$P < .01$), as were the following comparisons: SS versus NS, SS versus NN, SN versus NS, and SN versus NN ($\chi^2 = 4.21$, 11.2, 4.49, and 17.67, respectively; $P < .05$). Median gestation length in days (± range) was 24 ± 3, 23 ± 2, 22 ± 0, and 22 ± 0, respectively. The mean number of offspring per treatment (± standard error) was 8.2 ± 0.8, 8.0 ± 1.1, 11.7 ± 0.7, and 11.4 ± 0.4, respectively. The mean weight per pup in grams (± S.E.) was 5.8 ± 0.3, 5.5 ± 0.6, 7.6 ± 0.3, and 7.8 ± 0.2. The latter differences were significant ($F = 8.83$ and 11.40; $P < .01$), as were the following paired treatment means according to the Scheffé test: SS versus NS, SS versus NN, SN versus NS, and SN versus NN ($P < .05$).

Of the total number of litters born (6, 4, 7, and 14), the number of litters with neonatal deaths by day 10 postpartum was 4, 3, 0, and 0, respectively; the number that survived virtually intact was 1, 0, 7, and 14, respectively. Overall differences between the number of litters with neonatal deaths and the number surviving by postpartum day 10 were significant ($\chi^2 = 19.15$ and 26.94, respectively; $P < .001$), as were the following comparisons: SS versus NS, SS versus NN, SN versus NS, and SN versus NN ($\chi^2 = 6.74$, 11.67, 7.22, and 12.60, respectively, and 9.48, 15.56, 11.0, and 18.0; $P < .01$). Thus, in the cross-fostering experiment, the prenatally stressed groups differed from the nonstressed groups independent of rearing condition. Prenatal stress therefore seems to affect later reproduction not by disrupting postnatal rearing conditions but by altering the fetus, possibly by changing the hormonal milieu.

Under severe environmental stress, sexual differentiation in some mammalian species is believed to take place in the presence of large amounts of steroids, some of which are secreted by the adrenal glands (*7*). Disturbances in gonadal and adrenal hormones during perinatal sexual differentiation can disrupt reproduction in female offspring by decreasing sexual receptivity or by inducing gonadotropic or ovarian irregularities or by both means (*8*). Prenatal stress therefore may influence the exchange of gonadal and adrenal hormones between the mother and fetus or the balance of these hormones in the fetus alone during a critical stage of hypothalamic differentiation, thereby producing reproductive dysfunctions in adulthood.

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4 May 1979; revised 23 July 1979

Release of Luteinizing Hormone in Male Mice During Exposure to Females: Habituation of the Response

Abstract. *Male mice release luteinizing hormone when exposed for a short time to a female. In this experiment, multiple blood samples were withdrawn by atrial cannulas from tethered males during either continuous or intermittent exposure to nonreceptive females. After an immediate, transient release of luteinizing hormone, continuous exposure to the same female was accompanied by only random, spontaneous elevations in plasma levels of this hormone. Successive presentations of the same female at 2-hour intervals elicited gradually diminishing luteinizing hormone responses. Exposing such unresponsive males to novel, diestrous females, however, dramatically stimulated their release of the hormone. These results demonstrate habituation of a socially induced, neuroendocrine response involving reproductive hormones.*

Males of many mammalian species secrete increased amounts of reproductive hormones when exposed to females of the same species (*1*, *2*). The precise nature of the relevant cues from the females is unknown in most cases, and the reproductive function served by the males' responses is not understood in any. Although short-term exposure of male house mice to either females or female urinary odor provokes immediate release of luteinizing hormone (LH) and then secretion of testosterone, male mice do not show elevated titers of these hormones during sustained cohabitation with females (*3*, *4*). Thus, this particular neuroendocrine response must be subject to either sensory adaptation, hypothalamo-hypophyseal depletion, or habituation. By analogy with neuromuscular terminology, habituation is defined here as the absence of the other two phenomena (*5*). In the experiment reported here, we observed that progressively fewer male mice release LH in response to repeated exposure to the same female. The males' LH responses resumed, however, upon the introduction of a novel female. Therefore, our data establish that habituation can occur in a socially induced, neuroendocrine response involving reproductive hormones.

The design of the study involved sequentially sampling blood from cannulated but freely moving male mice (*6*) during continuous or intermittent exposure to individual females. In more detail, 45 cannulated CF-1 males (*7*) were allowed to interact with nonreceptive females in the males' home cages; 15 males were used for each of three patterns of female exposure. During the pattern of continuous exposure, a female remained with each male throughout the test period without being disturbed. During the two patterns of repetitive exposure, the same female was placed in each male's cage three times, and then either that individual or an unfamiliar female was presented during the fourth sequence. In the latter two experimental conditions, females were placed in the cages every 2 hours for 90 minutes and then removed for 30 minutes. Five blood samples were withdrawn from the males at 5-minute intervals every 2 hours, always beginning before females were placed in the cages. The first two samples established the individuals' baseline levels of LH and the

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next three assessed their secretion of LH in response to the females (*8*). Blood samples were obtained from males continuously exposed to females on the same time schedule. Thus, 20 blood samples were withdrawn from each male in each experimental condition during the 8 hours of testing.

All stimulus females used in the experiment were in diestrus, as verified by vaginal smearing. Diestrous females were chosen to stimulate the males because (i) samples of urine from diestrous, proestrous, ovariectomized, or pregnant females equally provoke LH release in male mice (*4*); (ii) ejaculation by the males was avoided; and (iii) the stimulus qualities of the females remained relatively constant throughout the 8-hour test period.

The data were selected before analysis and presentation. Male mice, like males of many other mammalian species, experience periodic episodes of spontaneous release of reproductive hormones (*2, 9*). Plasma levels of LH in mice may rise from a few to as many as 400 ng/ml within 1 or 2 minutes. We observed previously that such episodes of LH secretion are followed by a 20- to 30-minute refractory period, during which males do not release LH when presented with a female (*10*). Figure 1 shows an example of such interference in an animal that was not examined during the present experiment. To avoid interference of this type, the entire 20-sample record of a male was omitted from the final analysis if that male showed a markedly elevated LH titer in either of the two blood samples preceding female exposure. Of the 45 males examined, 29 showed an initial release of LH in response to the first stimulus female and no interference thereafter due to episodic release of LH; the data presented in this report are for these 29 males.

As shown in Fig. 2, the patterns of LH release varied considerably among the males of the three experimental groups [$P < .05$, analysis of variance (*11*)]. If the females were left in the males' cages continuously, infrequent and apparently random episodes of LH release were observed; nine such spontaneous pulses were observed in six males (the elevated mean at 240 minutes in Fig. 2A reflects such spontaneous release on the part of three males). During successive presentations of the same female (Fig. 2B), the proportion of males responding to the females, and hence the mean level of response for the group, decreased markedly ($P < .05$, t-test). Indeed, after the same female had been presented four

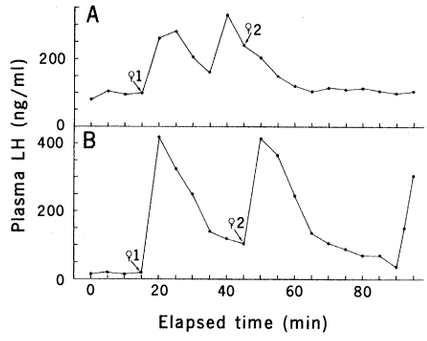

Fig. 1. Examples of LH release induced in two male mice in response to two different females for 30 minutes each (starting at the arrows). (A) Spontaneous release of LH preceded exposure to the second female and apparently inhibited the male's release of LH in response to that female. (B) The only episodic secretion of LH that was observed in this male occurred late in the sampling period and did not interfere with the two socially evoked responses.

times, plasma LH levels in the males did not differ from those in males continuously exposed to a female ($P > .10$, t-test; Fig. 2, A and B). The effect of presenting a novel female at this time was dramatic—unfamiliar females elicited LH secretion equal to the males' initial responses ($P > .40$, t-test; Fig. 2B).

A common observation in behavioral and physiological research is decreasing responsiveness of subjects to repeated or

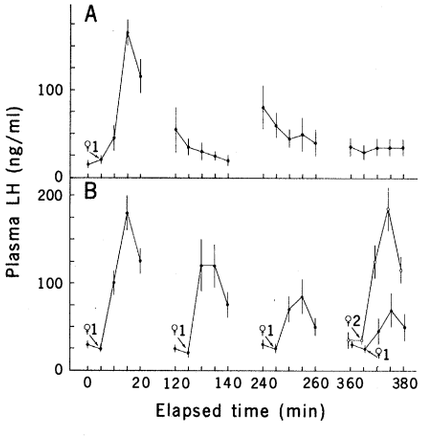

Fig. 2. Habituation of plasma LH secretion in male mice elicited by repeated or continuous female stimulation (denoted by arrows). (A) Mean (± standard error) LH concentrations for nine males during continuous exposure to one female that remained in the cage throughout the test period except for the first 5 minutes. (B) Mean LH concentrations for 20 males when one female was introduced into each male's home cage three times and then either (●) the same female ($N = 10$) or (○) a novel female ($N = 10$) was presented during the last sequence. Data are shown only for males who released LH after their first exposure to a female and did not spontaneously release LH immediately before succeeding female exposures.

continuous stimulation. If the response decrement occurs with neither adaptation of the sensory receptors nor fatigue of the effectors, habituation has taken place (*5*). The data reported here illustrate not only decreasing LH release in male mice after repeated exposure to females, but also resumption of secretion on the introduction of a novel female. Neither olfactory adaptation nor hypothalamo-hypophyseal depletion is involved. To our knowledge, this is the first fully verified description of habituation of a neuroendocrine response involving a reproductive hormone.

Regarding the function of the female-induced release of LH in a male mouse, our data suggest a correlation of this response with the arousal of sexual behavior. Two lines of evidence support this hypothesis. First, temporal alignment of the latencies of males' mounting behaviors and their LH responses to females have been observed, with maximum LH secretion occurring during the same period when the males began mounting females (*10*). Second, the characteristics of habituation described above for the LH response in male mice are markedly similar to the features of behavioral arousal exhibited by males of several mammalian species when they encounter sexually receptive females. For example, a novel female often elicits renewed mating behavior in a sexually satiated male; thus, this situation is also an example of habituation. Such resumption of sexual interest elicited by a new female, generally known as the Coolidge effect (*12*), has been described in numerous male mammals (*13*). Furthermore, in many of these species, short-term exposure to a female has been reported to increase circulating levels of LH or testosterone, or both (*1-4*). Despite these correlations, however, the parallel between the characteristics of the mouse's LH response and those of behavioral arousal is only suggestive of function. It must be stressed that the release of LH by male mice occurs in response to any genetically female mouse, whether she is gonadally intact or ovariectomized and regardless of her estrous state. Thus, although a surge of LH and testosterone secretion upon encountering a receptive female could in some way support the males' sexual arousal, it may merely reflect arousal and actually support another function.

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6. In our cannulation procedure, an atrial cannula (inner diameter, 0.30 mm; outer diameter, 0.64 mm; 45-cm Silastic tubing, Dow Corning Corp.) exits via a backpack sutured to the mouse and is protected by an extension spring. The entire unit is supported from above and swivels freely. During the 5-day surgical recovery period, 0.33 units of heparin in 0.3 ml of saline is continuously infused per 24 hours. Data to be presented elsewhere (*10*) document that cannulated males exhibit normal aggressive and sexual behaviors, when compared with males that do not have surgery.
7. The CF-1 mice used in this experiment were reared in our laboratory colony. At weaning (21 to 23 days of age), the males were isolated in 29 by 18 by 13 cm polypropylene cages in a room without females. The ambient conditions were 23° ± 1°C, 14:10 hour light-dark cycle, lights on at 0600 hours, and Purina mouse chow and water always available. Experimental males were 70 to 80 days old. After cannulation, they were housed in 29 by 14 by 14 cm wooden boxes and remained in the same room. All testing began between 0900 and 1100 hours.
8. After each 25-μl blood sample was obtained, the cannulas were rinsed and the blood was replaced with heparinized saline (10 units per milliliter). The 10-μl samples of plasma were stored in 40-μl radioimmunoassay (RIA) buffer at −80°C before assay. Plasma LH concentrations were determined with the NIAMDD rat radioimmunoassay kit verified for measuring mouse gonadotropins by W. G. Beamer, S. M. Murr, and I. I. Geschwind [*Endocrinology* **90**, 823 (1972)]. The reference curve was fitted and the unknown concentrations were interpolated by using the computer analysis described by D. Rodbard and D. M. Hutt [in *Symposium on Radioimmunoassay and Related Procedures in Medicine* (International Atomic Energy Agency, Vienna, 1974), p. 165]. Within-assay variation was 10.4 percent and between-assay variation 13.9 percent; the minimum detectable amount was 0.125 ng. Results are expressed as nanogram-equivalents of NIAMDD-RAT-LH-RP-1.
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30 January 1979; revised 11 May 1979

Inbreeding and Juvenile Mortality in Small Populations of Ungulates

Abstract. *Juvenile mortality of inbred young was higher than that of noninbred young in 15 of 16 species of captive ungulates. In 19 of 25 individual females, belonging to ten species, a larger percentage of young died when the female was mated to a related male than when she was mated to an unrelated male.*

An ever increasing number of the world's ungulate species exist only in relatively small populations in which some degree of inbreeding will inevitably occur. Extensive studies of laboratory and domestic mammals and birds indicate that inbreeding leads, in the majority of cases, to increased mortality in young animals and reduced fertility in adults (*1–3*). Inbred animals are usually "less able to cope with their environment than are noninbred animals" (*2*, p. 215) and are often more susceptible to various diseases and environmental stresses (*3*, *4*). The limited data from natural populations suggest that close inbreeding has the same deleterious consequences in the wild (*5*).

Table 1. Juvenile mortality in inbred and noninbred young.

Species	Non-inbred young	In-bred young	*N*	χ^2 test		Sign test*
				χ^2	*P*	
Elephas maximus (Indian elephant)						
Lived	11	2	19			+
Died	2	4		4.997	.025†	
Equus burchelli (zebra)						
Lived	20	3	32	.413	.528†	+
Died	7	2				
Choeropsis liberiensis (pygmy hippopotamus)						
Lived	139	23	235	17.28	.000†	+
Died	45	28				
Muntiacus reevesi (muntjac)						
Lived	18	12	40	1.212	.270	+
Died	4	6				
Cervus eldi thamin (Eld's deer)						
Lived	13	0	24	11.679	.001†	+
Died	4	7				
Elaphurus davidianus (Père David's deer)						
Lived	15	19	39	.030	.857	+
Died	2	3				
Rangifer tarandus (reindeer)						
Lived	19	9	50	2.538	.107	+
Died	10	12				
Giraffa camelopardalis (giraffe)						
Lived	11	2	19	2.537	.107	+
Died	3	3				
Tragelaphus strepsiceros (kudu)						
Lived	10	8	25	.005	.941	−
Died	4	3				
Tragelaphus spekei (sitatunga)						
Lived	15	31	75	9.012	.006†	+
Died	1	28				
Hippotragus niger (sable)						
Lived	18	3	32	8.183	.005†	+
Died	4	7				
Oryx dammah (scimitar-horned oryx)						
Lived	35	0	42	28.378	.000†	+
Died	2	5				
Connochaetes taurinus (wildebeest)						
Lived	6	29	48	.680	.419	+
Died	1	12				
Madoqua kirki (dik-dik)						
Lived	10	7	32	.473	.499	+
Died	7	8				
Gazella dorcas (Dorcas gazelle)						
Lived	36	17	92	9.288	.003†	+
Died	14	25				
Capricornis crispus (Japanese serow)						
Lived	52	27	135	10.585	.002†	+
Died	21	35				

*For the sign test, + = juvenile mortality higher in inbred than noninbred young ($P = .0003$). †Significant at .05 level; one degree of freedom in all cases; probabilities are rounded to three places.

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