

# Effect of hour of operation on feminization of neonatally castrated male rats<sup>1</sup>

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*Female sexual behavior of male rats castrated 1 h postpartum was qualitatively and quantitatively superior to that of males castrated 6, 12, or 48 h after birth. One-hour castrated males differed from spayed females only in that their lordotic responses were held less frequently after the male's dismount. A combined group of 1-day castrated males was found to be inferior to spayed females on all measures of receptivity. These data suggest that differences among various studies in the degree of feminization resulting from neonatal castration of the male are related to the percentage of Ss in each study castrated within a few hours of birth.*

Males castrated at 1 day of age and injected with estrogen and progesterone as adults have been reported by Grady, Phoenix, & Young (1965) to display as many lordotic responses as similarly injected spayed females. Gerall, Hendricks, Johnson, & Bounds (1968), using a method of scoring receptivity which included quality as well as frequency of lordosis and soliciting behaviors, found that 1-day castrated males' behavior was inferior to that of females spayed within a short time after birth. Neither study specified the time within the 24-h period after birth at which castration occurred. The present experiment was performed to determine whether degree of feminization of males is related to the time of gonadectomy within 48 h after birth.

## SUBJECTS

Sprague-Dawley (Rolfsmeyer) female rats mated during a 1-week period and maintained on a 12-h light and 12-h dark reversed illumination cycle were observed 22 days later at hourly intervals during the light phase and at 3- or 4-h intervals during the dark phase to determine the time and duration of birth of each litter. Male offspring were castrated at either 1 (N = 6), 6 (N = 7), 12 (N = 5), or 48 (N = 8) h after birth. The hour of birth was set at the midpoint of the delivery period. Castrated females (N = 9) were spayed 48 h after birth. Gonadectomy performed under ice anesthesia utilized a single midline incision between the phallus and naval in the 1-, 6-, and 12-h Ss and lateral incisions in the older groups. Ss were weaned at 28 days of age and

housed thereafter two to a cage. Food and water were available ad lib.

## PROCEDURE

The first of five test sessions was given when Ss were between 69 and 76 days old. Preliminary to each session, Ss were injected with 6.6 µg estradiol benzoate<sup>2</sup> followed 40 h later by 0.5-mg progesterone. Female behavior in response to vigorous males was observed in a 14-in. radius semicircular cage. Testing occurred 5 and 7 h after the progesterone injection, which was between 7 and 10 h after the beginning of the dark phase. Each of two tests during a daily session was terminated after the S was mounted six times by the male. Behavior was scored for the presence or absence of lordosis to obtain a receptivity quotient (RQ) and rated for quality to compute a receptivity score. A rating of 0 was given when S showed no response to the male's mount or actively rejected him, 1 when S slightly arched its back and raised its head when mounted, and 2 when S definitely arched its head and pelvis. In addition, incidences of holding and darting accompanying each response to the male were recorded. Thus, for the 12 mounts observed in the two tests comprising a session, the maximum scores were: for lordosis, 24; for holding, 12; for darting, 12; and total behavior, 48.

## RESULTS

The temporal characteristics of the 31 deliveries observed are shown in Table 1. Parturition was equally likely to be initiated any time in the last 9 h of the light period. Within litters, births might be separated by as much as 10 h. Ss selected for the 1- and 6-h castrated groups were taken from mothers requiring less than 2 h for delivery and those in the 12- and 48-h groups from mothers requiring up to 4 h. Litters which took longer than 4 h to be born were not included in this study.

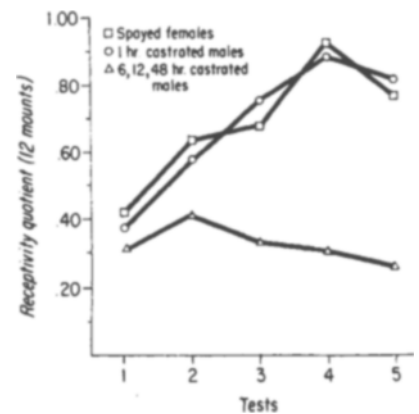


Fig. 1. Mean receptivity quotients (RQ) obtained in each session by spayed females, 1-h castrated males, and combined 6-, 12-, and 48-h castrated males.

Mean RQs obtained in each session by spayed females, 1-h castrated males, and combined 6-, 12-, and 48-h castrated males are shown in Fig. 1. An F test revealed that the latter three groups did not differ statistically from each other. Females' and 1-h castrated males' RQs were not significantly different from each other, but each group's quotient was greater than those from Ss castrated later than 1 h after birth ( $p < .05$ ).

Mean total receptivity scores obtained in each of the sessions are shown in Fig. 2. Again, a combined score was plotted for the 6-, 12-, and 48-h castrated Ss since their values did not differ significantly from each other. The 1-h castrated male and female control Ss' scores, while not different from each other, were reliably higher than those recorded for other Ss. Analysis of darting revealed the same pattern of results found with the total receptivity score. One-hour castrated males, while obtaining lower mean scores than females in each session, failed to do so with a sufficient magnitude to permit rejection of the null hypothesis. The t ratio failed to obtain significance by .19. Analysis of holding scores, however, revealed that females exhibited higher incidence of holding ( $p < .05$ ) than 1-day castrated males and both of the latter groups were different from those castrated at 6, 12, and 48 h after

Table 1  
Percentage of Mothers Initiating Parturition at 2-h Intervals after Beginning of Light Phase and Requiring Specified Durations for Completing Delivery

Time after light onset delivery started (h)	Percentage of mothers	Duration of delivery (h)	Percentage of mothers
<3	0	<2	34
3-4	9	2-3	47
5-6	19	3-4	6
7-8	22	4-5	6
9-10	22	7-10	6
11-12	22		
>12	6		

birth. Mean scores for the 1-h castrated males and females are presented in Table 2. Although the RQ score is equivalent in both groups, other measures of femininity are consistently lower in 1-h castrated males than in control females.

#### DISCUSSION

In the present study, total receptivity scores of the 1-, 6-, and 12-h castrated Ss were averaged to provide an estimate of a group gonadectomized within 24 h after birth. The mean RQ for such a 1-day castrated group in the present study was .397 (mean of five sessions). For 24-h castrated groups receiving identical amounts of estradiol benzoate, which was 6.6  $\mu$ g, the RQ was .280 (mean of four sessions) in Gerall et al's study, and .572 (one session of five tests) in Grady et al's experiment. In the present and Gerall et al's study, mean RQs and receptivity scores of 24-h castrated males were significantly lower than those obtained by control females.

Comparisons among different studies of the effect of gonadectomy within 1 day of birth are, however, of limited value because it is difficult to determine for at least two reasons the actual age of the organism at the time of operation. First, surgery could have occurred at any time within a 24-h period. Secondly, hour of birth is usually identified as when E, who might make one to three inspections per day, first finds the mother with her complete litter. As indicated in Table 1, actual age of individual Ss could differ considerably from age assigned by this method. Data from previous studies, therefore, represent degrees of feminization of a population whose assigned age could vary from actual age up to 24 h and differences among findings might be related to the total number of Ss castrated fewer than 6 h after birth.

Data from the present study also raise the question as to whether complete feminization is produced in the 1-h castrated male.

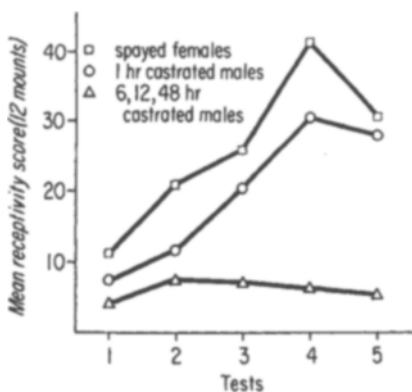


Fig. 2. Mean total receptivity scores obtained in each session by spayed females, 1-h castrated males, and combined 6-, 12-, and 48-h castrated males.

Table 2  
Receptivity Scores and Quotients of 1-h Castrated Males and Spayed Females

Groups	Receptivity Score			Receptivity Quotient
	Total	Lordosis	Holding	
Spayed female	26.9	16.2	6.3	.691
1-h castrated male	20.0	14.8	2.7	.680

The RQ score provides an affirmative, whereas the holding measure, a negative answer. Differences in darting also suggest that complete feminization has not been accomplished. It might be suggested that the lower degree of holding in the 1-h group is due to the absence of a vagina preventing the male's intromission pattern with its rapid dismount. Holding may have been less apparent following the slow dismount of an incomplete intromission. However, since a female given estrogen and progesterone will exhibit lordosis and hold in the absence of intromissions and even of full mounts, the difference between the females and 1-h castrated males on this measure probably represents true female behavior capacities. Although males castrated 1 h after birth could not be statistically distinguished from spayed females by either the mean RQ or the total qualitative receptivity score, the conclusion that postnatal castration effects complete feminization of the male must wait for future studies. The holding

measure, as noted above, was different between the two groups and there was a general tendency for frequency of darting and quality of lordosis to be lower in the males. Also, only one level of estrogen was used in the tests and lower dosages will have to be studied before a general conclusion is reached.

#### REFERENCES

- GERALL, A. A., HENDRICKS, S. E., JOHNSON, L. L., & BOUNDS, T. W. Effects of early castration in male rats on adult sexual behavior. *Journal of Comparative & Physiological Psychology*, 1967, 64, 206-212.
- GRADY, K. L., PHOENIX, C. H., & YOUNG, W. C. Role of the developing rat testis in differentiation of the neural tissues mediating mating behavior. *Journal of Comparative & Physiological Psychology*, 1965, 59, 176-182.

#### NOTES

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2. Estradiol benzoate was generously supplied by the Schering Corporation.

## Cortical polarization effects on consolidation of avoidance learning in rats

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*Anodal and cathodal cortical polarization, previously hypothesized to be effective in influencing learning consolidation, was given to 18 rats following a learning task. Results showed significant difference in performance as a function of the direction of current flow.*

Rusinov (1953), using anodal polarization in the motor cortex of the rabbit, found a "dominant focus of excitation"—a nonspecific hypersensitivity of a response to any sensory stimulation. This, he felt, demonstrated that extracellular current fields exerting an electronic influence on cell populations play a definite role in the cellular interactions within the brain. Morrell (1961), using rabbits and cats, found the same nonspecific hypersensitivity during