Ejaculatory Pattern in Female Rats Without Androgen Treatment

Abstract. *Adult female rats receiving long-term estrogen treatment displayed the species-typical motor pattern of ejaculation during copulation. This hormone treatment produced pituitary hypertrophy and concomitant pressure damage to brain areas dorsal to the pituitary, but did not cause clitoral hypertrophy. The demonstration of the ejaculatory pattern in perinatally untreated female rats indicates that the potential for the expression of the ejaculatory or "orgasmic" pattern is not dependent on exogenous androgen at any stage of development and is more widely represented among female mammals than previously believed.*

The acts of mounting, intromitting, and ejaculating displayed by rats during copulation are easily discriminated on the basis of their distinctive motor patterns. While intromissions and ejaculations are usually associated with phallic insertion by males and by perinatally androgenized females, the perinatally untreated female rat lacks the phallic development which may be necessary to achieve insertion. Therefore, the terms intromissive pattern and ejaculatory pattern have been adopted to refer only to the display of the respective motor components. The term ejaculatory pattern does not imply fluid emission.

Normal female rats have been observed displaying the typically masculine motor patterns of mounts and intromissions (*1*). Female rats treated with exogenous androgen or estrogen perinatally and androgen in adulthood are capable of displaying complete masculine copulatory behavior, including the motor pattern of ejaculation (*2*). It has been commonly assumed that perinatally untreated female rats do not have the potential for the ejaculatory pattern (*3*). This assumption has been variously attributed to deficits in phallic development (*4*) or to a failure of the potential for the ejaculatory pattern to be organized into the central nervous system (*5*).

However, we have observed the ejaculatory pattern in several perinatally untreated female rats. Independent and concurrent observations at another laboratory have further confirmed that perinatally untreated female rats are capable of displaying the ejaculatory pattern (*6*). These observations are the first evidence that the potential for the ejaculatory pattern exists in adult female rats receiving no perinatal treatment. There is now a need for a reevaluation of current views of the differentiation of sexual behavior.

The perinatally untreated ejaculating females in our laboratory were part of a group of 60 female Long-Evans rats that received subcutaneous implants of 5-mg pellets of fused estradiol benzoate (EB) in adulthood. The EB-implanted females were housed in groups and used as stimulus animals with males during copulatory tests. Approximately 7 months after EB implantation we began testing the 60 females for mounting behavior. The EB-implanted females were placed in an aquarium (50 by 30 by 30 cm) with wood shavings on the floor and allowed a 5-minute adaptation period before the introduction of a receptive stimulus female (*7*).

Twelve of the EB-implanted females displayed the ejaculatory pattern at least once during three 45-minute observation periods. The responses were clearly identifiable as ejaculatory patterns and even appeared exaggerated compared to the ejaculatory patterns of male rats. The deep ejaculatory thrust of the ejaculating females was maintained for several seconds while repeated flexures of the haunches occurred. Often the forepaw clasp was prolonged during the ejaculatory pattern, with the result that the stimulus female dragged the ejaculating female about the testing chamber. Occasionally the stimulus female would react defensively during and after an ejaculatory pattern, an indication that the forepaw clasp of the ejaculating female may have been painfully intense.

Although systematic measures of feminine behavior were not taken, all the ejaculating females displayed high levels of solicitation behaviors (hopping, darting, and crouching) when paired with receptive stimulus females. Stimulus females sometimes pursued and mounted the test females just after the latter displayed the ejaculatory pattern. In these cases too the test female displayed solicitation and lordosis.

The 12 ejaculating females were then individually housed and observed weekly in 90- to 120-minute copulatory tests. Again a 5-minute adaptation period preceded the introduction of the stimulus female. If the test females had not mounted within the first 30 minutes of a test, the stimulus female was changed. Pilot work had indicated that tail pinching of the test females potentiated the display of the ejaculatory pattern. During the last 30 minutes of a copulatory test in which the ejaculatory pattern had not occurred, the tails of the test females were pinched approximately every 5 minutes. Tail pinching was stopped when the ejaculatory pattern was displayed. Females that did not require tail pinches before the ejaculatory pattern occurred are referred to as spontaneously ejaculating females.

The data reported in Table 1 are based on the performances of spontaneously ejaculating females housed individually for at least 1 week (*8*). For comparative purposes, we have included data for control male rats from our laboratory (*9*). All statistical comparisons reported employed the Mann-Whitney U test.

The spontaneously ejaculating females did not differ significantly from the males in the number of mounts and intromissive patterns preceding the ejaculatory pattern. The females were significantly slower in initiating mounts and in the rate of displaying mount bouts (*10*). The most striking differences between the two groups were in the duration and variability of the postejaculatory intervals. While normal male rats are sexually inactive for at least 4 to 5 minutes after an ejaculation, the spontaneously ejaculating females would often begin mounting within 3 minutes of an ejaculatory pattern.

The spontaneously ejaculating females were also compared to the tail-pinched, ejaculating females ($N = 4$; one copulatory series) and to females from our laboratory receiving androgen perinatally and in adulthood ($N = 6$; three copulatory series) (*9*). The tail-pinched, ejaculating females had significantly more intromissive patterns (median, 23.5; range, 13.5 to 34.0; $P < .05$) than the spontaneously ejaculating females, but none of the other comparisons of copulatory measures between the two groups reached significance. The mount bout period for the tail-pinched, ejaculating females (median, 102.4 seconds; range, 63.6 to 212.1) was strikingly similar to the mount bout period of the spontaneously ejaculating females. The tail-pinched, ejaculating females also resembled the spontaneously ejaculating females in their postejaculatory intervals to the first mount (median, 253.5 seconds; range, 108.0 to 341.0 seconds) and first intromission (median, 253.5 seconds; range, 108.0 to 445.5 seconds).

Comparisons between the sexual behavior of the spontaneously ejaculating females and the perinatally androgenized females yielded a pattern almost identical to the differences between the spontaneously ejaculating females and control males. The perinatally androgenized females displayed the typically masculine pattern of lengthy postejaculatory intervals. The androgenized females' median intervals from the first, second, and third ejaculatory patterns to the first mount of the next copulatory series were 405.0, 496.8, and 567.5 seconds, respectively (*11*).

Table 1. Copulatory behavior of spontaneously ejaculating female rats and normal male rats during three copulatory series. Group medians and Mann-Whitney U tests were computed from the means of individual animals' copulatory behavior for one to three copulatory tests. A copulatory series includes the mounts and intromissive patterns preceding an ejaculatory pattern and the postejaculatory period that follows. Mount latency and intromissive pattern latency: intervals from introduction of stimulus female to first mount and intromissive pattern, respectively. Mount frequency and intromissive pattern frequency: number of mounts and intromissive patterns. Mount bout period: average interval between the first acts in bouts of copulatory activity. Postejaculatory mount latency and postejaculatory intromissive pattern latency: intervals from ejaculatory pattern to the first mount and intromissive pattern of the next copulatory series. Results are given as medians, and the ranges are shown below them.

Behavior	Series 1		Series 2		Series 3	
	Females (N = 5)	Males (N = 4)	Females (N = 4)	Males (N = 4)	Females (N = 3)	Males (N = 4)
Mount latency (seconds)	143.0†	22.5†				
	42.0–1299.5	16.0–120.3				
Intromissive pattern latency (seconds)	378.0*	44.5*				
	42.0–1398.5	17.3–161.3				
Mount frequency	10.0	6.2	5.0	4.7	2.5	5.0
	2.0–18.5	2.0–12.3	2.5–8.0	2.7–6.0	2.0–5.0	2.0–9.0
Intromissive pattern frequency	12.0	8.9	6.0	4.2	4.0*	5.3*
	6.0–16.5	6.7–11.0	3.0–9.0	3.0–5.3	1.0–4.0	4.0–13.7
Mount bout period (seconds)	110.0*	52.6*	106.2†	47.2†	60.4†	41.5†
	46.5–191.0	26.4–62.7	61.1–208.3	40.1–55.4	58.4–82.3	24.6–49.2
Postejaculatory mount latency (seconds)	180.0*	318.1*	177.8†	406.4†	176.0†	450.0†
	135.0–658.0	290.0–357.3	167.0–188.5	346.3–863.7	146.0–274.0	390.0–1212.7
Postejaculatory intromissive pattern latency (seconds)	283.5	339.4	216.8†	406.7†	296.0	476.4
	135.0–661.0	294.3–378.3	175.0–321.0	347.3–893.3	274.0–947.0	390.7–1232.0

*P < .10; †P < .05.

After we made behavioral observations, the animals were killed, and we made gross postmortem examinations of half the ejaculating females and six nonejaculating EB-implanted females. All had hypertrophied pituitaries that caused pressure damage to the ventral surface of the brain caudal to the anterior thalamus. Further histological examination of several of the pituitaries indicated a tumorous condition (*12*). The observed pathologies are interesting since both adrenocorticotrophic hormone (*13*) and ventral dimesencephalic junction lesions (*14*) have been implicated in the potentiation of ejaculation in male rats.

The presence of intact adrenals and ovaries in the ejaculating females makes it impossible to rule out the involvement of endogenous androgens in the display of the ejaculatory pattern. However, there is recent evidence that estrogen is capable of maintaining ejaculatory ability in adult castrate male rats (*15*).

Painful peripheral electric shock potentiates masculine copulatory behavior in male rats (*16*) and in female rats treated with androgen only in adulthood (*6, 17*). Shock also potentiates display of the ejaculatory pattern in neonatally untreated female rats (*6*). Our tail pinching of the females apparently functioned similarly to electric shock in potentiating the display of the ejaculatory pattern.

Also common to the control of mating in male and female rats is the influence of serotonin depletion on mounting behavior. Mounting behavior is increased in both male (*18*) and female (*19*) rats after systemic administration of parachlorophenylalanine (PCPA), a depleter of serotonin. We have observed potentiation of the display of the ejaculatory pattern in EB-implanted female rats after PCPA administration (*20*). Two months after the initial 45-minute copulatory tests mentioned above, 25 of the nonejaculating EB-implanted females were tested again for 45 minutes with receptive stimulus females. Seven nonejaculating females were selected from this group on the basis of the display of at least some mounting behavior. These females received four daily injections of PCPA methyl ester hydrochloride (100 mg/kg). Six hours after the last PCPA injection, six of the seven females displayed the ejaculatory pattern during a 90-minute copulatory test. When retested 2 weeks after PCPA administration, none of the females displayed the ejaculatory pattern during a 90-minute copulatory test. Serotonin returns to normal approximately 2 weeks after PCPA administration (*21*). Gross postmortem examination of four PCPA-ejaculating females indicated hypertrophied pituitaries and ventral brain damage.

Our observations of the ejaculatory pattern in neonatally untreated female rats indicate that the potential for the display of the ejaculatory pattern is present in both male and female rats. Hence, the two major views concerning differentiation of sexual behavior in rodents require modification. In regard to the hypothesis that normal females lack the central neural capacity for displaying the ejaculatory pattern, it now appears that perinatal administration of exogenous androgen or estrogen is not necessary to create the neural substrate of the ejaculatory pattern, but rather to potentiate the functional expression of that substrate (*22*). The absence of clitoral hypertrophy in our ejaculating females further indicates that masculine phallic development is not crucial for expression of the ejaculatory pattern. Views of sexual behavior differentiation which emphasize peripheral genital development as critical for ejaculatory pattern capacity could better accommodate our observations by focusing on genital sensitivity rather than on phallic development (*23*).

The orgasmic patterns of human and nonhuman primate females are very similar to the orgasmic or ejaculatory pattern of their conspecific males (*24*). This is the first report of a nonprimate species in which females displayed the conspecific masculine ejaculatory pattern without perinatal or adult androgen treatment. Thus, the potential for this response is more widely represented among female mammals than was previously thought. Further research on the factors controlling the potential and expression of the species-typical ejaculatory pattern in mammalian females may lead to a better understanding of both the evolution of the display of the ejaculatory pattern in females, and of the neuroendocrine mechanisms underlying orgasm in humans.

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6. M. S. Krieger, thesis, Rutgers University, New Brunswick, N.J. (1975); ——— and R. J. Barfield in preparation. Eight adult female rats received subcutaneous implants of fused pellets of testosterone propionate (TP). Two weeks after implantation the females were paired with receptive stimulus females for the first of three weekly tests. Throughout the testing periods the TP-implanted females received mildly painful shock to the flanks every 30 seconds. Three of the TP-implanted females displayed ejaculatory patterns during testing.
7. During the first three copulation tests stimulus females were made receptive by subcutaneous implants of EB. During all other tests the stimulus females were brought into estrus by injections of 50 μg of EB 48 hours before the test and 500 μg of progesterone 4 hours before the test (P. Perlman of Schering Corp., Bloomfield, N.J., contributed the hormone products).
8. Not all the spontaneously ejaculating females finished three copulatory series during individual observation periods. However, the data for the females completing less than three copulatory series were included, since deletion of the data for these females minimally altered the medians for the first and second copulatory series.
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10. Mount bouts are clusters of mounts and intromissions that are interrupted only by genital autogrooming or behavior oriented toward the stimulus female. Grooming any other part of the body or pointing the nose away from the stimulus female indicates that the preceding sexual act terminated the mount bout. For additional details, see B. D. Sachs and R. J. Barfield [*J. Comp. Physiol. Psychol.* **73**, 359 (1970)].
11. Male and perinatally androgenized female rats emit postejaculatory ultrasonic vocalizations [R. J. Barfield and L. A. Geyer, *Science* **176**, 1349 (1972); see also (*9*)]. Bat detector monitoring of the postejaculatory periods of three spontaneously ejaculating females indicated the absence of ultrasonic vocalizations. When the remainder of the spontaneously ejaculating females were observed during their postejaculatory periods, they did not display the stereotyped breathing pattern which normally accompanies postejaculatory vocalizations in the male rat.
12. Spontaneous pituitary tumors are common in older female rats, and the occurrence of pituitary tumors is further potentiated by chronic administration of estrogen [O. Mühlbock and H. G. Kwa, *Acta Unio Int. Contra Cancrum* **18**, 275 (1962)].
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22. Species of rodents differ in the periods in which exogenously administered androgens and estrogens are capable of potentiating the expression of the ejaculatory patterns in females. The postnatal period has been assumed to be the sensitive period for potentiation of the ejaculatory pattern in the rat [I. L. Ward, *Horm. Behav.* **1**, 25 (1969)]. Normal female rats may be exposed to prenatal androgen secreted by male siblings in utero. Female rats most proximal to male siblings in utero tended to show more mounting behavior in adulthood [L. G. Clemens, in *Reproductive Behavior*, W. Montagna and W. A. Sadler, Eds. (Plenum, New York, 1974), p. 23]. Natural in utero exposure to androgens may be involved in the reported adult display of the ejaculatory pattern in the prenatally untreated female guinea pig, a species in which the prenatal period is the sensitive period for differentiation of sexual behavior [R. W. Goy, C. H. Phoenix, R. Meidinger, *Anat. Rec.* **157**, 87 (1967)].
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25. Supported by NIH grant HD-04048 and a grant from the University of Connecticut Research Foundation.

27 November 1974; revised 29 April 1975

Auditory Fatigue: Retrocochlear Components

Abstract. *Changes in auditory sensitivity were measured at the VIII nerve, cochlear nucleus, and inferior colliculus after a fatiguing sound exposure. Losses in sensitivity progressively increased from peripheral to central auditory sites. The results suggest that there is a retrocochlear component to auditory fatigue when it is induced by low-level sounds of short duration.*

Excessive acoustic stimulation can lead to auditory fatigue or a temporary threshold shift (TTS) in hearing. Although the definition of TTS is straightforward, its physiological basis appears to be complex, and several different physiological disorders in the cochlea have been implicated in TTS (*1*). These disorders presumably lead to a depression of the cochlear potentials; for example, during asymptotic TTS, Benitez *et al.* (*2*) reported a reduction in cochlear microphonic sensitivity that was 24 and 48 db for the second and third turns of the cochlea, respectively. They were unable to elicit the VIII nerve action potential.

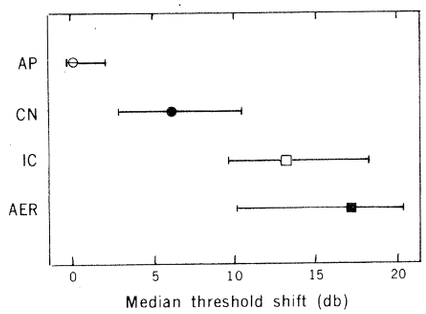

Fig. 1. The median threshold shift and interquartile range (horizontal bar) obtained after an 8-minute pure tone exposure at 95 db SPL. The open circle denotes the VIII nerve action potential (*AP*, $N = 41$); the closed square is the auditory evoked response (*AER*, $N = 30$). Threshold shifts in single neurons from the cochlear nucleus (*CN*, closed circle, $N = 51$) and inferior colliculus (*IC*, open square, $N = 12$) were based on the lateral shift in the function relating discharge rate to intensity.

Circumstantial evidence also links TTS to the cochlea. First, the TTS produced by a pure tone is distributed in a pattern that resembles the spread of mechanical vibration along the basilar membrane. Second, the audiological signs and symptoms associated with TTS (*3*) are consistent with a hearing loss of cochlear origin. Hence, the consensus has been that TTS is strictly a cochlear phenomenon.

A recent study, however, suggests that central auditory processes are involved with TTS (*4*). Our results not only show that there is a central component to TTS, but also demonstrate that there is a progressively larger loss in sensitivity from peripheral to central auditory sites.

Chinchillas were used as subjects in three series of experiments. Each animal was exposed to a standard TTS-producing tone 8 minutes in duration at 95 db SPL (sound pressure level relative to 0.0002 dyne/cm^2) near the tympanic membrane. The exposure was at a frequency between 0.4 and 8.0 khz.

In experiment 1, the chinchilla's auditory evoked response (AER) was recorded from a chronic electrode placed over the rudimentary tentorium. This potential was used to estimate the magnitude of TTS resulting from the exposure (*5*). Pre- and postexposure AER thresholds were measured at one of six test frequencies (0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 khz, five animals at each frequency). The TTS exposure was always at the test frequency.

In experiment 2, the VIII nerve action potential and neurons from the cochlear