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Effects of prior intrauterine position and housing on oestrous cycle length in adolescent mice

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Summary. Female mice that had developed *in utero* between 2 male (2M females) or 2 female (0M females) fetuses were housed individually at 32 days of age in the presence of a male. The 0M females had a significantly shorter cycle. When the females were housed in groups of 5 in the presence of a male, cycle length in 0M females was significantly longer than that of 2M females for the first cycle recorded, but this relationship reversed completely by the third and fourth cycles. These results are compatible with a hypothesis that former intrauterine proximity to male fetuses affects the intrinsic timing of the oestrous cycle and the capacity to emit oestrus-suppressing cues and/or the sensitivity to such cues.

Introduction

The sex of the nearest neighbours of a genetic female fetus in the uterus is a fundamental source of variation for a wide range of reproductively-important characteristics of that female during adulthood, at least in mice and rats (Clemens & Gladue, 1978; vom Saal & Bronson, 1980a). When tested as adults, for example, female mice that develop *in utero* between two male fetuses (2M females) are more aggressive, urine mark their environment at higher rates, and are relatively unattractive to males, when compared to females that develop in the uterus adjacent only to other female fetuses (0M females; vom Saal & Bronson, 1978). The proximal cause for such biases seems to be the direct diffusion of testosterone from male to female fetuses late in gestation (vom Saal & Bronson, 1980a).

Former intrauterine proximity to male fetuses also results in changes in the length of the oestrous cycle in mice, but the precise nature of this particular alteration is complex. An intrauterine position between 2 male fetuses is associated with a lengthening of the adult oestrous cycle that occurs under almost all social conditions (vom Saal & Bronson, 1980b). At puberty, however, 2M females ovulate and mate at a younger age than do 0M females when they are housed in groups consisting of several females and one male. But, when housed singly with a male, 0M females tend to mate sooner than do 2M females (vom Saal & Bronson, 1978). Such variation may be explicable on the basis of the regulation of gonadotrophin secretion in the mouse by social cues. It is well established that the ovulatory cycle of the mouse, pubertal, adolescent or adult, is heavily dependent upon three socio-developmental factors: (1) pheromonal and tactile cues associated with an adult male accelerate the timing of this cycle (Vandenbergh, 1969, 1973; Bronson & Maruniak, 1975); (b) female-originating cues decelerate the cycle in other females (Vandenbergh, Drickamer & Colby, 1972; Drickamer, 1977;

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McIntosh & Drickamer, 1977); and (c) the relative dominance of a female's sensitivities to male- and female-originating cues changes dramatically during adolescence; i.e. when peripubertal females are housed in groups, the accelerating action of a male on puberty and subsequent oestrous cycles is completely overridden by the inhibitory cues produced by the females. By adulthood, however, the cues produced by grouped females no longer override those of a male (Vandenbergh, 1973; Bronson, 1979). The potentially confusing results noted above are actually all compatible with the hypothesis that intrauterine proximity to male fetuses biases both the length of the oestrous cycle and a female's ability to transmit oestrus-suppressing cues and/or her response to such cues. The objective of the present study was to test this hypothesis by determining the relative lengths of the oestrous cycles of 0M and 2M females during adolescent development when the transition from the pubertal to the adult form of the suppressive response to grouping takes place.

Materials and Methods

To obtain females of known intrauterine position, 50 adult CF-1 females were placed with an adult male between 09:00 and 11:00 h on consecutive days until copulation was verified by the presence of a vaginal plug. Inseminated females then were housed individually in plastic 18 × 29 × 13 cm cages with food and water available *ad libitum*. Beginning at 08:00 h on Day 19 of pregnancy, just before parturition, about 75% of the females were killed by cervical dislocation, and the fetuses were removed from the uterus. The 0M and 2M female fetuses were saved and comprised the two groups of experimental animals. The remaining pregnant females were allowed to deliver normally; their young were removed, and 6–7 experimental young, all from the same intrauterine position relative to males, were placed with each foster mother.

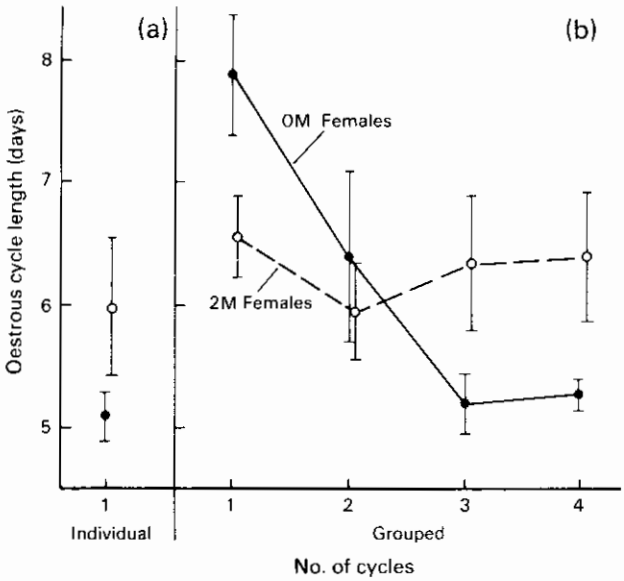
All the young were weaned on Day 23 after delivery but remained in foster-litter groups in a large colony room. At the start of each experiment, all females were 32 days old and weighed 20–24 g. Housing consisted of wooden cages that measured 30 × 30 × 15 cm and were divided equally into two living areas by a wire-mesh partition. The females were housed individually or in groups of 5 females of the same type on one side of the partition; in both conditions an adult male was placed on the other side. Oestrous cycles were assessed from daily vaginal lavages, and a complete oestrous cycle consisted of the number of days between oestrous smears, with the first day of oestrus being counted as Day 1 of the cycle. The animal rooms were maintained at 23 ± 1°C with 14 h light (06:00–20:00 h)/24 h.

Results

For the females housed in groups of 5, the between/within cage variance was first calculated for each group to determine whether there was a significant cage effect upon cycle length. The F ratios did not deviate significantly from 1, indicating that each animal was cycling independently of its cage mates. The analyses were therefore based on the data for each animal rather than the average cycle length of the females in each cage.

Twelve 2M and 12 0M females were housed individually across the barrier from a male in the boxes described above. The number of days to the first oestrus for both groups averaged 3–4 days, but the first cycle after the first oestrous smear tended to be longer in the 2M females (Text-fig. 1a). When 20 0M and 20 2M females were placed in the boxes in groups of 5 females from the same intrauterine position, there was again no difference in the number of days required for the 0M and 2M females to reach first vaginal oestrus from the start of the experiment (3–4 days average). The length of the first full cycle was again different, but the 0M females had the significantly longer cycles (Text-fig. 1b). A two-factor analysis of variance was performed on the

data for the first cycle when 0M and 2M females were housed either individually or in groups. This analysis revealed a significant interaction between prior intrauterine position and housing, $F(1,61) = 6{\cdot}1$, $P < 0{\cdot}05$. A test for homogeneity of variance performed on the data for the singly-housed animals revealed that the variance for the group of 2M females was significantly greater than that for the 0M females (F_{max} test; $F(12,13) = 16{\cdot}2$, $P < 0{\cdot}001$; Winer, 1971).



Text-fig. 1. Lengths of oestrous cycles of 0M and 2M females separated, at 32 days of age, from a male by a wire-mesh barrier and housed (a) individually and 1 complete cycle measured, or (b) in groups (5/cage) of similar females and 4 complete cycles measured.

A significant interaction between prior intrauterine position and age was observed in the group-housed condition with regard to cycle length during adolescence ($F(3,114) = 4{\cdot}08$, $P < 0{\cdot}001$). Specifically, while the mean cycle length for the 0M females was significantly longer than that for the 2M females for the first recorded cycle, by the third and fourth cycles (≥50 days old) 0M females had significantly shorter cycles than did 2M females (LSD, $P < 0{\cdot}05$; Winer, 1971). Neither the group mean nor the variance changed appreciably during adolescence for 2M females. However, both the group mean and variance for the 0M females decreased as these females matured. Specifically, the coefficient of variation, which provides a measure of the magnitude of the variance in relation to the magnitude of the mean, went from 30% for Cycle 1 to 11% for Cycle 4 for the 0M females. The coefficient of variation for the 2M females, on the other hand, increased from 22% for Cycle 1 to 36% for Cycle 4. Therefore, as 0M females approach adulthood, their oestrous cycles become shorter and more regular than those of 2M females housed in groups.

Discussion

As noted in the introduction, the oestrous cycle of the mouse is subject to acceleration by male cues and deceleration by female cues. Forming the experimental framework for the present study is the fact that the dominance relationship in a female's sensitivities to male- and female-originating cues changes dramatically during adolescence. All of our data, past and present, are compatible with the hypothesis that former intrauterine proximity to male fetuses

has two major effects with regard to the oestrous cycle: (1) there is an intrinsic lengthening of the oestrous cycle in 2M females, and (2) 0M females have a greater capacity to emit oestrus-suppressing cues and/or a greater sensitivity to such cues. The two types of females, on the other hand, do not seem to differ in their sensitivity to the accelerating action of a male's cues (vom Saal & Bronson, 1980b).

Table 1. The age of puberty as indicated by insemination and successful pregnancy and the length of oestrous cycles during adolescence and adulthood in 0M and 2M female mice housed differently

	Housing			
	Individual		Grouped (5♀/cage)	
Developmental stage	No male	Male present	No male	Male present
Puberty* (first ovulation)	—	0M < 2M	—	0M > 2M
Early adolescent oestrous cycle	Both prolonged†	0M < 2M‡	—	0M > 2M‡
Adult§ oestrous cycle	0M < 2M	0M < 2M	Both prolonged	0M < 2M

* From vom Saal & Bronson (1978).
† Unpublished observation.
‡ Refers to the first cycles examined in Text-fig. 1.
§ From vom Saal & Bronson (1980b) and the later cycles examined in Text-fig. 1.

All of the information relevant to the phenomena discussed above is presented in Table 1. Once a 2M female attains her pubertal ovulation, she displays a relatively constant 6- to 7-day cycle, whether housed individually or grouped, as long as she is in the presence of a male (vom Saal & Bronson, 1980b; Text-fig. 1). The peripubertal 0M female produces a more potent oestrus-suppressing cue(s) and/or is more sensitive to such a cue(s), and therefore exhibits a longer post-pubertal oestrous cycle relative to 2M females when placed in groups with other 0M females and with a male present. When there are no other females present, post-pubertal 0M females exhibit a 4–5-day cycle like adults, as long as a male is present to accelerate the cycle. During adolescence in 0M females, there is a gradual loss of the oestrous cycle-decelerating effect of grouping, such that by 50 days of age, grouped 0M females also exhibit the 4–5-day cycle typical of adults. During full adulthood, the cycles of 0M and 2M females are greatly lengthened when the females are housed in groups of 5/cage in the absence of a male, indicating that 2M females are not totally without sensitivity to the oestrus-suppressing actions of other females. When totally isolated from males and other females, the intrinsically faster cycle of the 0M females can be observed only when the females are well beyond adolescence (>150 days of age; vom Saal & Bronson, 1980b). The present results, when considered with our previous data, clarify the potentially confusing relationship between intrauterine position and the length of the oestrous cycle in mice.

It is generally recognized that the control of the timing of puberty in females by pheromonal cues produced by both sexes plays an important role in the regulation of population size in house mice (Bronson, 1979). The present findings suggest that a high density of females in the environment might have a greater inhibitory effect on the reproductive capacity of 0M females than on 2M females.

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