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An unknown male increases sexual incentive motivation and partner preference: Further evidence for the *Coolidge* effect in female rats

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HIGHLIGHTS

- We measure the effect of a novel sexual partner on Sexual Incentive Motivation (SIM).
- We also tested Partner Preference (PP) in female rats after mating with a known male.
- After mating, females spend more time close to the unknown male in the SIM test.
- In the PP test, females spent more time in the compartment of the unknown male.
- These results support the existence of the *Coolidge* effect in the female rat.

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ABSTRACT

The *Coolidge* effect is the resumption of copulatory behavior induced by a novel sexual partner that has been reported in several species. The term is also used in males when they resume mating when exposed to an unknown receptive female after they have reached sexual exhaustion. Only few studies have evaluated the *Coolidge* effect in females. In the present study we further evaluated this possibility using the sexual incentive motivation (SIM) and the partner preference (PP) tests. Ovariectomized rats were hormonally primed and allowed to mate for 1 h controlling the sexual interaction (paced mating) or in a condition where they were unable to pace the sexual encounters. In the SIM and PP tests, females were exposed to the male with whom they had mated before (known male) or with an unknown, sexually experienced one (unknown male). Regardless whether they paced the sexual interaction, all females showed clear preference for the unknown male but females that paced the sexual contacts spent more time in the incentive zone of the unknown male than females that could not pace the sexual interaction. Similar results were observed in the PP test. Both groups of females spent more time in the compartment of the previously unknown male than in that of the known one, but received the same amount of sexual stimulation, *i.e.*, mounts, intromissions and ejaculations from both males. No preference was found when the females were tested in the SIM test between an unknown male and a sexually receptive female. The results further support the existence of a *Coolidge* effect in female rats that is more apparent if they pace the sexual interaction.

1. Introduction

The *Coolidge* effect is a well described sex-related event that is observed in males of several species. When a male copulates *ad libitum* with a female, he will eventually reach sexual exhaustion and stop copulating, but if a new female is immediately presented, the male will resume mating [1,2]. The term *Coolidge* effect is also used when males, not sexually exhausted, show a shorter refractory period after an ejaculation when an unknown female is presented in the mating arena [3]. It has been proposed that the mating inhibition observed during exhaustion is the consequence of habituation to a sexual stimulus, and the resumption of mating (*Coolidge* effect) is the outcome of dishabituation [4]. Interpretations about the behavioral and/or biological significance of the *Coolidge* effect range from the opportunity to inseminate as many females as possible, to the removal of the seminal plug deposited by another male that had just mated with the female, preventing eventual fertilization [2,5].

Contrasting with reports in males, there are only few attempts to determine if females present the *Coolidge* effect. A pair of reports in the

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eighties in hamsters showed that receptivity decreases after mating with a given male and if an unknown one was presented, females resume lordosis behavior [6,7]. These research groups only explored receptivity, a reflexive component present over several hours, strongly dependent on hormone levels. Later, proceptive behaviors (hop-darting and ear wiggling), motivational elements of feminine sexual behavior [8–10] were analyzed in the context of sexual satiety and the *Coolidge* effect in female rats [4]. That study showed that a receptive female rat that had copulated *ad libitum*, under paced mating, with a male until reaching female sexual satiety (defined as at least 20 min of female inaccessibility to the male), resumes mating and increases proceptive behavior if an unknown male is presented. In a later report from our group, female rats in natural proestrus were allowed to mate with a sexually vigorous male for 90 min. At the end of that period the male was replaced by another one and the females were allowed to mate for 90 min more [11]. Females were tested under paced and non-paced-mating conditions. Maximal levels of receptivity were found in both mating conditions and did not change along the tests. However, females that paced their sexual interaction showed a decline in hop/darting behavior at the end of the test with the first male, and displayed a sharp increase in hop and darts when the second unknown male was presented. Females that mated in the non-paced condition showed no significant changes in proceptive behaviors when the second male was introduced, further supporting the notion that the *Coolidge* effect occurs under optimal mating conditions for the female (paced mating). The present study was designed to determine if female rats show a *Coolidge* effect, using experimental strategies that allow evaluating aspects of sexual motivation and partner preference that are affected by sexual novelty. We assessed sexual incentive motivation (SIM) and partner preference (PP) for an unknown male after females mated under paced and non-paced mating conditions.

The SIM test was evaluated in an arena where the female may approach, without physical contact, to either of two incentives and precopulatory elements, such as the incentive value and motivation for approaching the stimulus animals are inferred by quantifying the time spent in each incentive zone. A detailed description of the SIM test is found elsewhere [12]. We also evaluated PP in a three-compartment cage where the incentives were tethered and the experimental females could freely move between compartments, enabling the active seeking of sexual interaction. Previously, others have sustained that this paradigm clearly reveals sexual preference [13]. Using this test, we also evaluated if sexual novelty impacts partner preference, in conditions where copulation is feasible [14–17].

2. Materials and methods

2.1. Subjects

Twenty eight sexually naïve female Wistar rats weighting around 250 g were ovariectomized (OVX). Two weeks after surgery, they were randomly included in two independent groups: paced ($n = 14$) or non-paced mating ($n = 14$). Receptivity was induced by the sequential administration of estradiol (25 μg/rat) and progesterone (1 mg), administered 48 h and 4 h, respectively, before testing. Sexually experienced males (300 g) and steroid-primed OVX females of the same strain were used as stimuli. Animals were housed in groups of 2–4 per cage in a temperature controlled room under an inverted 12-h: 12-h light–dark cycle, starting the dark phase at 10:00 h. Food and water were available *ad libitum*. All procedures were done in accordance with the "Reglamento de la Ley General de Salud en Materia de Investigación para la Salud, NOM-062-ZOO-1999" of the Mexican Health Ministry that follows NIH guidelines and they were approved by the Institute of Neurobiology animal care committee.

2.2. Behavioral tests

2.2.1. Sexual behavior

All mating tests were performed during the first 4 h of the dark phase in acrylic cages with clean sawdust under dim red light. The female was gently placed in the mating cage, for habituation, 5 min before introducing a sexually experienced male. Females were randomly subdivided into two groups. One group mated under paced condition as previously described [18–21]. Briefly, the mating cage was divided by an acrylic barrier with a hole at the bottom. The hole was big enough to allow the female, but not the male, to go back and forth from the male compartment. Under these conditions the female controls or paces the sexual interaction. In the other group, subjects mated without the barrier, under this condition the female cannot control the sexual interaction (non-paced). The sexual behavior parameters registered were: latencies to the first mount and intromission, lordosis quotient (number of lordosis / number of mounts × 100), and the number of mounts, intromissions, and ejaculations. In the case of paced mating, we also registered the percentage of exits and return latencies following a mount and intromission.

2.2.2. Sexual incentive motivation

The test was performed as described by Ågmo [12]. Subjects were tested in a rectangular acrylic arena (100 × 50 cm) with openings covered with wire mesh at floor level on each long wall (100 cm long). A small acrylic compartment (25 × 25 × 15 cm) was assembled at each opening. For the test, a stimulus animal was placed in each compartment according to the behavioral testing procedure described below. A virtual zone in front of each compartment (20 × 30 cm) was called the incentive zone. The time spent by the experimental females in each incentive zone was recorded using a video camera connected to a computer software (EthoVision XT 10.1). Before the first test, the subjects were habituated in three, 10 min sessions performed on three successive days, placing each female alone in the SIM arena.

2.2.3. Partner preference

This test was carried out in a three-compartment box made of wood. The middle compartment (21 × 27 × 32 cm) communicated with two gated lateral compartments (36 × 27 × 32 cm). The lateral compartments contained the stimuli: a known sexually experienced male (that had just mated with the female) on one side and an unknown sexually experienced male (that had never mated with the female) on the other. The stimulus males were tethered to the rear of the compartment using a harness attached to a flexible rope. In this way, females were able to move freely and males were able to perform coital behaviors within their respective compartments. During testing, the female was gently placed in the central compartment, the gates were removed after one min and the time spent in each of the lateral compartments and the sexual behavior parameters were registered by two experienced observers during 15 min [14,17].

2.3. Behavioral testing procedure

Testing was initiated fifteen days after OVX; the mating tests were performed one every 4 days to mimic the length of the estrus cycle.

2.3.1. Sexual incentive motivation (SIM) test

On test 1, females mated for one hour under paced or non-paced mating conditions, depending on the group. Immediately after, subjects were tested in the SIM test. The incentives were: the male that had just mated the female (known male) and a sexually experienced unknown male (unknown male). Four days later, test 2 was done in the same sequence as test 1 but the position of the incentives was inverted. This counterbalanced procedure avoids the possibility of positional learning. In test 3 females mated during one hour with a sexually experienced male. Afterwards, the females were randomly divided in two subgroups