Mounting and Lordosis Behavior in Androgen Primed* Ovariectomized Rats: Effect of Dorsal Deafferentation of the Preoptic Area and Hypothalamus

KOREHITO YAMANOUCHI

*Department of Anatomy, Juntendo University School of Medicine,
Bunkyo-ku Tokyo 113, Japan*

Abstract

Daily injections of 500 μ g testosterone propionate (TP) for 21 days induced mounting behavior in all of 10 castrated male rats and the mean mount frequency (MF) for 30 min was 32.4 ± 6.6 (mean $\pm$ S.E.). The same treatment with TP induced mounting behavior in 6 out of 8 ovariectomized rats (female control), but the mean MF was 2.8 ± 0.7 . Eight out of 12 ovariectomized rats which received a horizontal half-circle cut anterior to the anterior commissure (anterior roof deafferentation, ARD) with an L-shaped Halász knife displayed mounting behavior more frequently and their MF was 14.5 ± 5.1 . The latent period between the introduction of a receptive female and the occurrence of the first mounting (mount latency, ML) in ARD rats was significantly shorter than that of the female controls. A half circle cut located posterior to the anterior commissure (posterior roof deafferentation, PRD) and sham deafferentation (SD) in the female rats had no effect on MF and ML.

On the day following the last test for male behavior, all female rats were injected with 0.5 mg progesterone in order to examine female sexual behavior. The mean lordosis quotient (LQ) of the ARD group was 62.5 ± 13.4 . In the female control, SD and PRD groups, however, LQs were significantly lower than that of ARD rats, being 15.1 ± 10.9 , 37.5 ± 14.7 and 23.8 ± 11.8 , respectively. These results suggest that the dorsal inputs to the POA and hypothalamus which pass anterior to the anterior commissure may exert an inhibitory influence not only on the female sexual behavior mediating system but also on the mounting mediating system in female rats.

The regulatory systems of sexual behavior in the central nervous system are differentiated between male and female rats. The differentiation of sexual behavioral patterns is thought to be achieved under the influence of the testicular activity during the perinatal period (see reviews, Arai and Gorski, 1974; Goldman, 1978). Female behavior is very rare in normal adult male rats even when castrated and treated with large doses of estrogen and progesterone

(Ward, 1974; Yamanouchi and Arai, 1976), and in normal adult female rats, the capacity to display male sexual behavior in response to exogenous androgen is very low (Pfaff, 1970; Hansen, 1977).

Recently, the interruption of dorsal inputs to the preoptic area (POA) and anterior hypothalamus (AH) by horizontal half-circle cut anterior to the anterior commissure has been reported to facilitate lordosis behavior in estrogen and progesterone treated castrated male rats (Yamanouchi and Arai, 1978). This suggests that the dorsal input to the POA and AH may exert an inhibitory influence on the display of lordosis

Received March 12, 1980.

* This study was supported by grants awarded to Prof. Y. Arai by the Ministry of Education of Japan.

in males. In the present study, the effect of transection of the dorsal inputs to the POA and AH on the induction of male sexual behavior was examined in testosterone propionate (TP) treated ovariectomized rats.

Materials and Methods

Adult Wistar female rats (220–250 g) were maintained under a controlled photoperiod (14:10 hr, light:dark) and temperature ($24 \pm 1.4^\circ\text{C}$) and given free access to a standard rat pellet diet and water. Two types of transection of dorsal inputs to the POA and AH were performed under ether anaesthesia. An L-shaped Halász knife having a horizontal blade of 2.5 mm was lowered into the brain through the sagittal sinus to the level of the anterior commissure and was rotated 180° anteriorly (anterior roof deafferentation, ARD) or 180° posteriorly (posterior roof deafferentation, PRD). In the sham operated group (SD), the knife was lowered to the same level of the ARD and removed without rotation. All operated females were castrated on the same day that the brain surgery was performed. A number of females and males without brain surgery were castrated as female or male controls.

Male sexual behavior

Four weeks after the operation, all females and males were injected with 500 μg TP (dissolved in 0.1 ml sesame oil) daily for 21 consecutive days. The observation of male sexual behavior was carried out on day 11 and day 21 following the initiation of daily injections of TP. Each experimental rat was allowed to become adapted to the observation cage (40 cm h, 60 cm w, 50 cm d) for 4–5 min. Then, a receptive female castrated and primed with 2 μg estradiol benzoate (in 0.1 ml oil) daily for 4–5 days and 0.5 mg progesterone (P, in 0.1 ml oil) on the day of the test was placed with the experimental rat. Observation of the masculine sexual behavior was carried out for 30 min. The following standard measures of male sexual behavior were recorded (see Table 1); mount frequency (MF, number of mounts with thrust, for 30 min), frequency of intromission pattern (number of mounts with intromission pattern, for 30 min), mount latency (ML, time from the introduction of the receptive female to the occurrence of the first mount).

Female sexual behavior

Soon after the end of the male sexual behavior test on the day 11 and day 22, each experimental female was put into the cage to which two vigorous

males had been adapted and the display of female sexual behavior by the subject was observed. On the next day (= day 22), female sexual behavior was also tested 5–7 hr after a 0.5 mg P injection. The observation was continued until the female had been mounted 10 times by males. A "lordosis quotient" (LQ) was computed for each animal and was indicated as the ratio of lordosis responses to 10 mounts times 100.

The animals were sacrificed at the end of the test series. Each brain was examined histologically to determine the precise localization of the cut.

Results

Male sexual behavior

Results are summarized in Table 1. Daily injections of 500 μg TP for 11 days induced mounting behavior and intromission pattern in all of 10 male controls. In female control rats, only 2 out of 8 females showed mounting behavior. In contrast, 7 out of 12 rats receiving ARD displayed mounting behavior. In the PRD and SD groups, the incidence of mounting behavior was very low; one of 8 and 2 of 8 rats, respectively. The mean MF of each female group was lower than that of the male control group. However, the mean MF of 7 ARD females which showed one or more mounts (14.4 ± 5.3) was not statistically lower than that of the male control group (18.0 ± 4.2). Intromission pattern was observed in 2 out of 12 ARD rats.

After daily injections of TP for 21 days, 6 out of 8 control females showed mounting but no intromission pattern. Both in SD and PRD group, 3 out of 8 rats exhibited mounting behavior. Intromission pattern was observed in only one rat in the SD or PRD group. In the ARD group, 8 out of 12 rats showed mounting and 3 rats displayed intromission pattern. The mean MF of this group (14.5 ± 5.1) was higher than those of other female groups (see Table 1). The average MF of the 8 ARD rats displaying mounting behavior was 21.6 ± 6.3 . This value was not significantly different

Table 1. Male sexual behavior in testosterone propionate (TP) treated ovariectomized rats with or without dorsal deafferentation of the preoptic area and hypothalamus

Treatment	Group	No. of rats	Mounting			Intromission Pattern	
			Incidence	Frequency (MF) (times/30 min)	Latency (ML) (sec)	Incidence	Frequency (times/20 min)
500 μ g TP $\times$ 11 days	Control	8	2/8	0.9 $\pm$ 0.6	674	0/8	0.0 $\pm$ 0.0
	SD	8	2/8	2.8 $\pm$ 1.8	1027	0/8	0.0 $\pm$ 0.0
	ARD	12	7/12	8.4 $\pm$ 3.7	448	2/12	0.2 $\pm$ 0.2
	PRD	8	1/8	1.8 $\pm$ 1.8	44	0/8	0.0 $\pm$ 0.0
	Male control	10	10/10	18.0 $\pm$ 4.2	246	10/10	9.6 $\pm$ 1.8
500 μ g TP $\times$ 21 days	Control	8	6/8	2.8 $\pm$ 0.7	947	0/8	0.0 $\pm$ 0.0
	SD	8	3/8	5.9 $\pm$ 3.4	462	0/8	0.0 $\pm$ 0.0
	ARD	12	8/12	14.0 $\pm$ 5.1*	175**	3/12	0.5 $\pm$ 0.3
	PRD	8	3/8	4.9 $\pm$ 3.5	365	1/8	0.3 $\pm$ 0.3
	Male control	9	9/9	32.4 $\pm$ 6.6	96	9/9	19.0 $\pm$ 4.2

* $P < 0.05$ vs. female control, $P < 0.005$ vs. male control (t -test).** $P < 0.005$ vs. female control (t -test).

Table 2. Female sexual behavior in testosterone propionate (TP) and progesterone (P) treated ovariectomized rats with or without dorsal deafferentation of the preoptic area and hypothalamus

Group	No. of rats	TP only*			TP+P*		
		Lordosis		Soliciting behavior	Lordosis		Soliciting behavior
		Incidence	Mean LQ $\pm$ S.E.	Incidence	Incidence	Mean LQ $\pm$ S.E.	Incidence
Control	8	1/8	5.0 $\pm$ 5.0	0/8	4/8	15.0 $\pm$ 10.9	0/8
SD	8	0/8	0.0 $\pm$ 0.0	0/8	4/8	37.5 $\pm$ 14.7	3/8
ARD	9	0/9	0.0 $\pm$ 0.0	0/9	8/12	62.5 $\pm$ 13.4	8/12
PRD	8	0/8	0.0 $\pm$ 0.0	0/8	4/8	23.8 $\pm$ 11.8	1/8

* The female rats were treated with 500 μ g TP daily for 21 days and injected with 0.5 mg P on the day 22. The behavioral test was performed on day 11, day 21 (TP only) and day 22 (TP+P). However, the results on day 11 are not noted in this table, because no females displayed lordosis.

from that of the male control group (32.4 $\pm$ 6.6), while the mean MF of 6 control females displaying mounting behavior was 3.7 $\pm$ 0.6. The mean ML of the ARD group (175 $\pm$ 82 sec) was shorter than that of the female controls (947 $\pm$ 114). Six out of 9 male control rats exhibited ejaculatory pattern but none of the experimental or control females showed this pattern through the test series.

Female sexual behavior

Results are summarized in Table 2. When examined just after the male behavior

tests, none of the TP injected females showed lordosis response on day 11. On day 21 only one control female displayed lordosis. When P was given on the day following the last injection of TP (=day 22), 4 out of 8 rats in the control, SD and PRD groups showed lordosis and their mean LQs were 15.1 $\pm$ 10.9, 37.5 $\pm$ 14.7 and 23.8 $\pm$ 11.8, respectively. In contrast, 8 out of 12 ARD rats displayed high levels of lordosis behavior and the mean LQ was 62.5 $\pm$ 13.4. In addition, soliciting behaviors such as ear wiggling and hopping were observed in one PRD, 8 ARD and 3 SD rats.

Fig. 1. Schematic representation of the surgical cuts in midsagittal section. ARD: anterior roof deafferentation, PRD: posterior roof deafferentation. AC: anterior commissure, AH: anterior hypothalamic nucleus. AL: anterior lobe of pituitary, AM: anteromedial thalamic nucleus, ARC: arcuate nucleus, CC: corpus callosum, DBB: nucleus and diagonal band of Broca, DM: dorso-medial nucleus, FX: fornix, HPC: hippocampus, IP: interpeduncular nucleus, LS: lateral septal nucleus, MB: Mammillary body, MD: mediodorsal thalamic nucleus, MS: medial septal nucleus, OC: optic chiasma, PH: posterior hypothalamic nucleus, PL: posterior lobe of pituitary, POA: preoptic area, PV: paraventricular nucleus, RE: nucleus reuniens, RH: nucleus rhomboideus thalami, SC: suprachiasmatic nucleus, VM: ventromedial nucleus.

Histological examination

As shown in Fig. 1, the typical ARD was located just below the anterior commissure and extended anteriorly from the anterior end of the anterior commissure to the middle level of the nucleus accumbens. Some ARD cuts were located at the level of the anterior commissure. There were slight individual differences in the location of the cuts, but the correlation between the variation in the cuts and the incidence of mounting was not significant. The PRD was located at the level of the anterior commissure and extended posteriorly to the anterior level of the dorsomedial hypothalamic nucleus. Considerable damage was caused to the corpus callosum in all rats which received brain surgery. The septal

area was also damaged to various degrees due to the passage of the knife in ARD and SD rats.

Discussion

In the present experiments, ARD increased MF and shortened the ML, but SD and PRD had no effect on either MF or ML in TP-treated ovariectomized rats. These results suggest that the dorsal inputs to the POA and/or AH which pass anteriorly to the anterior commissure may exert some form of inhibitory influence on the mounting behavior mediating system of female rats. In male rats, an extrahypothalamic inhibitory influence on the male sexual be-

havior regulatory system has been reported to exist in the hippocampus, based on the results showing that lesions of the hippocampus increased mounting behavior (Kim, 1960) or decreased post-ejaculatory intervals (Bermant *et al.*, 1968). Dewsebury *et al.* (1968) reported that resection of the hippocampal formation increased ML but did not influence other parameters of male sexual behaviors. Since the hippocampus projects some fibers to the septum and/or POA through the dorsal fornix and fimbria system (Raisman and Field, 1971), ARD might interrupt these fibers. However, it cannot be excluded that ARD may disconnect fibers from other extrahypothalamic areas, such as the amygdala. Thus, both in males and females, there seem to be extrahypothalamic inhibitory mechanisms involved in the display of masculine behavior. In males, the inhibitory influence on male sexual behavior may be released easily by androgen. Since the MF was significantly low in PRD, SD and control females, release of the inhibition by androgen may be difficult to occur in females. Such resistance to androgen of the inhibitory system of male behavior seems to be a characteristic feature of the female rat. This may be attenuated by perinatal exposure to androgen, because successive treatment with androgen from prenatal to neonatal periods had been found to cause all forms of characteristic male sexual behavior in female rats (Sachs *et al.*, 1977).

The ARD females showed high female sexual activity in response to TP and P in the present results. This is consistent with our previous report that the ARD was highly effective in potentiating female sexual behavior in estrogen-progesterone primed female rats (Yamanouchi and Arai, 1977). Nance *et al.* (1975) also showed lordosis facilitating effect of TP and P in female rats with septal lesion. The induction of lordosis by TP and P can be partly explained by aromatization of androgen to es-

trogen, because anti-estrogen inhibited testosterone-induced lordosis behavior (Whalen *et al.*, 1972) as well as estrogen induced lordosis (Arai and Gorski, 1968). From our unpublished data, however, the facilitation of lordosis by treatment of 500 μ g TP daily for 3 days and 0.5 mg P in ARD females was not suppressed by simultaneous treatment with 2 mg/kg anti-estrogen (CI-628). This finding corresponds to the results of Södersten (1974) that anti-estrogen (MER-25) did not inhibit the display of mounting behavior in female rats. However, it is still not conclusive that androgen can facilitate lordosis with or without conversion to estrogen in ARD females.

In summary, the results of male and female sexual behavior tests suggest that the dorsal afferent fibers of the POA and AH which pass anterior to the anterior commissure participate in exerting an inhibitory influence not only on the female sexual behavior mediating system but also on the mounting behavior mediating system in female rats. However, it is important to further clarify the neuroanatomical relationships between the lordosis inhibitory system and mounting inhibitory system in female rats.

Acknowledgements

The author wishes to express his hearty thanks Professor Yasumasa Arai, Juntendo University School of Medicine, for his guidance throughout the experiment and critical reading of this manuscript.

References

- Arai, Y. and R. A. Gorski (1968). *Physiol. Behav.* 3, 351.
- Arai, Y. and R. A. Gorski. Biological Rhythms in Neuroendocrine Activity (edited by M. Kawakami). Igaku Shoin, Tokyo, p. 232 (1974).
- Bermant, G., S. E. Glickman and J. M. Davidson (1968). *J. Comp. Physiol. Psychol.* 65, 118.

- Dewsbury, D. A., E. D. Goodman, P. J. Salis and B. N. Bunnell (1968). *Physiol. Behav.* 3, 651.
- Goldman, B. D. Biological Determinants of Sexual Behavior (edited by J. B. Hutchison). Wiley, New York, p. 127 (1978).
- Hansen, S. (1977). *Physiol. Behav.* 19, 749.
- Kim, C. (1960). *J. Comp. Physiol. Psychol.* 53, 553.
- Nance, D. M., J. E. Shryne and R. A. Gorski (1975). *Horm. Behav.* 6, 59.
- Pfaff, D. (1970). *J. Comp. Physiol. Psychol.* 73, 349.
- Raisman, G. and F. M. Field. Frontiers in Neuroendocrinology (edited by L. Martini and W. F. Ganong). Oxford University Press, New York, p. 3 (1971).
- Sachs, B. D., E. Pollak, M. S. Kriger and R. J. Barfield (1973). *Science* 181, 770.
- Södersten, P. (1974). *Horm. Behav.* 5, 111.
- Ward, I. L. Sex Difference in Behavior (edited by R. C. Friedman, R. M. Richart and R. L. Vande Wiele). Wiley, New York, p. 3 (1974).
- Whalen, R. E., C. Battie and W. G. Luttge (1972). *Behav. Biol.* 7, 311.
- Yamanouchi, K. and Y. Arai (1976). *Endocrinol. Japon.* 23, 179.
- Yamanouchi, K. and Y. Arai (1977). *Brain. Res.* 127, 296.
- Yamanouchi, K. and Y. Arai (1978). *J. Endocrinol.* 76, 381.