Brief communication

Mate Choice could be Random in Female Rats (*Rattus norvegicus*)

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Abstract

Female mate choice is often investigated in terms of reproductive success in order to understand how male characteristics contribute to sexual attractiveness. Previous studies have found that females rats prefer mating with their first encounter rather than males visited subsequently, suggesting that the rewarding value of this first encounter is enough to reinforce mating with the first partner. Using a multiple chambers paradigm, we allowed female rats to copulate freely with three males placed each in a different chamber. Then, we switched the males' position, and let the female interact with them freely again within the same session. We tested whether female mate choice was relying rather on a preferred male rat or on a preferred mating location. The results showed that females spent most time with the male in the chamber of 1st entry in the beginning, but as soon as male rats switched chambers, the female rat continued to copulate with the new male in the same chamber of 1st entry, instead of mating with her previously preferred male rat. This suggests that the male preference is an artefact of location preference. Therefore, female mate choice seems to be rather random than the consequence of an individual choice based on male characteristics. This finding, although contradictory with the intuitive feeling that mate choice is a crucial feature in sexual and reproductive behavior, is supported by several recent observations. In the coming years, behavioral neuroscience should bring light to the brain processes at work in random mate choice.

Keywords: female rats, multiple partner, sexual behavior, mate choice, location

1. Introduction

The concept of mate choice is a fascinating phenomenon, as the selection of the best mate partner for a sexual intercourse can increase the chances for reproductive success. When rats have the possibility to mate with several partners simultaneously, both male and female rats seem to prefer the conspecific that they approached first [1-4]. This preferred rat has, thus, a reproductive advantage over the others as well. This emphasizes the importance of the initial approach and raises the question of what induces this approach, or what makes a conspecific attractive.

In previous studies, we explored the role of odors and ultrasonic vocalizations in the induction of this initial approach, but without any success [1, 2]. The patterns of emitted ultrasonic vocalizations did not predict whether a rat became the preferred mate partner [1, 2]. This suggests that vocalizations do not play a role in mate choice, a theory that was strengthened by the observation that devocalized males were as likely preferred by females as vocalizing males [1]. Moreover, when exploring sniffing behavior towards the different mate options as an indicator for the involvement of odors, again, no correlation was found between sniffing and approach behavior [1, 2]. Therefore, we concluded that odors play limited to no role in mate choice as well.

Consequently, we hypothesized before that the continuous visits to the male of 1st entry might be induced by the rewarding effects of the first sexual interaction [1]. Other studies showing that partner preference can be learned support this idea [5-7]. Our previous studies did not allow us to investigate this hypothesis. Besides, the question remains whether the rewarding effect is linked to the sexual interaction itself, or to the location in which the copulation took place. In the present study, we investigated whether

female rats develop a preference for the chamber of 1st entry or for the male in the chamber of 1st entry. The females were able to copulate for 15 minutes in a multiple partner paradigm, before the males switched location. Then, the females were allowed to continue the copulation for another 15 minutes in which their new mating preference was investigated. If female mate choice is based on the preferred male, the females should follow the male to the other chamber, rather than continue to visit the chamber of 1st entry with another male.

2. Material and methods

2.1 Subjects

Sixteen female (250-300 gram at the start of this experiment) and nine male (300-380 gram) Wistar rats were obtained from Charles River (Sulzfeld, Germany). The rats were housed in same sex pairs in Macrolon IV® cages on a reversed 12 hours light/dark cycle (lights on 23:00 -11:00), in a room with controlled temperature (21±1°C) and relative humidity (55±10%). Standard rodent food and tap water were available *ad libitum*. Before this experiment, the rats were used in a different experiment in which they were housed in groups of three males and four females in a seminatural environment for 8 days, in which the rats could freely copulate. During this first experiment, the rats obtained sexual experience. However, the females and males used in the present study were still unfamiliar with each other. There were two weeks in between the two experiments. All experimentations were conducted in agreement with the European Union council directive 2010/63/EU and approved by the National Animal Research Authority in Norway.

All females were ovariectomized under isoflurane anesthesia two weeks before the previous experiment. The ovariectomized females received 25 μg/kg estradiol benzoate (EB), and 1 mg progesterone (P) 48 hours and 4 hours before the mate choice test, respectively. EB and P (Sigma, St. Louis, MO, USA) were dissolved in peanut oil (Apoteksproduksjon, Oslo, Norway), and injected subcutaneously in a volume of 0.2 ml/rat. This hormonal treatment ensures maximal receptivity and proceptivity [8, 9].

2.2. Apparatus

The experiments were conducted in a multiple chambers paradigm (as previously described [1], consisting of a middle large chamber (50 cm diameter) surrounded by three other small chambers (30 cm). The chambers were connected by holes of 4 cm diameter that were large enough for the females to move through, but not large enough for the males to enter. This allowed the females to pace their sexual interaction freely, without the males chasing her.

Above each chamber, a video camera was located to record the multiple partner tests on video. Event recording software Observer XT 10 (obtained from Noldus, Wageningen, The Netherlands) was used to score the rat behavior during the multiple partner test. The chambers were lightened with dim lights, which resulted in approximately 5 lux at the bottom of the cage.

2.3. Procedure

The procedures were similar to those previously described [1]. At the start of the experiment, a female rat was placed in the middle chamber of the multiple chambers set-

up. The subject was allowed to move freely and habituate to the chambers for 5 minutes. Then, for another 5 minutes, the female was placed in the middle chamber, while three male rats were positioned in the three surrounding small chambers. During this time, the openings were blocked with a wire mesh allowing the female subject to see, smell and hear the male rats without any possibility of physical contact (*habituation phase*). The number of times the female rat sniffed the opening of each of the male chambers was determined, as an indicator of olfactory preference [10, 11].

After the habituation phase, the openings were unblocked and the female rat was allowed to move freely between the chambers for another 15 minutes (*copulation phase A*). During this period, the following behaviors were scored for each male: order in which the males were visited, latency to first visit, number of visits, time spent in the chambers, number of sniff episodes to the openings of the chambers, and the number of mounts, intromissions and ejaculations per male. In addition, the number of paracopulatory behaviors the female performed in the chamber of each male were counted.

After the copulation phase A, the male rats were quickly randomly placed in a different chamber and the test continued for another 15 minutes (*copulation phase B*). During this period, the same parameters were scored as during copulation phase A.

2.4. Data Analysis

The data were analyzed in two ways: based on “location” and “preferred male”. First, the data was analyzed based on the location of the chamber that was entered first during copulation phase A. This was considered ‘chamber of 1st entry’ in both phase A and B, compared to the ‘other chambers’. All parameters that took place in the ‘chamber

of 1st entry' were compared to the mean of the behaviors from the second and third choice chamber in the 'other chambers'. Second, the same analysis was performed for copulation phase A and B, but now the male that was visited the longest during copulation phase A (based on time spent in the chamber) was considered the 'preferred male' and the other two males 'other males'.

The homogeneity of variances was checked with the Levene's test, after which all behavioral data were analyzed using the Welch's t-test. A comparison was made between the 'chamber of 1st entry' and the 'other chambers', or the 'preferred male' and the 'other males'. The level of significance was set at $P<.05$ and all probabilities mentioned are two tailed. All statistical analyses were performed using SPSS.

3. Results

The results were in line with previous findings that female rats prefer copulating with the male visited first in a multiple partners paradigm. Therefore, the 'preferred location' matched the 'preferred male' during copulation phase A.

3.1. Location

When the data was analyzed on the location, it was found that the females spent significantly more time in the chamber of 1st entry compared to the other chambers during copulation phase A ($t(19.914)=3.125$, $p=0.005$) and phase B ($t(17.657)=2.541$, $p=0.021$, Figure 1A). In addition, the females showed significantly more paracopulatory behaviors in the chamber of 1st entry compared to the other chambers during copulation

phase A (t(17.862)=2.409, p=0.027), but no differences were found on paracopulatory behavior during phase B (Figure 1C).

When the number of male behaviors that were received by the females in the different chambers was analyzed, it was found that female received significantly more mounts in the chamber of 1st entry during copulation phase A (t(21.245)=3.483, p=0.002) and a trend in more mounts during phase B (t(16.503)=1.754, p=0.098; Figure 1D). No significant difference was found for the number of intromissions during phase A, but the females received significantly more intromissions from the males in the chamber of 1st entry compared to the other chambers during phase B (t(18.734)=2.330, p=0.027; Figure 1E). A trend was found in both phase A (t(17.330)=1.884, p=0.076) and B (t(23.773)=1.718, p=0.099; Figure 1F) for the number of ejaculations received in the chamber of 1st entry and the other chambers

No significant differences were found in the number of sniffing behavior from the female chamber towards these chambers during the habituation phase, and both copulation phases.

It should be mentioned, though, that there was not a certain location of chamber preferred over the others. Different females chose different chambers for their first entry, containing every time different males as well.

3.2. Preferred male

When the data was analyzed on the preferred male instead (based on the most time spent with this male), it was found that the females spent significantly more time with the preferred male (t(15.345)=5.345, p<0.001), darted more in the chamber with the

preferred male (t(16.109)=3.419, p=0.003), and received more mounts (t(16.099)=3.840, p=0.001) and intromission (t(15.595)=3.051, p=0.008) from the preferred male compared to the other males. No effects were found on the number of ejaculations. These effects, however, were only found during copulation phase A. When the behaviors of the females with the same males during phase B were analyzed, no significant differences were found (Figure 2).

Again, no significant differences were found in the number of sniffing behavior from the female chamber towards the chambers containing the preferred male during the habituation phase, and both copulation phases.

4. Discussion

This study confirms previous findings that female rats prefer copulating with the male visited first in a multiple partners paradigm. However, the study also provides evidence that this mate selection is not based on the male rats, but on the location of the chamber in which the males are located. As soon as male rats switched chambers in the middle of the test, the female rat continued to copulate with the new male in the same chamber of 1st entry, instead of mating with her previously preferred male rat. Against all expectations, this could suggest that mate choice in female rats is less specific than expected, if not random. Several studies have shown that rats prefer one conspecific to the others in a multiple partner paradigm [1-4, 12-14]. Both male and female rats spend significantly more time with the preferred conspecific than with the others. Moreover, females perform more paracopulatory behaviors with the preferred male, and receive

more mounts and intromissions from this male [1, 3, 4, 12-14]. The preferred male is most often the male that is visited first (male of 1st entry) [1, 3, 4].

As mentioned before, the suggestion that the odor or ultrasonic vocalizations emitted by a preferred male could play a role in the initial approach behavior in females has been proven false [1]. No differences were found between the preferred males and the other males in the patterns of emitted ultrasonic vocalizations or in the interest for the odor of the different males [1]; a result that was replicated in male rats as well [2]. In our previous study [1], we therefore suggested that the cause for the continuous visits to the male of 1st entry could be the rewarding effects of the sexual interaction. The current study provides additional signs that this assumption was indeed correct. The females enter one chamber first, after which they continue to visit this chamber for copulation, independently of which male rat is in this chamber. This suggests that the location of the chamber is more important than the male itself.

It should be mentioned, though, that there was not a certain location of chamber preferred over the others. Different females chose different chambers for their first entry, containing every time different males as well. In addition, considering that the sniffing behavior during the habituation phase did not correlate with the choice of location for the first entry, we believe that female rats enter one chamber randomly to copulate with a male. The rewarding properties of this copulation might stimulate them to return to the same location. Our paradigm, however, did not investigate the rewarding properties of the sexual stimulation in the brain, meaning that another process than reward could be involved in the returning behavior as well.

The most interesting finding is that the females do not prefer one male across the complete test. This can only indicate the females are not attracted by the characteristics of a particular male during mating, they rather seem to be solely interested in the sexual interactions. Therefore, we hypothesize that female rat mate choice is random. This might sound controversial, whereas it has always been thought that sexual selection is crucial in 'survival of the fittest', but several other observations support this hypothesis. For example, no relationship was found between the females' preference for a male and the number of pups sired by him [14], showing that female preference is unrelated to male reproductive success. Curiously enough, in another study, the systematically preferred males sired even less offspring than non-preferred males [13]. This observation suggests that female rats prefer males with low fertility, a somewhat counterintuitive conclusion. - In the light of these previous studies and of our recent findings, we theorize that female's mate choice is purely random. In fact, in nature, rats mate with multiple males and females simultaneously [15]. In the moment of mate choice, a female can actually not predict what kind of sexual stimulation she will receive. Consequently, there is no reason to believe that she needs to actively select a male per sexual encounter. In addition, rats have a very short menstrual cycle (4-5 days) and a short gestation time (3 weeks), meaning that they can produce many offspring during their short lifespan. In species with so many offspring, sexual selection is much less relevant than in species with only a few. This supports our conclusion that female mate choice could be random. For sure, the preference for the chamber of 1st entry is based on a preference for the location of this chamber, not for the male that is in it. The basis for this first location preference is still to be investigated, and notably in terms of sexual interaction rewarding properties.

It is important to mention, though, that although female rats have a random mate choice independent on odors and ultrasonic vocalizations, these male characteristics still play a role in sexual behavior in general. It was shown multiple times that olfactory stimuli emitted by males are powerful attractants. The emission of these stimuli by males are androgen dependent since odor from castrated males are far less attractive than odor from intact or castrated, androgen treated males ([11]; reviewed in [16]). In addition, anosmic female rats do not distinguish between intact and castrated males [17]. Male approach odors of sexually active females in the same manner [18-20]. All these data show that olfactory stimuli are necessary and sufficient for giving a rat sexual incentive properties needed to induce approach behavior of the conspecific leading to sexual behavior. Thus, olfaction is definitely involved in the regulation of sexual behavior; it is just not a factor in mate choice.

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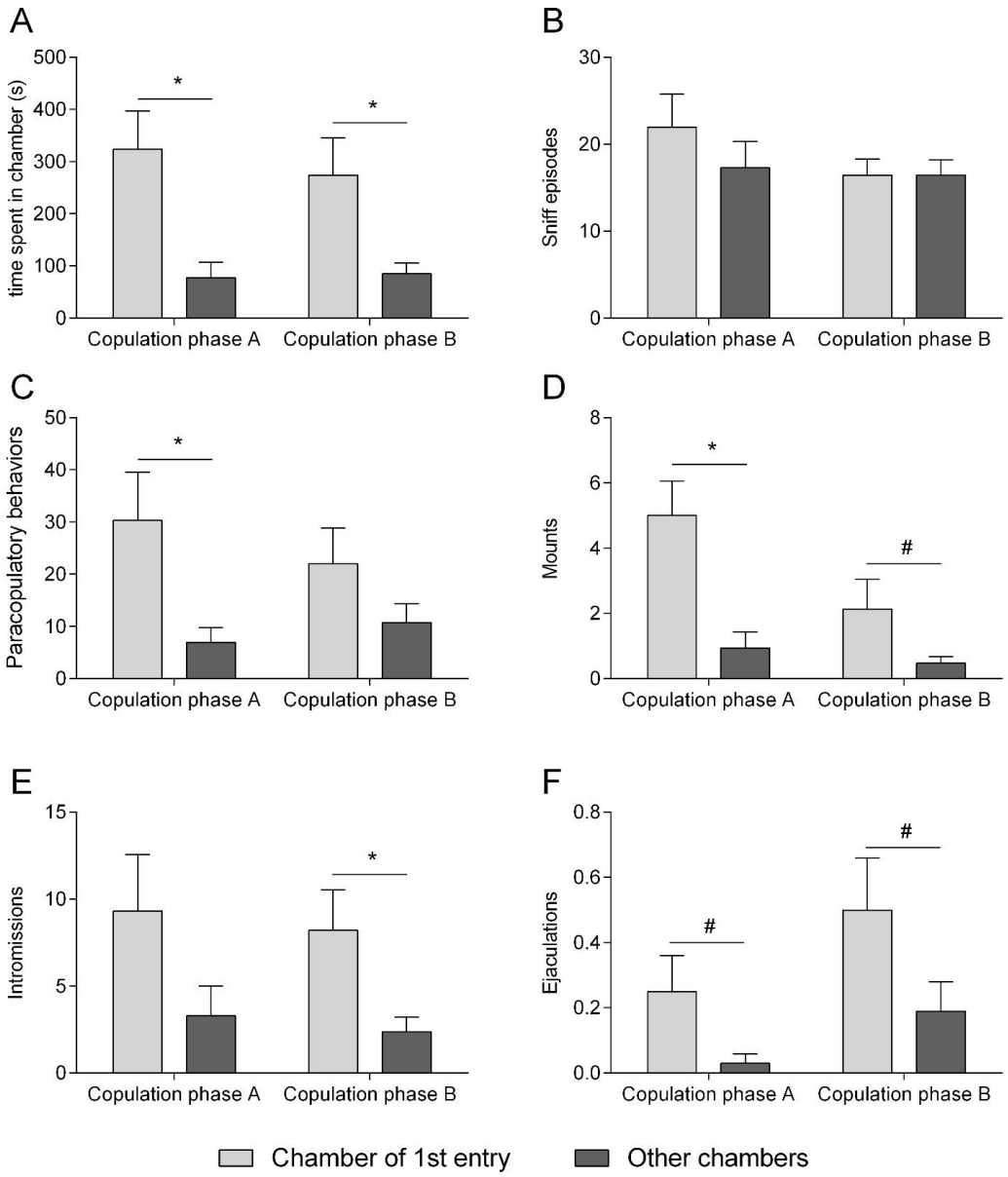

*Figure 1. Data analyzed on location. (A) time spent in the chamber (s), (B) number of sniff episodes, (C) number of paracopulatory behaviors, (D) number of mounts, (E) number of intromissions, and (F) number of ejaculation in the chamber of 1st entrance and the other chambers during copulation phase A and B. Data are shown in mean±standard error of the mean. * p<0.05, #p<0.1*

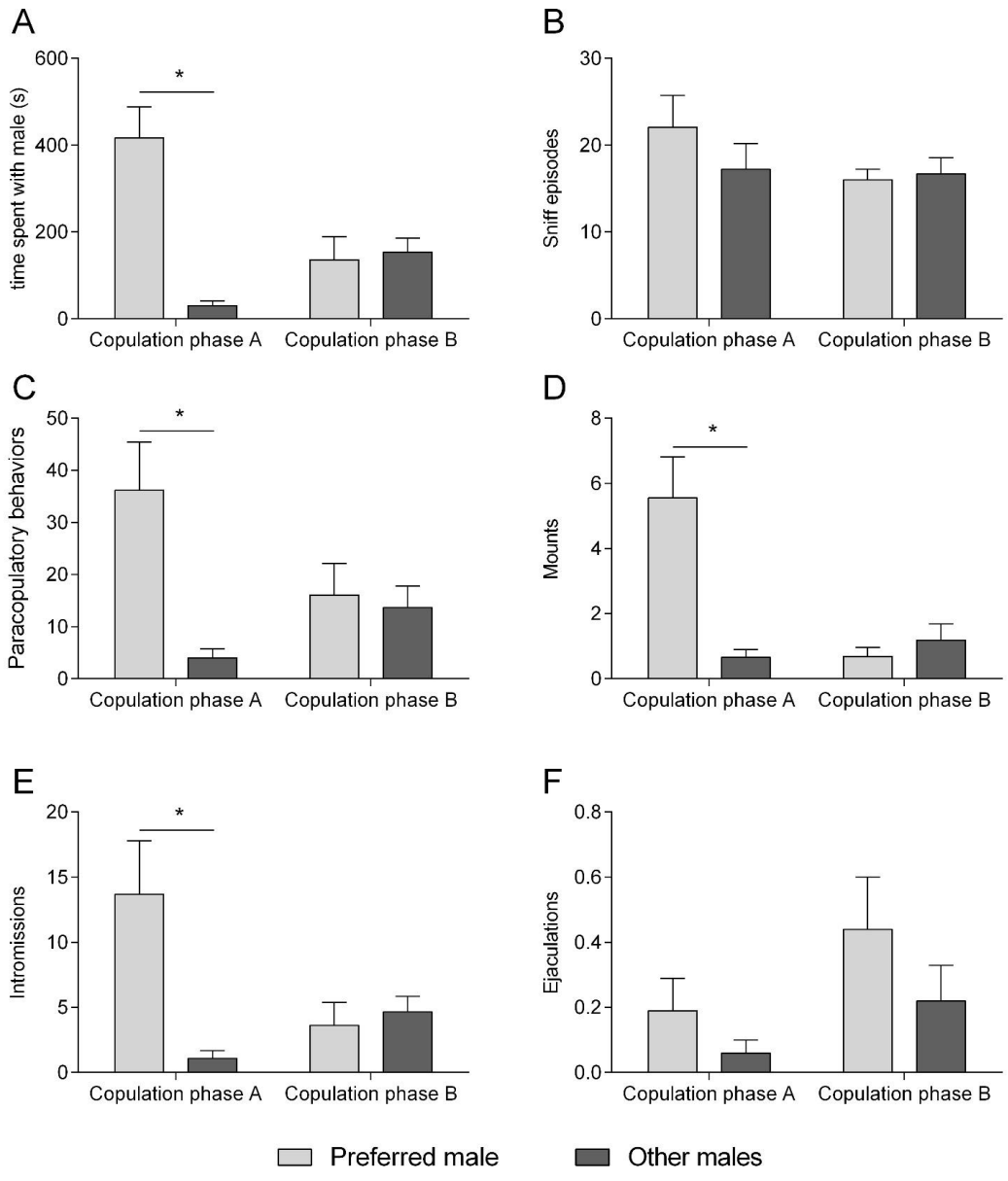

*Figure 2. Data analyzed on preferred male. (A) time spent with the male (s), (B) number of sniff episodes, (C) number of paracopulatory behaviors, (D) number of mounts, (E) number of intromissions, and (F) number of ejaculation with the preferred male and the other males during copulation phase A and B. Data are shown in mean±standard error of the mean. * $p<0.05$*