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Adult female hamsters require long and sustained exposures to heterospecific males to avoid interspecific mating

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Abstract

Abstract Interspecific mating normally decreases female fitness. In many species, females avoid heterospecific males innately or by imprinting on their parents. Alternatively, adult females could learn to discriminate against heterospecific males after exposure to such males. For example, Syrian hamster (*Mesocricetus auratus*) females learn to discriminate between conspecific males and Turkish hamster (*M. brandti*) males during adulthood by exposure to males of both species. Adult females not previously exposed to Turkish hamster males will mate similarly with conspecific and heterospecific males. However, in a previous study we showed that exposure to a heterospecific male and a conspecific male for 8 days led to mating avoidance and aggression towards the heterospecific male. Here we conducted two experiments to investigate how much exposure to the heterospecific male was required for females to avoid mating with the heterospecific male (Experiment 1) and how long that avoidance lasted in the absence of continuous exposure to heterospecific stimuli (Experiment 2). Fast and durable learning would indicate the evolution of an efficient avoidance response. In Experiment 1, females were exposed to a heterospecific male for 1, 4 h, 4 or 8 days and then paired with that male. We found more avoidance of interspecific mating after 4 or 8 days of exposure than after 1 or 4 h of exposure. In Experiment 2, females were exposed to a heterospecific male for 8 days and then paired with that male either 10 min later or 8 days later. We found that after an 8-day delay females were highly sexually receptive to the heterospecific male. Additionally, a comparison between the current experiments and a previous study indicates that female Syrian hamsters do not require concurrent exposure to a conspecific male and a heterospecific male to learn to avoid interspecific mating; exposure to a heterospecific male is sufficient.

Keywords

Interspecific mating; Species discrimination; Learning; Memory; Lordosis; Aggression

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Conflict of interest The authors declare that they have no conflict of interest.

Introduction

When females mate with a heterospecific male (i.e., a male from a different species), they normally produce no offspring or unfit offspring (Gröning and Hochkirch 2008); thus there is strong selective pressure on females to avoid interspecific mating. To avoid interspecific mating, females may reduce their sexual receptivity and/or increase their aggression towards males of closely-related species (Andersson 1994). This discriminative behavior against heterospecifics can be innately determined (Hebets 2007) or it may require some type of learning during early development, e.g. imprinting (Irwin and Price 1999; Servedio et al. 2009). Learning to discriminate between conspecific and heterospecific individuals also occurs during adulthood in male fruit flies and male guppies (Dukas 2004; Magurran and Ramnarine 2004; Dukas 2008), but not in female guppies (Haskins and Haskins 1949). In fact, learning to avoid interspecific mating by females during adulthood has only been shown in Syrian hamsters, *Mesocricetus auratus* (delBarco-Trillo et al. 2010).

Estrous female Syrian hamsters show a preference for the scent of conspecific males over the scent of Turkish hamster males, *M. brandti* (delBarco-Trillo et al. 2009a, b). In addition, activity in the posterior medial amygdala, a brain region that has been implicated in species discrimination (Meredith and Westberry 2004), is higher in estrous female Syrian hamsters exposed to the scent of conspecific males than in females exposed to the scent of heterospecific males (delBarco-Trillo et al. 2009a). These findings indicate that females can discriminate between conspecific and heterospecific males. However, Syrian hamster females do not show an innate mating avoidance of heterospecific males (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010). Such interspecific mating would be costly to Syrian hamster females because the failure to produce hybrids seems to occur after fertilization due to differences in the number of chromosomes between both species. Consequently, females mating with a heterospecific male may lose not only a reproductive event but the next estrous cycle can also be significantly delayed (Todd et al. 1972; Murphy 1977).

Syrian hamster females that have never encountered heterospecific males are receptive to and do not show any sign of aggression towards such males (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010). However, when Syrian hamster females were exposed for 8 days to a conspecific male and a heterospecific male across wire-mesh partitions and then paired sequentially with those same two males, the females were aggressive and not receptive when paired with the heterospecific male whereas females were always receptive when paired with the conspecific male (delBarco-Trillo et al. 2010). We do not know whether this learned response necessarily requires a relatively long time (8 days) or whether a much shorter time (e.g., a few hours of exposure) can be equally effective.

The amount of time required for females to learn to discriminate against heterospecific males may help us understand the evolution of such a learned response. We argue that learning to avoid mating with heterospecific males through direct interactions or exposure to stimuli from heterospecific males, instead of innately avoiding heterospecific males, can be an adaptive strategy in groups of emergent or recently formed species which distributions repeatedly fluctuate over time so that there are recurring episodes of overlapping and no-

overlapping distributions among pairs of those species. Under this evolutionary scenario, each different species will be evolving separately and interactions between pairs of species will be possible only during periods of overlapping distributions. Any innate response toward heterospecific males established during a period of overlapping distributions may not be adaptive during the following episodes of overlapping distributions if the stimuli used by females to discriminate against heterospecific males have significantly evolved during the time that both species were separated (Magurran and Ramnarine 2005). In addition, an innate heterospecific avoidance may cause the rejection of some conspecific males. Thus, during long periods of allopatry, innate preferences might be selected against because they would reduce mating opportunities or increase mating costs. An alternative and possibly more stable strategy is an adaptive response in which females learn to discriminate against males that are significantly different than conspecific males (Bergman and Feldman 1995; Dukas 2009). Such a strategy would remain adaptive independently of how much males of the other species change during non-overlapping periods. How fast females learn to discriminate against heterospecific males may depend on the rate of interactions between any two species. If interactions are relatively frequent, females are predicted to require shorter exposures to heterospecific males in order to discriminate against them and to maintain such a learned response once in place. Alternatively, if interactions between the two species are relatively infrequent, females are predicted to require longer exposures to heterospecific males to discriminate against them and to be more likely to forget the learned response in the absence of continuous heterospecific stimuli. We base our predictions on the assumption that infrequent episodes of overlapping distributions will provide (a) fewer opportunities for the adaptive establishment of a rapid and persistent response, and (b) more opportunities for the evolved response to become less efficient during the long episodes of non-overlapping distributions, i.e., when there is not an adaptive value for that response.

The *Mesocricetus* genus is a good model to test the above predictions because it consists of species which current distributions and phylogenetic relationships indicate that such distributions have fluctuated over time (Neumann et al. 2006). For example, even though *M. auratus* is more closely related to *M. raddei* than to *M. brandti*, the distribution of *M. brandti* is between those of *M. auratus* (to the South) and *M. raddei* (to the Northeast) (Neumann et al. 2006). Also, there are two separate populations of *M. brandti* separated by the distribution of *M. raddei*. The two species considered in this study, *M. auratus* and *M. brandti*, at present seem to have non-overlapping geographical distributions (Neumann et al. 2006) even though *M. auratus* distribution has not yet been fully clarified (Gattermann et al. 2001); however, it is unclear what even quite recent distributions were, given that *M. auratus* distribution has severely declined very recently (in evolutionary terms) due to loss of habitat (caused by agriculture) and deliberate destruction by humans (Gattermann et al. 2001). In addition, *M. brandti* has been reported to be distributed not only to the North but also to the Southwest of the *M. auratus* known distribution (Hamar and Schutowa 1966), offering more opportunities for episodes of overlapping distributions since their inception as separate species, approximately 2.7 million years ago (Neumann et al. 2006).

We conducted two experiments to determine how much exposure to a heterospecific male is necessary for Syrian hamster females to avoid interspecific mating (Experiment 1) and to

determine whether females that were initially exposed to a heterospecific male still discriminate against that male after a long period of no exposure to heterospecific stimuli (Experiment 2). In Experiment 1, females were exposed to a heterospecific male across a wire-mesh partition for 1, 4 h, 4 or 8 days and then paired with the same heterospecific male. We measured sexual receptivity and aggression towards the heterospecific male. We predicted that longer exposures would lead to higher degrees of avoidance against the heterospecific male. In Experiment 2, female subjects were first exposed to a heterospecific male across a wire-mesh partition for 8 days. Then, these females were paired with the familiar heterospecific male after either 10 min of separation or 8 days of separation. We predicted that females would avoid mating with the heterospecific male even after a period without exposure to the heterospecific male.

We also compared the results of the present study (in which females were exposed for 8 days only to a heterospecific male) to those of our previous study, in which females were concurrently exposed for 8 days to a conspecific male and a heterospecific male (delBarco-Trillo et al. 2010). Liley (1966) proposed that in order to learn to avoid interspecific mating, an individual must be simultaneously exposed to both conspecifics and heterospecifics (i.e., as in a sympatric context). Alternatively, exposure to a heterospecific male could be sufficient for females to learn to avoid interspecific mating.

Methods

Animals

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 × 24 × 14.5 cm) with sani-chip bedding material and constant access to water and food (Prolab 1000, Agway, Syracuse, NY). Turkish and Syrian hamsters were maintained in separate rooms with independent air intake and exhaust. Turkish hamsters were maintained on a 16L:8D light–dark schedule with lights off between 10:00 and 18:00 h (Eastern Standard Time). Syrian hamsters were maintained on a 14L:10D 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 one meter from the cage. To avoid family effects, no siblings (of either sex) were used in the same group.

To determine that 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. Given the regular, 4-day estrous cycle in Syrian hamsters (Lisk 1985), once the day of estrus was determined, we could always determine the subsequent estrous

days. In this study, females were always tested 4 days after determining the timing of their estrous cycles (i.e., during their next estrous day).

Experiment 1. Different exposure times

We used arenas ($50 \times 38.1 \times 21.5$ cm; Allentown, model# PCT4SHT) divided into two, similarly-sized sections ($24.85 \times 38.1 \times 21.5$ cm) by a wire-mesh partition with 1-cm^2 openings. This type of barrier is sufficient for hamsters to learn the multi-odor individual identity of other conspecifics (Johnston and Peng 2008). We started each test by placing one Turkish hamster male in one of the two sections and then placed a Syrian hamster female in the other section. The female could perceive and interact in a limited manner with the male through 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 either 1 h ($N = 9$), 4 h ($N = 11$), 4 days ($N = 9$), or 8 days ($N = 10$). The mismatched sample sizes across treatments were unintentional and due to a counting error. Arenas were placed in a room different than the colony and testing rooms; the light cycle in this room was the same as in the rooms in which the females were housed (i.e., 14L:10D light–dark). After the exposure treatment (1, 4 h, 4, or 8 days), the female and the male were transferred to individual cages lined with clean bedding and both animals 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. We videotaped all trials. We 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. If the female was aggressive and not receptive to the heterospecific male, the female was immediately paired with an unfamiliar conspecific male for 5 min to determine that the female was indeed in estrus. Such estrus-checking trials were not used for any statistical analyses.

Experiment 2. Delaying the time between exposure and testing

In this experiment, after a female and a heterospecific male had lived in the adjacent sections of the arena for 8 days, the female and the male were separated and transferred to individual cages lined with clean bedding. Females were tested with the familiar heterospecific male after 10 min of separation ('10-min delay group'; $N = 10$) or 8 days of separation ('8-day delay group'; $N = 10$). The 10-min delay group was the 8-day group in Experiment 1. We interspersed testing of the 10-min delay and 8-day delay groups to avoid any order effects. In the '8-day delay group' ($N = 10$), after the male and the female were transferred to individual cages, they were then returned to their original colony rooms. Eight days later, the two animals were moved to the testing room. After 10 min in the testing room, the male was transferred to the female's cage in a clean, 500-ml plastic beaker. That is, there was a delay of 8 days from the time that the female and the male were separated to the time that they were tested together. Trials were videotaped and scored as indicated in Experiment 1.

In Experiment 1 and Experiment 2 we did not use a control in which a female Syrian hamster was housed with a conspecific male and then paired with that same conspecific male. We considered that the additional number of animals required for such controls was not warranted because in a previous study (delBarco-Trillo et al. 2010) we housed female Syrian hamsters next to two conspecific males for 8 days and then sequentially paired the female with both conspecific males; in all pairings the female was as sexually receptive and not aggressive as when a receptive female is paired with an unfamiliar conspecific male. We did not have any reason to expect a different response by females when housed with only one conspecific male.

Statistics

In Experiment 1 we used an ANOVA to determine if any of the variables (lordosis latency, lordosis duration, aggression latency, and aggression duration) differed among the four groups (1-hour, 4-hours, 4-days, and 8-days of exposure). If so, we used Tukey pairwise comparisons for the post-hoc tests.

In Experiment 2 we used independent *t*-tests to determine if any of the variables (lordosis latency, lordosis duration, aggression latency, and aggression duration) differed between the two groups (10-min delay and 8-day delay). We used the Levene's test for equality of variances; when equal variances were not assumed, we report the estimated degrees of freedom.

We tested the assumption of normality using the Kolmogorov–Smirnov test. When necessary, we log transformed data to meet normality. In both experiments values are shown as means $\pm$ SEM. We used SPSS 14 for Windows for all statistical analyses.

Results

Experiment 1. Different exposure times

Lordosis latency was greater with longer durations of exposure of females to heterospecific males (1 h: 67.89 ± 30.71 s; 4 h: 68.64 ± 24.9 s; 4 days: 186.33 ± 39.89 s; 8 days: 225.3 ± 38.09 s; $F_{3,38} = 6.98$, $P = 0.001$). Significant differences were found in 1-hour vs. 4-day groups ($P = 0.028$), 1-hour vs. 8-day groups ($P = 0.007$), 4-hour vs. 4-day groups ($P = 0.032$), and 4-hour vs. 8-day groups ($P = 0.007$) using Tukey pairwise comparisons.

The duration of lordosis towards a heterospecific male was significantly longer after 1 or 4 h of exposure to heterospecific males than it was in females exposed to heterospecific males for 4 or 8 days ($F_{3,38} = 5.94$, $P = 0.002$; Fig. 1a). The duration of lordosis in naïve females (i.e., females that were never exposed to heterospecific males before testing) measured in previous studies (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010) was similar to the duration of lordosis in females in the 1-hour group ($t_{9,8} = 1.47$, $P = 0.17$) and the 4-hour group ($t_{11,8} = 1.66$, $P = 0.12$; Fig. 1a); but higher compared to females in the 4-day and 8-day groups ($P < 0.05$; Fig. 1a).

Copulation within the 5 min trials occurred in 55.6% of the trials (5 out of 9) in the 1-hour group, in 54.6% of the trials (6 out of 11) in the 4-hour group, in 11.1% of the trials (1 out of

9) in the 4-day group, and in 10% of the trials (1 out of 10) in the 8-day group. These proportions were not significantly different between groups (two-tailed Fisher's exact test: $P > 0.05$). No lordosis during the 5 min trials was observed in 11.1% of the trials (1 out of 9) in the 1-hour group, in 9.1% of the trials (1 out of 11) in the 4-hour group, in 33.3% of the trials (3 out of 9) in the 4-day group, and in 70% of the trials (7 out of 10) in the 8-day group. Such absence of lordosis differed statistically between the 8-day group and both the 1-hour group (two-tailed Fisher's exact test: $P = 0.02$) and the 4-hour group ($P = 0.008$). In all of the trials in which the female did not exhibit lordosis towards the heterospecific male, the female was highly receptive when she was paired immediately afterwards with an unfamiliar conspecific male (274.6 ± 3.15 s of lordosis out of 300 s).

The latency to engage in aggressive behavior by female Syrian hamsters towards male Turkish hamsters did not significantly differ in the 1-hour (175 ± 43.67 s), 4-hour (160.73 ± 39.06 s), 4-day (72 ± 33.1 s), or 8-day groups (115.9 ± 38.74 s; $F_{3,38} = 1.37$, $P = 0.27$). The duration of aggression increased progressively in the 1-hour (29.16 ± 13.67 s), 4-hour (35.81 ± 13.95 s), 4-day (63.93 ± 18.46 s), and 8-day groups (79.84 ± 31 s) but there were no significant differences among the four groups ($F_{3,38} = 1.33$, $P = 0.28$; Fig. 1b). All these durations were significantly higher than those of naïve females in previous studies (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010; Mann–Whitney tests: $P < 0.05$; Fig. 1b).

Experiment 2. Delaying the time between exposure and testing

Lordosis latency was significantly shorter in the group with a 8-day delay between exposure to the heterospecific male and pairing with that male (67.5 ± 26.8 s; $t_{18} = 3.39$, $P = 0.003$) than in the group with a 10-min delay (225.3 ± 38.09 s). The duration of lordosis was significantly longer in the 8-day delay group (169.4 ± 30.24 s; $t_{18} = -3.35$, $P = 0.004$; Fig. 2a) than in the 10-min delay group (40.3 ± 23.98 s). The duration of lordosis in naïve females measured in previous studies (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010) was similar than for females in the 8-day delay group ($t_{10,9} = 1.15$, $P = 0.28$; Fig. 2a).

Copulation within the 5 min trials occurred in 10% of the trials (1 out of 10 trials) in the group with a 10-min delay between exposure to the heterospecific male and pairing with that male whereas it occurred in 70% of the trials (7 out of 10) in the 8-day delay group (two-tailed Fisher's exact test: $P = 0.02$). No lordosis occurred in 10% of the trials in the 8-day delay group compared to 70% of the trials in the 10-min delay group ($P = 0.02$). In the only trial in the 8-day delay group in which the female did not show lordosis towards the heterospecific male, the female was highly receptive when she was immediately paired with an unfamiliar conspecific male (256 s of lordosis out of 300 s).

The latency to fight did not differ significantly between the 10-min delay group (115.9 ± 38.73 s) and the 8-day delay group (189.2 ± 45.32 s; $t_{18} = -1.23$, $P = 0.24$). The duration of aggression was shorter in the 8-day delay group (17.26 ± 9.42 s) than in the 10-min delay group (79.84 ± 31 s), but this difference was not significantly different ($t_{10,7} = 1.93$, $P = 0.08$; Fig. 2b). The duration of aggression in both groups was significantly higher than for

naïve females in previous studies (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010; Mann–Whitney tests: $P < 0.05$; Fig. 2b).

Discussion

Our results indicate that, in female Syrian hamsters, a longer exposure to a heterospecific male leads to a greater avoidance of interspecific mating (Experiment 1). Furthermore, avoidance of interspecific mating can be lost if females are not periodically exposed to stimuli from heterospecific males (Experiment 2). These experiments reinforce the results of a previous study in which we showed that exposure to heterospecific males during adulthood can influence subsequent female behavior towards such males (delBarco-Trillo et al. 2010).

Our results agree with an evolutionary scenario in which the two species have had (a) few interactions during their evolutionary history, and consequently few opportunities for a faster and more resilient learned response to evolve, and/or (b) a relatively long period of isolation since their last episode of overlapping distributions, resulting in a reduced efficiency of that response (i.e., long time to start and reduction of the response when the heterospecific stimuli disappears).

An alternative explanation is that evolution has not favored the learned response that we suggest, and that instead females respond negatively to the abnormal behavior of the heterospecific male. However, if this was the case, we should not have seen any significant differences among groups (since heterospecific males appeared to behave similarly in all groups). We can also rule out the possibility that females will become unreceptive toward any male after a long exposure to him because in a previous study females exposed to conspecific males (either kin, non-kin, losers or winners) for 8 days were sexually receptive to them (delBarco-Trillo et al. 2010).

Experiments using pairs of closely-related *Drosophila* species have shown that male flies exposed for 1 h to a heterospecific female later did not court other heterospecific females (Dukas 2004, 2008). Interestingly, in another study in which the exposure phase was only 10 min, male fruit flies did not learn to avoid heterospecific females (Kandul et al. 2006), even though there were several methodological differences between these studies. The only other vertebrate system in which learning to avoid interspecific mating during adulthood has been investigated was carried out with guppies (*Poecilia* spp.) and these studies indicate that exposure to heterospecifics during several days is required for accurate avoidance of interspecific mating (Haskins and Haskins 1949; Liley 1966; Magurran and Ramnarine 2004). For example, *Poecilia reticulata* males will show courtship towards both conspecific and heterospecific (*P. picta*) females at random in allopatric populations (Magurran and Ramnarine 2004). Up to 3 days of exposure to heterospecific females does not have any significant effect on males, but after 4–6 days of exposure to heterospecific females, males learn to avoid courting such females (Magurran and Ramnarine 2004). In contrast to our study, female guppies seem unable to learn to avoid interspecific mating, and behave similarly towards conspecific and heterospecific males even after several days of exposure to heterospecific males (Haskins and Haskins 1949).

We predicted that female hamsters that had been exposed to a heterospecific male for 8 days would discriminate against this heterospecific male even after a period without exposure to the heterospecific male. In contrast, we found that the learned avoidance of interspecific mating requires maintained exposure to heterospecific males. A significant period of time without such exposure (8 days in our study) resulted in the duration of lordosis being similar to that shown by females that had never been exposed to heterospecific males. These results can be interpreted in two ways. First, females exposed to heterospecific males may be forming a short-term discriminative memory for heterospecific males. As long as females are exposed to heterospecific males, that type of memory can be sustained. However, if heterospecific stimuli are not available to females for some duration of time (8 days in this study), memory for such stimuli can be lost. Syrian hamsters can retain memories of conspecific individuals at least for 10 days (Johnston 1993); however, we do not know if memories about heterospecific individuals are retained that long or for a shorter amount of time. Alternatively, exposure to a heterospecific male may change the physiological state of the female, for example by altering concentrations of hormones in blood or neurotransmitters and receptors in the brain that modulate behavior (Mucignat-Caretta et al. 2006). After 8 days without any heterospecific stimuli, physiological levels of the relevant substances may return to baseline levels so that females behave again as naïve females do, that is, responding similarly towards conspecific and heterospecific males. Further research on the physiological mechanisms underlying the results obtained in this study will be important for advancing our understanding of avoidance of interspecific mating.

Our results indicate that lordosis and aggressive behaviors directed towards heterospecific males can be independent of one another. For example, lordosis duration in females after 1 or 4 h of exposure did not differ compared to females without any previous exposure to heterospecific males (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010; Fig. 1a). In contrast, aggression was elicited in females after 1 or 4 h of exposure to a heterospecific male compared to naïve females (which do not show any signs of aggression towards heterospecific males; Fig. 1b). That is, short exposures to heterospecific males are sufficient to elicit aggression in females but such exposures may not influence lordosis behavior.

Liley (1966) proposed that in order to learn to avoid interspecific mating, an individual must be simultaneously exposed to both conspecifics and heterospecifics. In a previous study we showed that female Syrian hamsters that were exposed to both a conspecific male and a heterospecific male for 8 days learned to avoid interspecific mating (delBarco-Trillo et al. 2010). However, we did not show whether exposure to a heterospecific male could be sufficient for females to learn to avoid interspecific mating. In the present study, females were exposed only to a heterospecific male and we also found that 8 days of exposure significantly reduced lordosis behavior and increased aggressive behavior towards such males. Thus these results show that females do not discriminate against heterospecific males by making a comparison between conspecific males and heterospecific males. Instead, it is the exposure to the stimuli from heterospecific males that results in females learning to avoid interspecific mating.

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Fig. 1.

Duration of lordosis (a) and aggression (b) by female Syrian hamsters (*Mesocricetus auratus*) after different durations of exposure to a heterospecific male (Turkish hamster, *M. brandti*): 1, 4 h, 4, or 8 days. As a reference, the *dashed lines* indicate the average duration of lordosis and aggression shown in previous studies by naïve females, i.e., females that were never exposed to heterospecific males before testing (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010). *Different letters* denote a statistically significant difference. Values represent mean $\pm$ SEM

Fig. 2.

Duration of lordosis (a) and aggression (b) by female Syrian hamsters (*Mesocricetus auratus*) which, after being exposed to a heterospecific male (Turkish hamster, *M. brandti*) for 8 days, were then tested with that male 10 min later or 8 days later. As a reference, the *dashed lines* indicate the average duration of lordosis and aggression shown in previous studies by naïve females, i.e., females that were never exposed to heterospecific males before testing (delBarco-Trillo et al. 2009b; delBarco-Trillo and Johnston 2010). *Different letters* denote a statistically significant difference. Values represent mean $\pm$ SEM