



## Kin discrimination in prepubescent and adult Long–Evans rats

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### ABSTRACT

The present study investigated the preference of prepubescent and adult rats for an unrelated conspecific over a closely related conspecific (e.g., father, mother). Preference was measured by the amount of time spent in the vicinity of the stimulus animals as well as who was visited first. To prevent mating behavior, stimulus animals were housed behind wire-mesh. Experiment 1 determined if adult female offspring prefer an unrelated, unfamiliar adult male or their father. The preference of adult female rats was independent of kinship. Experiment 2 evaluated the preference of prepubescent female and male offspring for an unrelated, unfamiliar adult male or their father. The preference of prepubescent female and male rats was also independent of kinship. Experiment 3 evaluated the preference of adult male offspring for an unrelated, unfamiliar adult female or their mother. The preference of adult male rats was independent of kinship. In summary, prepubescent and adult rats do not demonstrate preference for kin vs. non-kin (as measured by time spent near stimulus animals or who was visited first). Although kin recognition provides a mechanism for inbreeding avoidance (Wilson, 1987), in the present study adult rats show no evidence of inbreeding avoidance.

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### 1. Introduction

Kin discrimination, or the differential treatment of kin and non-kin, has adaptive significance for many animals because it provides a mechanism for inbreeding avoidance, parental care, and altruistic behavior (Wilson, 1987). Incest avoidance is important to many species across the animal kingdom because inbreeding can reduce reproductive fitness (i.e., increase homozygosity and expression of deleterious alleles) (Pillay, 2002). For example, in mice, litters of more closely related parents were significantly smaller than litters of less closely related or unrelated parents (Barnard and Fitzsimons, 1989). Furthermore, male mice born to closely related parents (e.g., full or half siblings) were less aggressive than male offspring born to second cousins (Barnard and Fitzsimons, 1989).

Rodents can distinguish close kin from unrelated conspecifics, as well as different degrees of relatedness, using olfactory cues, suggesting the existence of “kinship odors” (Hepper, 1987; Todrank et al., 1998). However, differences between rodent species and the necessity of prior experience with kin for recognition to occur contribute to discrepancies found between studies. For example, golden hamsters must live with kin during early life (i.e., from birth to weaning at one month old) in order to discriminate between kin

in adulthood using olfactory cues (Todrank et al., 1998). Several other rodent species, including the striped mouse (Pillay, 2002), the Mongolian gerbil (Agren, 1984), and the deer mouse (Dewsbury, 1982), will avoid their kin when choosing mates, but only when they are familiar with their kin. In addition, both male and female juvenile house mice appear to avoid their fathers in the wild, perhaps as a mechanism of inbreeding avoidance, as evidenced by the observation that they are never caught in the same trap, but they are often caught with their siblings or mother (Drickamer et al., 2003). On the other hand, Hepper (1987) found that rats can recognize kin without any prior experience with kin. Specifically, the time spent investigating same-sex conspecifics was inversely correlated with the degree of relatedness of the subject with the stimulus animal (i.e., siblings, half-siblings, cousins, and unrelated conspecifics). Therefore, it is possible that rodents can use olfactory cues to discriminate between kin and non-kin in both conditions (i.e., when experience is necessary, as well as when only genetic information is available).

Hayashi and Kimura (1983) systematically investigated the use of odors for kin discrimination in male and female mice. They used a two-way choice apparatus with wire mesh limiting contact between subjects (i.e., male and female mice, 2.5–4.5 months old) and stimulus animals (i.e., fathers, mothers, sisters, brothers, and unrelated conspecifics). Mice were given the opportunity to spend time near kin or an unrelated conspecific of the same sex as the kin. Hayashi and Kimura found that adult male mice spent more time near unrelated, unfamiliar females than with their

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litter sisters or mother. In contrast, adult female mice spent equal time near unrelated and related (i.e., brothers or father) males. In addition, both male and female mice showed no preference for unrelated conspecifics over their same-sex parents (i.e., males for father and females for mother). From their results, they concluded that adult male mice, rather than adult females, are the sex responsible for inbreeding avoidance in populations of mice containing close relatives. However, it was noted that female mice may be more sensitive to kinship under specific circumstances, because others have found that female mice prefer an unrelated, unfamiliar male to their father when they lived with their father until adulthood or at least until weaning (Gilder and Slater, 1978; Yanai and McClearn, 1972).

Hepper (1986) conducted a similar kin recognition study using rat pups. He used a two-way choice test to determine the preference of pre-weanling male and female rat pups (starting when pups were 10 days old) for stimulus animals, which consisted of their mother vs. an unrelated female in the first experiment, and their father vs. an unrelated male in the second experiment. During tests, fans at either end of a T-maze blew odors of stimulus animals, housed behind wire mesh, toward the pup in the center of the maze. Subjects were tested daily until the pups were 22 days old (age of weaning). Preference was operationally defined as the stimulus animal the subject visited first. In the first experiment, pups visited their mother first instead of an unrelated female for the duration of the experiment (days 10–22). However, in the second experiment 10–14 day old pups visited their father first, even if they had never seen him before. When the pups were 18–22 days old, they switched to visit the unrelated male first (if they had lived with their father throughout the experiment) or had no significant preference (if they had no previous experience with their father). Hepper concluded from this experiment that rat pups, not surprisingly, prefer the odors of their mother while they are still suckling. Furthermore, rat pups use olfactory cues to recognize their father, even if they have had no previous experience with him. The fact that very young pups prefer their father and then switch or lose this preference, suggests that there is a developmental aspect to paternal recognition in rat pups. Hepper hypothesized that paternal recognition by young rat pups may be advantageous or adaptive because it is not uncommon for adult male rats to kill unrelated neonatal pups and even preweaning pups (Paul and Kupferschmidt, 1975). Similarly, young female mice avoid odors of adult male rats, but as they mature, their preference changes to be attracted to adult male odors, reflecting their increasing sexual motivation (Drickamer and Brown, 1998).

The present study was designed to investigate whether pre-pubescent female and male rats (24–25 days old) or adult female and male rats (38–41 days old) can discriminate between a parent and an unrelated stimulus animal in a two-way choice test. Although Hepper (1986) investigated the preference of preweanling rat pups, to the best of our knowledge, the present study is the first to report on the preference of older rats for their mother or father vs. non-kin. Preference for kin vs. non-kin was determined using two measures of preference (i.e., time spent in the vicinity of stimulus animals; stimulus animal visited first) in two different age groups of rats (i.e., adult, prepubescent). Understanding kin discrimination provides insight into the factors that may contribute to reproductive success of rats and what role preference has on mate choice and the evolution of rats.

## 2. Methods

### 2.1. Production of offspring for kin discrimination experiments

Nine sexually naïve female Long–Evans rats (200–300 g) and 18 sexually experienced male Long–Evans rats (400–600 g) were

purchased from Harlan Sprague-Dawley (Indianapolis, IN) and used to produce offspring as subjects in the present study. None of the purchased animals were related to one another. All rats were housed in hanging plastic cages with aspen wood shavings for bedding and food and water available ad libitum. Male rats were housed in pairs upon arrival from Harlan. Female rats were housed three to a cage until successful mating and then they were housed individually. All rats were weighed weekly. Temperature and humidity in the rat colony room were monitored, and the lights were maintained on a reversed 12:12 h light–dark cycle (lights off at 10:00 a.m.). All experiments were conducted during the dark cycle under dim red light.

Following successful mating, female rats were monitored daily for signs of pregnancy and parturition. All of the females became pregnant and gave birth approximately 22 days after mating, at which time pups were counted. Pups were weaned and separated by sex at 21 days old.

### 2.2. Behavioral procedure

#### 2.2.1. Kin Discrimination Experiment 1: adult female offspring and fathers

Experimental subjects included 28 sexually naïve, adult (38–41 days old) female offspring from 7 litters (described above in Section 2.1). Stimulus animals consisted of two age-matched, unfamiliar male rats, one of which was the father for each subject.

#### 2.2.2. Acclimation

All subjects were habituated to the two-way choice apparatus on two separate occasions, for 15 min each session, prior to any behavioral testing. Each two-way choice apparatus consisted of a Plexiglas arena (101.0 cm long × 32.0 cm height × 37.0 cm wide) divided into three equal compartments using clear Plexiglas dividers. Each of the dividers had a 5.0 cm hole in each of the two bottom corners. Wood shavings covered the floor of each compartment. Each experimental subject was placed into the chamber alone and allowed to freely explore the three compartments.

#### 2.2.3. Hormones

All adult female offspring received 10.0 µg of estradiol benzoate (EB) 48 h and 1.0 mg of progesterone (P) 4 h before each mating test. Hormones were dissolved in a sesame seed oil vehicle and administered subcutaneously in the flank. These doses of EB and P have been shown to produce high levels of sexual receptivity in ovariectomized rats (Zipse et al., 2000). Hormones were administered to adult female experimental subjects in the present study to synchronize the estrous cycles of the naturally cycling females so they would all be in behavioral estrus on the day of the kin preference test. Consequently, all adult female offspring were sexually receptive on the day of the kin preference test. All hormones were purchased from the Sigma Chemical Company (St. Louis, MO).

#### 2.2.4. Kin preference test

Stimulus animals (father and unrelated male rat) were placed in either of the outer compartments of the two-way choice apparatus, but behind wire mesh partitions (30.5 cm wide × 35.6 cm height), which were positioned 15 cm from each of the clear Plexiglas dividers. These wire mesh partitions allowed the transmission of visual, auditory, and olfactory cues, but prohibited mating or physical contact with the male stimulus animals. The experimental subjects were placed in the apparatus and confined to the center compartment with opaque dividers blocking their view of the stimulus animals. Five min after the experimental subjects had been placed in the apparatus, the opaque dividers were removed and the test started. Observers, who were unaware of the genetic relationship between subjects and stimulus animals, sat approximately

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