Published in final edited form as:

Behav Neurosci. 2009 August ; 123(4): 758–763. doi:10.1037/a0016439.

Medial amygdala involvement in discrimination of same-species and closely-related-species male stimuli in estrous female *Mesocricetus* hamsters

Javier delBarco-Trillo, Kara Gulewicz, and Robert E. Johnston

Department of Psychology, Cornell University, Uris Hall, Ithaca, NY, 14853

Abstract

Efficient discrimination between individuals of closely related species is important to maximize reproductive potential. Some studies using males as subjects indicate that the medial amygdala (MeA) is involved in discrimination between odors of conspecific females and females from distantly-related species. We investigated the involvement of the MeA in discrimination by females between odors of conspecific males and odors of males of a closely-related species. We exposed estrous or diestrous females (*Mesocricetus auratus*) to saline, conspecific male odors, or heterospecific (*M. brandti*) male odors, and quantified the expression of FRAs (fos-related antigens) in the anterior and posterior MeA. We found that estrous (but not diestrous) females investigated conspecific male odors longer than heterospecific male odors. Neural activity in both the anterior and posterior MeA was higher in estrous than in diestrous females. In the anterior MeA, there were no significant differences in response to odors of conspecific and heterospecific males. In the posterior MeA, however, neural activity was higher when estrous females were exposed to conspecific odors than when they were exposed to heterospecific odors. No such difference was observed in diestrous females.

Keywords

Mesocricetus; species discrimination; early-immediate genes; medial amygdala; chemical signal

Introduction

Efficient species discrimination is important to maximize reproduction. The reproductive success of females decreases significantly if they mate with males of another species (heterospecific males) because such matings nearly always produce no or infertile offspring. Consequently, females should be able to discriminate between conspecific (same species) and heterospecific males and show a behavioral preference for conspecific males. For example, in *Mesocricetus spp.* hamsters, females exposed at the same time to a conspecific male and a closely-related heterospecific male show a preference for the conspecific male (Murphy, 1977). When estrous Turkish hamsters (*M. brandti*) were paired with either a conspecific or a heterospecific male, they displayed lordosis towards the conspecific male but attacked the heterospecific male (Murphy, 1978). These results indicate a clear, dichotomous response towards conspecific and heterospecific males. Such a behavioral preference for conspecific

Publisher's Disclaimer: The following manuscript is the final accepted manuscript. It has not been subjected to the final copyediting, fact-checking, and proofreading required for formal publication. It is not the definitive, publisher-authenticated version. The American Psychological Association and its Council of Editors disclaim any responsibility or liabilities for errors or omissions of this manuscript version, any version derived from this manuscript by NIH, or other third parties. The published version is available at www.apa.org/journals/bne.

males is especially important when females are in estrus, since mating with a heterospecific male may decrease a female's reproductive success (Johnston, 1983). When females are not in estrus, a lack of discrimination between conspecific and heterospecific males may not carry reproductive costs. Consequently, a preference between conspecific and heterospecific males may not be observed in non-estrous females (Johnston, 1983).

Given the importance of chemical communication in most mammals, especially in rodents, females may use male odors to discriminate between conspecific and heterospecific males (Johnston, 1983). Several studies have shown that female rodents in estrus prefer odors of conspecific males over those of heterospecific males but that when females are not in estrus there is no preference for odors of conspecific males (Johnston, 1983; Johnston & delBarco-Trillo, in press). Examples include the mouse *Peromyscus maniculatus* (Doty, 1972), and the Syrian hamster *Mesocricetus auratus* (delBarco-Trillo, Gulewicz, Segal, McPhee, & Johnston, in press; Johnston & Brenner, 1982). In these studies, heterospecific males belonged to a closely-related species of the same genus. Even more precise discriminations have been reported in several species when subjects were tested with individuals from two populations of the same species, e.g. in the mole rat *Spalax ehrenbergi* (Nevo, Bodmer, & Heth, 1976), the striped mouse *Rhabdomys pumilio* (Pillay, 2004), and in the murid rodent *Graomys griseoflavus* (Theiler & Blanco, 1996). In all of these species, estrous females preferred odors of males of the same population over odors from males of a different population, whereas diestrous females showed no preference.

An area of research that has not received much attention is the neural basis of species discrimination among mammals. In one study, male Syrian hamsters were exposed to vaginal secretions of conspecific females or vaginal secretions of female Djungarian hamsters (*Phodopus campbelli*) (Fiber, Adames, & Swann, 1993). Fos-immunoreactivity in the anterior medial amygdala (MeA) was similar in response to odors of both types of female. However, neural activity in the posterior MeA was higher in males in response to vaginal secretions of conspecific females than to vaginal secretions of Djungarian hamster females (Fiber *et al.*, 1993). There were no differences in response to the two stimuli in the olfactory bulbs, suggesting that the discrimination between the two types of stimuli occurred in more central areas such as the MeA (Fiber *et al.*, 1993). In a similar study, male Syrian hamsters were exposed to vaginal secretions of conspecific females, flank-gland secretions of conspecific males and females, male and female mouse urine, or urine from castrated male cats (Meredith & Westberry, 2004). Both conspecific and heterospecific stimuli similarly increased Fos activity in the accessory olfactory bulb, but a difference in response was found at the level of the amygdala. The anterior MeA was activated by both conspecific and heterospecific stimuli, but the posterior MeA was only significantly activated by conspecific stimuli (both male and female) (Meredith & Westberry, 2004). This laboratory conducted a similar study exposing male mice to conspecific (male and female urine) and heterospecific (hamster vaginal secretion, steer urine, and cat collar) stimuli (Samuelsen & Meredith, 2009). They supported their previous findings, i.e., activation in the MeA occurred in response to both conspecific and heterospecific stimuli, whereas the MeP was activated over control values only in response to biologically relevant stimuli (conspecific odors, and cat collar wore for 2 weeks but not for 12 hours) (Samuelsen & Meredith, 2009). In another study, the nose of female prairie voles (*Microtus ochrogaster*) was painted with urine of a conspecific male or urine of a male mouse (Tubbiola & Wysocki, 1997). Fos immunoreactivity in the accessory olfactory bulb was higher in female voles exposed to conspecific urine than in females exposed to mouse urine or water (Tubbiola & Wysocki, 1997). In this study, more central areas such as the MeA were not investigated (Tubbiola & Wysocki, 1997).

Overall, most of the studies mentioned above (Fiber *et al.*, 1993; Meredith & Westberry, 2004; Samuelsen & Meredith, 2009) point to the involvement of the MeA in the discrimination

between conspecific and heterospecific individuals (or between biologically relevant and irrelevant individuals). We wanted to investigate further this discriminatory involvement of the MeA, considering two new aspects. First, we wanted to determine whether the MeA plays the same role in females than in males. Previous studies have mostly used males as subjects (Fiber *et al.*, 1993; Meredith & Westberry, 2004; Samuelsen & Meredith, 2009), so it is not clear whether the MeA plays the same role in males and females. Females are normally the sex involved in mate choice (Trivers, 1972), so given that previous studies have shown a discriminatory role of the MeA in males, we expected a similar role in females. An interesting aspect in estrous cycling females is that discrimination between conspecific and heterospecific individuals may differ in different phases of the estrous cycle. That is, females may be more discriminative when they are sexually receptive (estrus) than when they are not sexually receptive. Second, subjects and donors in previous studies did not belong to closely-related species (Fiber *et al.*, 1993; Meredith & Westberry, 2004; Samuelsen & Meredith, 2009; Tubbiola & Wysocki, 1997). We wanted to determine whether the discriminatory role of the MeA already observed between conspecific and distantly-related heterospecific individuals also applies to the discrimination between conspecific and closely-related heterospecific individuals.

We thus examined in this study the hypothesis that neural activity in the MeA in females exposed to male stimuli differs across different phases of the estrous cycle and that such activity also depends on whether the male stimuli come from conspecific or closely-related heterospecific males. More specifically, we tested the prediction that the anterior MeA is activated similarly in females in response to conspecific and heterospecific male odors, whereas the posterior MeA is activated significantly more when estrous females (but not necessarily diestrous females) are exposed to odors of conspecific males than when they are exposed to odors of heterospecific males. In order to test this prediction, we exposed female Syrian hamsters in different stages of the estrous cycle (estrus and diestrus-2) to odors of conspecific and heterospecific males, using odors of male Turkish hamsters as the heterospecific stimuli. We then measured immediate early-gene activity (FRAs, fos-related antigens) in the anterior and posterior MeA.

Materials & Methods

Animals

We used 82 female Syrian hamsters as subjects: 46 were tested only in behavioral experiments (363.5 ± 7.5 days of age), 16 were used to obtain neural data but were not tested behaviorally (295.6 ± 17.35 age), and 20 were tested behaviorally and were also perfused to obtain neural data (373.4 ± 6.8 days). Sample sizes were $n = 11$ per group for the behavioral data, and $n = 6$ per group for the neural data (except in the estrus-conspecific group, $n = 5$). We used both sexually naïve and sexually experienced females, but data were not significantly affected by sexual experience. Female subjects were not related to, or previously exposed to, the odor donors.

All hamsters were born in our laboratory colony, weaned at 30 days, and housed individually after weaning. They were maintained on a reversed 14:10 light:dark cycle. Food and water were available *ad libitum*.

Female hamsters have a very consistent 4-day estrous cycle (Carter, 1985; Lisk, 1985; Orsini, 1961). To monitor the estrous state of a female, a male was placed in the female's cage and her back was rubbed longitudinally. If lordosis was elicited, the female was categorized as being in estrus. Once the estrous state of a female was determined, she was tested two or six days later (when she was in diestrus-2), or four days later (when she was again in estrus).

Testing Procedure

Female hamsters were tested during the middle 4 hours of the dark portion of their light:dark cycle. We tested females in one of two estrous states (estrus or diestrus-2). For all females, a glass plate (17.8 × 7.6 cm) containing a single stimulus odor was clipped vertically on the short side of the female's cage, flush against the wall. The stimulus odor was (1) flank gland secretions of a male golden hamster (conspecific odor), (2) flank gland secretions of a male Turkish hamster (heterospecific odor), or (3) saline (control odor). To obtain flank gland secretions, each flank gland of a male golden or a male Turkish hamster was rubbed 15 times (i.e., 30 marks per animal) in a circular motion against the glass plate immediately prior to a test. As for the saline, a cotton swab was submerged in a saline container, dried until the swab was not saturated, and then rubbed 30 times against the glass plate. All stimulus odors were rubbed onto the same location on the glass plate, 2 cm above the end closest to the ground of the female's cage. The glass plate was left in the female's cage for ten minutes after the female first investigated the plate. We measured the amount of time that the female investigated the stimulus odor during that 10-min period. The criteria for "investigation" were sniffing the glass plate within 2 cm, or licking or biting the glass plate (all subjects contacted the scented area on the glass plate). After removal of the glass plate, females were left undisturbed for 40 minutes in their home cage.

Tissue processing and immunohistochemistry

Forty minutes after removing the glass plate, a subset of female subjects was sacrificed and perfused with phosphate buffered saline (PBS), followed by 4% paraformaldehyde. Brains were postfixed in fresh 4% paraformaldehyde for 1 h and then transferred to 30% sucrose solution for 1–2 days. Using a sliding microtome, 40- μ m coronal sections were obtained from a frozen brain starting from the rostral end. The first of every set of three sections was used for FRA (Fos-related antigen) immunolabeling (1:1000 rabbit polyclonal antiserum, SC-253, Santa Cruz Biotechnology, CA) (Meredith & Westberry, 2004). After washing in KPBS and incubating in 20% normal goat serum (Vector Laboratories, CA), sections were incubated in primary antibody solution for 65 h on a shaker at 4–8°C. The free-floating sections were then labeled using biotinylated goat antirabbit secondary antibody (1:500, Vector Laboratories) for 1 h at room temperature and then incubated in avidin–biotin complex (ABC kit, Vector Laboratories) for 1 h at room temperature. Labeling was visualized using DAB (SIGMAFAST™, Sigma) with 0.5 M nickel chloride for 5 min. The stained sections were mounted on gelatin-coated slides, air-dried overnight and coverslipped (using increasing concentrations of alcohol and then 5 drops of HistoClear (National Diagnostics)).

Brain areas of interest were located under light microscopy using the golden hamster brain atlas (Morin & Wood, 2001) and local landmarks in each brain section. The brain areas that we analyzed were the anterior and the posterior MeA (Meredith & Westberry, 2004). These brain areas were digitally photographed (Spot RT Camera, Diagnostic Instruments, Inc.) at 200× magnification (Nikon E800 microscope). We used the public domain NIH Image program (V1.61, US National Institutes of Health; available at <http://rsb.info.nih.gov/nih-image/>) for cell counting. For each brain area, we selected a standard area and manually counted all the immunoreactive cells in that area as shown in the digital photographs. We counted all cells that were clearly stained over the background staining level. For each brain area, we averaged the counting of two sections 120 μ m apart. For each section, we counted only one side, determined at random. The same criteria were used for all six conditions, so statistical differences among those conditions cannot be attributed to a counting bias. Immunoreactivity values are given as stained cells / μ m².

Statistical analysis

We used three separate GLMs (General Linear Model), one for the behavioral data, one for the anterior MeA data, and a third one for the posterior MeA data. For the behavioral data, the dependent variable was time (in seconds) that the female spent investigating the glass plate. For the neural data, the dependent variable was the density of stained neurons (cells / μm^2). For each of these three GLMs, the two factors were the female's estrous state (estrus or diestrus-2), and the stimulus odor (conspecific, heterospecific, or saline). For pairwise comparisons we used Tukey post-hoc tests for the behavioral data and nonparametric tests for the neural data (due to smaller samples). We used SPSS 14 for Windows for statistical analyses. All values are shown as mean $\pm$ sem.

Results

The time that females investigated the stimulus was affected by estrous state ($F_{1,60} = 31.43$, $p < 0.0005$) and type of stimulus ($F_{2,60} = 26.36$, $p < 0.0005$). When considering all three stimuli together, estrous females showed greater interest in odors of males than diestrous females did (Fig. 1). We also found a significant interaction between estrous state and type of stimulus ($F_{2,60} = 9.33$, $p < 0.0005$). When considering separately diestrous and estrous females, we found significant differences in the time spent investigating the three stimuli for both the diestrous females ($F_{2,30} = 9.08$, $p = 0.001$) and the estrous females ($F_{2,30} = 20.47$, $p < 0.0005$). However, pairwise comparisons differed for diestrous and estrous females. Diestrous females investigated conspecific and heterospecific odors more than saline ($p < 0.018$), but there was no significant difference in investigation of the conspecific and heterospecific odors ($p = 0.45$; Fig. 1, left side). Estrous females also investigated conspecific and heterospecific odors more than saline ($p < 0.0005$), but, in addition, estrous females investigated conspecific odors more than heterospecific odors ($p = 0.026$; Fig. 1, right side).

In the anterior MeA there were no significant differences in the density of stained cells in response to the different odor stimuli ($F_{2,29} = 0.99$, $p = 0.39$; Fig. 2 and 3a). However, the reproductive state of the female did influence neural activity in the anterior MeA in that there was a greater density of stained cells in this area when females were in estrus than when they were in diestrus ($F_{1,29} = 9.38$, $p = 0.005$; Fig. 3a, left side vs right side).

In the posterior MeA we also found that activity was higher in estrous females than in diestrous females ($F_{1,29} = 6.31$, $p = 0.018$; Fig 3b). In addition, we found a significant effect of type of male stimulus on brain activity in the posterior MeA ($F_{2,29} = 3.53$, $p = 0.042$). Immunoreactivity was higher when estrous females were exposed to conspecific male odors than when they were exposed to heterospecific male odors (Mann Whitney U test, $Z = -2.01$, $p = 0.045$). Indeed, staining to heterospecific male odors was no different than in the saline control condition.

Discussion

While diestrous females did not show a preference for flank gland odors of either conspecific or heterospecific males, estrous females did show a preference for conspecific flank-gland odors over those of heterospecific males when presented with one or the other. The lack of a preference for conspecific male odors in diestrous females is consistent with prior studies and indicates the reduced importance of identifying the species of a male when females are not sexually receptive. Functionally, the lack of a significant difference in response by diestrous females may be due to a low interest in male stimuli. That is, diestrous females may be capable of discriminating between conspecific and heterospecific males, but their low interest in males or male stimuli may result in no significant preference.

In contrast, the preference of estrous females for conspecific male flank-gland secretion over that for heterospecific male flank-gland secretion indicates that this type of odor alone carries cues sufficient for differentiating between conspecific and heterospecific individuals.

Our results agree with previous studies (Fiber *et al.*, 1993; Meredith & Westberry, 2004; Mucignat-Caretta *et al.*, 2006; Samuelsen & Meredith, 2009) that have shown a role for the central projection targets of the vomeronasal chemosensory system in the discrimination of stimuli from different species. It has been shown that main olfactory input is not necessary for discrimination of stimuli from different species, since the categorical discrimination in the posterior MeA is similar in anosmic and sham-operated male hamsters (Meredith & Westberry, 2004). The role of the accessory olfactory bulb as a sorting area between conspecific and heterospecific stimuli seems to differ in different species. In Syrian hamsters, c-fos immunoreactivity in the mitral and granule cell layers of the accessory olfactory bulb was similar in response to conspecific and heterospecific stimuli (Fiber *et al.*, 1993). However, in prairie voles, activity in the accessory olfactory bulb was much higher in response to conspecific odors than to heterospecific odors (Tubbiola & Wysocki, 1997). It would be valuable to have more species to compare in order to determine what factors may explain such species differences.

Our study offers two important contributions to the study of the neural mechanisms underlying discrimination of stimuli from different species. First, we used females as subjects and found that female reproductive state had an important influence on behavioral and neural responses to conspecific versus heterospecific males. We found that activity in both the anterior and the posterior MeA was higher in estrous females than in diestrous females. This may be due to the fact that estrous females showed higher levels of odor investigation (similarly, the higher activity in the posterior MeA in response to conspecific odors could be related to longer investigation of conspecific odors by estrous females). Differences in activity between estrous and diestrous females may also be due to inherent differences in excitability or morphology of the MeA across the estrous cycle. In female rats, for example, the anteroventral MeA has a larger volume and contains more neurons in estrous females than in diestrous females, indicating that the MeA is affected by estrous state (Carrillo, Pinos, Guillaumon, Panzica, & Collado, 2007). Differences in neural activity in the MeA between estrous and diestrous females may be due to the hormonal changes that occur across the estrous cycle (Carter, 1985; Leavitt & Blaha, 1970). The effect of different hormonal milieus may be especially important in the posterior MeA, which is affected by gonadal hormone inputs (Gomez & Newman, 1992). In another study where females were used as subjects, *in vivo* neurotransmitter release in the posteromedial cortical nucleus of the amygdala (PMCo) was monitored at different times after female rats were exposed to odors of either a male rat or a male mouse (Mucignat-Caretta *et al.*, 2006). The accessory olfactory bulb projects to the PMCo. The release of glutamate, aspartate, GABA and taurine soon after exposure to the stimuli did not differ depending on the stimulus type. However, after about two hours, only conspecific odor induced a significant net release of glutamate and aspartate (Mucignat-Caretta *et al.*, 2006). Such a delayed differential response to conspecific and heterospecific stimuli may be involved in long-term processes such as learning but not in the immediate investigation of or the attraction toward different types of males or of stimuli from males.

Another important aspect of our study is that for the heterospecific stimuli we used a closely-related species belonging to the same genus. Previous studies that investigated responses to stimuli from heterospecific animals used much more distantly related species (Fiber *et al.*, 1993; Meredith, Samuelsen, Blake, & Westberry, 2008; Meredith & Westberry, 2004; Samuelsen & Meredith, 2009). However, using distantly related species, these previous studies obtained results similar to ours, i.e., activity of the posterior MeA was higher in response to biologically relevant odors than in response to non-biologically relevant odors. This general

agreement across studies indicates that a function of the medial amygdala may be to categorize the relative biologically relevance of different odors (Samuelsen & Meredith, 2009).

The differential activation to conspecific versus heterospecific odors in the posterior MeA may be due to GABA inhibition from the caudal intercalated nucleus upon exposure to heterospecific odors (Meredith *et al.*, 2008; Meredith & Westberry, 2004). This inhibitory mechanism may cause a significant decrease in neural activity in the posterior MeA compared to the anterior MeA in response to heterospecific stimuli. Other areas that may be involved in species discrimination are the bed nucleus of the stria terminalis and the medial preoptic area (Fiber *et al.*, 1993). These two areas, together with the posterior MeA, had higher fos-immunoreactivity when male golden hamsters were exposed to conspecific female odors than when they were exposed to Djungarian hamster female odors (Fiber *et al.*, 1993). Especially relevant may be the hypothalamic areas to which the posterior MeA projects (Choi *et al.*, 2005; Gomez & Newman, 1992). The discrimination between conspecific and heterospecific stimuli in the posterior MeA may ultimately result in differential activity in those hypothalamic nuclei (Meredith *et al.*, 2008).

Acknowledgments

This work was supported by NIMH grant NIMH 5 R01 MHO58001-09 and NSF grant IBN-0318073 to R. E. Johnston. The experiments here described comply with the current laws of the USA. All research was conducted with approval from Cornell University's Institutional Animal Care and Use Committee (protocol #1993-0120).

References

- Carrillo B, Pinos H, Guillamon A, Panzica G, Collado P. Morphometrical and neurochemical changes in the anteroventral subdivision of the rat medial amygdala during estrous cycle. *Brain Research* 2007;1150:83–93. [PubMed: 17425950]
- Carter, CS. Female sexual behavior. In: Siegel, HL., editor. *The Hamster: Reproduction and Behavior*. New York: Plenum Press; 1985. p. 173–189.
- Choi GB, Dong HW, Murphy AJ, Valenzuela DM, Yancopoulos GD, Swanson LW, Anderson DJ. Lhx6 delineates a pathway mediating innate reproductive behaviors from the amygdala to the hypothalamus. *Neuron* 2005;46:647–660. [PubMed: 15944132]
- delBarco-Trillo J, Gulewicz K, Segal A, McPhee ME, Johnston RE. Can captivity lead to inter-species mating in two *Mesocricetus* hamster species? *Journal of Zoology*. in press
- Doty RL. Odor preferences of female *Peromyscus maniculatus bairdi* for male mouse odors of *P. m. bairdi* and *P. leucopus noveboracensis* as a function of estrous state. *Journal of Comparative and Physiological Psychology* 1972;81:191–197. [PubMed: 4673475]
- Fiber JM, Adames P, Swann JM. Pheromones induce c-fos in limbic areas regulating male hamster mating behavior. *Neuroreport* 1993;4:871–874. [PubMed: 8369476]
- Gomez DM, Newman SW. Differential projections of the anterior and posterior regions of the medial amygdaloid nucleus in the Syrian hamster. *Journal of Comparative Neurology* 1992;317:195–218. [PubMed: 1573064]
- Johnston, RE. Chemical signals and reproductive behavior. In: Vandenberg, JG., editor. *Pheromones and Reproduction in Mammals*. New York: Academic Press; 1983. p. 3–37.
- Johnston RE, Brenner D. Species specificity of scent marking in hamsters. *Behavioral and Neural Biology* 1982;35:46–55.
- Johnston, RE.; delBarco-Trillo, J. Communication by chemical signals: Behavior, social recognition, hormones and the role of the vomeronasal and olfactory systems. In: Etgen, A.; Pfaff, D., editors. *Hormones, Brain and Behavior*. Vol. 2nd. New York: Elsevier; in press
- Leavitt WW, Blaha GC. Circulating progesterone levels in the golden hamster during the estrous cycle, pregnancy, and lactation. *Biology of Reproduction* 1970;3:353–361. [PubMed: 5522862]
- Lisk, RD. The estrous cycle. In: Siegel, HL., editor. *The Hamster: Reproduction and Behavior*. New York: Plenum Press; 1985. p. 23–51.

- Meredith, M.; Samuelsen, C.; Blake, C.; Westberry, J. Selective response of medial amygdala subregions to reproductive and defensive chemosignals from conspecific and heterospecific species. In: Hurst, J.L.; Beynon, R.J.; Roberts, S.C.; Wyatt, T.D., editors. *Chemical Signals in Vertebrates*. Vol. 11. New York: Springer; 2008. p. 367-378.
- Meredith M, Westberry JM. Distinctive responses in the medial amygdala to same-species and different-species pheromones. *Journal of Neuroscience* 2004;24:5719–5725. [PubMed: 15215294]
- Morin, L.P.; Wood, R.I. A stereotaxic atlas of the golden hamster brain. New York: Academic Press; 2001.
- Mucignat-Caretta C, Colivicchi MA, Fattori M, Ballini C, Bianchi L, Gabai G, Cavaggioni A, Della Corte L. Species-specific chemosignals evoke delayed excitation of the vomeronasal amygdala in freely-moving female rats. *Journal of Neurochemistry* 2006;99:881–891. [PubMed: 16956376]
- Murphy MR. Intraspecific sexual preferences of female hamsters. *Journal of Comparative and Physiological Psychology* 1977;91:1337–1346.
- Murphy MR. Oestrous Turkish hamsters display lordosis toward conspecific males but attack heterospecific males. *Animal Behaviour* 1978;26:311–312.
- Nevo E, Bodmer M, Heth G. Olfactory discrimination as an isolating mechanism in speciating mole rats. *Experientia* 1976;32:1511–1512. [PubMed: 1035542]
- Orsini MW. The external vaginal phenomena characterizing the stages of the estrous cycle, pregnancy, pseudopregnancy, lactation, and the anestrous hamster, *Mesocricetus auratus* Waterhouse. *Proceedings of the Animal Care Panel* 1961;11:193–206.
- Pillay N. Female mate preference and reproductive isolation in populations of the striped mouse *Rhabdomys pumilio*. *Behaviour* 2004;137:1431–1441.
- Samuelsen CL, Meredith M. Categorization of biologically relevant chemical signals in the medial amygdala. *Brain Research* 2009;1263:33–42. [PubMed: 19368822]
- Theiler GR, Blanco A. Patterns of evolution in *Graomys griseoflavus* (Rodentia, Muridae). III. Olfactory discrimination as a premating isolation mechanism between cytotypes. *Journal of Experimental Zoology* 1996;274:346–350.
- Trivers, R.L. Parental investment and sexual selection. In: Campbell, B., editor. *Sexual Selection and the descent of Man: 1871-1971*. Chicago: Aldine; 1972. p. 136-179.
- Tubbiola MI, Wysocki CJ. FOS immunoreactivity after exposure to conspecific or heterospecific urine: Where are chemosensory cues sorted? *Physiology and Behavior* 1997;62:867–870. [PubMed: 9284509]

Fig. 1.

Mean investigation time in seconds ($\pm$ SEM) for *Mesocricetus auratus* females exposed to conspecific male flank-gland secretions, heterospecific (*M. brandti*) male flank-gland secretions or saline. Data are shown for estrous (receptive) and diestrous-2 (non-receptive) females. Different letters indicate statistically significant differences in investigation by non-receptive females (lowercase letters) or receptive females (capital letters). ***, $p < 0.001$ for the comparison across all three stimuli between non-receptive and receptive females.

Fig. 2a

Fig. 2b

Fig. 2c

Fig. 2d

Fig. 2.

Representative sections showing FRAs expression in anterior MeA (a and c) and posterior MeA (b and d) when estrous females were exposed to odors of a conspecific male (a and b) or a heterospecific male (c and d). The sections shown represent the range of anterior MeA and posterior MeA that were sampled. Outlines show the area counted.

Fig. 3a

Fig. 3b

Fig. 3.

Mean FRA immunoreactivity (FRA-ir) in the anterior medial amygdala (a) and posterior medial amygdala (b) for estrous (receptive) and diestrous-2 (non-receptive) *Mesocricetus auratus* females exposed to conspecific male flank-gland secretions, heterospecific (*M. brandti*) male flank-gland secretions or saline. Values are given as stained cells / μm^2 . Different letters indicate statistically significant differences for non-receptive females (lowercase letters) or receptive females (capital letters). *, $p < 0.05$; **, $p < 0.01$ for the comparison across all the three stimuli between non-receptive and receptive females.