Short-latency effect of testosterone on copulatory behaviour and ejaculation in sexually experienced intact male rats

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Summary. The effect of a single injection of testosterone on the number of stimuli required to trigger ejaculation and on copulatory behaviour was investigated in sexually experienced intact male rats. An intraperitoneal injection of 100 µg free testosterone 1 h before testing facilitated the copulatory behaviour and decreased the number of mounts and intromissions needed for ejaculation; the latter effect was obtained in a test in which a constant interval was kept between each intromission.

Introduction

Copulatory behaviour, as well as anticipated copulation, elevates plasma levels of LH and testosterone in male rats (Taleisnik, Caligaris & Astrada, 1966; Purvis & Haynes, 1974; Kamel, Mock, Wright & Frankel, 1975). The rise in testosterone levels is more pronounced in sexually experienced than in sexually naïve males (Kamel *et al.*, 1975), but the physiological significance of such a rise in testosterone levels associated with heterosexual contact is not fully known.

In the present study, the effect of testosterone on the number of stimuli required to trigger ejaculation, and on the copulatory behaviour, was investigated in sexually experienced intact male rats.

Materials and Methods

Intact male rats, of about 150 days of age and weighing 350–450 g, were kept in laboratory conditions as previously described (Malmnäs, 1973). The rats were sexually experienced and had achieved one observed ejaculation weekly during the 2 weeks preceding the experiments. Stimulus partners were spayed female rats treated s.c. with 10 µg (Exps I and II) or 25 µg (Exp. III) oestradiol benzoate followed 48 h later by 1 mg progesterone s.c. They were tested with the males 4–7 h later.

The copulatory tests took place during the dark phase of the reversed light: darkness cycle. The male was allowed 5 min to adapt to the observation cage before a sexually receptive female rat was introduced (= zero time).

In Exp. I a comparison was made between the copulatory scores of rats allowed to copulate *ad libitum* until ejaculation and rats with artificially prolonged intervals between intromissions because the female was immediately withdrawn from the observation cage when intromission was achieved. The female was replaced gently in the observation cage every 3 min after each withdrawal until ejaculation occurred. In Exp. II the effect of testosterone on the number of mounts and intromissions needed for ejaculation was studied in rats in which the interintromission interval was artificially prolonged. In Exp. III the effect of testosterone on copulatory behaviour of rats allowed to copulate freely was studied.

In Exps II and III one group of rats was given an i.p. injection of 100 µg free testosterone dissolved in 0.1 ml propylene glycol 1 h before the onset of testing while a control group was given the same volume of the vehicle alone.

The measurements recorded were (1) the number of mounts without intromission ('mounts') before ejaculation; (2) the number of mounts with intromission ('intromissions') before ejaculation; (3) the intromission latency, i.e. the time from the introduction of the female to the first mount with intromission; (4) the average intromission interval, i.e. ejaculation latency divided by the no. of

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intromissions, minus one, before ejaculation, (5) the ejaculation latency, i.e. the time from the first intromission to ejaculation, and (6) the postejaculatory interval, i.e. the time from ejaculation to the next intromission.

Results

Experiment I: effects of enforced interintromission intervals on copulatory behaviour

All males initiated copulation and achieved ejaculation. Intromission occurred within 1.5 min from the introduction of the female. As shown in Table 1, the intromission intervals of freely copulating rats were about one-fifth of those of rats with enforced interintromission intervals, the calculated figure of which falls slightly below 3 min because the first intromission latency was significantly longer than the subsequent ones ($P < 0.001$ by sign test). Ejaculation latencies were significantly increased by artificial prolongation of the interintromission intervals; this increase, however, was not in proportion to the increase in interintromission intervals because the numbers of mounts and intromissions required for ejaculation were considerably reduced by prolongation of the interintromission intervals.

Experiment II: effects of testosterone on the number of mounts and intromissions needed for ejaculation

All males initiated copulation and achieved ejaculation. Testosterone had a significant effect on the number of mounts and intromissions required for ejaculation under conditions of enforced 3-min interintromission intervals. The number of mounts and intromissions preceding ejaculation in the 24 controls was 5.5 ± 1.5 and 6.0 ± 0.5 , respectively. In the 24 testosterone-treated animals, the

Table 1. Comparison of copulatory scores (mean $\pm$ S.E.M.) in male rats (15/group) under conditions of free copulation and enforced interintromission intervals

	Free copulation	Enforced interintromission intervals	<i>P</i> *
No. of mounts before ejaculation	13.00 $\pm$ 2.29	5.07 $\pm$ 1.31	<0.001
No. of intromissions before ejaculation	12.87 $\pm$ 1.66	5.80 $\pm$ 0.47	<0.001
Intromission latency (min) at			
0 min	0.33 $\pm$ 0.10	0.35 $\pm$ 0.09	
3 min		0.06 $\pm$ 0.01	
6 min		0.06 $\pm$ 0.01	
9 min		0.06 $\pm$ 0.01	
Average interintromission interval (min)	0.61 $\pm$ 0.08	2.94 $\pm$ 0.02	<0.001
Ejaculation latency (min)	7.26 $\pm$ 0.86	14.11 $\pm$ 1.41	<0.001

* Calculated by means of the Mann-Whitney U test.

Table 2. Effects (mean $\pm$ S.E.M.) of a single injection of 100 μ g free testosterone on copulatory behaviour in sexually experienced intact male rats 1 h later

	Treatment		<i>P</i> *
	Vehicle only (23 rats)	Testosterone (24 rats)	
Intromission latency (min)	0.78 $\pm$ 0.18	0.29 $\pm$ 0.11	<0.01
No. of intromissions before ejaculation	12.39 $\pm$ 1.24	10.78 $\pm$ 0.63	N.S.
Intromission ratio	44 $\pm$ 3	50 $\pm$ 2	N.S.
Average interintromission interval (min)	0.93 $\pm$ 0.11	0.57 $\pm$ 0.04	<0.01
Ejaculation latency (min)	12.03 $\pm$ 1.54	5.63 $\pm$ 0.50	<0.01
Postejaculatory interval (min)	6.73 $\pm$ 0.44	5.27 $\pm$ 0.27	<0.01

* Calculated by means of the Mann-Whitney U test. N.S. = $P > 0.05$.

number of mounts and intromissions preceding ejaculation was 2.9 ± 0.5 and 4.4 ± 0.4 , respectively, i.e. 53 and 73 % of the corresponding values of controls.

Experiment III: effects of testosterone on copulatory behaviour

All males initiated copulation and achieved ejaculation. Compared to controls, the testosterone-treated rats had significantly shorter intromission and ejaculation latencies as well as postejaculatory intervals (Table 2). Furthermore, testosterone treatment decreased the interintromission intervals but had no significant effect on the number of intromissions before ejaculation and intromission ratio.

Discussion

The stimuli required for ejaculation in the male rat are obtained during the mounting phase of the copulatory act. Mounts with intromission are necessary for ejaculation (Kaufman, 1953) but some additional facilitation is also provided by mounts without intromission (Hård & Larsson, 1968). The achievement of intromission during mounting is characterized by a vigorous backward lunge during dismount and by subsequent genital grooming. The sensory inflow obtained by the insertion, not by the grooming, is essential for ejaculation because prevention of grooming does not affect ejaculation (Hart & Haugen, 1971) whereas local anaesthetization of the glans penis abolishes ejaculation (Carlsson & Larsson, 1964; Adler & Bermant, 1966).

In accordance with previous studies (Larsson, 1956; Bermant, 1964), the results of Exp. I show that artificial prolongation of the interintromission intervals considerably reduces the number of mounts and intromissions required for ejaculation. This suggests that any experimental treatment which affects the timing of intromission behaviour will also secondarily affect the number of intromissions preceding ejaculation.

From studies of copulatory behaviour it is well known that the occurrence of ejaculation in the male rat gradually disappears after castration, an effect readily reversed, or prevented, by treatment with testosterone propionate (Stone, 1939; Beach & Holz-Tucker, 1949). However, the disappearance rate of ejaculations after castration follows closely that of intromissions (Davidson, 1966), in that ejaculation will no longer be achieved when the appropriate stimulus is no longer obtained. In Exp. II, the effect of a single injection of free testosterone on ejaculation was investigated in sexually experienced intact adult male rats. An enforced 3-min interval was kept between each intromission to standardize the stimulus interval. The results obtained clearly demonstrate that the number of mounts with and without intromission required for ejaculation was decreased within 1 h after the injection. This is the first demonstration that testosterone facilitates ejaculation in these conditions. This finding accords with the fact that testosterone propionate facilitates ejaculation-related reflexes in the castrated spinal male rat (Hart, 1967).

The Exp. III results demonstrate that the facilitatory effects of testosterone on sexual behaviour in intact male rats are not restricted to the ejaculation reflex but also include other components of the copulatory behaviour. When the data of Exp. III are compared with those of Exp. II it is seen that testosterone did not significantly affect the number of intromissions preceding ejaculation in freely copulating rats. The testosterone-induced decrease of the interintromission intervals is probably the cause of the relative increase in the number of intromissions before ejaculation in Exp. III compared to that in Exp. II, in which the interintromission intervals were kept constant.

In non-mounting castrated male rats, 30–54 h elapsed before mounting was activated after a single injection of free testosterone (Malmnäs, 1973). The results of the present study demonstrate that the latency for testosterone to facilitate copulatory behaviour in the intact male rat is considerably shorter than that previously found in the castrate and suggest that short-term changes in plasma levels of testosterone may be important for copulatory behaviour in normal rats.

The hormones were kindly supplied by Organon, Netherlands, through Erco, Sweden. This work was supported by the Swedish Medical Research Council, grant no. B73-04X-64-09B.

References

- ADLER, N. & BERMANT, G. (1966) Sexual behavior of male rats: effects of reduced sensory feedback. *J. comp. physiol. Psychol.* **61**, 240-243.
- BEACH, F.A. & HOLZ-TUCKER, A.M. (1949) Effects of different concentrations of androgen upon sexual behavior in castrated male rats. *J. comp. physiol. Psychol.* **42**, 433-453.
- BERMANT, G. (1964) Effects of single and multiple enforced intercopulatory intervals on the sexual behavior of male rats. *J. comp. physiol. Psychol.* **57**, 398-403.
- CARLSSON, S.G. & LARSSON, K. (1964) Mating in male rats after local anesthetization of the glans penis. *Z. Tierpsychol.* **21**, 854-856.
- DAVIDSON, J.M. (1966) Characteristics of sex behaviour in male rats following castration. *Anim. Behav.* **14**, 266-272.
- HARD, E. & LARSSON, K. (1968) Effects of mounts without intromission upon sexual behavior in male rats. *Anim. Behav.* **16**, 538-540.
- HART, B.L. (1967) Testosterone regulation of sexual reflexes in spinal male rats. *Science, N. Y.* **155**, 1283-1284.
- HART, B.L. & HAUGEN, C.M. (1971) Prevention of genital grooming in mating behaviour of male rats (*Rattus norvegicus*). *Anim. Behav.* **19**, 230-232.
- KAMEL, F., MOCK, E.J., WRIGHT, W.W. & FRANKEL, A.I. (1975) Alterations in plasma concentrations of testosterone, LH, and prolactin associated with mating in the male rat. *Horm. & Behav.* **6**, 277-288.
- KAUFMAN, R.S. (1953) Effects of preventing intromission upon sexual behavior of rats. *J. comp. physiol. Psychol.* **46**, 209-211.
- LARSSON, K. (1956) *Conditioning and Sexual Behavior in the Male Albino Rat*. Almqvist & Wiksell, Stockholm.
- MALMNÄS, C.O. (1973) Monoaminergic influence on testosterone-activated copulatory behavior in the castrated male rat. *Acta physiol. scand., Suppl.* **395**, 1-128.
- PURVIS, K. & HAYNES, N.B. (1974) Short-term effects of copulation, human chorionic gonadotrophin injection and non-tactile association with a female on testosterone levels in the male rat. *J. Endocr.* **60**, 429-439.
- STONE, C.P. (1939) Copulatory activity in adult male rats following castration and injections of testosterone propionate. *Endocrinology* **24**, 165-174.
- TALEISNIK, S., CALIGARIS, L. & ASTRADA, J.J. (1966) Effect of copulation on the release of pituitary gonadotropins in male and female rats. *Endocrinology* **79**, 49-54.

Received 17 January 1977