confluent vaginal smears, with the first observation of such a smear being counted as Day 1 of the cycle being assessed. Under these housing conditions 0M females were found to have significantly shorter estrous cycles than did 2M females; 0M females, mean cycle length in days ± SEM = 4.3 ± 0.1 vs 6.4 ± 0.5 for 2M females; t(90) = 2.2, P < 0.05.

The next experiment involved a partial replication and an extension of the previous study in that it measured estrous cycle length between another set of 0M and 2M females when housed individually or in like-groups as adults in the presence of a male. These females were 90 days old at the start of the experiment and had been housed since weaning in a room free of male odors. The barrier-type cages described above were used again in this experiment. Twenty females from each intrastene position were housed as previously described in like-groups of 3 across a barrier from a male. Ten other females of each type were housed individually across the barrier from a male. The first complete estrous cycle subsequent to the first estrous smear was determined for all females. When a female was housed in the presence of a male, 90-day-old 0M females again had significantly shorter estrous cycles than did 2M females; 0M females, mean cycle length in days ± SEM = 2.7 ± 0.05 vs 2.7 ± 0.1 for 2M females; t(58) = 2.07, P < 0.05.

When housed individually in the presence of a male, 0M females also exhibited shorter cycles than did 2M females, but with only 10 animals/group, this difference did not reach statistical significance; t(10) = 2.04, P = 0.06, Fig. 1A.

The final experiment examined estrous cycle length in 150-day-old 0M and 2M females when housed individually or in foster-litter groups of 5 per cage. Each foster-litter group contained 2M females that had been raised in groups in a male-free environment would not exhibit regular estrous cycles when reared while individually housed. However, by 150 days of age, such females exhibited regular cycles in the absence of male stimulation when housed individually (cf. Vaudenby et al., 1972). This experiment that assessed the possibility that 0M and 2M females might have estrous cycles that were intrinsically different in length, independent of any potential differences due to regulatory cues emanating from males of one or both sexes. Each female’s mean cycle length was first calculated and the data then were analyzed by t test. All females grouped in the absence of a male exhibited prolonged cycles, with no significant differences between 0M and 2M groups being noted. When housed individually in the absence of males, however, 0M females again exhibited shorter estrous cycles than did 2M females; t(30) = 2.1, P < 0.05, Fig. 1B.

Ovulation was known to be a relatively constant component of vaginal smears when adult mice are housed with males, but this is not always true when adult females are housed away from males (Stoff et al., 1974). To verify that the individually-housed females studied in this experiment were experiencing ovulatory cycles, 7 isolated females of each type were killed on the morning of the first estrus that was observed after the 4 week test period. 7/7 0M females and 6/7 2M females had tubal eggs at this time, indicative of normal, adult cycles.

DISCUSSION

Given the well established importance of social cues to the estrous cycle of the mouse,
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Individual variation in the length of this cycle could be marked either to the intrinsic control of the oestrous regulation by social rules or to both. The present experiments were designed to separate these two potential sources of variation. To summarize the data presented in Fig. 1, grouping of adult female mice in the absence of a male, regardless of the female’s former intraspecific position, resulted in cycles that were greatly prolonged. Thus, the production of and/or the sensitivity to the oestrous hormone originating from females that interfere with the exhibition of regular cycles in mice is not limited to adult females or other intraspecific position in relation to the male. This suggests a fundamental basis of the length of the adult estrous cycle by factors other than those affecting the cycle length of males and suggests an intraspecific effect on the intraspecific mechanisms that regulate cycle length.

Previously, both morphological and behavioral differences between OM and 2M females have been reported. Importantly, 2M females have larger allogestational spaces at birth (a possible factor for steroid exposure), they are more aggressive in a variety of situations and temperament their environment at a higher rate. OM females, on the other hand, are highly preferred by males that are allowed to choose between the two types of females (von Saal and Bruns, 1979).

We recently determined that 2M female fetuses have significantly higher levels of testosterone in their blood and amniotic fluid than do OM female fetuses. Importantly, the mother’s circulation does not appear to be involved in this difference (von Saal and Bruns, 1980). This finding supports the hypothesis that OM and 2M females differ in their morphology, stimulator characteristics and behavior as a result of the 2M female fetuses having been exposed to elevated levels of androgens produced by continuous male fetuses. It is well documented that exposure to elevated levels of testosterone within the first few days after birth via exogenous administration can have profound effects on a female mouse’s reproductive capacity. Specifically, such females are incapable of ovulating during later life (Brañas-Léchon and Leachon, 1954; Götz, 1979). It is interesting to note that the period during which the developing brain of a mouse becomes maximally sensitive to the influence of testosterone on capacity to exhibit the “female” pattern or cycle biomorphic production in adulthood is just after birth. Thus, this sensitive period exists just after the time that females that develop next to male fetuses around 70% of all females cease being exposed to elevated levels of androgens produced by the male fetuses. The present as well as previous findings indicate that while female mice that develop in utero between male fetuses have adult estrous cycles that are prolonged, there is no decrement in the capacity to ovulate, pregnancy rates, or normal numbers of healthy young when these females are housed singly (von Saal and Bruns, 1980).

The present data extend the range of reproductive differences that appear to be linked to estrogenic exposure. These include a neurobehavioral effect on reproductive potential. While the full range of reproductive correlations of greater intraspecific gonadotropin activity is not yet realized, the picture that is emerging suggests that an ultrasonic pressure of female mice to male fetuses may yield variation.

FIG. 1. Mean length of the oestrous cycle (in days) of OM and 2M females housed individually or grouped. A) when measured continuously with a male present on the other side of a wire mesh barrier. B) when measured over a 6-week test period in a multi-cage environment.
in reproductive potential during later life by way of several pathways. Whether such effects are common in other species in which more than one fetus is present in the uterus remains to be established.

REFERENCES


RECOMMENDED REVIEWS

