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Individual Recognition and Odor in Rat-Like Hamsters: Behavioral Responses and Chemical Properties

Dingzhen Liu[1], Ke-Jian Huang[1] and Jian-Xu Zhang[2]

[1]Ministry of Education, Key Laboratory of Biodiversity Science and Ecological Engineering, College of Life Sciences, Beijing Normal University, 100875 Beijing, China and [2]State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, 1-5 Beichenxi Road, 100101 Beijing, China

Correspondence to be sent to: Jian-Xu Zhang, Institute of Zoology, Chinese Academy of Sciences, 100101 Beijing, China. e-mail: zhangjx@ioz.ac.cn

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Abstract

Individual recognition has been studied across a number of taxa and modalities; however, few attempts have been made to combine chemical and biological approaches and arrive at a more complete understanding of the use of secretions as signals. We combined behavioral habituation experiments with gas chromatography–mass spectrometry of glandular secretions from the left and right flank gland and midventral gland of the rat-like hamster, *Tscheskia triton*. We found that females became habituated to one scent and then could discriminate individuals via another scent source from the same individual only when familiar with the scent donor. However, this prior social interaction was not required for females to discriminate different individuals in single-stimulus habituation–dishabituation tests. Chemical analyses revealed a similarity in volatile compounds between the left and right flank gland and midventral gland scents. It appears that individually distinctive cues are integratively coded by a combination of both flank gland and midventral gland secretions, instead of a single scent, albeit animals show different preferences to the novel scent. Our results suggest that odors from the flank and midventral glands may provide information related to individuality and aid individual recognition in this species and confirm that prior interaction between individuals is a prerequisite for rat-like hamsters to form multi-odor memory of a particular conspecific.

Key words: cross-habituation, flank gland, glandular secretion, individual recognition, midventral gland, rat-like hamsters

Introduction

The ability for social and solitary animals to recognize individuals is important in the maintenance of stable social groups, parent–offspring dynamics, inbreeding avoidance, and the regulation of competitive relationships (Brown and MacDonald 1985; Tibbetts and Dale 2007). Animals discriminate individuals using nonspecific cues such as spatial location or specific signals such as vocalizations, scents, and behavior (Holmes 2004; Thom and Hurst 2004). Individualistic cues can be coded in scent markings from glands (Smith et al. 2001; Johnston 2003; Mateo 2004; Beauchamp and Yamazaki 2005; Zhang, Liu, et al. 2008), urine (Hurst et al. 2001, 2005), vocalizations (Beer 1970; Espmark 1971; Petrinovich 1974; Snowdon and Cleveland 1980; Kroodsma and Miller 1996; Wanker et al. 2005; Barton 2006; Janik et al. 2006; Charlton et al. 2009), and facial features (Campanella and Belin 2007). How animals integrate these different sources of signals to learn a memory of individual identity and discriminate/recognize individuals remains unknown.

As an important mode of communication in mammals, chemical signals play a vital role in individual discrimination/recognition systems (Halpin 1980; Brown and MacDonald 1985; Johnston 2003). The scent of glandular secretions (apocrine, sebaceous, and eccrine gland secretions) and excretions (urine and feces) provide cues related to individual identity, reproductive state, age, gender, and social rank and may elicit specific behaviors and physiological responses in receivers (Vandenbergh 1983; Halpin 1986; Lai et al. 1996). For example, male golden hamsters (*Mesocricetus auratus*) use 5 different sources of scent to discriminate different conspecifics (Johnston et al. 1993). In addition, golden hamsters (Johnston and Jernigan 1994) and Belding's ground squirrels (*Spermophilus beldingi*) are found to recognize individually distinctive cues on the basis

of the referent (or meaning) of one scent and form a multifactor representation of individuals (Mateo 2006). To realize this task of discrimination, a limited interaction between subjects and scent donors is essential in order to generalize across sources of scent from the same individual (cross-habituation effect) (Johnston and Bullock 2001). A vast number of studies into individual differences in auditory, olfactory, and visual signals available for individual discrimination/recognition have been conducted (Halpin 1980, 1986; Colgan 1983; Johnston 1993; Johnston and Jernigan 1994); yet attempts to investigate the complete repertoire of cues from both a behavioral and chemical perspective are scant (Johnston 2005). Here, we combine behavioral observation with analyses of the chemical composition of different sources of scent in rat-like hamsters (*Tscheskia triton*) to test the following hypotheses: 1) similar to golden hamsters, mice, and Belding's ground squirrels, a limited interaction between the scent donor and receiver is required for a cross-habituation effect; 2) individually distinctive information is coded across more than one source of scent in rat-like hamsters; and 3) different sourced scents share common characteristics either in chemical composition and/or same compounds with different abundances in coding individual identity.

The rat-like hamster is a solitary and polygynous species distributed across farmland in northern China. Males are dispersal prone, and females are philopatric. A single midventral gland is located along the middle line of the abdomen. Secretions from this sebaceous gland are thought to be used in mate attraction, choice, and individual recognition by females (Doty and Kart 1972; Vasilieva and Sokolov 1994). In addition, rat-like hamsters also possess a pair of dark-colored flank glands either side of the waist that produce a yellow sticky secretion. Flank gland size increase with body size is larger in dominant males and becomes larger during the breeding season (Zhang et al. 1999, 2001). Previous evidence has shown that estrous females are able to discriminate between the flank gland scents of males of different social status (Zhang et al. 2001), and no other sources of body odor such as urine, tears, or saliva appear to have this function (Zhang JX, personal communication). Given these 2 glands and their secretions, wild-derived rat-like hamsters are an excellent model species to answer questions regarding signal generalization and individual discrimination.

To test the above hypotheses, we conducted a series of behavioral tests and gas chromatography and mass spectrometry (GC-MS) analysis in female wild-derived rat-like hamsters. Specifically, we asked: is prior social interaction required for female rat-like hamsters to learn individual information from flank to midventral gland secretions of the same individual? Is previous social interaction needed for female rat-like hamsters to learn individual information from flank glands of the same individual? Is prior social interaction required for hamsters to discriminate a new conspecific in a single-stimulus test under a habituation–dishabituation paradigm? Are there differences in chemical compositions between the midventral and flank gland secretions and between right and left flank gland secretions? Are these individualistic cues commonly coded by all 3 scents?

Materials and methods

Subjects and housing

All subjects were F_2 generation animals from 28 wild-caught rat-like hamsters from farmland in Hebei, China. Animals were housed individually in plastic cages (40 × 25 × 15 cm) lined with wood shavings as bedding material. The room was maintained at a reversed light:dark regime of 14:10 h (lights on at 1900) and at approximately 20 °C. Standard mouse chow and water were provided ad libitum. Protocols for maintenance and handling were in accordance with guidelines for animal care established by the Institute of Zoology, Chinese Academy of Sciences. We regularly allowed females and males to interact through wire mesh in a wooden box (60 × 60 × 40 cm) for 1 h per week, imitating the meeting of wild conspecifics. Animals that have this regular social interaction were permitted to have an interval of rest for 2–3 days before being used in behavioral tests. All animals used were healthy and between 8 and 12 months old. Since this wild-derived colony is small, due to the fact that it is difficult to breed in the laboratory, 22 of the females used in test 3 with a single stimulus either flank–flank glands (11 individuals) or flank–midventral glands (11 individuals) were previously used in test 1 and test 2. However, when a female subject was used more than once, an interval of 2–3 days was permitted. All other female subjects were naive animals. All females were virgin, and tests were run during their diestrus period. Female estrous cycles were examined and confirmed by microscopical examination of the vaginal secretion and by referring to animal management records. In total, 44 females were used as subjects and 34 males were used as odor donors. One male donor familiar to one female may have been used as an unfamiliar donor to another female in the same tests due to the limited supply of male hamsters.

Test 1: is prior social interaction necessary for cross-habituation to scent from different glands?

We adopted a cross-habituation discrimination paradigm to test whether female rat-like hamsters discriminate male flank scents and midventral scents from familiar and unfamiliar donors. This method has been used in several studies of golden hamsters (Johnston and Jernigan 1994; Johnston and Bullock 2001), a species also belonging to the Cricetidae that shares characteristics with rat-like hamsters such as being solitary, possessing flank glands, and in body size and behavior. Detailed information on the cross-habituation discrimination can be found elsewhere (Johnston and Bullock 2001). In brief, we repeatedly provided the focal animal with a fresh scent from one donor and then simultaneously

provided 2 different sourced scents (one from the previous donor and one from a novel donor). We used 12 naive adult healthy female hamsters randomly chosen as test animals and 24 adult male hamsters as odor donors. We further divided them into 12 subgroups containing 1 female and 2 males that were of similar body mass. To familiarize females with males, on each day for 4 days, each male from the subgroup was allowed to interact with the female for one 3-min period. The order of males was chosen at random, and an interval of 20 min between tests was allowed. Animals were rapidly separated for several seconds if aggression or sexual behaviors were observed. On the fifth day following familiarization, we conducted 4 consecutive habituation trials using females and flank gland odor from one randomly selected familiar male. We used a glass rod (20 cm long × 4 mm diameter) to collect flank gland residue by rubbing it across the surface of the gland 10 times (Lai et al. 1996) and then inserted the glass rod 4 cm into the female housing for 3 min. This was repeated 4 times with a 3-min interval between trials. For the test trial, we presented midventral gland scent–emitting glass rods to females from both the habituated odor donor and another familiar male. The rods were presented simultaneously for 3 min and were held approximately 3 cm apart. During all trials, the scents used were freshly collected, and we recorded the amount of time a female spent sniffing the scent. Sniffing behavior was defined when a female's nose was within 1 cm of the scent end of the rod. All behavioral tests were conducted during the dark phase. Throughout all experiments, we wore disposable plastic gloves and cleaned glass rods using 75% ethanol.

To determine if females habituated to flank gland scent can discriminate unfamiliar males based on midventral scent, we used 11 females and 22 males divided into 11 subgroups following the protocols above. The experimental procedure was identical to the familiar group in test 1 except the absence of any familiarization phase for the female subjects prior to habituation trials.

Wilcoxon signed-rank tests (2 tailed) were used for comparing the time female hamsters spent sniffing scents between the first trial and the fourth trial on the habituation phase and the time hamsters spent sniffing odors on the test (dishabituation) trials. All statistical tests were conducted using SPSS v10.0 for Windows (SPSS Inc., Chicago). The significance level was set at 0.05.

Test 2: is prior social interaction necessary for cross-habituation to scent from the left and right flank glands?

To determine if females habituated to scent arising from the left flank gland can discriminate familiar males based on scent from the right flank gland, we used 10 naive female hamsters as test subjects and 20 adult male hamsters as odor donors matched by body mass. Familiarization was carried out over 4 days and testing conducted on the fifth day. During habituation on the fifth day, we used a glass rod to collect left flank gland residue from one of the familiar males and presented it to females for 3 min, repeating this process 4 times with freshly collected odor. For the test trial, we presented a glass rod containing fresh right flank gland residue from the habituated male and a second rod containing fresh residue from the right flank gland of the other familiar but not habituated male following the protocols outlined above.

To determine if females habituated to scent arising from the left flank gland are able to discriminate unfamiliar males based on scent from the right flank gland, we used 11 females and 22 males divided into 11 subgroups containing 1 female and 2 males. The protocol was identical to the one used for the familiar group in test 2 except the absence of any familiarization phase for the females prior to habituation trials. We conducted habituation followed by test trials using these animals. We repeatedly provided females with left flank gland scent from an unfamiliar male in the habituation phase on 4 trials for 3 min with an interval of 3 min and then simultaneously exposed the females to 2 right flank gland scents from the habituated male and a novel male.

Test 3: the effect of scent source on habituation and dishabituation in female hamsters

To determine if female hamsters become habituated and dishabituated to flank gland secretions from different individuals, we chose 11 females previously used in test 1 as test subjects and 22 males as donors, divided into 11 subgroups. Focal females and male odor donors were not familiarized prior to habituation testing. Females were first habituated to the fresh flank scents of one male during a habituation phase that included 4 trials with an interval of 3 min and then tested with flank gland scent from a novel male during the test trial.

To determine if female hamsters become habituated and dishabituated to different sources of scent from the same individual, we used 11 females previously used in test 2 and 11 males and allocated each to a dyad based on body mass. Each female was presented with fresh flank scent from a male during the habituation phase 4 times with an interval of 3 min. This was followed by a test trial whereby a female was exposed to midventral scent from the same male.

Test 4: chemical analysis of secretions from left and right flank glands and midventral gland

Five males were randomly chosen and decapitated 1 day after all behavioral tests were finished. The left flank gland, right flank gland, and midventral gland were collected and weighed (milligrams). They were frozen immediately, transferred to the laboratory, and stored at –20 °C until analysis. We thawed samples and then dissolved them directly in dichloromethane at a ratio of 1 mg gland: 2.5 mL dichloromethane without any mechanical treatment and stored them at –20 °C for GC-MS analysis. GC-MS procedures

are described in detail elsewhere (Zhang et al. 2002; Yuan et al. 2004). The analytical GC-MS was performed on an Agilent Technologies Network 6890N GC system coupled with 5973 Mass Selective Detector with the library National Institute of Standards and Technology (NIST 2002). Xcalibur (Windows XP) was equipped with a 30-m glass capillary column (internal diameter 0.25 mm × 0.25 μm film) coated with HP5MS. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed as follows: 100 °C initial temperature, which was increased by 5 °C/min up to 240 °C and then by 10 °C/min up to 280 °C and held for 6 min. The amount of sample injected was 2 μL each time.

Chemical compound identification was undertaken by comparing the mass spectra of GC peaks with those in the MS library (NIST 2002), previous results on golden hamsters and house mice (Zhang et al. 2007), and the authentic standards ordered from Sigma-Aldrich (Sigma-Aldrich Co., Shanghai, China). Four small volatiles were identified unknown and used for subsequent analysis (Table 1). We used the method developed by Sun and Müller-Schwarze (1998b) and Zhang et al. (2003) to quantify the absolute and relative abundance of relevant compounds in both sexes. We first used the peak area as absolute abundance and then converted the peak area of each compound into the percentage of the sum peak areas of a total of 11 GC peaks as relative abundance.

The relative abundances of the volatiles in the 3 gland secretions were subject to a unit-sum constraint (the ratios summed to 1). In response to this constraint, the original data were transformed by natural logarithm before analysis (Aitchison 1986) followed by distribution examination. The general linear model (GLM) of multiple variate analysis was used for the analysis of relative abundance of the 11 compounds (see Table 1 in Results) within individuals. The post hoc test of Least significant difference (LSD) was used if a significant result was found. To examine the similarity between the chemical profiles of the 3 sourced scents from the same animal, we also used principal component analysis (PCA) to classify the relative abundance (percentage of the peak areas) of 11 compounds from the 3 gland secretions. The PCA is a multivariate statistical method to reduce the dimensions of a group of data by producing a smaller number of extracted variables. We conducted PCA in SPSS using transformed relative abundance data of 11 identified chemical compounds (4 were unknown compounds, Table 1) in the 3 kinds of glandular secretions of the 5 male rat-like hamsters and calculated all factors on the basis of a correlation matrix without rotation (Salamon and Davies 1998). Then simple scatter plots were drawn based on different combinations of the 4 extracted principal factors (see Table 3). In addition, to determine the variability of volatile constituents between individuals, a relative standard deviation (RSD) was calculated as follows:

$$\mathrm{RSD} = (\mathrm{SD}/\mathrm{mean}) \times 100.$$

SD is the standard deviation for all individuals, and the mean was the average of each volatile peak area percentage across all individuals. All statistical analyses were conducted using SPSS, and significance was set at 0.05.

Table 1 Relative abundance of volatiles emitted from the flank and midventral glands

Peak number	Retention time (min)	Compounds	Match rate (%)	Relative abundance		
				Males (N = 5)		
				LFG	RFG	MVG
1	5.184	Unknown	?	0.69 ± 0.34	0.51 ± 0.33	0.62 ± 0.36
2	5.325	Unknown	?	0.81 ± 0.40	0.63 ± 0.41	0.80 ± 0.46
3	5.467	Unknown	?	0.75 ± 0.58	0.47 ± 0.32	0.65 ± 0.40
4	7.806	Unknown	?	1.25 ± 0.28*	0.86 ± 0.17	0.70 ± 0.32**
5	10.586	Dodecanoic acid	98.0	0.80 ± 0.60	0.75 ± 0.67	1.53 ± 2.46
6	14.689	Tetradecanoic acid	64.3	1.93 ± 1.19	1.82 ± 1.49	2.45 ± 2.11
7	18.302	*Z*9-hexadecenoic acid	99.0	15.19 ± 3.80	16.73 ± 4.05	13.04 ± 3.91
8	18.676	Hexadecanoic acid	96.0	18.19 ± 2.86	17.75 ± 3.72	17.80 ± 2.09
9	20.990	5-dodecyl-dihydro-2(3H) furanone	90.0	7.18 ± 4.66	6.85 ± 3.05	3.15 ± 1.33
10	21.914	Oleic acid	80.4	44.53 ± 7.18	45.32 ± 9.78	51.15 ± 10.28
11	22.289	Octadecanoic acid	98.0	8.71 ± 2.44	8.32 ± 1.48	8.12 ± 2.34

"?" Indicates compounds not found in the MS library. LFG, left flank gland; RFG, right flank gland; MVG, midventral gland. Data shown are mean ± SD.
*Significant difference ($P < 0.05$) in relative abundance of volatiles from the left and right flank glands.
**Significant difference ($P < 0.05$) in relative abundance of volatiles from the left flank and midventral glands (GLM multivariate analysis followed by post hoc test of LSD).

Table 2 Individual variation in relative abundance of volatiles emitted from secretions from the flank and midventral glands

Peak number	Males (RSD, $N = 5$)		
	Left flank gland	Right flank gland	Midventral gland
1	49.28	64.71	58.06 (1)[a]
2	49.38	65.08	57.50 (1)
3	77.33	68.09	61.50 (1)
4	22.40	19.77	45.71
5	75.00	89.33 (1)	160.78
6	61.66	81.87	86.12
7	25.02	24.21	29.98
8	15.72	20.96	11.74
9	64.90	44.53	42.22
10	16.12	21.58	20.10
11	28.01	17.79	28.82
Mean ± SD	44.08 ± 23.53	47.08 ± 27.45	54.78 ± 41.07

[a]Figures in parentheses indicate the numbers of individuals from which that compound was not detected.

Table 3 Component loadings for first 4 PCs of GC-MS peak area in secretions of left, right, and midventral glands

Compound	Rotated component loadings[a]			
	PC_1	PC_2	PC_3	PC_4
Unknown	0.965	0.068	0.226	0.093
Unknown	0.960	0.076	0.217	0.045
Unknown	0.860	−0.081	0.134	0.342
Unknown	0.165	0.173	−0.027	0.930
Dodecanoic acid	−0.119	0.081	0.965	−0.033
Tetradecanoic acid	0.376	0.093	0.902	0.034
Z-11-hexadecenoic acid	0.034	0.971	0.113	−0.044
Hexadecanoic acid	0.795	0.134	−0.240	−0.410
5-dodecyl-dihydro-2(3H) furanone	−0.332	0.844	−0.119	0.355
Oleic acid	−0.489	−0.831	−0.246	−0.066
Octadecanoic acid	0.881	−0.035	−0.065	0.049
% Variance	41.22	22.06	18.29	11.80

[a]Rotation converged in 5 iterations.

Results

Is prior social interaction necessary for cross-habituation to scents from different glands of the same individual?

Female hamsters with prior social contact with male odor donors quickly became habituated to flank gland scents from the familiarized male during habituation trials with significant decline in sniffing odor ($Z = 3.059$, $N = 12$, $P = 0.002$; Figure 1a). During the test trial, the time females spent investigating midventral gland scent from the male used during habituation was significantly shorter than for the other familiar male ($Z = 2.510$, $N = 12$, $P = 0.012$; Figure 1a). Female hamsters without prior social interaction with male odor donors also became habituated to flank gland scent of unfamiliar males with a significant decline in sniffing time ($Z = 2.578$, $N = 11$, $P = 0.010$; Figure 1b); however, they did not discriminate between the 2 scents from the habituated male and a novel male during the test trial ($Z = 0.267$, $N = 11$, $P = 0.790$; Figure 1b).

Is prior social interaction necessary for cross-habituation to scents from the left and right flank glands of the same individual?

Following familiarization with males, female hamsters showed habituation to left flank gland scent repeatedly provided ($Z = 2.497$, $N = 10$, $P = 0.013$; Figure 2a). During the test trial, females discriminated the 2 male scent donors by spending less time investigating right flank gland scent from the male donor used during habituation than right flank gland scent from the other familiar male ($Z = 2.803$, $N = 10$, $P = 0.005$; Figure 2a). Female hamsters without prior interaction with male hamsters, however, did not discriminate the right flank gland scent of a novel male from the one they habituated to ($Z = 0.356$, $N = 11$, $P = 0.722$; Figure 2b), despite the fact that they showed signs of habituation to left flank gland scent repeatedly provided during the habituation phase ($Z = 2.401$, $N = 11$, $P = 0.016$; Figure 2b).

The effects of scent source and glands on habituation and dishabituation in female hamsters during a single-stimulus test

Females became habituated to repeatedly presented flank gland scent of unfamiliar males ($Z = 2.934$, $N = 11$, $P = 0.003$; Figure 3a,b) and discriminated the scent from a novel male ($Z = 2.490$, $N = 11$, $P = 0.013$; Figure 3a) or midventral gland scent from the same male habituated during the habituation trials ($Z = 2.934$, $N = 11$, $P = 0.003$; Figure 3b) with an increasing sniffing time.

Chemical analysis of secretions from left and right flank glands and midventral gland

We found similarities in the chemical profiles of all 3 sources of odor (Figure 4). GC-MS analyses of individual samples revealed 11 total ion chromatogram peaks found in the left flank, right flank, and midventral gland secretions of males (Figure 4, Table 1). All compounds except compound #5, #6, and #10 in the midventral gland secretions were less abundant compared with the left flank gland. In particular, compound #4 was significantly higher in concentration in secretions from the left flank gland than from the right flank

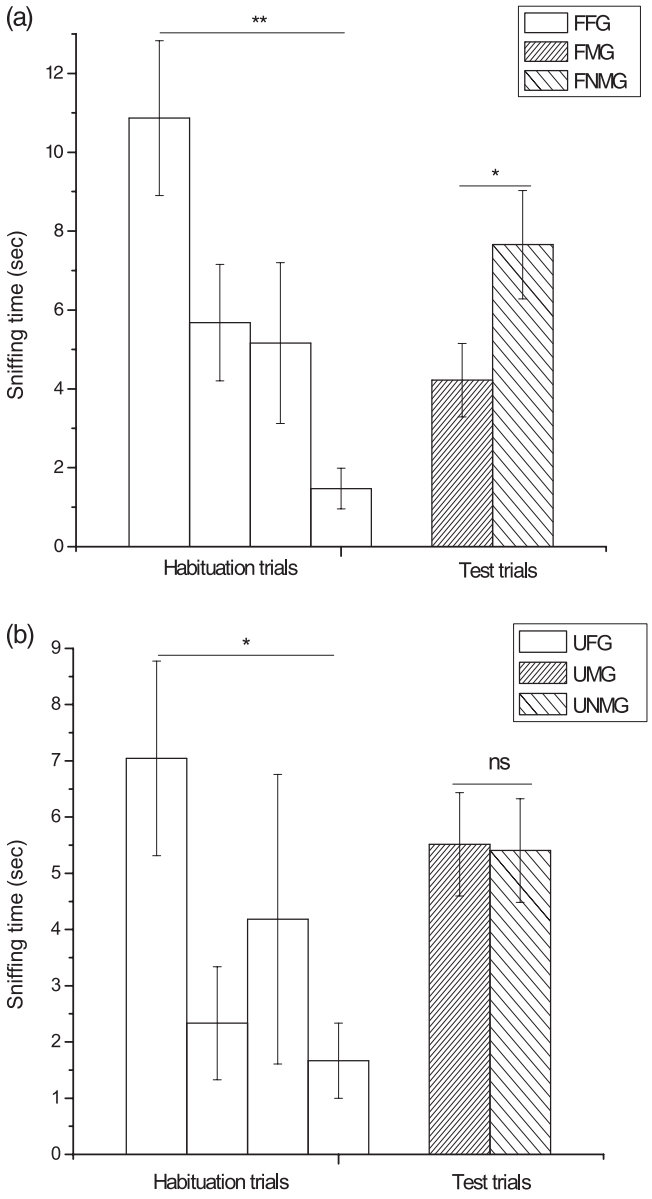

Figure 1 Prior social contact is necessary for female hamsters to generalize individual information from the flank gland scent to ventral gland scent of the same male. **(a)** Mean (±standard error [SE]) time that female hamsters (N = 12) spent sniffing the flank gland scent of familiar (FFG) male hamsters during 4 habituation trails and midventral gland scent of the habituated familiar male (FMG) and familiar novel male hamsters (FNMG) during the test trial. **(b)** Mean (±SE) time that female hamsters (N = 11) spent sniffing flank gland scent from unfamiliar (UFG) males during 4 habituation trials and midventral gland scents from the habituated unfamiliar males (UMG) and unfamiliar novel males (UNMG) during the test trial. *P < 0.05, **P < 0.01, ns, not significant.

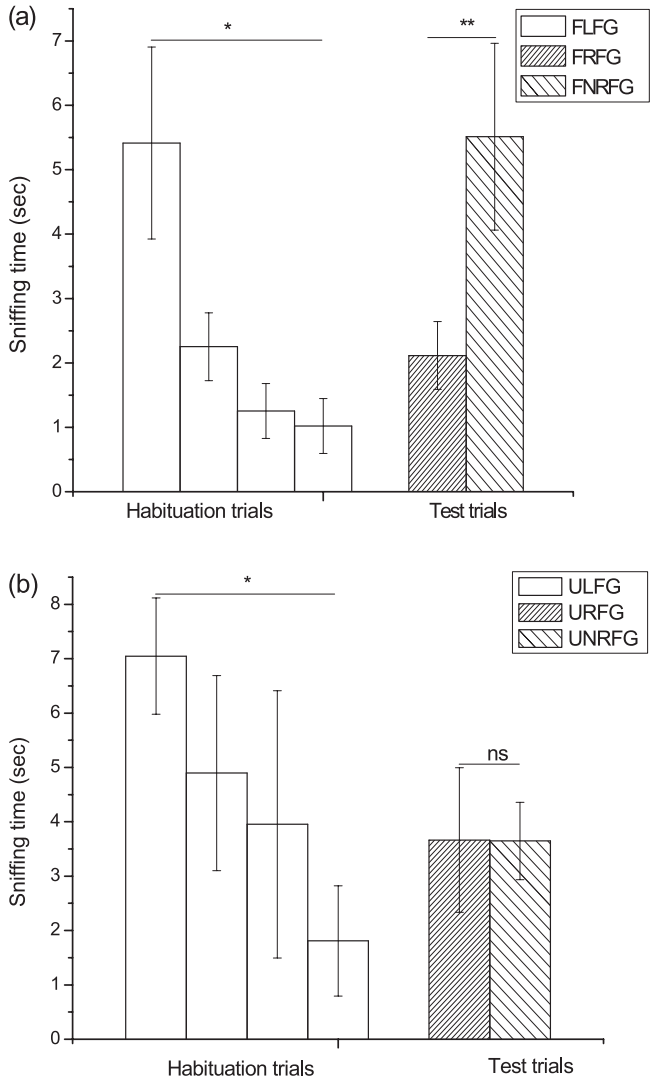

Figure 2 Prior social contact is necessary for female hamsters to generalize individual information from the left flank gland scent to right flank gland scent. **(a)** Mean (±standard error [SE]) time that female hamsters (N = 10) spent sniffing the left flank gland scent of familiar male hamsters (FLFG) during 4 habituation trials and right flank gland scents from habituated male hamsters (FRFG) and familiar novel male hamsters (FNRFG) during the test trial. **(b)** Mean (±SE) time that female hamsters (N = 11) spent sniffing the left flank gland scent of unfamiliar male hamsters (ULFG) during 4 habituation trials and right flank gland scents from habituated males (URFG) and unfamiliar novel male hamsters (UNRFG) during the test trial. *P < 0.05, **P < 0.01, ns-not significant.

gland and midventral gland (GLM, multivariate analysis followed by post hoc test of LSD, $F_{(2,12)} = 0.017$ for compound #4) (Table 1).

We also calculated the RSD for individual differences (Table 2). First, patterns in the types of chemical compounds present differ between secretions of the left and right flank glands and the midventral gland. Three compounds (#1, #2, and #3) were absent in secretions from the midventral gland of one male (#28), and one compound (#5) was absent from the right flank gland of another male (#12, Table 2). Second, RSDs of 6 compounds from secretions from the left flank gland were larger than average (44.08 ± 23.53), and 5 compounds in the right flank gland and midventral gland were larger than average (Table 2). The RSD of all compounds predominantly fell between 20.10 and 160.78 except for compound #4 (right flank gland = 19.77), compound #8 (left flank gland = 15.72, midventral gland = 11.74),

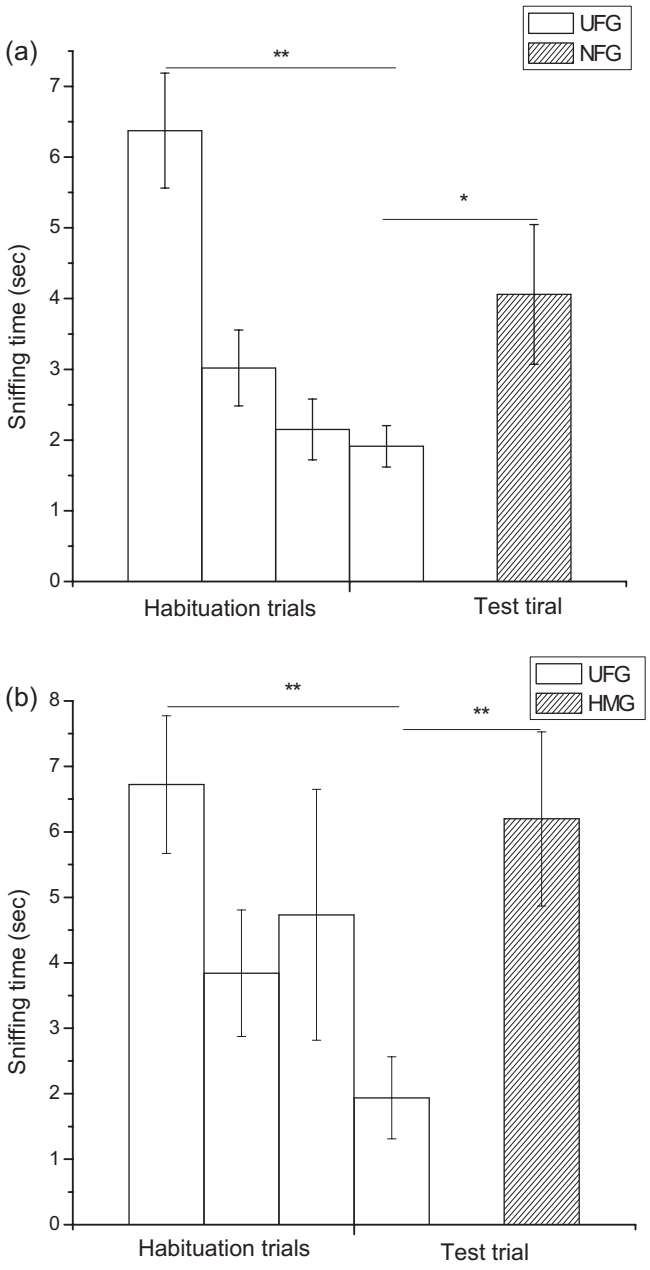

Figure 3 Mean (±standard error) time female hamsters (N = 11) spent sniffing flank gland scent from unfamiliar males (UFG) during 4 habituation trials and different sourced glands (**a**, flank gland scents of novel males [NFG]; **b**, midventral gland scents of the habituated males [HMG]) during the test trial. $*P < 0.05$, $**P < 0.01$.

compound #10 (left flank gland = 16.12), and compound #11 (right flank gland = 17.79).

PCA based on the relative abundance of the chemical compounds present in the 3 gland scents extracted 4 principal components (PCs). The compounds' values for the component loading for PCs 1 (PC_1), 2 (PC_2), 3 (PC_3), and 4 (PC_4) are listed in Table 3. The percentage of variance explained by each of the 4 PCs was 41.22, 22.06, 18.29, and 11.80, respectively. The 4 factors accounted for a total of 93% of the cumulative total variance. The scatter plot revealed no clear division between the left and the right flank glands or between either flank gland or midventral gland secretions (Figure 5a–f). However, we found that individuals were clearly and individually separated based on PC_2 versus PC_1 and PC_3 versus PC_1 (Figure 5a,b). No similar results were found in other combinations of plots of PCs (Figure 5c–f).

Discussion

From our experiments, we found that female rat-like hamsters are able to learn individual cues across scents from the right flank, left flank, and midventral glands and discriminate individuals provided that female rat-like hamsters have prior social interaction with the male scent donor. However, this prior social familiarization is unnecessary for rat-like hamsters to discriminate individuals with a paradigm of habituation–discrimination by a single and same-sourced scent. Chemical analyses of the glandular secretions from the 3 glands provide further support for our hypotheses. Individually distinctive cues are coded in the midventral, left, and right flank gland secretions in the rat-like hamster. Our current results are consistent with previous studies in golden hamsters (Johnston and Bullock 2001), wild-derived house mice (Hurst et al. 2001; Cheetham et al. 2007), and Belding's ground squirrels (Mateo 2006). This study provides a novel and useful addition to the literature of individual recognition/discrimination study integrating behavioral measurements and chemical analysis.

Johnston (2005) proposed that individual cues in the golden hamster may be a mixture of scents from different skin glands, urine, and feces. Our analyses on the chemical composition of 3 gland secretions in the rat-like hamster (Table 1, Figure 5a,b) show that 3 sourced scents provide a profile of individual cues. Scents from the left, right, and midventral glands from the same individual were clearly grouped together by the PCA. There was also overlap between different individuals, indicating the similarity of chemical compounds and composition in the 3 scents across different individuals (Figure 5a,b, Tables 1 and 2). This result indicates that 3 sources of scent may be enough to present an individual profile of odor, albeit the more sources of scent the better for coding individual cues.

One may argue that the results of previous studies using a habituation–dishabituation protocol with same sourced scents (Johnston 1993; Johnston and Bullock 2001; Murdock and Randall 2001; Tang-Martinez 2001) did show individual discrimination. These studies and our results (test 3, Figure 3a,b) seem to indicate that individual cues are coded in one scent only. However, individual recognition/discrimination under those experimental designs has been questioned due to the animal's unequal familiarity to the 2 testing scents (Thom et al. 2005). Previous study results (Hurst et al. 2001; Johnston and Bullock 2001; Mateo 2004; Johnston

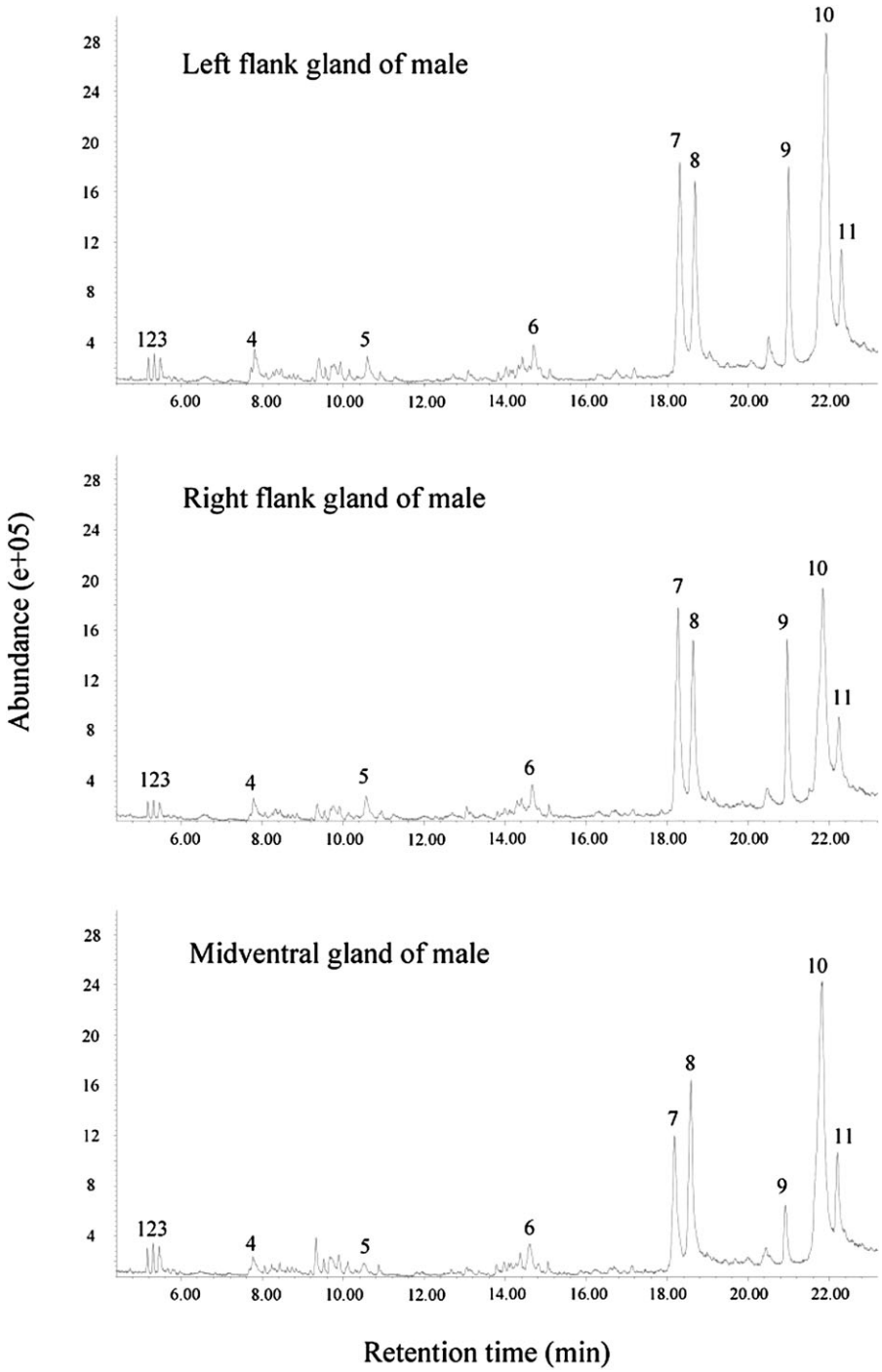

Figure 4 Representative of typical GC profiles of dichloromethane extracts from the left flank gland, right flank gland, and midventral glands of male rat-like hamsters. Numbered GC peaks correspond to compounds listed in Table 1.

2005) and those presented here (behavioral and chemical) on scents from different sources suggest that a single cue alone is not equivalent to cues generalized across 2 or more scents even though animals show different preference to the novel scent. Forming an integrated representation or "concept" of an individual is a complicated process dependent on neural and hormonal regulation (Albone and Shirley 1984; Thody and Shuster 1989; Johnston 2007) and, based on our results and previous results (Johnston and Bullock 2001), requires a prior social interaction in rat-like hamsters. Social interaction may provide female hamsters with the opportunity to collect, compare, and form the profile of the whole individual instead of a single scent (Johnston and Jernigan 1994). Johnston and Peng (2008) and Ramm et al. (2008) defined

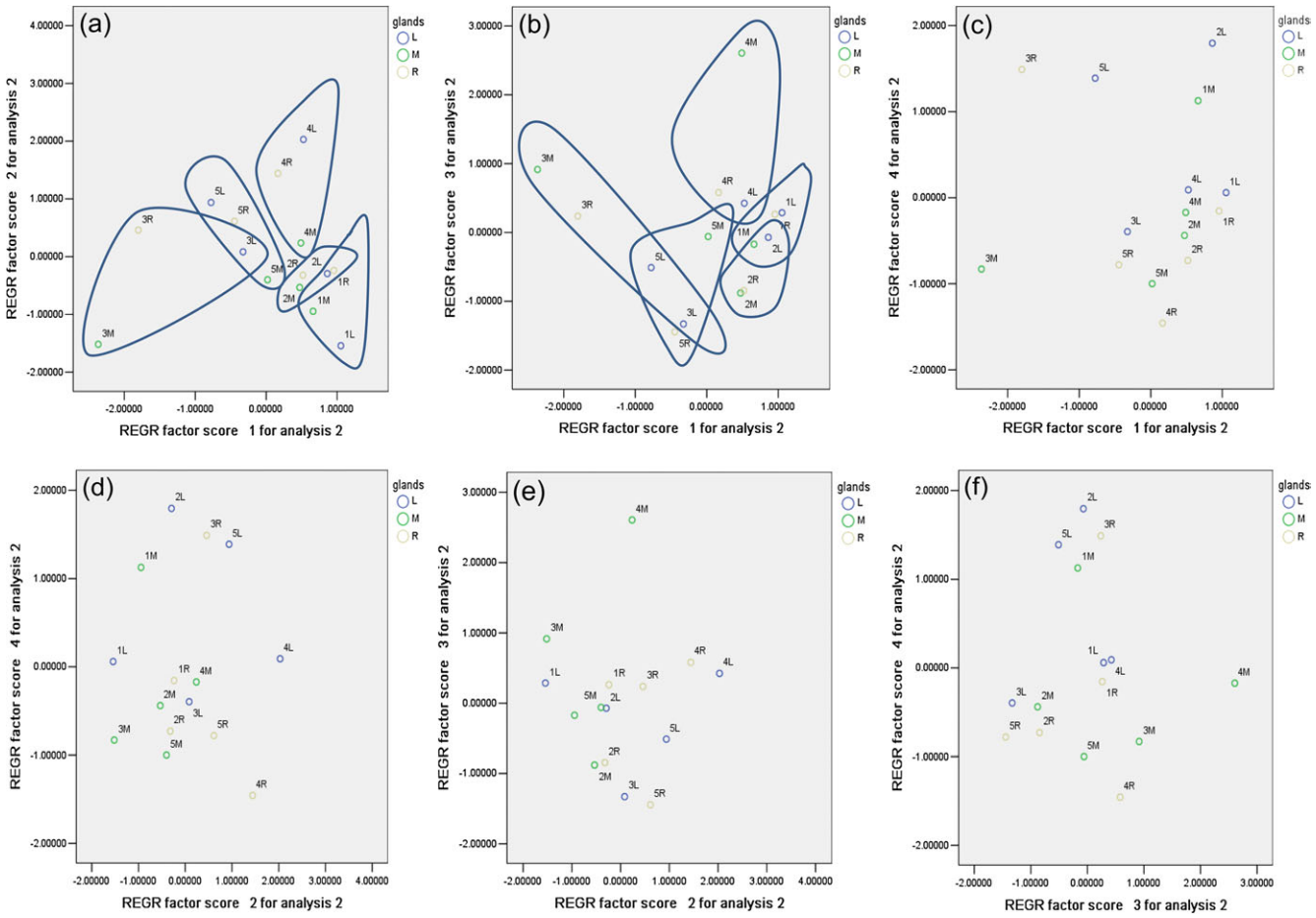

Figure 5 Results of PCAs of 11 compounds putatively identified in the secretions of left, right, and midventral glands from 5 male rat-like hamsters and scatter plots based on different combinations of the first 4 components. **(a)** Regression factor score 1 versus 2; **(b)** regression factor score 1 versus 3; **(c)** regression factor score 1 versus 4; **(d)** regression factor score 2 versus 4; **(e)** regression factor score 2 versus 3; **(f)** regression factor score 3 versus 4. L, R, and M beside a circle indicate left flank gland secretions (L), right flank gland secretions (R), and midventral gland secretions (M). Number in front of a letter represents the animal number. REGR indicates regression. Each solid line circle encircling the 3 numbers–letters represents one individual.

this prior social interaction as physical contact. This social contact may be a common prerequisite for animals to generalize individual information from one scent to another from the same individual.

A criticism of tests of individual recognition via the habituation–dishabituation paradigm with single stimulus is that they do not reveal real recognition but simple discrimination because the 2 scents are of unequal familiarity to the subjects (Thom et al. 2005). The habituation–dishabituation paradigm itself has been greatly criticized because of the risk of unequal odor familiarity (Halpin 1986), scent location effects, and novel scent effects (Thom et al. 2005). Prior social contact and habituation trials in our design did cause unequal familiarity between the 2 scents; however, our results showed that female hamsters in tests 1 and 2 treated the 2 testing scents from unfamiliar males as novel scents even though they were habituated to one of them (flank gland scent or left flank gland scent) (Figures 1b and 2b). Unlike the regular weekly social interaction, a recent social interaction between male and female hamsters caused unequal familiarity and also facilitated the learning process of individual information from different sourced cues (Figures 1a and 2a). The effects of unequal familiarity in the cross-habituation discrimination tests may not be a simple familiarity to one single scent but the mixed scent or body odor. For example, subjects have probably generalized and learnt the body odor and then discriminate between the 2 testing scents by using the referent created during social contact. This is real individual discrimination and recognition rather than simple scent discrimination as seen in the single stimulus tests (Figure 3a,b).

For solitary and polygynous mammal species such as the rat-like hamster, recognizing conspecifics is essential to many aspects of their social behavior, including parent–offspring relationships, mate choice, and recognition and territory defense (Brown and MacDonald 1985; Sherman et al. 1997). When males do not provide parental care, females are the choosier sex due to an unequal investment in reproduction

(Anderssen 1994; Dugatkin and Godin 1998). For females, however, there is a trade-off between choosing a superior mate and the possibility of missing the reproductive season due to the short window (1–2 days), during which they are receptive (Krebs and Davies 1993; Dugatkin and Godin 1998). The ability of females to learn individual cues from one scent to another from the same individual undoubtedly saves time and energy when identifying mates and avoiding inbreeding. From the point of an individual, it is essential to remember neighbors, rivals, and potential mates so as to promote one's own reproductive success and fitness (Johnston 2005). Individuals may gain direct benefits if they are able to learn and discriminate conspecifics using different sources of scent encountered in their social lives (Brown and MacDonald 1985; Jennions and Petrie 2000; Hurst et al. 2001). In addition, wild animals may deposit scent markings using different sources of odor (e.g., urine, skin gland secretions, and feces) at different times and social contexts. Laboratory male rat-like hamsters frequently deposit scent from the flank gland or midventral gland (Zhang et al. 2001), and female and male home ranges overlap in the field (Zhang et al. 1998). Females were easily able to learn from flank gland to discriminate midventral gland secretions or learn from left to discriminate right flank gland secretions of the same male provided that they had a prior social interaction. Our results suggest that the capability of learning individual information from one scent to another from the same individual may be common in solitary and polygynous mammals.

Moreover, our chemical analysis results indicate that the coding system for individual distinctive cues in rat-like hamsters may be analog and not digital (Sun and Müller-Schwarze 1998a) whereby individual distinctive cues are based on qualitative (variance) and not quantitative patterns (presence or absence) of constituents (Sun and Müller-Schwarze 1998a, 1998b; Zhang et al. 2007; Zhang, Liu, et al. 2008). We identified 7 compounds from rat-like hamster secretions (dodecanoic acid [5], tetradecanoic acid [6], *Z*-11-hexadecenoic acid [7], hexadecanoic acid [8], 5-dodecyldihydro-2(3H) furanone [9], oleic acid [10], and octadecanoic acid [11]; Table 1). Four peaks were identified as "unknown" compounds due to no matching compounds in the MS library. The compounds tetradecanoic acid (6), hexadecanoic acid (8), and octadecanoic acid (11) have also been identified in the closely related golden hamster (Zhang, Rao, et al. 2008) and primates such as female cotton-top tamarins, *Saguinus oedipus oedipus* (Belcher et al. 1988). Tetradecanoic acid (6) and hexadecanoic acid (8) are potential female pheromones, and octadecanoic acid (11) is male specific. Examples of these compounds in other species include the anal gland secretions of *Mustela eversmanni* and *M. sibirica* (Zhang et al. 2003; Zhang, Rao, et al. 2008). We did neither find species-specific compounds nor clear differences between secretions from the right and left flank glands and between the flank and midventral glands. Overlap in chemical composition in secretions from different glands is common in small mammals due to the presence of more than one gland of the same type such as apocrine or sebaceous (Albone and Shirley 1984; Brown and MacDonald 1985; Thody and Shuster 1989; Johnston 2003, 2005). It is possible that the similarity in chemical composition found here was the result of odors mixing across the body (Johnston 2005).

It is important to note a caveat in our experimental design. Ideally, the manipulation of females should be identical across all conditions except the familiarization process because motivation might be affected after being exposed to male scent. In other words, female control subjects should also interact with a male during the familiarization process and then be tested using scents from 2 other males. Fortunately, our experimental results were not greatly impacted because of the regular exposure of subjects to male donors each week before our experiment. A previous study on golden hamsters showed that one male loser learnt to recognize individuals during brief interactions and remembered this information in the short term (30 min) and long term (1 week) (Lai and Johnston 2002). Differences in the intensity of interaction and subject gender may affect one animal's memory to the inter-actor and thus the final behavioral results. Further research is needed that considers all potential factors, including behavioral assay of chemical compounds in the gland secretions. Studies that amalgamate behavioral and chemical data are an exciting avenue for future research, and understanding both these elements is necessary in order to garner a complete understanding of how animals perceive those around them and how chemical information contributes to individual recognition.

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