

## The role of mounts and intromissions in triggering ejaculation in rats

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**Abstract**. Eight male rats copulated with an estrus female with an open vagina (NF) and an estrus female with the vagina covered by adhesive tape (CF). Experimental sessions were composed of two phases. During Phase 1 the males copulated with NF or with CF females until 4 intromissions or 4 mount bouts were displayed respectively. In Phase 2 the males copulated alternately with NF or CF according to three schedules: (1) a sequence two mount bouts with a CF female followed by one intromission, (2) a sequence one intromission followed by two mount bouts with a CF female, and (3) a sequence one intromission followed by four mount bouts with a CF female. All of these sequences were repeated until ejaculation. Ejaculation occurred in 95.8% of cases within one minute after the last intromission during copulation with the CF female. The results of this study indicate that: (1) the number of intromissions displayed during Phase 2 does not depend on the character of the copulatory behaviour displayed during Phase 1, (2) at least two intromissions are displayed before ejaculation, and (3) the tactile stimulation of the glans of penis plays a crucial role in the ejaculatory seminal reflex.



Copulatory behaviour of the male rat consists of a series of separate copulations accompanied (intromission) or not accompanied (mount) with insertion of the penis into the vagina, occurring in intervals ranging from several seconds to one minute or more, and terminated by ejaculation (Larsson 1956, Dewsbury 1967). These copulatory events occur in clusters of 1-5 within short period (2-10 s) separated by longer intervals (Sachs and Barfield 1970, Sachs and Barfield 1976, Meisel and Sachs 1994). Each copulatory event leads to the increase of the sexual excitation, which in turn continues up to the moment when the ejaculatory threshold is exceeded and ejaculation occurs (Kurtz and Adler 1973).

Intromission as well as ejaculation are preceded by insertion of the glans penis into vaginal orifice. Then the penile sheath exerts the pressure on the base of the penis where the reflexive field for the penile reflexes (erections, cups and flips) is localized (Hart 1975, Lumia et al. 1979). During intromission the erection is displayed, whereas ejaculation is accompanied by the penile cup (Pollak and Sachs 1976).

Penile reflexes can be tested also during *ex copula* tests. With this aim the male is held in a supine position, and the penile sheath is continuously retracted by maintaining light pressure at the base of the penis. After several minutes the cluster of penile reflexes is displayed in 1–3 min intervals. Ejaculation in such experimental situations occurs very seldom (Sachs and Garinello 1978).

In previous study (Beck and Biały 1993) using the method of response contingent presentation of the stimulus female with an intact or surgically closed vagina, we found out, that the mount bout had the same rewarding values in spite of the quantity or the quality of copulatory events of which it is composed. Moreover mount bouts have the same influence on sexual arousal estimated by the rate of bar pressing. We suggested that the temporal patterning of copulatory behaviour in male rats depends on the fluctuation of sexual reward. These results are in good agreement with the observation of Wahlen (1961) that male rats in a T-maze did not exhibit a clear tendency to select an intact female over a female with a closed vagina.

However, the mounts are insufficient to trigger ejaculation. Hord and Larsson (1968) found out that the male rats did not ejaculated after 40 min of copulation with a female with a surgically closed vagina. But during subsequent copulation with intact females these males had significantly fewer intromissions before ejaculation then the control males.

The purpose of this study was to analyse the role of the mounts in the triggering ejaculation. Accordingly male rats copulated alternately with normal females or with females with the vagina covered by adhesive tape.

Eight sexually experienced male rats from the Wistar strain were the subjects of this study. Eight female Wistar rats were used as the stimulus females. At the beginning of the testing the animals were 180 days old. They were maintained on ad lib. food and water and a reversed 12L:12D cycle, with light off from 9 am to 9 p.m. Males as well as females were housed 3 per standard laboratory cage.

Estrus in previously spayed females was induced by sc. injection of 25  $\mu g$  of estradiol benzoate (Oestradiolum benzoicum JELFA Jelenia Góra SA) and 500  $\mu g$  of progesterone (Progesteronum JELFA Jelenia Góra SA), and 48 and 3-6 h before each session respectively. After this treatment all females were sexually receptive and displayed the symptoms of behavioural estrus. Two kinds of stimulus females were used: normal estrus female with open vagina female (NF), and estrus female with vagina covered by adhesive tape (CF). In order to cover the vagina, a 4 x 5 cm piece of adhesive tape was attached to the lower region of the abdomen and fixed by a 2 x 8 cm band of adhesive tape to the base of the tail.

The test were carried out in glass arena measuring 55 x 65 x 20 cm. The floor of the arena was covered with absorbent paper. Sessions started from 4 to 6 h after the beginning of the dark period. During session, the male was placed into the arena and the stimulus female was introduced 5 min later. During control sessions the males copulated with NF up to the first intromission after the first ejaculation. During experimental sessions, the NF and CF were used alternately according to the various experimental schedules. During the contact with CF various number of mount bouts (Sachs and Barfield 1970) was allowed. A mount bout (MB) is a sequence of one or more copulatory events (mounts or intromission) that is not interrupted by acts other than genital autogrooming or orienting of the males nose toward the female. The experimental sessions were carried out until the male achieved the first intromission after the first ejaculation. Hence if the ejaculation occurred with a CF, she was replaced by a NF. Sessions were conducted at 1 week intervals. One control session (Session 1) and three experimental sessions (Sessions 2-4) were conducted with each subject. The purpose of the control session was to estimate the number of intromissions preceding the ejaculation. Table I represents the schedule of the experiment.



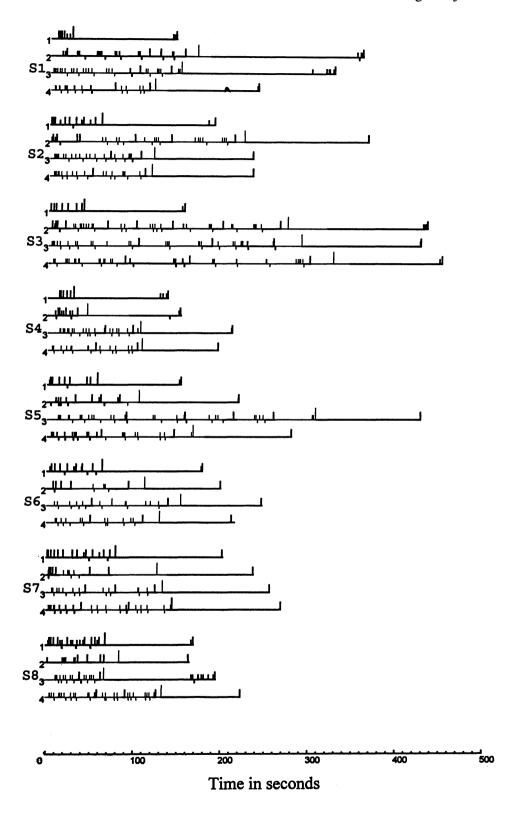


Fig. 1. The records of events during control and experimental sessions, for subjects S1 - S8. Line 1 refers to the control session; lines 2-4 to the experimental session. Thick line indicates use of normal females; thin line to females with covered vaginas; Long upward vertical lines refer to ejaculation; middle length line: intromission; short lines to mounts; downward lines to end of mount bouts.

TABLE I

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Schedule o	of the	experimental	SESSIONS
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Session	Phase 1	Phase 2
2	4I	Sequences (2MB + 1I) repeated up to E
3	4MB	Sequences (1I + 2MB) repeated up to E
4	4MB	Sequences (1I + 4MB) repeated up to E

I, intromission; MB, mount bout; E, ejaculation.

The behavioural events as well as the ultrasonic vocalization were recorded using a program for PC/AT. Ultrasonic vocalizations were registered with Bat S-25 ultra sound detector Ultra Sound Advice. Sexual behaviour was recorded on Sony video using a Philips observation system VSS 3440.

The Fishers exact test. Kruskal-Wallis Nonparameteric ANOVA test, the Mann-Whitney Test, and the Bartletts test for homogeneity of variances were used for the statistical analysis of the experimental data.

Figure 1 represents the records of events from all sessions. It is evident that during the control sessions ejaculation was achieved after 5-13 intromissions, whereas during the experimental sessions it occurred after 2-9 intromissions. As during the control session the ejaculation occurred after at least 5 intromissions, the subjects were allowed to copulate up to 4 intromissions during the Phase 1 of Session 2. In Session 3 and Session 4 instead 4 I, 4 MBs were displayed during Phase 1. The analysis of the number of intromissions using Bartlett's test for homogeneity of variances, revealed the significant difference among the variances (Bartlett statistic = 18.796, P=0.0003, N1 = 8, N2 = 8, N3 = 8, N4 = 8). The analysis of the number of intromissions during the control and experimental sessions with the Mann-Whitney Test revealed that the numbers of intromissions observed during control session were significantly higher then during the experimental sessions (Median = 7 (4-12), Median = 3(2-9), U = 16.000, U = 176.00, P=0.0002, N1 = 8, N2 = 24).

Table II represents the median number of intromissions displayed by the subjects during Phase 2 of the experimental sessions. The analysis of these data with Kruskal – Wal-

**TABLE II** 

The number of intromissions displayed during Phase 2 of the experimental sessions

Session	Median	Minimum	Maximum
2	0.5	0	5.0
3	2.5	2.0	4.0
4	2.0	2.0	3.0

lace Nonparametric ANOVA Test revealed no significant difference (KW = 4.536, P=0.1035, N1 = 8, N2 = 8, N3 = 8) between the numbers of intromissions displayed in Phase 2 of all experimental sessions.

Ejaculations with CF occurred during 23 out of 24 experimental sessions (95.8%). The median interval between extravaginal ejaculation and immediately preceding intromission was 12 s and ranged from 3 to 60 s. The extravaginal ejaculatory pattern was very similar to mount, but was longer (0.5 to 1 s), and sometimes a slight extension of the fore legs was observed.

Postejaculatory ultrasonic vocalizations in 22 kHz band were displayed by seven subjects. These occurred more often (8 out of 8 cases) after ejaculation with NF then after the ejaculation with CF (14 out of 20 cases). This difference was statistically significant (Fishers Exact Test, P=0.0019).

The occurrence of ejaculations in 95.8% of cases during copulation with the females with covered vagina was the most amazing result of this study. These ejaculations occurred within one minute after the last intromission. The fact that the ejaculation did not occur during the insertion of the penis into the vagina but during the pressing of the glans of the penis against the surface of the adhesive tape, indicates that the genital receptors localized on the glans of the penis play a crucial role in the ejaculatory seminal reflex. This suggestion is in good agreement with the finding of Sachs (1985) who reported the occurrence of ejaculations during *ex copula* tests if the penile sheath was not retracted. He related the occurrence of these ejaculations with the stimulation of the glans penis by the sheath.

The fact that at least two intromissions were displayed during the copulatory series before ejaculation and that ejaculation occurred within 60 s after the last intromission indicates that the sensory stimulation received during the intromission is necessary for ejaculation to occur.

Another phenomenon observed in this study was the significant decrease of the number of intromissions preceding the ejaculation during the experimental sessions. The fact that the number of intromissions performed during Phase 2 of the experimental sessions did not depend on the character of the copulatory behaviour presented by the male during Phase 1 (mount bouts or intromissions) may be a result of the influence of the mount bouts on sexual arousal or the effect of the enforced intervals (Larsson 1959).

In conclusion, the results of this study indicate that the genital receptors localized on the glans of the penis play an important role in triggering of the ejaculatory seminal reflex. However at least two intromissions during the copulatory series are necessary for ejaculation.

- Beck J., Biały M. (1993) The role of sexual reward in the temporal patterning of copulatory behaviour in male rats. Acta Neurobiol. Exp.53: 451-456.
- Dewsbury D.A. (1967) A quantitative description of the behaviour of rats during copulation. Behaviour 29: 154-
- Hord E., Larsson K. (1968) Effects of mounts without intromissions upon sexual behaviour in male rats. Anim. Behav. 16: 538-540.
- Kurtz R.G., Adler N.T. (1973) Electrophysiological correlates of copulatory behavior in the male rats: evidence for a sex-

- ual inhibitory process. J. Comp. Physiol. Psychol. 84: 225-
- Larsson K. (1956) Conditioning and sexual behaviour in the male albino rats. Acta Psychol. Ghothoburgensia 1: 1-269.
- Larsson K. (1959) The effect of restrain upon copulatory behaviour in the rat. Anim. Behav. 7: 23-25.
- Lumia A.R, Sachs B.D, Meisel R.L. (1979) Sexual reflexes in male rats: restoration by ejaculation following suppression by penile sheath removal. Physiol. Behav. 25: 89-92.
- Meisel R.L, Sachs B.D (1994) The physiology of male sexual behavior. The physiology of reproduction. Second edition. (Eds. E. Knobil and J.D. Neil). Raven Press Ltd., New York, p. 3-105.
- Pollak E.I, Sachs B.D. (1976) Penile movements and the sensory control of copulation in the rat. Behav. Biol. 17: 177-186.
- Sachs B.D., Barfield R.J. (1970) Temporal patterning of sexual behavior in male rats. J. Comp. Physiol. Psychol. 73: 359-364.
- Sachs B.D., Barfield R.J. (1976) Functional analysis of masculine copulatory behavior in the rat. Adv. Study. Behav. 7: 91-154.
- Sachs B.D., Garinello L.D. (1978) Interaction between penile reflexes and copulation in male rats. J. Comp. Physiol. Psychol. 92: 759-767.
- Whalen R.E. (1961) Effects of mounting without intromission and intromission without ejaculation on sexual behavior and maze learning. J. Comp. Physiol. Psychol. 54: 409-415

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