Conspecific and heterospecific behavioural discrimination of individual odours by mound-building mice

Discrimination comportementale des odeurs individuelles dans un contexte homo- et hétérospécifique par la souris des tumulus

Patrick Gouat [a]*, Bruno Patris [a], Cyril Lalande [b]

[a] Laboratoire d'éthologie expérimentale et comparée, UPRES-A 7025, université Paris-Nord, 93430 Villetaneuse, France

[b] Département de psychophysiologie, UFR lettres, sciences humaines et sociétés, université Paris-Nord, 93430 Villetaneuse, France

(Received 19 December 1997, accepted after revision 24 April 1998)

Abstract – We have tested the ability of male mound-building mice, *Mus spicilegus*, to discriminate on the basis of their social odours a) two males of their own species; and b) two males of the house mouse, *Mus musculus domesticus*. An habituation–dishabituation procedure was used. An experimental animal was presented with the scent from the same stimulus animal for four trials; on the fifth trial, scent from a second stimulus animal was presented. Male *Mus spicilegus* were able to discriminate the olfactory signatures of two mound-building mouse males but did not discriminate between the olfactory signatures of two house mouse males. The lack of inter-specific individual recognition is discussed in terms of specificity and attractive value of odour cues. (© Académie des sciences / Elsevier, Paris.)

***Mus spicilegus* / *Mus musculus domesticus* / individual recognition / olfactory signature**

Résumé – Nous avons testé la capacité de mâles de souris des tumulus, *Mus spicilegus*, à discriminer les signatures chimiques a) de deux mâles de cette même espèce ; b) de deux mâles de la souris domestique *Mus musculus domesticus*. Un protocole d'habituation–déshabituation a été utilisé. La signature chimique d'un même individu est présentée au sujet expérimental lors de quatre tests successifs. Au cinquième test l'animal donneur est changé. Les mâles de souris des tumulus discriminent les deux mâles de leur propre espèce mais pas les deux mâles de souris domestique. Cette absence de discrimination individuelle hétérospécifique est discutée en termes de spécificité et de valeur attractive des signaux olfactifs. (© Académie des sciences / Elsevier, Paris.)

***Mus spicilegus* / *Mus musculus domesticus* / reconnaissance individuelle / signature olfactive**

Note communicated by Pierre Buser

* Correspondence and reprints
E-mail: pgouat@leec.univ-paris13.fr

Version abrégée

La capacité de discrimination de signatures olfactives individuelles a été étudiée chez de nombreuses espèces de rongeurs. Nous avons testé la capacité de mâles de souris des tumulus, *Mus spicilegus*, à discriminer les signatures chimiques a) de deux mâles de cette même espèce ; b) de deux mâles de la souris domestique *Mus musculus domesticus*. Un protocole d'habituation–déshabituation a été utilisé. La signature chimique d'un même individu est présentée au sujet expérimental lors de quatre tests successifs. Au cinquième test l'animal donneur est changé. Lors de chaque test une coupelle contenant de la sciure propre est présentée simultanément avec la coupelle contenant la sciure souillée (signature chimique). La durée de flairage de chaque coupelle est mesurée. La diminution de la durée de flairage de la coupelle souillée du premier au quatrième test rend compte d'un phénomène d'habituation. La détection du changement de donneur au cinquième test est mise en évidence par l'augmentation du temps de flairage de la coupelle souillée. Afin d'évaluer l'intérêt porté par l'animal test à l'odeur sociale, nous avons comparé le temps passé à flairer la coupelle emplie de sciure propre et celle emplie de sciure souillée. Dans les deux types d'expérimentation, le temps passé à flairer la coupelle souillée diminue du premier au quatrième test. Par contre, ce n'est que lors des tests homospécifiques que le temps de flairage de la coupelle souillée augmente lors du cinquième test (changement de donneur). Nous en déduisons que les mâles de souris des tumulus discriminent les deux mâles de leur propre espèce mais pas les deux mâles de souris domestique. Par discrimination nous entendons la réaction comportementale qui découle d'une reconnaissance individuelle correspondant à un processus neurophysiologique. L'absence de discrimination individuelle lors des tests hétérospécifiques peut donc avoir deux origines distinctes : soit être la conséquence de l'absence d'une reconnaissance individuelle, soit la reconnaissance individuelle a bien eu lieu mais la valeur attractive du signal est trop faible pour entraîner une réponse comportementale. Cette deuxième hypothèse est privilégiée car on constate que lors du premier test et au contraire de l'expérience homospécifique, la souris des tumulus ne passe pas plus de temps à flairer la coupelle souillée que la coupelle de sciure propre.

1. Introduction

Behavioural discrimination of individual chemical signatures has been studied in numerous rodent species (see review in [1]). Studies revealed that mice have great capacities of olfactory discrimination. On the basis of olfactory cues, mice are able to discriminate between two male mice of the same inbred strain [2]. Mice are able to detect the difference in two mice that are genetically identical except for the major histocompatibility complex [3]. After training, these abilities of discrimination can be used in an heterospecific context in different mammal species (e.g. [4–6]). Two studies report on a spontaneous cross-species discrimination of individual odours in rodents. In two chromosomal species of the subterranean mole rat, *Spalax ehrenbergi*, animals are able to discriminate between the individual specific odour cues from pairs of conspecifics and pairs of heterospecific donors [7]. A similar result was found between two less closely related species of hamsters, the golden hamster, *Mesocricetus auratus*, and the Djungarian hamster, *Phodopus campbelli* [8].

In this paper we report the results of a preliminary study of the ability of male mound-building mice, *Mus spicilegus*, to discriminate on the basis of their social odours a) two males of their own species; and b) two males of the house mouse, *Mus musculus domesticus*. Discrimination is considered here to be a behavioural response based on individual recognition which is a neural process [9]. The lack of individual recognition prevents individual discrimination, but the inverse proposition, however, is not true.

Mus spicilegus and *Mus musculus domesticus* constitute, on the basis of numerous genetic markers, well differentiated biochemical groups [10, 11]. These two species nevertheless, are closely related and, in the *Mus* species, are classified into the same sub-genus *Mus* [12]. *Mus spicilegus* is an outdoor mouse characterized by its ability to build large mounds of dirt containing piles of seeds, a nest chamber and numerous tunnels in which a group of mice overwinter [9]. This species occurs in the steppe grassland habitat from south-eastern Austria to Romania north into Ukraine (review in [13]). *Mus musculus domesticus* is a commensal species that occurs in western Europe and in the Mediterranean region. A small sympatric zone is described in the south of Bulgaria but according to the difference in habitat use, the probability of direct encounters between the two species in the field is low [14].

According to the great capacity of mice for olfactory discrimination of individual odours [2, 3], and to the existence of complex social groups during winter [15], we expect that individual recognition by olfactory cues will occur in *Mus spicilegus*. Studies of cross-species discrimination of individual odours [7, 8] and the phyletic proximity of the mound-building and the house mice lead us to predict that male *M. spicilegus* will discriminate the individual odours of male house mice.

2. Materials and methods

2.1. Animals

Thirteen male *Mus spicilegus* were used as experimental subjects and as scent donors in homospecific experiments. Animals were 85–176 d old at the beginning of

the experiments. These mice originated from a population in Yugoslavia and were raised in captivity for at least 14 generations. Twelve of the previous 13 mound-building mice were the experimental animals in the heterospecific experiments. A minimum 5-d period separated the two types of experiments. Six *Mus musculus domesticus* males were used as scent donors. House mice were 100–150 d old. These house mice originated from a population in Denmark and were raised in captivity for 17 or 18 generations. Animals were maintained at 21 ± 3 °C on a 14:10-h light/dark cycle. Animals were placed in individual cages (26 × 16 cm) 2 weeks before the beginning of experiments. Prior to isolation, animals were maintained in brother groups from weaning at 21 d of age.

2.2. Procedure

An habituation–dishabituation procedure was used according to a protocol derived from Johnston et al. [16]. An experimental animal was presented with the scent from the same stimulus animal for four trials; on the fifth trial, scent from a second stimulus animal was presented. The time spent by the experimental animal investigating the scent stimulus was measured during each trial. We checked whether or not an habituation process had occurred by comparing the investigation duration between the first and the fourth trial. During the fifth trial, a significant increase in investigation indicated that the experimental animal perceived the change of scent donor. The two donors were randomly assigned to an experimental animal following the rule that the three animals were not brothers and were unfamiliar.

Tests were performed in the home-cage of the experimental animal during the first half of the dark phase. Two small plastic dishes (3 cm in diameter) were placed in the cage of the experimental animal on the opposite side of the nest for a 5-min period. One dish was filled with soiled bedding (shavings with urine and droppings) collected in the cage of a scent donor just prior to the habituation experiment; the second dish was filled with clean shavings. The two dishes were removed and cleaned after the trial. After 2–3 min, two new dishes were placed in the cage of the experimental animal for a new trial. The position of each type of stimulus (i.e. right or left) alternated between each trial and the initial position of the dishes was randomly designated for each test. Tests were video-recorded.

2.3. Data and statistical analysis

From the video-recordings and with the use of a stopwatch, we measured the time spent by an experimental animal investigating each dish. The observer of video-recordings was unaware of the nature of the stimulus in each dish.

Two types of comparisons were made using a Wilcoxon matched-pairs signed-ranks test. In order to check the existence of an habituation process, we compared the time spent investigating the dish filled with soiled bedding in the first and in the fourth trial. The effect of donor change was studied by a comparison of the investigating duration between the fourth and the fifth trial. A comparison between the soiled and the clean bedding was made for the first trial in order to evaluate the attractiveness of the social odour.

3. Results

3.1. Conspecific tests (*figure 1*)

During the first trial, experimental males spent more time investigating the soiled dish than the dish filled with clean shavings (n = 13, T = 3, P = 0.003). An habituation process occurred as shown by the decrease in investigating time of the soiled dish between the first and the fourth trial (n = 13, T = 0, P = 0.001). Experimental animals clearly detected the change of donor in the fifth test and increased their investigating time of the soiled dish (n = 13, T = 3, P = 0.005)

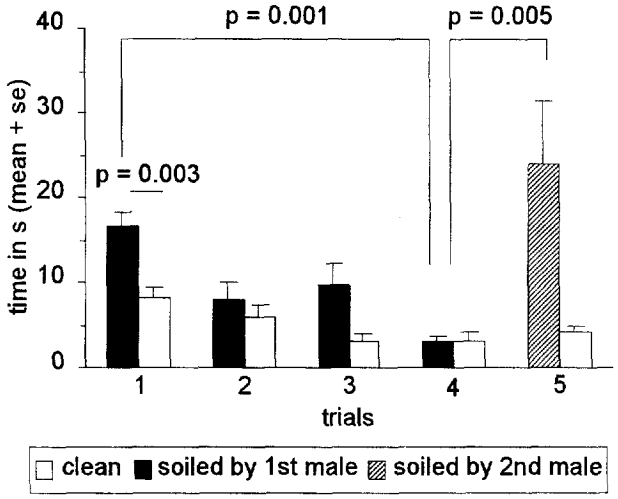

Figure 1. Mean (+ standard error) time that male mound-building mice spent investigating the dishes filled with clean shavings or with soiled bedding from a male mound-building mouse.

3.2. Heterospecific tests (*figure 2*)

During the first trial, *Mus spicilegus* males did not spend more time investigating the dish filled with bedding soiled by male *M. musculus domesticus* than the clean dish (n = 12, T = 36.5, P = 0.845). An habituation process occurred between the first and fourth trial (n = 12, T = 5, P = 0.008) but no increase was detected in the fifth trial (n = 12, T = 22, P = 0.575).

4. Discussion

As predicted, male *Mus spicilegus* were able to discriminate the olfactory signatures of two mound-building mouse males. On the other hand, contrary to our predic-

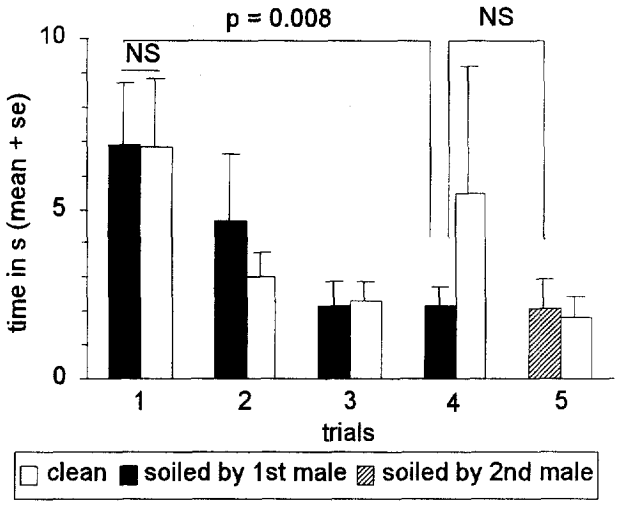

Figure 2. Mean (+ standard error) time that male mound-building mice spent investigating the dishes filled with clean shavings or with soiled bedding from a male house mouse.

tion, male mound-building mice did not discriminate the olfactory signatures of two *Mus musculus domesticus* males. We propose two hypotheses to explain why male mound-building mice failed to discriminate male house mice. First, as suggested by Tubbiola and Wysocki [17] in the prairie vole, chemical cue used for individual recognition may be functionally species specific because of the specificity of receptors. Species specificity of chemosignals, nevertheless, is not strictly absolute between closed species [8, 18, 19]. According to the phyletic proximity of the two species, a more likely alternative hypothesis is that a recognition process occurred during our heterospecific experiments but did not elicit a behavioural discrimination. In other words, mound-building mouse males were able to distinguish between the olfactory signatures of the two house mouse males, whereas the attractive value of these heterospecific odour cues was too low to elicit a behavioural discrimination. This lack of attractiveness was indicated by male mound-building mice during the first trial since they did not spend more time investigating the dish filled with shavings soiled by a house mouse. Such a result is rather unusual. In the well-studied golden hamster, the odour stimulus is always more investigated than the clean one, during the first trial, even if the odour source does not allow for individual recognition [16].

To test the hypothesis of a neural recognition without a behavioural discrimination, three types of experiments could be carried out. a) The existence of a neural recognition may be established by the observation of a similar increase in neural activity after exposure to conspecific or heterospecific odour cues. b) We might promote a behavioural discrimination by using males *Mus musculus musculus* as donors. This sub-species of the house mouse is a sympatric potential competitor as shown by its capture inside mounds built by *Mus spicilegus* [20]. c) Behavioural discrimination might be induced by increasing the attractiveness of the heterospecific odour by a social contact between the two species prior to the test. Krasnov and Khokhlova [21] showed that a direct contact is necessary for house mice to avoid the odour of midday jirds, *Meriones meridianus*, which are potential competitors.

Acknowledgements: The two strains of mice were kindly provided by François Bonhomme and Annie Orth (UPR 9060 CNRS 1493, Montpellier, France). The authors would like to thank Sévane Maslak, Janine Gouat, Gilles Gheusi, Christophe Féron, Claude Baudoin and an anonymous referee for comments on the original manuscript, Lavinia Bruneau for her revision of the English, Nathalie Christophe for providing fresh samples of soiled bedding from male house mice and Simone Demouron for her help in caring for animals.

5. References

[1] Gheusi G., Bluthé R.-M., Goodall G., Dantzer R., Social and individual recognition in rodents: methodological aspects and neurobiological bases, Behavioural Processes 33 (1994) 59–88.

[2] Bowers J.M., Alexander B.K., Mice: individual recognition by olfactory cues, Science 158 (1967) 1208–1210.

[3] Yamazaki K., Yamaguchi M., Baranoski L., Bard J., Boyse E.A., Thomas L., Recognition among mice: evidence from the use of a Y-maze differentially scented by congenic mice of different major histocompatibility types, J. Exp. Med. 150 (1979) 755–760.

[4] Beauchamps G.K., Yamazaki K., Wysocki C., Slotnick B., Thomas L., Boyse E.A., Chemosensory recognition of mouse major histocompatibility types by another species, Proc. Natl. Acad. Sci. USA 82 (1985) 4186–4188.

[5] Beauchamp G.K., Katahira K., Yamazaki K., Mennela J.A., Bard J., Boyse E.A., Evidence suggesting that the odortypes of pregnant women are a compound of maternal and fetal odortypes, Proc. Natl. Acad. Sci. USA 92 (1995) 2617–2621.

[6] Schoon G.A.A., Scent identifications by dogs (*Canis familiaris*): a new experimental design, Behaviour 134 (1997) 531–550.

[7] Todrank J., Heth G., Individual odours in two chromosomal species of blind, subterranean mole rat (*Spalax ehrenbergi*): conspecific and cross-species discrimination, Ethology 102 (1996) 806–811.

[8] Johnston R.E., Robinson T.A., Cross-species discrimination of individual odors by hamsters (Muridae: *Mesocricetus auratus, Phodopus campbelli*), Ethology 94 (1993) 317–325.

[9] Waldman B., Frumhoff P.C., Sherman P.W., Problems of kin recognition, Trends Ecol. Evol. 3 (1988) 8–13.

[10] Thaler L., Bonhomme F., Britton-Davidian J., Processes of speciation and semi-speciation in the house mouse, Symp. Zool. Soc. Lond. 47 (1981) 27–41.

[11] Boursot P., Auffray J.-C., Britton-Davidian J., Bonhomme F., The evolution of house mice, Annu. Rev. Ecol. Syst. 24 (1993) 119–152.

[12] Bonhomme F., Genetic diversity and evolution in the genus *Mus*, in: D. Goldowitz, D. Whalsten, R. Wimer (eds.), Techniques for the Analysis of Brain and Behaviour: Focus on the Mouse, P. Hudson (ed.), Techniques in the Behavioral Neural Sciences, Elsevier, Amsterdam, 1992, 8, 41–56.

[13] Orsini P., Bonhomme F., Britton-Davidian J., Croset H., Gerasimov S., Thaler L., Le complexe d'epèces du genre *Mus* en Europe Centrale et Orientale. II. Critères d'identification, répartition et caractéristiques écologiques, Z. Säugetierkunde 48 (1983) 86–95.

[14] Thaler L., Approche paléontologique et génétique de l'évolution des rongeurs, La Vie des Sciences 3 (1985) 105–120.

[15] Garza J.C., Dallas J., Duryadi D., Gerasimov S., Croset H., Boursot P., Social structure of the mound-building mouse *Mus spicilegus* revealed by genetic analysis with microsatellites, Mol. Ecol. 6 (1997) 1009–1017.

[16] Johnston R.E., Derzie A., Chiang G., Jernigan P., Lee H.C., Individual scent signatures in golden hamsters: evidence for specialization of function, Anim. Behav. 45 (1993) 1061–1070.

[17] Tubbiola M.I., Wysocki C.J., FOS immunoreactivity after exposure to conspecific or heterospecific urine: where are chemosensory cues sorted?, Physiol. Behav. 62 (1997) 867–870.

[18] Fiber J.M., Adames P., Swann J.M., Pheromones induce c-fos in limbic areas regulating male hamster mating behavior, NeuroReport 4 (1993) 871–874.

[19] Hildebrand J.G., Shepherd G.M., Mechanisms ol olfactory discrimination: converging evidence for common principles across phyla, Annu. Rev. Neurosci. 20 (1997) 595–631.

[20] Duryadi D., Rôle possible du comportement dans l'évolution de deux souris *Mus macedonicus* et *Mus spicilegus* en Europe centrale, thèse de doctorat, université Montpellier-II, 1993.

[21] Krasnov B., Khokhlova I., Discrimination of midday jird's odour by house mice, Anim. Behav. 52 (1996) 659–665.