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Cohabitation impaired physiology, fitness and sex-related chemosignals in golden hamsters

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Abstract

This study investigated the impact of long-term paternal presence (cohabitation) on several physiological parameters such as body weight, adrenal weight, cortisol of parents, and the survival of pups compared with brief daily encounters (isolation) of male–female pairs in golden hamsters (*Mesocricetus auratus*). We showed that females were affected more by cohabitation as evidenced by increased body and adrenal weights, elevated cortisol concentrations, and heavier uteri and spleens as compared with cohabiting male and isolated females. Furthermore, we found that tetradecanoic and hexadecanoic acids of the flank glands were sexually dimorphic, for which they were putative female pheromones. These two compounds were suppressed in females and elevated in males by cohabitation, suggesting that cohabitation impaired sex chemosignals. Overall, we concluded that housing females and males together had deleterious effects on adults and the survival of their pups in the golden hamster.

Keywords: Biparental care; Fitness; Cortisol; Stress; Chemosignal; Flank gland

1. Introduction

Adult golden hamsters (*Mesocricetus auratus*) live a solitary life in both breeding and nonbreeding conditions except copulation of a half hour when females are sexually receptive [25]. Such a lifestyle suggests that individual caging is optimum to laboratory golden hamsters and, indeed, it has already been shown in golden hamsters and ratlike hamsters (*Tscheskia triton*), also a solitary rodent, that caging individuals of the same sexes in pairs has stressful effects [13,17,36]. In contrast, caging gregarious female mice (*Mus musculus*) or female brown rats (*Rattus norvegicus*) in groups of moderate size is advantageous [3,5]. Thus, artificially caging solitary rodent conspecifics in pairs has created a form of social stress for scientific studies in the laboratory [13,36]. Such a social stress form might be used as models to study human diseases such as obesity and depression and regulating mechanism of rodent population [13, 16]. However, little is known about what exact effects it may have to put solitary rodents of opposite sexes in pairs, and whether such effects are sexually different. Only one relevant study has shown the effects of paternal presence on survival of pups. Namely, Siberian hamsters (*Phodopus sungorus*) perform maternal care, whereas Djungarian hamsters (*P. campbelli*) do biparental care in nature; hence the absence of males makes survival of pups fall to 47% in Djungarian hamsters but does not affect that in Siberian hamsters [6,7,32,33]. Our recent work also showed that paternal presence imparted stressful effects on female partners indicated by their obesity and decrease in the survival of pups in ratlike hamsters (Authors' unpublished data). In addition, if group-housed male and female rodents of solitary species are stressed, the survival of pups may decrease, because maternal infanticide is usually associated with stress [9,25].

Adrenal glands are the anti-stress glands and produce glucocorticoids in response to social stress such as overcrowdings and social defeats, namely, social interactions can affect the adrenal gland and social victory often atrophies and social defeat

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hypertrophies adrenals, and chronic release of glucocorticoid hormones may suppress reproduction and androgen production [2,6]. Therefore, they are regarded as most efficient indicators of animals' responses to social stresses [2,6].

Since many male pheromones are hormone-dependent, it was rational to explore the relationship between social stress and pheromones in golden hamsters. For example, 2,5-dimethylpyrazine, a female-specific urinary component, is dependent on adrenal glands and glucocorticoids and vitally responsible for the Lee–Boot effect, in which groups of female mice housed together or a solitary female mouse is kept in a cage supplied with bedding from a cage full of female mice experience increases and synchrony in their estrus cycles [22, 23]. In addition, social conditions virtually always change behavioral features of rodents. For example, isolated golden hamsters are more aggressive than those housed in groups [25].

In the current study, we aimed at examining how permanently caging male and female golden hamsters in pairs affect adult pairs and their pups. We hypothesized that cohabitation of male–female pairs would impart negative effects on behavior and/or physiology of them and their pups. In testing the hypothesis, we paired adult males and females and let them cohabit since to examine their behavior, physiological status, flank gland secretion composition, and survival of pups in comparison with the control (treated with brief daily encounters).

In particular, it has been well documented in golden hamsters that the flank gland is larger in males than in females and can release olfactory signals of sex, social and physiological status [9,20,25]. Sexual attractiveness and the size of flank glands are also affected by social stimulus, namely, dominant male hamsters have larger flank glands that have higher attractiveness to females [9,20]. We would conduct gas chromatographic-mass spectrometry (GC-MS) analysis to examine whether forced long-term cohabitation would impair sex-related chemosignals excreted by flank glands.

2. Materials and methods

2.1. Experimental animals

Fourteen sex-naïve male and female golden hamsters at 8 weeks of age were purchased from Weilitong-Lihua Laboratory Animal Company, Beijing, China. They were individually kept in plastic cages (measuring 37×26×17 cm) in a reversed light:dark regime of 14L:10D (lights off at 9:00 am) at 22±2 °C for 4 weeks prior to housing treatment in the Institute of Zoology, Chinese Academy of Sciences, China. Food and water were always available. Wood shavings were used as bedding material and changed biweekly. Females with perforated vaginas and scrotal males were classified as potential breeders and used in our experiments.

2.2. Procedures of housing treatment

The body weight-matched males and females were randomly paired and assigned to either the cohabitation group, where a male was paired with a female and kept in the same cage for 46 consecutive days, or the control group, where we allowed a male to interact a female for 30 min a day between 9:00 and 11:00 AM (during the dark phase) for 46 days. To remove the handling effect, we also handled (taking out and returning) the cohabitation males once a day, exactly as we did on the males in the isolation group.

2.3. Quantification of aggressive behavior

We defined chasing, biting and side-way posture as aggressive behavior, and fleeing, standing upright and lying on back as defensive behavior as previously described [25,31,39]. Aggressive behavior and defensive behavior were collectively named agonistic behavior. When we were conducting the experimental manipulation of the hamsters on days 14, 21 and 28, we used scanning as our sampling method (with 5 min as the inter-scan period) to tally the frequency of agonistic behavior between male and female pairs for 30 min between 9:00 and 11:00 of the dark phase.

2.4. Physiological and hormonal determination

We weighed the subjects before the first day and after the last day of the experimental manipulation. We checked the numbers of scars on the entire body surface on the 13th day. These scars should have been accumulated during previous 12 days of the experiments. The number of offspring was recorded on the parturition day for each female (the 16th or 17th day of the experiment) and on the last day (46th day), and was used for calculating litter size and survival of pups.

On the 47th day, the pups were separated with their parents and continuously kept in our laboratory. We killed all experimental adults at 09:00–11:00 AM by decapitation, and the trunk blood was individually collected within 3 min. We then autopsied carcasses, weighed the organs including the spleen, adrenals, flank glands, testis, epididymis, seminal vesicles, ovaries and uteri (to the accuracy of 0.1 mg). The quantity of fetuses of each female was counted as the second litter size. Each pair of flank glands was sealed in a clear vial and frozen at −20 °C. Relative organ weights were calculated in milligrams of organ weight per 100 g body weight [36].

To collect the flank glands, we first shaved the fur on the flank glands, and then cut off the glands along their outlines on the inner skin as previously described by Zhang et al. [34,39].

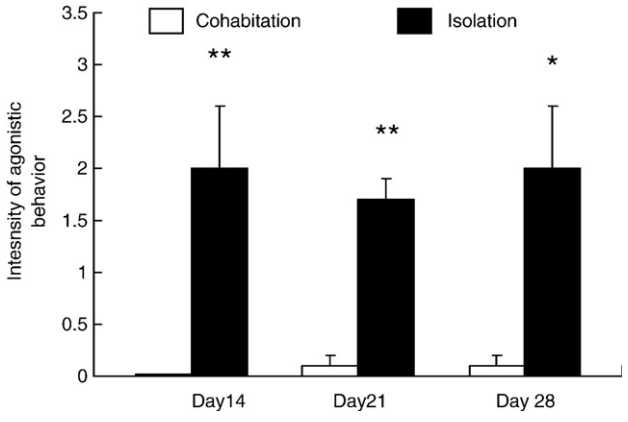

Fig. 1. Agonistic behavior between male and female pairs subjected to cohabitation or isolation. $^{**}p<0.01$, $^{*}p<0.05$ (Mann–Whitney U test).

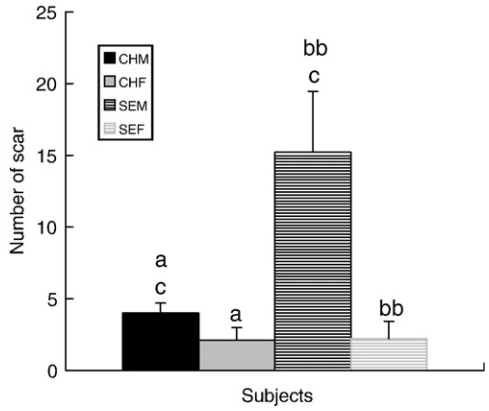

Fig. 2. Comparison of scar numbers between golden hamsters of the same sexes subjected to different housing conditions (CHM = cohabiting males, CHF = cohabiting females, SEM = singly caged males, SEF = singly caged females). The same-lettered bars refer to significant differences at 0.05 (single-letter) or at 0.01 (double-letters).

The paired glands from one hamster were sealed in a vial and stored at −20 °C until dichloromethane extraction.

The blood samples were centrifuged at 4000 rpm, and the serum was collected and stored at –20 °C for radioimmunoassay to measure serum hormone levels such as cortisol (ng/ml), testosterone (ng/ml) (only in males), estradiol (pg/ml) (only in females) and progesterone (only in females).

2.5. Radioimmunoassay of serum hormone levels

All samples for each hormone were quantified in a single radioimmunoassay (RIA) by ^{125}I RIA kits. All hormone kits were provided and measures were carried out by the Kemei-Dongya Biotechnological Company, Inc. Beijing, China. The human antiserum used was highly specific: cross-reactivity with other steroid hormones was <0.01%; intra- and inter-assay variability was <7.4% and <10.0%, respectively, for all measured hormones. The detectable sensitivities of cortisol, testosterone, estradiol and progesterone were respective <1 ng/ml, <2 ng/dl, <5 pg/ml, and <0.05 ng/ml, respectively [15,31,34,39].

2.6. Chemical analysis

We used dichloromethane to extract flank gland tissue. We first thawed and weighed each pair of flank glands at room temperature. Then, we added dichloromethane into the vial containing the glands in the proportion of 1 mg flank gland material in 5 μl dichloromethane. After 24 h, we removed the flank glands, sealed and stored the remaining solution at −20 °C until GC-MS assay within 1 week.

Analytical GC-MS was performed on an Agilent Technologies Network 6890N GC system coupled with 5973 Mass Selective Detector with the library NIST 2002. Xcalibur (Windows XP) was used for data acquisition and processing. The GC 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 the flow rate of 1.0 ml/min. The temperature of the injector was set at 280 °C. The oven temperature was programmed as follows: 100 °C as the initial temperature, which was increased by 5 °C/min up to 180 °C, then by 1 °C/min up to 260 °C and held for 15 min. Finally, the temperature was increased to 280 °C and held for 10 min for post run to clean the column. Electron impact ionization was used at 70 eV.

Table 1
Comparison of reproductive organs and serum hormone levels in male and female golden hamsters subjected to cohabitation or isolation (Mean±SD)

Physiological indexes		Males		Females	
		Cohabitation	Isolation	Cohabitation	Isolation
Body weight (g)	Initial	112.8±8.3	114.7±17.5	114.6±9.3	104.2±16.6
	Final	161.3±9.8	143.8±25.8	156.5±12.3a	129.0±15.9a
Adrenal	Absolute (mg)	44.1±12.2	35.9±5.5bb	33.3±10.6a	20.6±4.1a,bb
	Relative (mg/g)	0.28±0.09	0.26±0.04bb	0.21±0.07	0.17±0.04bb
Spleen	Absolute (mg)	110.7±21.9bb	111.2±24.0cc	377.4±122.9a,bb	209.8±78.5a,cc
	Relative (mg/g)	0.69±0.17bb	0.77±0.08cc	2.42±0.82a,bb	1.62±0.57a,cc
Flank gland	Absolute (mg)	140.4±17.6a,bb	105.0±31.2a,cc	21.8±8.0bb	16.4±4.3,cc
	Relative (mg/g)	0.87±0.12bb	0.73±0.18cc	0.14±0.05bb	0.13±0.04cc
Testis	Absolute (mg)	3490.2±333.4	3353.8±356.6	–	–
	Relative (mg/g)	21.68±2.09	23.70±3.01	–	–
Epididymis	Absolute (mg)	948.5±256.0	937.7±223.7	–	–
	Relative (mg/g)	5.87±1.44	6.54±1.38	–	–
Seminal vesicle	Absolute (mg)	1298.0±419.3	1690.2±274.4	–	–
	Relative (mg/g)	8.17±2.97a	11.89±1.63a	–	–
Ovary	Absolute (mg)	–	–	42.4±18.2	31.2±8.0
	Relative (mg/g)	–	–	0.28±0.13	0.24±0.04
Uterus	Absolute (mg)	–	–	2471.0±1725.7a	477.3±180.4a
	Relative (mg/g)	–	–	15.66±10.73	7.38±4.32
Serum hormones	Cortisol (ng/ml)	40.9±10.8bb	50.7±35.5	146.6±91.7a,bb	52.2±32.28a
	Testosterone (ng/ml)	4.5±1.6	3.1±2.3	–	–
	Estradiol (pg/ml)	–	–	4.4±2.1	3.9±2.4
	Progesterone (ng/ml)	–	–	6.8±4.7	6.3±6.1

The means in a row same-lettered singly or doubly are significant at the 0.05 or 0.01 level, respectively (*t* test).

Table 2
Comparison of reproductive success of female golden hamsters subjected to cohabitation or isolation (Mean±SD)

Experimental groups	Litter size at age of 0 day (n=14)	Survival of pups at age of 4 weeks (n=14)	Second litter size (numbers of fetuses) (n=13)
Cohabitation females	11.71±2.75	2.29±4.11*	13.00±6.00
Isolation females	11.88±3.14	7.25±3.92*	7.57±7.23

The means in a column marked by asterisk are significant at the 0.05 (t test).

Transfer line temperature was 280 °C. Scanning mass ranged from 30 to 350 amu. The amount of sample injected was 1 μl every time in a split mode (10:1).

Tentative identification was undertaken by matching the mass spectra of GC peaks with those in the MS library (NIST2002) and literatures [40]. The six compounds so identified were verified by authentic analogs (saturated acids, viz. compounds 2, 5 and 8 in Table 3) and/or corresponding methyl esters after methylation of samples (unsaturated acids, viz. compounds 4, 6 and 7). Two major male-specific compounds (1 and 3 in Table 3) were identified using NIST2002 library and previous literature [21] (W., Ma, D., Wiesler and M., Novotny, unpublished data).

Finally, we converted the peak area of a particular compound into the percentage (relative abundance) of the summed peak areas of all GC peaks to quantify the relative abundance of relevant compounds [28,34].

2.7. Statistical tests

To test the statistical significance, we first explored the normality of the data. Normal data were tested by parametric tests whereas non-normal data were tested by nonparametric tests. Specifically, we used Mann–Whitney U test for aggressive behavior, and independent t test for the number of body scars, reproductive success, organ indexes, serum hormone levels and relative abundances of the compounds of flank glands except those between the opposite sexes of different groups. All statistical analyses were conducted using SPSS (Version 10.0), and the level of significance was set at α=0.05 for all tests.

3. Results

We observed that all male and female pairs displayed more copulatory behavior in the first 2 days. This result was confirmed by consequent parturition on days 16 and 17, and thereafter, more aggressive behavior was observed. Furthermore, we found that the pairs subjected to the cohabitation treatment showed significantly less agonistic behavior than those subjected to the isolation control (Z=3.173, p=0.002 on day 14; Z=3.381, p=0.001 on day 21, Z=2.561, p=0.010 on day 28, Fig. 1). Males bore more scars than females in both cohabitation group (t=3.350, p=0.010) and isolation control with a brief daily encounter (t=3.980, p=0.004). Males in the control group bore more scars than those in the treatment group (t=2.615, p=0.019). However, females did not show any significant difference in the number of scares between the two groups (t=0.075, p=0.941) (Fig. 2).

Females in the treatment group became significantly heavier than females in the control group (t=3.621, p=0.004) at the end of the experiment whereas males showed no such difference (Table 1). Furthermore, compared with the control, cohabitation treatment increased the absolute weights of the adrenals (t=2.974, p=0.012), uteri (t=2.294, p=0.041) and spleen (t=3.041, p=0.010), and cortisol level (t=3.301, p=0.010) in females. Cohabitation-treated males had heavier flank glands in absolute weight (t=2.611, p=0.023) and lighter seminal vesicle in relative weight than control males (t=2.908, p=0.013). Control males had heavier adrenals (t=5.933, p=0.000 for absolute weight; t=4.153, p=0.001 for relative weight) than control females, but the sexual differences disappeared when they cohabited. Cortisol level was significantly elevated in females compared with that in cohabitation-treated males (t=2.571, p=0.024), but this sexual difference did not occur in the control group. Compared with their female partners, the spleens of both treatment and control males were lighter in absolute weight (t=5.653, p=0.000 for treatment, t=3.179, p=0.008 for control) and relative weight (t=5.476, p=0.000 for treatment, t=3.928, p=0.002 for control), and flank glands were heavier in absolute weight (t=16.21, p=0.000 for treatment; t=7.741, p=0.000 for control) and relative weight (t=14.96, p=0.000 for treatment; t=8.493, p=0.000 for control, Table 1). Also, cohabitation did not affect litter size

Table 3
Comparison of relative abundances of flank gland volatiles between male and female golden hamsters subjected to cohabitation or isolation (Mean±SD)

GE peak number	Retention time (min)	Compounds	Relative abundance of flank gland volatiles			
			Cohabiting		Separation	
			Males	Females	Males	Females
1	8.10	*E*5-Dodecen-2-one*	15.54±3.574	–	21.55±8.390	–
2	16.80	Tetradecanoic acid	3.743±0.657aa	9.487±2.838b,aa	4.061±1.119cc,	16.28±6.779b,cc
3	18.35	*E*14-Pentadecenoic acid**	4.900±1.570	–	5.126±1.807	–
4	20.37	*Z*9-Hexadecenoic acid	13.91±3.112aa,	8.124±3.383aa,	13.18±3.864b	7.277±3.357b
5	20.77	Hexadecanoic acid	18.89±2.429a,bb	29.30±4.248,bb	15.21±2.705a,cc	29.72±4.158cc
6	23.96	*Z*9,*Z*12-Octadecadienoic acid	12.47±2.911	17.27±5.847	11.88±6.430	16.66±7.806
7	24.07	*Z*9-Octadecenoic acid	27.13±5.391	35.83±6.980	28.65±7.525	30.06±6.917
8	24.41	Octadecanoic acid	0.286±0.443	–	0.366±0.556	–

* or ** indicate that the compounds were identified in previous study or in the NIST2001 library; other compounds were verified by authentic analogs in the present study. The means in a row same-lettered singly or doubly are significant at the 0.05 or 0.01 level, respectively (t test).

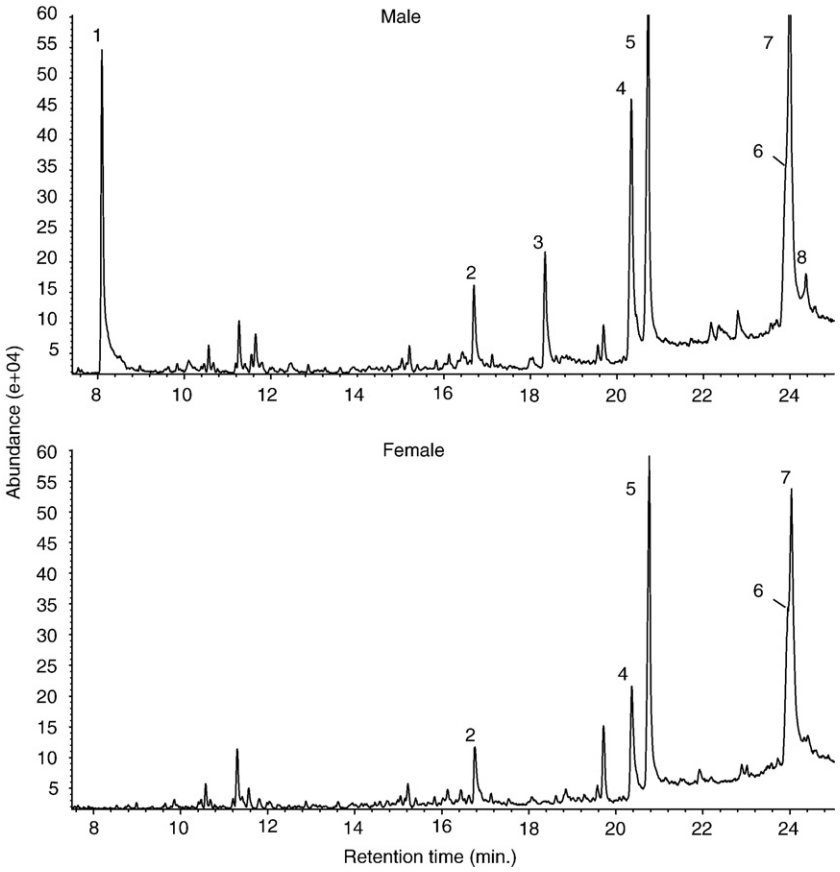

Fig. 3. Representative GC profiles of the dichloromethane extracts from the flank glands of isolated male (upper panel) and female (bottom panel) golden hamsters. The numbered GC peaks correspond to the compounds listed in Table 3.

(t=0.105, p=0.918) and the number of fetuses for females (second litter size) (t=1.529, p=0.152), but reduced the survival of pups (t=2.393, p=0.033, Table 2).

GC-MS analysis detected eight compounds in males and five compounds in females from the flank glands. Three compounds (1, 3 and 8) were unique to males and the other five (2, 4, 5, 6 and 7) were common to both sexes. The relative abundances of compounds 2 (t=5.217, p=0.000 for treatment, and t=4.704, p=0.001 for control) and 5 (t=5.627, p=0.000 for treatment, and t=7.742, p=0.000 for control) were significantly higher in females, whereas compound 4 (t=3.333, p=0.006 for treatment, and t=3.049, p=0.010 for control) was significantly higher in males as compared with their female partners. Compound 5 was more abundant in treatment males (t=2.679, p=0.020), whereas compound 2 was less abundant in treatment females (t=2.445, p=0.031) than the control for the same sexes (Fig. 3; Table 3).

4. Discussion

Our results indicated that agonistic behavior between male and female pairs was affected by housing condition, namely, cohabiting hamsters displayed less agonistic interactions than isolated ones in the golden hamster. Concurrently, scars on body surface were fewer in the hamsters after long-term cohabitation than those only allowed a brief daily encounter. The increases in body weight, adrenal mass and cortisol level of cohabiting females suggested that forced cohabitation had a stressful effect especially on the females. These results were in consensus with previous studies, which also show that females accelerate their body weight gain in response to social stress [13,16,18]. Furthermore, our study revealed all females had less scars, indicating that females were behaviorally dominated over their males during their interaction. This was corroborated by our observation that almost all females initiated attacks on and defeated their male partners. This result also suggested that females might be more sensitive in physiological response to social stress than males. Such sensitivity might be attributable to pregnancy and lactation [17,18]. This contention was supported by our recent results, which showed that putting intact female and spermaduct-ligated male golden hamsters together for a long time did not yield such a stressful effect (Pan et al., unpublished data). On the one hand, this result differentiated the effects of social defeats between the same sexes and opposite sexes [14,30]. On the other hand, it added a case of a dissociation between aggressiveness and physiological response between males and females that females were more aggressive but more physiologically stressed. Such dissociation occurs between persistent avoidances and brief corticosterone responses to repeated exposures to cat odor in rats [12]. In addition, cohabitation led to uterus hypertrophy in stressed females whereas in males, it led to seminal vesicle hypertrophy in the golden hamster. This result was converse to the finding in the house mouse (*M. musculus*), a gregarious rodent species, in which caging females alone (social

isolation) enlarges adrenal glands and enhances reproduction by shortening the estrous cycle, whereas caging female mice in groups suppresses both adrenals and reproduction [3].

Furthermore, because litter size did not differ between cohabitation and isolation groups and fathers did not show hostility to their infants after the removal of the mothers, the large survival reduction in pups in the cohabitation group could be due to elevated cortisol levels, which negatively affected maternal behavior by especially increasing the likelihood of infanticide as a result of stress [7–9,25]. In ratlike hamsters, a behaviorally and ecologically similar species with the golden hamster, the survival of pups is also considerably reduced by the presence of their fathers (Authors' unpublished data). Such different effects on the survival of pups due to the presence/absence of males have also been shown in Djungarian and Siberian hamsters albeit different lifestyles (more social) [33].

The flank gland is sexually dimorphic and secretes chemicals to signal sexual and social cues. Its size, sexual attractiveness and marking frequency are closely related to hormonal levels, social rank, mate choice, and aggression [1,2,11,25,20]. It has been found that the flank glands of male and female ratlike hamsters exhibit a reverse variation in response to certain social stress such as paired housing and predator exposure [35,36,39]. In the current study, isolation male golden hamsters that were most intensively attacked by female partners had smaller flank glands than cohabiting males, but females exposed to different housing conditions did not show such a difference.

Thus far, although numerous studies have reported the biological functions and proximate factors affecting the flank glands [25], only one has investigated the volatiles from the flank glands in the golden hamster and found male-specific compound (*E*5-dodecen-2-one) to attracts estrous females [21] (W., Ma, D., Wiesler and M., Novotny, unpublished data). In this study, we have found seven new compounds from the flank glands, two of which were male-specific.

Several studies have demonstrated that odorant compounds either unique to one sex or common to both sexes but varying in relative ratios can be considered putative chemical signals to communicate corresponding information [22,26,37,38]. In our study, the sex-common compounds tetradecanoic acid and hexadecanoic acid were richer in females and consequently might be potential female pheromones in the golden hamster. Indeed, our recent study confirmed their pheromonal activities by their attractiveness to and suppression of agonistic behavior between males (Authors' unpublished data). As such, the male-specific *E*14-pencadecenoic acid and male-elevated *Z*9-hexadecenoic acid might be potential male pheromones besides the male-specific *E*5-dodecen-2-one. This indicated that golden hamsters could communicate information about sex using either digital coding (viz. qualitative differences in unique compounds) or analog (viz. quantitative difference in shared compounds) [26–29]. However, ratlike hamsters did not have sex-specific compounds in their flank glands and therefore might only use analog coding for sex information (Author's unpublished data).

*Z*9-hexadecenoic and hexadecanoic acids have been identified as pheromonal components in insects and found in the vaginal secretion of golden hamsters [4,10,24,40]. Furthermore, our study demonstrated that tetradecanoic and hexadecanoic acids, both characteristic of femaleness, were increased in cohabiting males and suppressed in cohabiting females, indicating that chemical signals characteristic of a specific sex were somewhat impaired by cohabitation. Similar effects of housing condition on pheromonal composition have been substantiated in female house mice [23]. In rats, it has been shown that pheromones of pregnant females can reduce the likelihood of infanticide by nulliparous conspecifics [19], but we are not sure whether and how the changes in female pheromones affect the rate of infanticide and consequent survival of pups in the golden hamster.

In conclusion, although the male and female hamsters that were artificially caged in pair lived a behaviorally peaceful life, an array of physiological parameters including adrenal mass, serum cortisol level and sexual dimorphism in volatiles of flank glands and survival of pups suggested that caging them in pair could be a form of stress, which yielded sexual differences in behavioral and physiological response to cohabitation. These results should provided an insight into physiological reasons why some mammals are solitary whereas others are gregarious, in addition to the significance in designing optimal housing conditions for golden hamsters in the laboratory. In addition, this is the first report about putative pheromones from the flank glands of the golden hamster.

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