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### Sexual behavior of female rats in a multiple-partner preference test

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#### Abstract

In this study, sexually experienced female rats were tested in a multiple-partner preference test (MPPT) in which they were allowed to pace their sexual contacts with four sexually active males. Four cylinders, with a small hole through which only the female could move freely from one cylinder to another, were assembled forming in the center an empty compartment. An intact female was placed in the central compartment and a sexually active male in each cylinder. Female sexual behavior was analyzed throughout the estrus cycle in four consecutive days. Each daily test lasted 15 min. The percentage of exits after intromission or ejaculation was significantly higher than the percentage of exits after each mount. The female spent significantly longer time with one of the males. We designated this male as the preferred male (PM). Although in each of the 4 days studied, females spent significantly longer time with the PM, however, the male selected was not the same throughout the estrus cycle. The number of entries into the compartment of the PM was significantly higher and increased around proestrus. Compared to previous studies, pacing behavior was notably lower in the conditions of the MPPT. No significant differences were observed during the estrous cycle concerning the other parameters recorded. The present results suggest that the MPPT could be a good model to study partner preference in the female rat.

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#### Introduction

It is clearly established that if given the opportunity female rats control ("pace") the rate of sexual stimulation they receive when placed with a sexually active male (Bermant and Westbrook, 1966; Blaustein and Erskine, 2002; Erskine, 1989; Gans and Erskine, 2003; Gilman and Westbrook, 1978). We have repeatedly demonstrated that the rewarding components of sexual behavior for the female rat are enhanced when they are allowed to pace their sexual contacts with the male (García-Horsman and Paredes, 2004; Martinez and Paredes, 2001; Paredes and Vazquez, 1999). Several methodologies have been used to evaluate the ability of female rats to control their sexual interactions including pressing a lever to reach the male (Bermant, 1961;

Bermant and Westbrook, 1966; Matthews et al., 1997; Peirce and Nuttall, 1961), mating in a bilevel chamber in which the female can move between levels, thus pacing the sexual interaction (Agmo and Soria, 1997; Mendelson and Gorzalka, 1987; Pfaus et al., 1999), mating with a tethered male (Broekman et al., 1988; De Jonge and van de Poll, 1986; Edwards and Pfeifle, 1983), or escaping from the male through a small hole of a size that only the female can pass through it (Blaustein and Erskine, 2002; Erskine, 1989; Erskine et al., 1989; Gans and Erskine, 2003; Paredes and Alonso, 1997). It must be noted that all of the abovementioned studies have been performed using only one male and one female rat. In natural conditions, however, females mate in groups and may receive sexual stimulation from different males before receiving an ejaculation (Calhoun, 1962; Robitaille and Bouvet, 1976). Similar observations have been described in the few studies in which female sexual behavior has been analyzed in seminatural conditions (McClintock and Anisko, 1982; McClintock et al., 1982).

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To our knowledge, no systematic attempt to study partner preference in a multiple choice test in which a female can choose between several sexually active males has been performed. In most of the studies, in which the appetitive aspects of feminine sexual behavior have been evaluated using the partner preference test, the female is allowed to choose between two conditions: a sexually active male versus either a receptive female or a non-copulating male. (De Jonge and van de Poll, 1986; Dudley and Moss, 1985; Edwards and Pfeifle, 1983). In the present study, pacing and other aspects of feminine sexual behavior were analyzed in a situation in which the female was allowed to choose among four active males. In order to analyze the hormonal influences on this behavior, female were tested throughout the estrus cycle.

#### Methods

#### Animals

Adult Wistar rats from our own vivarium were used in this study. Subjects were housed 2-3 per cage in acrylic cages and were kept in a room with a reversed 12/12-h light-dark cycle (lights off: 14 h) and temperature controlled. Food and water were available ad lib. Adult, sexually experienced active males (weight: 400-450 g) (N=4) were used as stimuli. Selected males were those who showed at least two ejaculations in less than 15 min during five consecutive sexual behavior

tests. Intact adult females (weight: 250–300 g) (N=15) were selected after verifying through vaginal smears that they had a normal estrus cycle. All experiments were carried out following the "Regulations of the General Law for Health of the Mexican Health Ministry" and approved by the Animal Care Committee from the Universidad Autónoma Metropolitana.

#### Apparatus and procedures

Four transparent Plexiglas cylinders (diameter 55 cm, height 40 cm) were assembled together, forming a compartment in the center where the female was placed at the beginning of the test (Fig. 1). The bottom of each cylinder had a small hole (5 cm height, 3.5 cm width), the size of which allowed only the female to freely move across them and enter the different compartments. Before the first session, females were habituated to the apparatus for 30 min allowing them to explore the empty compartments.

All sessions were conducted during the first 2 h of the dark period, under a dim red light. At the beginning of the test, a sexually active male was placed in each cylinder and a female was placed in the central compartment with the holes of the cylinders closed. Ten minutes later, the holes were opened allowing the females to freely move to any compartment during the 15 min test. A total of four tests were conducted for each female in consecutive days. Vaginal smears were taken daily and the first test was done when the female was in diestrus.



Fig. 1. The multiple-partner choice test arena.

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