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Asymmetric learning to avoid heterospecific males in *Mesocricetus* hamsters

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Abstract

If a female mates with a male of a closely related species, her fitness is likely to decline. Consequently, females may develop behavioral mechanisms to avoid mating with heterospecific males. In some species, one such mechanism is for adult females to learn to discriminate against heterospecific males after exposure to such males. We have previously shown that adult, female Syrian hamsters (*Mesocricetus auratus*) learn to discriminate against male Turkish hamsters (*Mesocricetus brandti*) after exposure to a single heterospecific male during 8 days across a wire-mesh barrier. Here we repeated that experiment but this time we exposed female Turkish hamsters to a male Syrian hamster for 8 days and then measured sexual and aggressive behaviors towards that heterospecific male and towards a conspecific male. In contrast to female Syrian hamsters, female Turkish hamsters did not differ in their latency to go into lordosis or in any measure of aggression towards either type of male. Female Turkish hamsters spent less time in lordosis with the heterospecific male, but the percentage of trials in which females copulated with conspecific and heterospecific males did not differ. When comparing females from both species that had been exposed to a heterospecific male for 8 days, female Syrian hamsters copulated less and were more aggressive towards the heterospecific male compared to the behavior of female Turkish hamsters. We discuss how this asymmetric response between females of the two species may be due to the much larger geographical range of Turkish hamsters compared to Syrian hamsters.

Keywords

Mesocricetus; Interspecific mating; Heterospecific aggression; Lordosis; Adult learning

1. Introduction

When interspecific mating between two species results in no or subfertile offspring (Gröning and Hochkirch, 2008), many species will develop pre-mating isolating mechanisms (Mayr, 1970). A recurrent mechanism resulting in assortative mating is behavioral isolation, whereby an individual will avoid mating with heterospecific, opposite-sex individuals. The

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avoidance of interspecific mating can be innately determined (Hebets, 2007). In other species, juveniles learn to prefer conspecifics as future mates via imprinting, normally on the parents (Irwin and Price, 1999; Servedio et al., 2009; ten Cate and Vos, 1999). In both cases (innate predisposition and imprinting) when individuals reach adulthood they are prepared to discriminate against heterospecific individuals. In some species this is not the case, and learning to discriminate against heterospecific individuals occurs during adulthood after exposure to or interactions with heterospecifics. This type of learning has been reported in three, very dissimilar taxonomic groups: *Drosophila* fruit flies (Dukas, 2004, 2008), *Poecilia* fish (Magurran and Ramnarine, 2004), and *Mesocricetus* hamsters (delBarco-Trillo and Johnston, 2011a; delBarco-Trillo et al., 2010). The fact that this response occurs in such dissimilar groups indicates that it may be prevalent in many other species as well.

If Syrian hamsters (*Mesocricetus auratus*) and Turkish hamsters (*M. brandti*) do mate, they do not produce viable offspring (Murphy, 1977; Todd et al., 1972). Female Syrian hamsters show an innate preference for odors of conspecific males compared to odors of heterospecific males (delBarco-Trillo et al., 2009a, b). In addition, *c-fos* activity in a brain area involved in species discrimination (Meredith and Westberry, 2004) differs in female Syrian hamsters exposed to odors of conspecific males versus odors of heterospecific males (delBarco-Trillo et al., 2009a). However, female Syrian hamsters do not innately avoid mating with male Turkish hamsters (delBarco-Trillo et al., 2009b; delBarco-Trillo and Johnston, 2010). That is, when female Syrian hamsters without any previous experience with heterospecific males were paired with a male Turkish hamster, these females behaved as if paired with a conspecific male, i.e. they were highly sexually receptive and they showed no aggression towards these males (delBarco-Trillo et al., 2009b; delBarco-Trillo and Johnston, 2010, 2011b). In contrast, when female Syrian hamsters were exposed to a male Turkish hamster for 8 days across a wire-mesh partition and were then paired with that heterospecific male, these females were highly aggressive and not sexually receptive (delBarco-Trillo et al., 2010). Shorter exposures to the heterospecific male resulted in less sexual avoidance of such heterospecific males (delBarco-Trillo and Johnston, 2011a). Female Turkish hamsters that were not previously exposed to male Syrian hamsters behave similarly towards conspecific and heterospecific males (delBarco-Trillo et al., 2009b). However, female Turkish hamsters have been reported to be aggressive towards male Syrian hamsters (Murphy, 1978), possibly because those females had been previously exposed to male Syrian hamsters. We thus predicted that the same type of adult learning against heterospecific males that we observed in female Syrian hamsters may also occur in female Turkish hamsters. Here we tested this prediction by exposing female Turkish hamsters to a male Syrian hamster across a wire-mesh partition for 8 days and then measuring sexual and aggressive behavior towards that heterospecific male. Importantly, by using the same experimental design as in previous studies we were able to compare the learned responses of female Turkish and Syrian hamsters. Specifically, we evaluated two alternative hypotheses: (i) when females of both species are given the same type of exposure to a heterospecific male they will show the same types of responses towards the heterospecific male; or (ii) females from one species will behave more aggressively and less sexually receptive towards the heterospecific male than females from the other species. Female Turkish hamsters may learn to avoid heterospecific males more effectively than female Syrian hamsters due to the fact that the geographical distribution of Turkish hamsters is between that of Syrian hamsters to the south and that of *Mesocricetus raddei* to the north, whereas the distribution of Syrian hamsters is only adjacent to that of Turkish hamsters (Neumann et al., 2006); this difference may have resulted in more interspecific interactions for Turkish hamsters since the inception of the different species and thus more opportunities for the establishment of an effective behavioral isolating mechanism. Alternatively, one reason why female Syrian hamsters may discriminate against heterospecific males more effectively than female Turkish hamsters is because of the striking difference in the size of the geographical range

of the two species. The geographical range of the Turkish hamster is several orders of magnitude larger than that of the Syrian hamster (Neumann et al., 2006). Consequently, the within-species variability of the traits used by females to choose mates is likely to be much higher among male Turkish hamsters than among male Syrian hamsters. Females are more likely to accept heterospecific males as mates when variation among conspecific males is high than when variation among conspecific males is very low, especially if the between-species differences are relatively small (Pfennig and Pfennig, 2009).

2. Materials and methods

2.1. Animals

We used 10 female Turkish hamsters, 10 male Turkish hamsters, and 10 male Syrian hamsters. All animals were born and raised in captivity at Cornell University, Ithaca, NY. Hamsters were weaned at 30 days of age and housed individually in solid bottom polycarbonate cages (45 cm x 24 cm x 14.5 cm) with sani-chip bedding material and constant access to water and food (Prolab 1000; Agway, Syracuse, NY, USA). Turkish and Syrian hamsters were maintained in separate rooms with independent air intake and exhaust. Turkish hamsters were maintained on a 16:8 light dark schedule with lights off between 10:00 and 18:00 h (Eastern Standard Time). Syrian hamsters were maintained on a 14:10 light-dark schedule with lights off between 09:00 and 19:00 h. Different light cycles were simply due to husbandry reasons (Turkish hamsters seem to require longer light cycles to reproduce successfully in captivity). Experiments were run between 10:00 and 13:00 h. We used dim and indirect light to allow videotaping and observations. The video camera was located approximately 1 m from the cage in which the interactions took place. There is a 4-day estrous cycle in both Turkish and Syrian hamsters. To determine whether a female was in estrus on a specific day, a conspecific male hamster was placed inside the female's home cage. If lordosis occurred within 15 s, the female was considered in estrus (no mounting occurred during estrous testing). Lordosis is a stereotypical position in *Mesocricetus* spp. in which the female arches her back (down in the center), spreads her legs, raises her tail, and remains immobile from seconds to minutes. If no lordosis was observed, the female was retested on the following days (up to 3 days) until lordosis occurred. Females were always tested 4 days after determining the timing of their estrous cycles (i.e., on their next estrous day). Once a test started no more checking of estrous took place.

2.2. Procedure

We used arenas (50 cm x 38.1 cm x 21.5 cm; model# PCT4SHT; Allentown, Inc., Allentown, NJ, USA) divided into two similarly-sized sections (24.85 cm x 38.1 cm x 21.5 cm) by a wire-mesh partition with 1-cm² openings. This type of barrier is sufficient for hamsters to learn the individual identity of other conspecifics (Johnston and Peng, 2008). We started a test by placing one estrous female Turkish hamster in one of the two sections and then placed a male Syrian hamster in the other section. The female could perceive and interact in a limited manner with the male across the wire-mesh partition. We used soiled bedding from each animal's home cage as substrate for its respective section of the arena. Each animal had constant access to its own water and food. Animals lived undisturbed in this arena for 8 days. The arenas were placed in a separate room from the colony rooms and the testing rooms; the light cycle in this room was a 14:10 light-dark schedule with lights off between 09:00 and 19:00 h. After the 8 days of exposure, the female and the male in the same arena were transferred to individual cages lined with clean bedding and the cages were moved to the testing room. The pairing trial started 10 min after the animals had been transferred to their individual cages. The male was transferred to the female's cage in a clean, 500-ml plastic beaker. The trial lasted 5 min. We did not interfere with the behavior of animals during these trials. 5 min after the trial with the heterospecific male, the same

female was paired for 5 min with an unfamiliar conspecific male in her own cage. We videotaped all trials and scored the recorded videos to determine the latency to lordosis, the duration of lordosis, the latency to aggression and the duration of aggression. We considered any attempted biting or tumbling fights as signs of aggression. If a female did not show lordosis or aggression during a test, we scored the latency for that behavior as 300 s.

We have previously shown that female Syrian hamsters show more avoidance of mating with a heterospecific male if they first interact with a heterospecific male and then interact with a conspecific male than if they first interact with a conspecific male and then interact with a heterospecific male (delBarco-Trillo et al., 2010). Given that we observed a low level of avoidance of interspecific mating using the condition that should have maximized it (i.e., a trial with the heterospecific male followed by a trial with a conspecific male), we decided not to add a condition in which the female was tested first with a conspecific male followed by a trial with the heterospecific male.

We have previously shown that female Syrian hamsters will behave similarly towards familiar conspecific males (delBarco-Trillo and Johnston, 2010) and towards unfamiliar conspecific males (delBarco-Trillo et al., 2010). That is, in these studies, all receptive females showed lordosis and displayed no aggressive behavior towards conspecific males, regardless of whether those conspecific males were familiar or unfamiliar to a female. Therefore, we argue that the behavior towards the conspecific male in this study would not have differed had the female been familiarized with that male.

2.3. Statistics

Since lordosis latency, lordosis duration, aggression latency, and aggression duration were not normally distributed (Shapiro–Wilk normality test: $p < 0.0005$ in all cases), we used the Wilcoxon signed-rank test to determine whether these variables differed between trials in which females were paired with a heterospecific male and those in which females were paired with a conspecific male.

We used Fisher's exact tests to determine whether the percentage of trials in which female Turkish hamsters were aggressive and the percentage of trials in which copulation occurred differed between trials with a heterospecific male and trials with a conspecific male. For species-comparison purposes, we used non-analyzed data from a previous study (delBarco-Trillo and Johnston, 2011b), specifically from trials in that study in which female Syrian hamsters were paired with a familiar male Turkish hamster and then with a conspecific male. We used Fisher's exact tests to determine whether the percentage of trials in which these female Syrian hamsters were aggressive and the percentage of trials in which copulation occurred differed between trials with a heterospecific male and trials with a conspecific male. To directly compare the responses of female Turkish and Syrian hamsters to heterospecific males after the same type of exposure (8 days in the same type of arena), we used Fisher's exact test to compare the percentage of trials without aggression and the percentage of trials with copulation when females of either species were paired with their respective heterospecific male. Results are given as means $\pm$ SD unless otherwise noted. We used R 2.11.1 (R-Development-Core-Team, 2010) for all statistical analyses.

3. Results

The latency to lordosis by females paired with a heterospecific male (36.8 ± 51.79 s) and with a conspecific male (16.2 ± 3.39 s) was not significantly different (Wilcoxon signed-rank test: $V = 39.5$, $p = 0.24$; Fig. 1A). However, the duration of lordosis was significantly shorter with a heterospecific male (219.52 ± 84.31 s) than with a conspecific male (271.7 ± 9.7 s; $V = 8$, $p = 0.0488$; Fig. 1B).

Female Turkish hamsters copulated with the heterospecific male during 70% (7 of 10) of the trials and with the conspecific male during 100% of the trials but this difference was not statistically significant (Fisher's exact test: $p = 0.21$; Fig. 2). In contrast, female Syrian hamsters copulated with the heterospecific male during just 10% (1 of 10) of the trials but they mated with the conspecific male during 100% of the trials (Fisher's exact test: $p = 0.0001$; Fig. 2). The percentage of trials in which female Turkish hamsters copulated with the heterospecific male (70%) was significantly higher than the percentage of trials in which female Syrian hamsters did (10%; Fisher's exact test: $p = 0.02$; Fig. 2).

The latencies to aggression with the heterospecific male (227.5 ± 121.07 s) and the conspecific male (300 ± 0 s) were not significantly different (Wilcoxon signed-rank test: $V = 0$, $p = 0.18$). Similarly, the duration of aggression did not differ towards the heterospecific male (21.32 ± 47.55 s) and the conspecific male (0 ± 0 s; Wilcoxon signed-rank test: $V = 6$, $p = 0.18$). Female Turkish hamsters were not aggressive towards heterospecific males in 70% (7 of 10) trials, which did not significantly differ from their lack of aggression towards conspecific males in 100% trials (Fisher's exact test: $p = 0.21$). In contrast, female Syrian hamsters were not aggressive towards heterospecific males in 10% (1 of 10) trials compared to 100% trials towards conspecific males (Fisher's exact test: $p = 0.0001$; Fig. 2). The percentage of trials in which female Turkish hamsters were not aggressive towards heterospecific males (70%) was significantly higher than for female Syrian hamsters (10%; Fisher's exact test: $p = 0.02$; Fig. 2).

4. Discussion

We investigated whether female Turkish hamsters learn to avoid mating with a heterospecific male after they have been exposed to that heterospecific male for 8 days. We found that female Turkish hamsters reduced the duration of lordosis when paired with a heterospecific male compared to when they were paired with a conspecific male. However, the latency to lordosis, the percentage of trials in which the female copulated, and measures of aggression did not differ when female Turkish hamsters were paired with a conspecific or a heterospecific male. In contrast, all of these measures did differ significantly for female Syrian hamsters. That is, after female Syrian hamsters were exposed for 8 days to a heterospecific male they were much less receptive and much more aggressive towards that heterospecific male than towards a conspecific male.

Murphy (1978) found much higher levels of aggression and lower levels of sexual receptivity from female Turkish hamsters towards male Syrian hamsters than we did. Since we have determined that longer exposures to a heterospecific male are more effective than shorter exposures in increasing a female's ability to discriminate against heterospecific males (delBarco-Trillo and Johnston, 2011a), we propose that Murphy (1978) may have used females that had had more exposure to heterospecific males prior to testing than the females that we used. Unfortunately, we do not know how much exposure the female Turkish hamsters that Murphy (1978) used had had with heterospecific males. It still needs to be determined whether exposures longer than 8 days to a heterospecific male or exposure to several heterospecific males for an undetermined amount of time is required for female Turkish hamsters to avoid heterospecific males at the same level that we have previously observed in female Syrian hamsters.

In other species in which crosses in both directions between two closely related species were investigated, there were relatively symmetrical learned responses towards heterospecifics. This was the case in two species of fish, *Poecilia reticulata* and *Poecilia picta* (Magurran and Ramnarine, 2004) and two species of fruit flies, *Drosophila pseudoobscura* and *Drosophila persimilis* (Dukas, 2009). An asymmetric response was found between two species of three-

spined sticklebacks (*Gasterosteus* spp.), possibly due to the fact that one of these two species is more social than the other (Kozak and Boughman, 2009). However, all *Mesocricetus* species are asocial, so differences in sociality cannot explain the differences in behavior that we observed between female Turkish and Syrian hamsters. An asymmetric response was also found between two subspecies of *Mus musculus* from the border of a hybrid zone (Smadja and Ganem, 2002); while female *M. m. musculus* prefer conspecific males over male *M. m. domesticus*, female *M. m. domesticus* do not show any preference between conspecific and heterospecific males (Smadja and Ganem, 2002).

The asymmetry in how females of both hamster species respond to heterospecific males could be explained by the nature of the different geographical distributions of the two species. The geographical distribution of the Turkish hamster (encompassing Turkey, Georgia, Armenia, Azerbaijan, Dagestan, and Iran) is much larger than that of the Syrian hamster (restricted to a small area on the Turkish/Syrian border) (Neumann et al., 2006). Given that geographical range and population size correlate positively with the total genetic variation of a species (Frankham, 1996; Leimu et al., 2006), two male Turkish hamsters in any location within the species distribution are likely to differ more (genetically and/or morphologically) than two male Syrian hamsters will. The higher within-species variation in Turkish hamsters could lead to females being more lax in their mating decisions. Otherwise, females that show a very stringent preference for a type of male could reject suitable conspecific males as mates if such males are outside of the range of female preference (Pfennig and Pfennig, 2009). A lower within-species variation in Syrian hamsters should ensure that a female's stringent preference for conspecific males will in most cases lead to acceptance of conspecific males and rejection of heterospecific males as mates. As a result, female Syrian hamsters may have been selected to be more stringently discriminative than female Turkish hamsters. If so, given the same type of information (8 days of exposure to a heterospecific male in our experiments), female Syrian hamsters should show higher levels of aggression and lower levels of sexual receptivity against heterospecific males than female Turkish hamsters do.

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Fig. 1a

Fig. 1b

Fig. 1. (A) Latency to lordosis and (B) lordosis duration in female Turkish hamsters when paired with a heterospecific male or a conspecific male. The bar within each box represents the sample median, each box represents 50% of the data around the median, and the two whiskers around each box represent the 95% confidence interval. Circles represent outliers.

Fig. 2.

Percentage of trials in which female Turkish or Syrian hamsters copulated with or showed no aggression towards a conspecific male or a heterospecific male. Data for trials without aggression and for trials with copulation were identical. Different letters denote a statistically significant difference. NS, non-significant; ***, $p = 0.0001$.