Influence of Female Hormonal State on Rhesus Sexual Behavior Varies with Space for Social Interaction

Abstract. *The sexual behavior of rhesus monkeys in 15 male-female pairings was observed in both a large and a small area during the follicular and luteal phases of the female's cycle. Males ejaculated in all tests at the follicular phase of the female's cycle and in 53 percent of tests at the luteal phase. However, a significant decline in ejaculation during tests at the luteal phase occurred in the large, but not in the small area. Thus the degree to which the pair's sexual behavior was influenced by the female's hormonal state depended on the spatial conditions of the test.*

The extent to which the occurrence of copulation in rhesus monkeys is influenced by hormonal changes in the female is unclear. Studies of free-ranging or large captive groups of rhesus describe discrete periods of heterosexual activity occurring at approximately 29-day intervals for each female and lasting for about 8 days (*1*). These recurring periods of regular copulation are generally followed by an absence of copulation after the midcycle estradiol peak and are associated with peak luteal progesterone (*2*). Although these findings suggest a well-defined relation between the female's hormonal state and copulation, laboratory studies of pairs of rhesus in cages suggest a less clear-cut relation between the female's ovarian cycle and copulation. Pairs copulate throughout the female's ovarian cycle, and changes in copulatory activity are generally limited to periovulatory increases in the occurrence of multiple ejaculations during a time-limited test and shorter times before the first ejaculation (*3*). As many as 50 percent of the pairs show no cyclic changes in copulatory activity (*4*). These contradictory findings have led to the viewpoint that rhesus monkeys, and possibly all primates, differ substantially from other mammalian species in that there is no consistent relation between the female's hormonal state and the occurrence of copulation (*5*). However, an alternative possibility is that a consistent relation exists, but its demonstration depends on the specific social and environmental conditions of observation. In support of the second viewpoint, I have found that the female's hormonal condition has more influence on sexual behavior of a paired male and female when the pair is observed in a large area than when the same pair is observed in a small area.

Five intact adult female rhesus were paired with two intact and one vasectomized adult male rhesus to produce the 15 pairs studied. Males were housed individually indoors between tests, and females lived together in an indoor-outdoor run. Subjects were adapted to handling and testing procedures for 12 weeks before data were collected, and females displayed at least one menstrual cycle before data collection (*6*). Females were tested 8 to 10 days after the onset of menstruation (follicular tests) and 10 to 15 days later (luteal tests). At each phase of the cycle, females were tested outdoors with all three males on two consecutive days, three tests taking place in a 1.4 by 1.2 m cage and the other three in a 15.2 by 15.2 m compound (*7*). For each test, first the female, and then the male, was introduced into the testing area; the pair were confined together for 30 minutes during which observations of the sequence and duration of selected behaviors were collected by means of a data recorder (Datamyte 900). Saphenous vein blood was collected from each female at the end of the test day and analyzed for estradiol and progesterone (*8*). Estradiol did not vary significantly between follicular and luteal tests [follicular, 175 ± 48 pg/ml, and luteal, 113 ± 15 pg/ml; $t = 1.02$, d.f. = 4, not significant (N.S.)], but follicular progesterone was significantly lower than luteal progesterone (follicular, 0.8 ± 0.6 ng/ml, and luteal, 4.5 ± 1.2 ng/ml; $t = 2.89$, d.f. = 4, $P < .05$).

Males ejaculated during all follicular tests, but not during all luteal tests (Fig. 1). During the female's luteal phase, males ejaculated in significantly fewer tests taking place in the compound (33 percent) than in those taking place in the cage (73 percent) [$\chi^2 = 4.2$, $P < .05$ (*9*)]. For tests taking place in the cage, the percentage of tests resulting in ejaculation did not differ significantly across the female's cycle ($\chi^2 = 2.3$, N.S.), whereas for tests in the compound the percentage differed significantly ($\chi^2 = 8.1$, $P < .01$). The number of ejaculations per test was significantly less for luteal tests than for follicular tests in both cage and compound, but was always greater during cage tests (Table 1). In the compound, latency to ejaculation was significantly shorter during follicular tests than during luteal tests for the five pairs (two males and three females) for which ejaculation occurred at both cycle phases [follicular, 5.4 ± 0.8 minutes, and luteal, 12.5 ± 2.3 minutes; $t = 2.83$, d.f. = 4, $P < .05$ (*9*)]. In cage tests, latency to ejaculation did not change significantly for the 11 pairs (three males and five females) with ejaculation occurring at both cycle phases (follicular, 4.8 ± 0.9 minutes, and luteal, 6.3 ± 1.4 minutes; $t = 0.99$, d.f. = 10, N.S.). Thus, when the pairs were tested in the 231-m^2 compound, they showed luteal decreases in the number of tests resulting in ejaculation and in the frequency of ejaculation and luteal increases in latency to ejaculation, as compared with results at the follicular phase of the cycle. In contrast, when the same pairs were tested in a 1.7-m^2 cage, only the frequency of ejaculation varied significantly with the female's cycle.

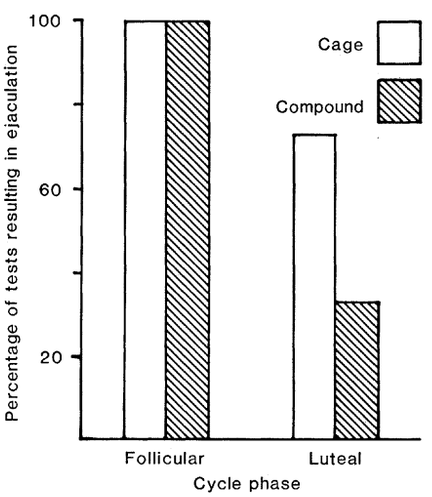

Fig. 1. Percentage of tests resulting in ejaculation for 15 pairings of three male and five female rhesus monkeys tested in two spatial conditions during the follicular and luteal phases of the female's ovarian cycle.

Spatial conditions of testing influenced the frequency with which males and females approached each other and the amount of time the females spent in back proximity to the male (*10*) (Table 1). Males approached females significantly less often during tests taking place in the compound than during those taking place in the cage at both cycle phases. In addition, male approaches declined significantly during luteal tests in the compound. Females approached males at comparable frequencies during follicular tests in the cage and the compound, but they approached males significantly less often during luteal tests in the compound than during luteal tests in the cage. During cage tests, females spent comparable times in back proximity to the male at both cycle phases. However, during tests in the compound, females spent significantly longer times in back proximity to the male during follicular than during luteal tests; they spent significant-

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Table 1. Frequencies and durations of behavior displayed by rhesus monkeys during heterosexual pair tests in two different-sized areas at two phases of the female's menstrual cycle. Frequencies and durations are given as means ± standard errors per pair.

Behavior	Area	Cycle phase		*F* ratios* by variance source		
		Follicular	Luteal	Phase	Area	Phase by area
		Frequencies				
Ejaculation	Cage	1.6 ± 0.6	0.9 ± 0.6†	31.4	26.3	0.1
	Compound	1.1 ± 0.4‡	0.3 ± 0.5†‡	$P < .001$	$P < .001$	N.S.
Male approach	Cage	9.3 ± 1.6	7.5 ± 1.9	4.7	23.3	0.3
	Compound	4.9 ± 1.0‡	1.4 ± 0.8†‡	$P < .05$	$P < .001$	N.S.
Female approach	Cage	15.8 ± 2.0	14.5 ± 3.2	0.9	5.2	1.1
	Compound	11.8 ± 2.4	7.7 ± 1.2‡	N.S.	$P < .05$	N.S.
		Durations				
Back proximity time (minutes)	Cage	1.8 ± 0.9	1.3 ± 0.4	1.0	1.0	17.2
	Compound	1.2 ± 0.4	0.3 ± 0.1†‡	N.S.	N.S.	$P < .01$

*In all instances, d.f. = 1,14. †Value differs significantly ($P < .05$) from follicular value in the same test area. ‡Value differs significantly ($P < .05$) from cage value at the same cycle phase.

ly shorter times in back proximity to the male during luteal tests in the compound than during luteal tests in the cage. Females responded negatively to males (*11*) 18 times in the 60 tests with no apparent relation either to the female's cycle or to the spatial conditions of testing. Thus, in the compound, male approaches and the time the female spent in back proximity to the male varied with the female's cycle; this did not occur when the same pairs were tested in the cage. Moreover, in tests taking place in the compound, the decline in proximity behavior during the luteal phase was associated with a significant decline in the occurrence of ejaculation.

These results demonstrate that the space available for social interaction affects the degree to which the female's hormonal state influences the behavior of both the male and the female. Even though females were in the same hormonal state during both cage and compound tests, the behavior of the pair varied more clearly with the female's cycle in the larger area. Several possible explanations for this finding are suggested.

The limited area available to the pair in the cage may interfere with the system of spatial cues used by rhesus to communicate sexual interest (*12*). Thus, if a female indicates willingness to copulate by approaching the male, a cage that limits separation to less than 2 m may increase the frequency of female approach, independent of her sexual interest. Forcing the pair into more intimate spatial contact may also increase the arousal level of both the male and the female. This notion is supported by the consistently higher frequencies of ejaculation and male approach in cage tests at both phases of the menstrual cycle, and by the absence of luteal decreases in female approach and duration of back proximity in cage tests. According to this viewpoint, female sexual interest is normally stimulated by the hormonal changes preceding ovulation, which causes her, under free-ranging conditions, to initiate and maintain contact with the male until her presence sexually arouses him. In the cage, the limited spatial separation makes female maintenance of contact less critical for initiating male arousal and therefore copulation occurs relatively independent of the female's cycle. Another possibility is that the cage prevents the female from avoiding the male, thus reducing her control over the sexual interaction. Although this possibility seems unlikely because there was no increase in female negative responses or decrease in female approaches in cage tests during the luteal phase, the cage may so limit the female's behavioral options that she simply accommodates the male sexually.

The fact that some copulation was observed during luteal tests in the compound, instead of the complete cessation reported for captive groups (*2*), suggests that the procedure of testing an isolated male-female pair may itself modify the importance of the female's hormonal state in determining copulation in rhesus. Males used in pair tests are provided few opportunities to copulate, even though they are capable of copulating several times per day. Under these circumstances, the hormonal state of the female may be less important to the male than the sexual opportunity that the female represents. Alternatively, in a group, females at the luteal phase might be unable to copulate with the males, either as a result of competition from females closer to midcycle or because of male preference for females at midcycle.

The demonstration that decreasing the amount of space for social interaction reduces the importance of the female's hormonal state as a determinant of the sexual activity of rhesus suggests that hormones play a less crucial behavioral role in rhesus than in rodents; in the latter, hormones appear to be obligatory to the expression of female sexual behavior when the subjects are interacting freely. However, my results and those of others (*2*) suggest that as the conditions of observation more closely reflect the social and environmental natural history of rhesus monkeys, the extent to which hormones influence the expression of sexual behavior becomes more pronounced. It remains to be seen whether this is a characteristic of primates in general or specific to rhesus.

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6. Menstruation was detected by swabbing each female's vagina with a moistened sterile cotton applicator on Mondays, Wednesdays, and Fridays. The first day of bleeding was considered day 1 of the cycle.
7. The cage had bars on its sides, top, and bottom, with no platforms dividing the vertical space. Subjects used the top and sides of the cage providing a maximum separation of 2.0 m. The compound had three 1-m-high culvert sections and 2-m mesh walls. Maximum subject separation was 21.6 m. The three daily tests were conducted as two cage and one compound tests, or as one cage and two compound tests. On the second test day, subjects received the complementary number of cage and compound tests to produce three tests in each space within the 2 days. Testing order was counterbalanced so that males and females received an equal number of first tests in the cage and compound across both cycle phases. Each of the 15 possible pairs was tested once in each spatial condition at each cycle phase for a total of four tests per pair.
8. Estradiol was assayed according to the procedure of K. Wright, D. C. Collins, and J. R. K. Preedy [*J. Clin. Endocrinol. Metab.* **47**, 1084 (1978)]. Progesterone was assayed according to the procedure of M. E. Wilson, T. P. Gordon, and D. C. Collins [*Horm. Behav.* **16**, 94 (1982)].
9. Due to the repeated measures design of the study, the McNemar test for the significance of changes, with Yates's correction for continuity, was used for comparing proportions [S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956)]. Ejaculation latencies were compared with a paired *t*-test [J. Bruning and B. Kintz, *Computational Handbook of Statistics* (Scott, Foresman, Glenview, Ill., 1977)]. Two-way analyses of variance with cycle phase and testing location as repeated measures were used for all other data analyses [B. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962)], with the Newman-Keuls procedure used for comparisons between means.
10. Approach was defined as one animal moving directly toward and stopping within 15 cm of another animal. Back proximity was defined as

the female sitting within 15 cm of the male with her spinal column directly facing the male's ventrum. Back proximity is thought to be a female proceptive behavior in rhesus monkeys [C. G. Cochran, *Behav. Neurol. Biol.* **27**, 342 (1979); K. Wallen and R. W. Goy, *Horm. Behav.* **9**, 228 (1977)].

11. Negative responses included threatening the male, pulling away, or jerking, in response to a behavior initiated by the male.
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13. I thank S. A. Higgins and L. A. Winston for assistance in data collection, M. E. Wilson for performing the steroid assays, and T. P. Gordon for valuable discussions. This work was supported by NIH grants RR-00165 and MH35835 and by a grant from the Emory University research committee.

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Nerve Growth Factor Attenuates Neurotoxic Effects of Taxol on Spinal Cord–Ganglion Explants from Fetal Mice

Abstract. *Most neurons in organotypic cultures of dorsal root ganglia from 13-day-old fetal mice require high concentrations of nerve growth factor for survival during the first week after explantation. These nerve growth factor–enhanced sensory neurons mature and innervate the dorsal regions of attached spinal cord tissue even after the removal of exogenous growth factor after 4 days. In cultures exposed for 4 days to nerve growth factor and taxol (a plant alkaloid that promotes the assembly of microtubules) and returned to medium without growth factor, > 95 percent of the ganglionic neurons degenerated and the spinal cord tissues were reduced almost to monolayers. In contrast, when the recovery medium was supplemented with nerve growth factor, the ganglionic neurons and dorsal (but not ventral) cord tissue survived remarkably well. Dorsal cord neurons do not normally require an input from dorsal root ganglia for long-term maintenance in vitro, but during and after taxol exposure they become dependent for survival and recovery on the presence of neurite projections from nerve growth factor–enhanced dorsal root ganglia.*

Taxol, an antitumor drug (*1*) isolated from the plant *Taxus brevifolia*, promotes the assembly of microtubules and binds to them in cell-free systems; such microtubules resist depolymerization by cold or calcium (*2*, *3*). In fibroblasts and other cells exposed to taxol, abnormal bundles of microtubules form throughout the cytoplasm (*4*, *5*). Taxol exposure also leads to the formation of unusual numbers and arrays of microtubules in neurons and supporting cells in explants of spinal cord tissue with attached dorsal root ganglia (DRG's) (*6*). The primary effects of taxol appear to be on the tubulin-microtubule system; no other specific mode of action has been reported.

This study demonstrates that nerve growth factor (NGF), a hormone required for the maintenance of sympathetic and DRG neurons (*7*, *8*), markedly attenuates the cytotoxic effects of taxol on DRG neurons explanted with spinal cord tissue from fetal mice. Our data suggest that taxol prolongs the critical developmental period during which fetal DRG neurons are dependent on NGF for survival and maturation. Furthermore, exposure of these cultures to taxol (*9–11*) in the presence of NGF permits survival and recovery of many dorsal cord neurons with a large DRG input under culture conditions resulting in extensive degeneration of ventral cord neurons (*12*). Dorsal cord neurons do not normally require a DRG input for long-term maintenance in vitro (*10*, *11*, *13*), but during and after taxol exposure they become remarkably dependent on factors provided by NGF-enhanced DRG neurites. Some target tissues in the central nervous system have been shown to degenerate after deafferentation during critical periods of embryonic development in vivo (*14–16*), but this, to the best of our knowledge, is the first demonstration of in vitro conditions under which neurons in central nervous system explants become dependent on their afferent inputs (*12*).

Spinal cord tissue with attached DRG's was removed from 13-day-old fetal mice and cross-sectioned. The sections were placed on collagen-coated cover slips (*9*, *17*) and incubated in Maximow depression-slide chambers at 35°C in a medium containing human placental serum and chick embryo extract (*17*, *18*). The medium was changed twice weekly. Nerve growth factor (300 U/ml) was added to the medium to ensure optimal survival and growth of a large fraction of the DRG neurons (>1000 per ganglion). This led to enhanced innervation of dorsal regions of the attached spinal cord tissue (*9–11*), even when NGF was withdrawn after 4 days (*9*, *18*). In the absence of exogenous NGF, > 90 percent of these DRG neurons degenerated. These results agree with the recent finding that exposure of rats and guinea pigs in utero to antibodies to NGF results in the selective destruction of up to 85 percent of DRG neurons and the destruction of sympathetic neurons (*19*). In contrast, injection of antibodies to NGF into postnatal rats leads to destruction only of sympathetic neurons (*7*).

In cultures exposed for several days to 1 to 2 μM taxol (*20*) in the absence of exogenous NGF, most neurons degenerated, whereas in the presence of the NGF (300 U/ml), survival of DRG neu-

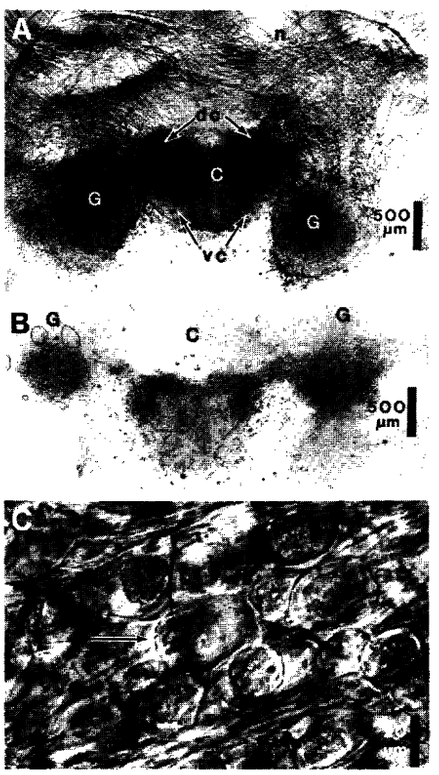

Fig. 1. Photomicrographs of cross sections of living spinal cord (~ 0.5 mm thick) with attached DRG's, explanted from 13-day-old fetal mice. (A) Explant cultured for 5 weeks in medium with NGF (300 U/ml) and exposed to 1 μM taxol for 24 hours during days 4 to 5 in vitro. Neuronal development in DRG (*G*) and cord (*C*) tissues was not altered, although growth of Schwann cells was severely depressed. Dorsal (*d*) and ventral (*v*) regions of the cord contain abundant neurons. Note the profuse outgrowth of neurites (*n*) without Schwann cells. (B and C) Extensive degeneration of cord and DRG's in an explant where NGF was withdrawn from the culture medium for 3 weeks following the exposure to 1 μM taxol during the fourth to eighth days in vitro. Nerve growth factor was present in the medium for 4 days prior to and during the exposure to taxol. The cord and DRG tissues have been reduced to thin layers and neuritic outgrowth is sparse. The number of neurons per DRG has been reduced from more than 1000 [see (A)] to less than 100. A surviving DRG neuron is indicated by the arrow in (C). The damage would have been even more severe had taxol been added at the time of explantation.

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