Physiology & Behavior 201 (2019) 184–190

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Brief communication

Behavioral response is absent under the mating competition in rats (*Rattus norvegicus*)

Xi Chu[a,*], Anders Ågmo[b]

[a] Department of Psychology, Norwegian University of Science and Technology, Norway
[b] Department of Psychology, University of Tromsø, Norway

ARTICLE INFO

Keywords:
Sperm competition
Simultaneous copulation
Multiple males
Sexual behavior
Social interaction

ABSTRACT

Sexually receptive female rats normally copulate with several males during estrus, and multiple paternity is common. Sperm competition is therefore likely to occur. One response to competitive mating is to enhance sperm output per ejaculation and another is to augment the number of ejaculations. The latter alternative requires more intense copulatory behavior. In studies in a seminatural environment we observed that male rats did not modify their behavior according to the intensity of competition, whereas observations from standard observation cages suggested that they do so. In order to further evaluate the potential response to competitive mating, we observed male rats copulating in a pair situation, i.e. one male and one female, and in a situation where three males simultaneously copulated with one female. In addition to sexual behavior, social interactions were quantified. It was found that the males in the multiple male condition prolonged mount and intromission latencies, and displayed a reduced number of mounts. There was no change in the number of preejaculatory intromissions or the ejaculation latency. The multiple mating did not affect non-sexual interactions with the female, whereas the female displayed more nose-offs and rejections when copulating with three males. It is concluded that mating competition does alter the initiation of copulation in the male rat, whereas copulatory behavior, i.e. intromission and ejaculation, remains unchanged.

1. Introduction

Both male and female rats are promiscuous in the way that they simultaneously copulate with several partners when it is possible (e.g. [1–3]. Multiple paternity is consequently a rule in *Rattus rattus* as well as in *Rattus norvegicus*, both in the wild and in the laboratory (e.g. [4–7]). Thus, either ejaculatory competition during the sexual encounters, or postcopulatory sperm competition, or both, are likely to occur.

In rats, the presence of a potential competitor has been reported to increase the number of spermatozoa found in the female reproductive tract after ejaculation [8]. In this study, the “competitor” was separated from the copulating couple by a screen. Sperm competition has not been evaluated in studies where two or more males simultaneously copulate with a female. This means that we only have data concerning sperm output from studies of potential, but not actual, sperm competition.

It is also possible that rats modify copulatory behavior when sperm competition is likely. When several male-female pairs copulate in the same arena, the increasing number of ejaculations [9], suggest that males intensify their copulatory behavior and reach ejaculation faster when competition is likely. Similar results were reported when a one-male, one-female copulatory session was compared with a two-male, one-female session [10]. Another study showed that male rats reduced the ejaculation latency when allowed to copulate with females that had been mated with another male 30 min or 6 h earlier [11].

In a seminatural environment containing three sexually active males and either one, two or three sexually receptive females, it was found that the males' behavior was unaffected by the number of available females [12]. Competition between males should have been at a maximum when only one receptive female was available for the three males and at a minimum when three females were available. The absence of modifications of copulatory behavior suggests that male rats do not alter their copulatory behavior in response to increasing sperm competition in a seminatural environment. This is different from the rat studies mentioned earlier.

One possible explanation for the contradictory observations mentioned above may be the different physical characteristics of the experimental setup, a large, seminatural environment (for a detailed description see [3]) versus a standard observation cage. In order to test

* Corresponding author.
E-mail address: xi.chu@ntnu.no (X. Chu).

https://doi.org/10.1016/j.physbeh.2019.01.010
Received 24 October 2018; Received in revised form 11 January 2019; Accepted 11 January 2019
Available online 14 January 2019

Table 1
Description of recorded behaviors [3,12] and parameters.

	Data collected as	Behavior/parameter description
Male and female behavior		
Sniffing	Duration and frequency	The rat places its snout close to any body part, except the anogenital region, of another rat while its whiskers move briskly.
Anogenital sniffing	Duration and frequency	The rat sniffs, occasionally grooms and licks, another rats' anogenital region.
Pursuit	Duration and frequency	The rat runs closely behind another rat.
Nose-off	Duration and frequency	Facing another rat either standing on 4 legs or while rearing; it includes boxing and teeth showing.
Male copulatory behavior		
Mount	Frequency	The rat stands on its hind legs and places its forepaws on another rat's rump from behind and displays pelvic thrusting.
Intromission	Frequency	Mount associated with penile insertion. The mount is ended by a backward thrust and is followed by genital grooming.
Ejaculation	Frequency	Penile insertion lasts longer than at intromission and is associated with rhythmic abdominal contractions. Dismount is slow and associated with an open arm posture.
Female behavior		
Rejection	Frequency	The rat kicks, bites or turns around against its suitor.
Behavioral parameters		
Mount latency	Time	Time from introduction of the female until the first mount.
Intromission latency	Time	Time from the introduction of the female until the first intromission.
Ejaculation latency	Time	Time between the first intromission and ejaculation.
Postejaculatory interval (PEI)	Time	Time between ejaculation and the next intromission.
Intercopulatory interval (ICI)	Time	Mean interval between two adjacent copulatory acts (regardless of whether they were mounts or intromissions); the interval following ejaculation was excluded.
Interintromission interval (III)	Time	Mean interval between two adjacent intromissions; the interval following ejaculation was excluded.
Intromission ratio (IR)	Ratio	The number of intromissions/(number of mounts + number of intromissions).

this possibility we compared copulatory behavior in males either copulating in a heterosexual pair or in a group consisting of 3 males and 1 female in a standard observation cage. In addition to recording standard items of male copulatory behavior, we also registered social interactions among the males and with the female as well as the female's responses to male approaches. This would give a rather complete description of the potential behavioral changes caused by competitive mating.

2. Methods and materials

2.1. Subjects

Twelve male (300 g upon arrival) and eight female Wistar rats (about 250 g upon arrival) Wistar rats were obtained from Charles River WIGA (Sulzfeld, Germany). They were housed in same sex pairs in Macrolon® IV cages in a room with controlled temperature (21 ± 1 °C) and humidity (55 ± 10%) and a 12:12 h light/dark cycle (lights on 23:00) withcommercial rat pellets (RM1, Special Diets Services, Witham, UK) and tap water. Females were ovariectomized under isoflurane anesthesia 2 weeks before the beginning of experiments. Behavioral estrus was induced by administration of estradiol benzoate, 25 μg/rat, followed by progesterone, 1 mg/rat, 48 h later. Females were used between 4 and 8 h after the progesterone injection. Both steroids were from Sigma (St. Louis, MO, USA).

2.2. Mating test cages

The tests for copulatory behavior were made under dim white light (about 25 lx).The test arena was a rectangular box (40 × 60 × 40 cm high) made of sheet steel and with a Plexiglas front and wire mesh top. All behaviors were recorded for analyses. The cages were identical to those employed in many earlier experiments (see [13]).

2.3. Procedure and design

The rats were given a sexual experience trial 4 weeks before the experiment. One male and one sexually receptive female were placed in the mating test arena until the first postejaculatory intromission. If no ejaculation was reached, the test was terminated 30 min after the first intromission. If there was no intromission, the test was terminated after 15 min. During this test, all male subjects displayed mounts and intromissions and about a third of the animals ejaculated. Each male was then tested in two trials; copulating with a receptive female alone (Single Trial) and copulating with another female together with two other males (Multiple Trial). A sexually receptive female was introduced one minute after the male(s) were placed into the observation arena. Subjects were observed until the end of the postejaculatory interval. In case a male did not ejaculate, the observation was ended when one of the criteria mentioned earlier was satisfied. Six of the 12 males were tested under Single Trial first, while the six others were exposed to the Multiple Trial first. The intertrial interval was seven days.

2.4. Data preparation and statistics

The Observer XT 10 (Noldus, Wageningen, Netherlands) was used to determine the frequency and/or the duration of the behaviors. The statistical analyses of copulatory activity were based on the behavioral data from the first ejaculatory series. Relevant behaviors and behavioral parameters are described in Table 1. Since the observation durations varied from individual to individual (detail see Fig. 1C–D), the absolute value of frequency or duration of non-sexual behaviors lacked comparative value. To examine the difference in non-sexual behaviors between Multiple Trial and Single Trial, we therefore calculated the behavior rate (total duration of a behavior expressed in seconds or total frequency of a behavior displayed within an observation/observation duration in minutes).

The majority of the behavioral data failed to follow a normal distribution according to the Shapiro-Wilk test. Therefore, the nonparametric Wilcoxon test was employed for comparing the two experimental conditions. However, comparisons of the proportion of time invested in the different behaviors as well as the intromission ratio were made with the paired samples *t*-test. Data are reported as median ± IQR for Wilcoxon tests, whereas mean ± standard error for *t*-test. The effect size (*r*) for the Wilcoxon signed rank test was calculated by dividing the test statistic by the square root of the number of observations (see [14]). All probabilities given are two-tailed. SPSS, version 24, was used for statistical analysis.

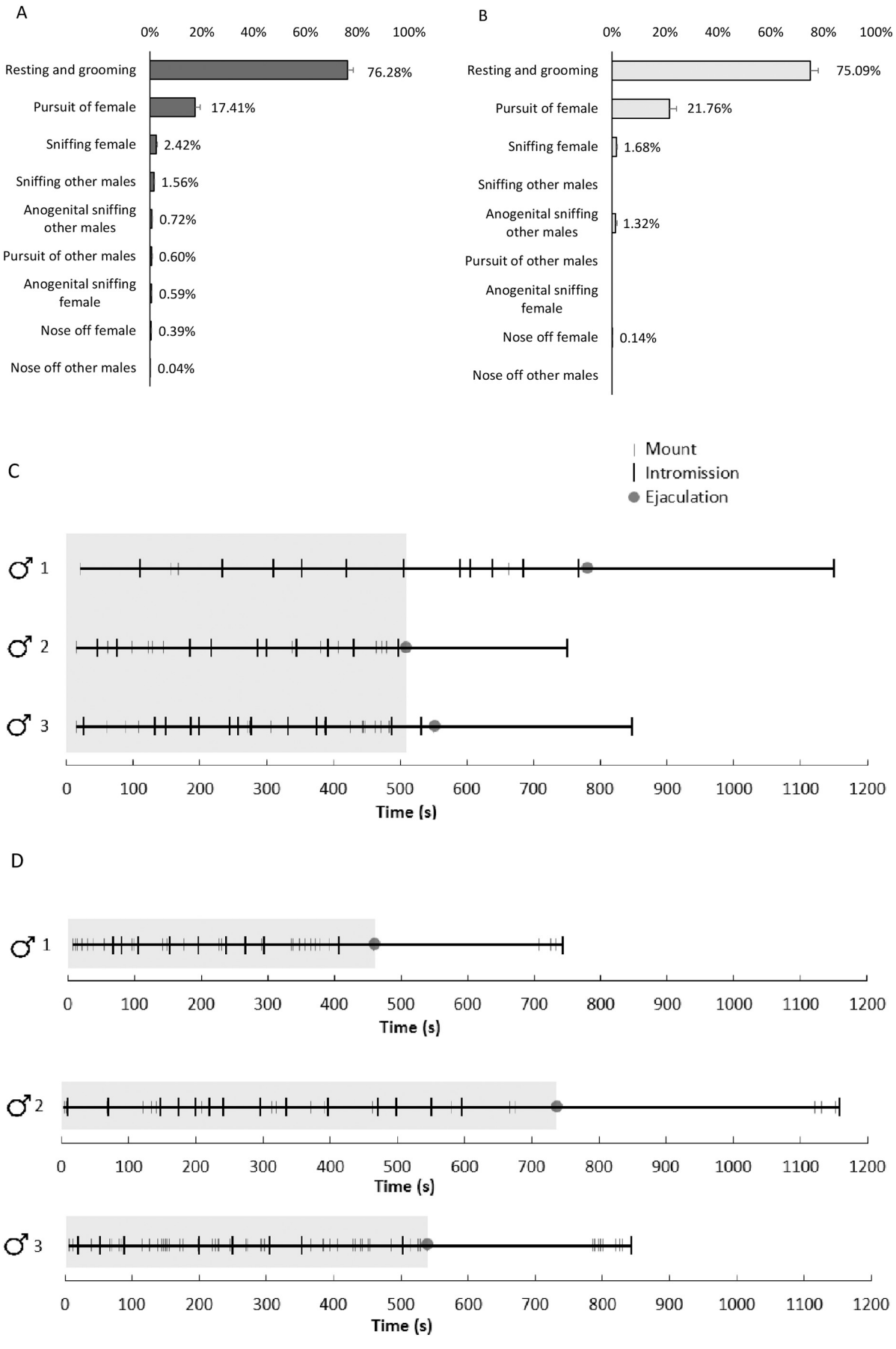

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Fig. 1. Illustration of the temporal distribution of sociosexual activities in two trials. (A–B) Proportion of time the males invested in different behaviors during the Multiple Trial (A) and the Single Trial (B), data are represented as mean ± SEM. (C–D) Distribution of copulatory acts in the same three males observed either in the Multiple Trial (C) or in the Single Trial (D). Each black horizontal line represents an individual male. The observation of copulatory actions (mount, intromission and ejaculation) was made from the beginning of the test until the end of the postejaculatory interval. In case a male failed to ejaculate, the test was ended 30 min after the first intromission. This applies to one male in each of the experimental conditions. The grey rectangles demonstrate the observation periods of non-sexual interactions in each individual, which was recorded from the moment the female was introduced into the arena until the first ejaculation. In the Multiple Trial, this means that observation was ended as soon as the first of the three males ejaculated. The reason for ending the observation at this moment was that a male in the postejaculatory interval neither displays much sexual behavior nor much interest in the female. Thus, potential copulatory competition with the other 2 males is absent. By ending the observation of non-sexual interactions at the first ejaculation, we assured that the data stemmed exclusively from the period in which all three males were actively engaged in competitive copulation.

3. Results

3.1. General description

All males displayed mounts and intromissions under both experimental conditions. One of the males failed to ejaculate in the Single Trial. However, he achieved an ejaculation in the Multiple Trial. Another male ejaculated in the Single Trial but not in the Multiple Trial. With the exception of these two subjects, all males ejaculated in both trials. All females responded with lordosis to every mount received.

In order to keep the results reasonably short, only the significant data obtained at the test are shown. The relative time the males were engaged in non-sexual activities before the first ejaculation in the Multiple and Single Trial is shown in Fig. 1A–B, respectively. No difference was found between the two conditions (*t*-test, *ps* > .07).

During the Multiple Trial, no fighting, boxing or sidling was observed. The only antagonistic behavior was nose-off, which kept very low intensity (0.04% of the observation time). In fact, only 4 of 12 males displayed such activity. Examples of the distribution of copulatory activity in time are shown in Fig. 1C–D. In the Multiple Trial, the distribution of copulatory activity was rather even among the three males, indicating the absence of competition for access to the female.

3.2. Comparisons between the single and multiple trial: copulatory behaviors

We report both the comparisons between all 12 males and between the ten males that ejaculated in both conditions. As illustrated in Fig. 2, the mount latency and intromission latency were significantly longer in the Multiple Trial than in the Single Trial when data from all the males were used or when data only from males that displayed ejaculation were used.

The ejaculation latency and the postejaculatory interval were not different between the Single and Multiple trials (Fig. 2B). There was no difference in the number of mounts and intromissions when all males are included. However, more mounts were performed by the males displaying ejaculation both in the Single and the Multiple Trial (Fig. 2C). When the males copulated in the Multiple Trial, they presented the same intromission ratio as well as the same interintromission interval as in the Single Trial (Fig. 2D-E). The intercopulatory interval was longer in the Multiple Trial than in the Single Trial. Statistical data are shown in Fig. 2F.

3.3. Comparisons between the single and multiple trial: non-sexual interactions

The duration of the observation period was similar in the Single and Multiple trials ($p = .64$, Fig. 3A). Nevertheless, in order to make accurate comparisons of the duration and frequency of behaviors in the two experimental conditions, duration or occurrences per unit time (behavioral rates) were used. The males sniffed the female body and the female anogenital region equally in both conditions (*ps* > .08; Fig. 3B–C). The duration and frequency per unit of time of male pursuit of the female were also similar in the Single and Multiple trials (*ps* > .06; Fig. 3D). The females sniffed the male's body more often and for longer time in the Single Trial than in the Multiple Trial.There was no difference between conditions with regard to the females' sniffing of the male anogenital region (*ps* > .57). Data are illustrated in Fig. 3E–F.

The female displayed more nose-off and rejections in the Multiple Trial than in the Single Trial (Fig. 3G). Statistical results are shown in Fig. 3H.

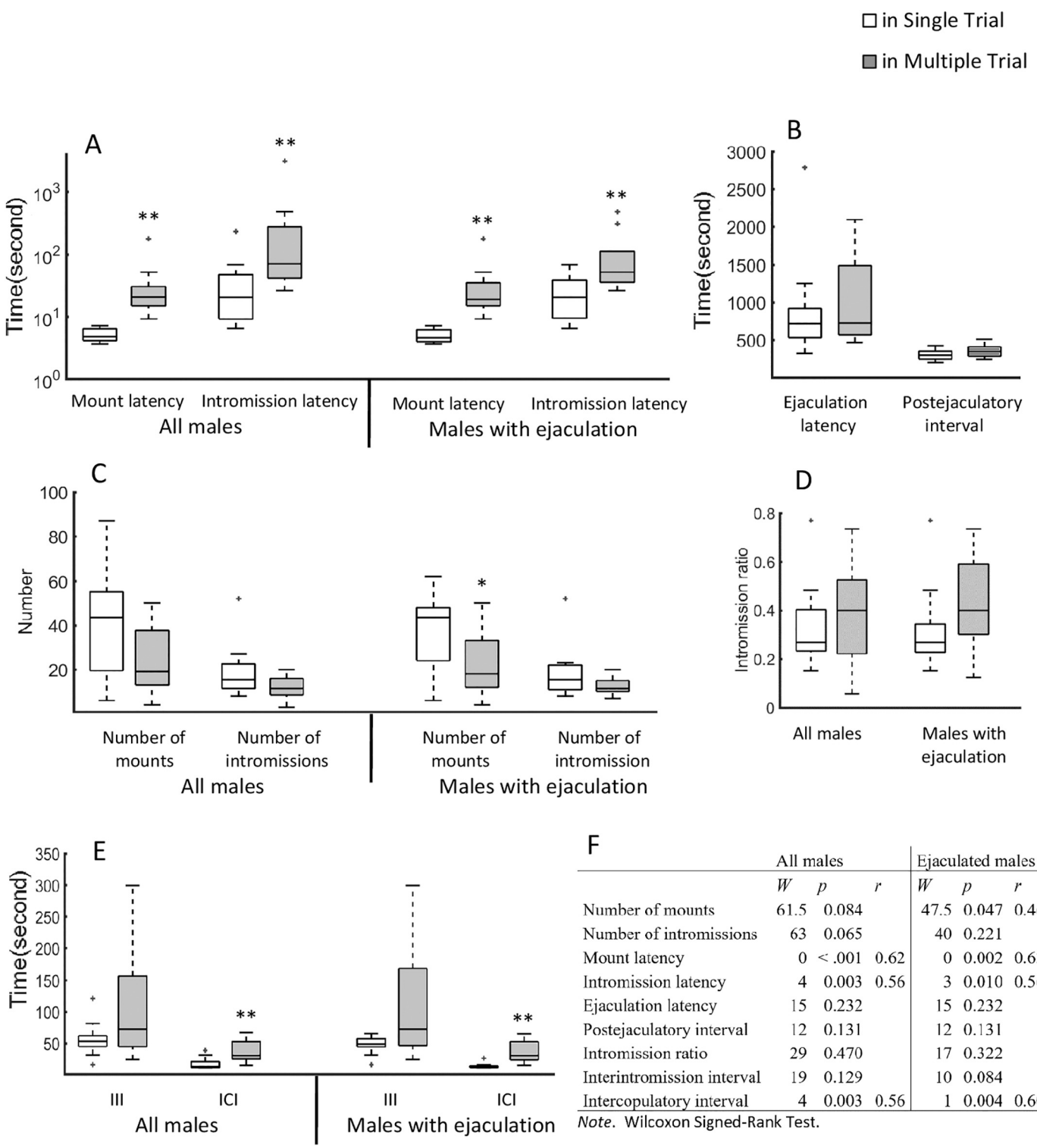
4. Discussion

Male rat sexual behavior in the Multiple Trial was not much different from the behavior observed in the Single Trial, i.e. a standard pair test. The absence of competition for access to the female during copulation is in agreement with data reported for wild rats [15]. However, the competitive condition slowed the initiation of copulatory behavior, and prolonged the intercopulatory interval. It appears, then, that potential sperm competition makes the initiation of copulation slower, and reduces the intensity of copulatory behavior without altering the ease by which ejaculation is achieved.

Little is known about the behavioral determinants of copulatory success in terms of generating offspring in male rats. However, in an earlier study we analyzed the relationship between male copulatory behavior and fertility in groups of rats living in a seminatural environment [4]. It was found that the only observable determinant of male reproductive success was the amount of sperm deposited in the female, expressed as either the number of ejaculations with a particular female, or the proportion of all ejaculations performed by the male received by that female. These observations would coincide with the "raffle principle" ([16], see also, [17]). The increase in sperm output in the first ejaculate when competition is possible reported earlier [8] would also coincide with this principle.

The prolonged latencies to mount and intromission in the Multiple Trial are difficult to explain. The males used only about 2.9% of the observation period (about 30 s per male) to interact with other males. In fact, interaction among males was very unlikely to occur before the beginning of mating, and cannot, therefore, explain the long latencies. Nevertheless, it is possible that the males required some time to identify the receptive female among the males in the Multiple Trial. We have no data to confirm that proposition, though.

An issue raised in the Introduction was whether the physical characteristics of the observation environment were important for the effects of competitive mating on male behavior. The size and shape of the observation arena has indeed been reported to modify some aspects of male rat sexual behavior, particularly the number of mounts and the ejaculation latency [18]. These changes are not necessarily relevant for the response to competitive mating, though. It seems, in fact, that they are not, since we obtained similar data in the present experiment, in which a standard observation arena was employed, and in a seminatural environment [3,12]. The absence of a behavioral response to competitive mating can perhaps be explained by the long duration of estrus in female rats. Estrus lasts for > 9 h [19], and we have earlier reported that females remain sexually active during the entire period of estrus ([3], see also [20]). Males, on the other hand, show relatively short bouts of copulatory activity interrupted by long periods of inactivity [12]. In view of the prolonged sexual activity of females and the rather short periods of male sexual activity, acceleration of ejaculation



F

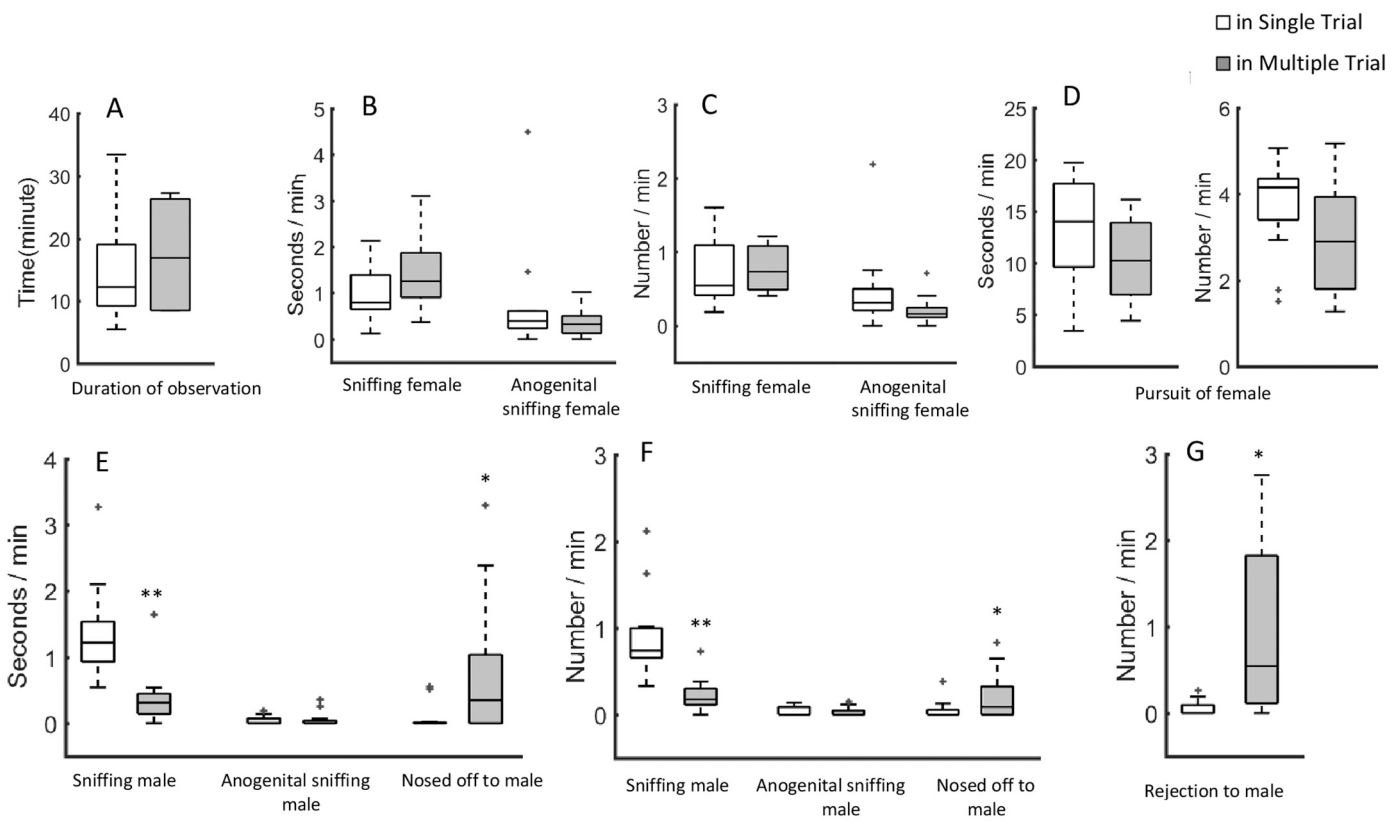
	All males			Ejaculated males		
	W	*p*	*r*	*W*	*p*	*r*
Number of mounts	61.5	0.084		47.5	0.047	0.46
Number of intromissions	63	0.065		40	0.221	
Mount latency	0	< .001	0.62	0	0.002	0.63
Intromission latency	4	0.003	0.56	3	0.010	0.56
Ejaculation latency	15	0.232		15	0.232	
Postejaculatory interval	12	0.131		12	0.131	
Intromission ratio	29	0.470		17	0.322	
Interintromission interval	19	0.129		10	0.084	
Intercopulatory interval	4	0.003	0.56	1	0.004	0.60

Note. Wilcoxon Signed-Rank Test.

Fig. 2. Copulatory activities in the Single and Multiple Trials. (A) Mount and intromission latency in semi-logarithmic plot; (B) Number of preejaculatory mounts and intromissions; (C) Ejaculation latency and postejaculatory interval; (D) Intromission ratio and (E) Interintromission interval and intercopulatory interval. (F) Statistical data. The data marked as "All males" include the 2 males that failed to ejaculate whereas those marked as "Males with ejaculation" represent only the 10 males that ejaculated in both experimental conditions.
*, Wilcoxon test $p < .05$, **$p < .01$.

would probably have little consequence for the amount of sperm deposited by a male. The long female estrus also assures ample opportunity to copulate with other males, and competition between males for access to females is, as already pointed out, not common in rats. In view of this, it is not evident that there has been any selective pressure for males to accelerate copulation when other males are present.

Similar behavioral responses to potential competition are observed in mice: Exposure to odors, sounds and visual stimuli from a potential competitor reduced number of preejaculatory intromissions and reduced ejaculation latency [21]. In meadow voles, exposure to odors of a



H

	Duration			Frequency		
	W	*p*	*r*	*W*	*p*	*r*
Female sniffing male(s)	4	0.006	0.56	1	0.003	0.61
Female nose-off male(s)	40	0.040	0.42	39	0.050	0.40
Female rejection				6	0.010	0.53

Note. Wilcoxon Signed-Rank Test.

Fig. 3. Male and female social interactions in the Single and Multiple Trials. (A) Duration of observation; (B) Male sniffing and anogenital sniffing of the female in s per minute of observation; (C) The number of episodes per min of observation of male sniffing and anogenital sniffing of the female; (D) Duration and frequency of male pursuit of the female expressed as s per minute of observation; (E) Female sniffing and anogenital sniffing of the male or males as well as nose-off directed towards the male or males expressed in s per minute of observation; (F) Number of episodes of female sniffing and anogenital sniffing of the male or males as well as of nose-off directed towards the male or males expressed as episodes per minute of observation; (G) The number of rejections of the male or males displayed by the females; (H) Statistical data.
*, Wilcoxon test $p < .05$; **, $p < .01$.

conspecific male does not modify copulatory behavior [22,23].

Finally, it could be argued that the domesticated Wistar rats employed here could have lost their natural response to competitive mating. However, the differences in behavioral interaction and copulatory patterns are relatively scant between the domestic and wild rats [24–26]. It seems, therefore, unlikely that the modest response to mating competition found here is a result of domestication.

In sum, present data show that rats mating in a competitive context do not alter their copulatory behavior in a way promoting sperm transfer. Perhaps postcopulatory events are more important for determining reproductive success than elements of copulatory behavior.

Acknowledgements

This study was supported by Faculty of Health Sciences, University of Tromsø. We thank Carina Sørensen, Katrine Harjo, and Nina Løvhaug, who provided excellent care of the rats.

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