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ARTICLE

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Effects of social conditions on adult and subadult female rat-like hamsters (*Cricetulus triton*)

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Abstract As a solitary species, rat-like hamsters (*Cricetulus triton*) still live in family groups before they become mature and leave their families for a solitary life. This study aimed to investigate by a laboratory experiment if housing conditions have a different effect on physiological aspects of immature and mature females. We found that paired caged adult females became significantly heavier than their original weights; whereas the singly caged did not show significant change in their body weight. Although the subadults' body weights increased significantly compared to their initial weights in both paired or singly caged groups, significant changes in body weight did not occur between the two groups. Although spleen and adrenal gland sizes were not significantly different between the two adult groups, the cortisol levels were significantly elevated by paired caging. In subadults, the adrenal size of the singly caged group was larger than that in the paired caged group despite there being no significant difference in cortisol level. Flank glands became significantly larger in paired caged adults than in singly caged adults, and there were no significant differences in subadults between the two groups. Additionally, ovaries and uteri of the paired caged adult females were comparatively lighter than those of the singly caged group; in contrast, ovaries and uteri of the paired caged group were larger than those of the singly caged group in subadults, although progesterone and estradiol levels did not show significant differences between the two adult groups. These different changes in physiological traits caused by housing conditions indicated that paired caging depressed adults and facilitated subadults; isolation facilitated adults and depressed subadults.

Key words Rat-like hamster · *Cricetulus triton* · Housing condition · Adult · Subadult · Physiology

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Introduction

The effects of social conditions on the physiology of rodents are closely related to their lifestyles. For example, paired housing of the solitary-living golden hamsters (*Mesocricetus auratus*) caused an increase in body weight in adult females, but this did not occur in Siberian hamsters (*Phodopus sungorus sungrous*), a relatively social animal compared to golden hamsters (Bartness 1996; Fritzsche et al. 2000). In social female mice (*Mus domestics*), individual caging (separation) resulted in enlarged adrenal glands and shortened estrous cycle, whereas moderate group caging suppressed both adrenals and reproduction (Bronson and Champman 1968). However, in solitary male mice, separation decreased their adrenals (Palanza et al. 2001). Subadulthood is a period characterized by a transition of social behavior from family groups to a solitary life. It is unknown whether the effects of social conditions on solitary rodents produce a difference between subadults and adults. As subadults, animals undergo sex maturation, natally disperse and establish territory. So the consequences of social interactions of nonsibling subadults are valuable for us in order to understand the adaptation and population regulation of the animal (Wolff 1997).

Rat-like hamsters (*Cricetulus triton*) lead a solitary life throughout the year in farmlands in northern China and breed in spring and summer (Wang et al. 1996; Yang et al. 1996; Zhang et al. 2001a). Two unfamiliar males housed in the same cage quickly establish a stable social relationship marked by behavioral and physiological differences between dominants and subordinates (Zhang et al. 2001b). In contrast, two unfamiliar females caged together did not form a stable dominant–subordinate relationship during the

breeding season (Zhang et al. 2001a, unpublished data). The objective of the present study was to examine whether changes in physiological conditions caused by changes in housing conditions differed for subadult and adult female rat-like hamsters.

Materials and methods

We used 36 adult and 27 subadult female rat-like hamsters as test subjects. The adult hamsters were captured by live traps in farmlands in central Hebei Province, North China, in April (breeding season) 2001. The subadults were born to females that were pregnant when caught in the field.

All captured adult females were housed individually in plastic cages ($40 \times 25 \times 15$ cm) containing wood shavings and cotton nesting materials and fed with rat chow and water ad libitum. The colony was maintained on a 14L:10D light cycle (lights on at 17:00 h) at approximately 20°C. Unfamiliar adults came from different areas with a distance of more than 1 km, greater than the maximum activity range of males (the average range of females was less than that of the males and averaged about 300 m) (Wang et al. 1996). After 1 month of acclimation, two unfamiliar adults with similar body weight (within 10% difference) and estrous cycles of 4 days were housed in one cage; 12 pairs were formed. The remaining 12 adults were caged individually. There was no significant difference in initial body weights between the two groups (Table 1, $t = 0.119$, $df = 34$, $P = 0.906$). Estrous cycles were determined by extra-vaginal examination (Yang et al. 1999).

Hamsters caught in the wild and weighing between 40 and 80 g were classified as subadults (Yang et al. 1999). In the laboratory, hamsters are weaned from 23 to 33 days after birth (with ca. 50 g of body weight). The vagina opens and first estrus begins at ca. 85 g of body weight (Yang et al. 1999). Thus, it is legitimate to define subadult females as those weighing from 50–85 g. Nine pairs of females of 5 weeks of age from six different litters (litter size: 7–12) were formed by matching for body weight (10% difference) by placing two females from different litters in the same cage; the other nine females were singly caged. There was no significant difference in initial body weight between the two groups (54.88 ± 8.38 and 53.28 ± 10.65, $t = 1.387$, $df = 25$, $P = 0.178$).

Table 1. Changes in the body weight of adult female rat-like hamsters. Values given are mean ± SD. The means in a column marked by the same superscript letter are significantly different at 0.05: a denotes use of dependent *t*-test or 0.01, aa denotes use of paired-sample *t*-test

Group	Absolute body weight (g)	Percent increase in body weight
Paired ($N = 24$)		
Initial	153.38 ± 21.51^{aa}	7.99 ± 9.59^{a}
Final	164.79 ± 20.86^{aa}	
Single ($N = 12$)		
Initial	154.31 ± 23.40	2.97 ± 8.30^{a}
Final	158.40 ± 24.42	

Between 10:00 and 11:00 a.m. of the 31st and 32nd days, all animals were sacrificed during their diestrous days. Blood samples were taken and centrifuged at 4,000 rpm, and the serum was stored at –20°C for radioimmunoassay to measure estradiol, progesterone and cortisol levels. The spleen, adrenal gland, flank gland, uterus and ovary were weighed (± 0.1 mg) and dissected. Relative organ weights given as milligram organ weights per 100 g body weight were calculated by the following formula: ROW(mg) = AOW(mg) × 100/BW(g), where ROW, AOW and BW were relative organ weights, absolute organ weight, and body weight, respectively.

Radioimmunoassays were performed using the methods developed by Li (1985). Specifically, we used commercially available testing kits (Beijing Institute of Radioimmunoassay, China) for ^{125}I-cortisol, ^{125}I-estradiol, ^{125}I-progesterone and human antiserum. For cortisol, the detectable range of the assay was 0–500 ng/ml and the sensitivity was higher than 1 ng/ml; for estradiol, the detectable range of the assay was 0–200 pg/ml and the sensitivity was higher than 0.2 pg/ml; for progesterone, the detectable range of the assay was 0–20 ng/ml and the sensitivity was higher than 0.02 ng/ml.

Statistically, the differences between initial and final body weights were tested using paired *t*-test; differences in organ weights and hormone levels of two groups were tested using independent two-tailed *t*-test. All tests were performed using the computer software SPSS 6.0. The level of significance was set at $\alpha = 0.05$.

Results

Body weights

In adult females caged in pairs, the final body weights were significantly greater than their initial body weights [$t(23) = 4.508$, $P < 0.01$]; whereas, in adult females caged alone, there was no significant difference between initial and final body weights [$t(11) = 1.035$, $P > 0.05$]. The percent increase in body weight in the paired group was significantly higher than that in the singly caged group [$t(34) = 2.203$, $P < 0.05$; Table 1].

In subadults, the final body weights in both paired and single groups were significantly greater than their initial body weights (the paired group: $t(17) = 15.60$, $P < 0.01$; the single group: $t(8) = 13.40$, $P < 0.01$). There was no significant difference in the percent increase in body weight between subadults housed alone and those housed in pairs ($t(25) = 0.180$, $P = 0.859$; Table 2).

Organ weights

There was no difference in absolute ovary weight between paired caged and singly caged adult females [$t(34) = 1.669$, $P < 0.104$], but the relative ovary weights of paired caged adults were significantly smaller than those of singly caged adults [$t(34) = 2.127$, $P < 0.050$]. For the uterus, both abso-

lute [$t(34) = 2.303$, $P < 0.05$] and relative weights [$t(34) = 2.689$, $P < 0.05$] of the paired caged adults were significantly smaller than those of the singly caged adults (see Table 3). The pattern differed for subadult females. Paired caged subadults had both heavier absolute ovary weight [$t(25) = 2.162$, $P < 0.05$] and relative ovary weight [$t(25) = 2.182$, $P < 0.05$]. This was also true for uterus weights with paired caged females having heavier uteri (absolute uterus weight: $t(25) = 2.951$, $P < 0.01$; relative uterus weight: $t(25) = 2.588$, $P < 0.05$) than singly caged subadults (see Table 4). The absolute and relative weights of the flank glands of paired caged adult females were significantly greater than those of singly caged adults [$t(34) = 2.449$, $P < 0.05$; $t(34) = 2.539$, $P < 0.05$; Table 3]. There were no differences between paired caged and singly caged subadults in flank gland weight [absolute: $t(25) = 0.679$, $P > 0.05$; relative: $t(25) = 0.638$, $P > 0.05$; Table 4].

There were no significant differences in either absolute or relative spleen weights or adrenal gland weights between paired and singly caged adults [absolute weight: spleen: $t(34) = 0.391$, $P > 0.05$; adrenal: $t(34) = 0.303$, $P > 0.05$; relative weight: spleen: $t(34) = 0.221$, $P > 0.05$; adrenal: $t(34) = 0.037$, $P > 0.05$; Table 3]. Paired caged subadults had significantly higher absolute spleen weights [$t(25) = 2.272$, $P < 0.059$ and lower absolute adrenal weights [$t(25) = 2.274$, $P < 0.05$] than singly caged subadults. There were no differences in relative spleen or adrenal weight between paired and singly caged subadults (Table 4).

Table 2. Changes in the body weight of subadult female rat-like hamsters. Values given are mean ± SD. The means in a column marked by the same superscript letter are significantly different at 0.01 (double letters) using paired-sample *t*-test. Relative organ weights (organ-weight-to-body ratios) are given as milligram organ weight per 100 g body weight

Group	Absolute body weight (g)	Percent increase in body weight
Paired ($n = 18$)		
Initial	54.88 ± 8.38^{aa}	59.11 ± 16.02
Final	87.12 ± 14.06^{aa}	
Single ($n = 9$)		
Initial	53.28 ± 10.65^{bb}	60.36 ± 18.89
Final	84.17 ± 10.37^{bb}	

Hormone levels

Paired caged adults had higher cortisol levels than singly caged adults [$t(34) = 2.63$, $P < 0.05$]; there were no differences in progesterone [$t(34) = 0.26$, $P > 0.05$] or estradiol levels [$t(35) = 1.27$, $P > 0.05$]. Paired caged subadults had higher progesterone levels than singly caged subadults [$t(35) = 2.48$, $P = 0.021$]; but there were no differences in cortisol [$t(35) = 0.97$, $p > 0.342$] or estradiol levels [$t(35) = 0.01$, $P = 0.994$] (see Table 5).

Table 3. Differences in organ weights of adult females. Values given are mean ± SD. The means in a column marked by the same superscript letter are significantly different at 0.05 using dependent *t*-test. Relative organ weights (organ-weight-to-body ratios) are given as milligram organ weight per 100 g body weight

Organ	Group	*N*	Absolute weight of organs (mg)	Relative organ weight in 100 g body weight (mg)
Ovary	Paired	24	15.50 ± 4.27	9.52 ± 2.74^{a}
	Single	12	19.20 ± 9.15	11.99 ± 4.58^{a}
Uterus	Paired	24	87.65 ± 31.38^{a}	53.02 ± 16.53^{b}
	Single	12	119.94 ± 52.96^{a}	77.71 ± 38.90^{b}
Flank gland	Paired	24	126.23 ± 47.96^{b}	77.59 ± 31.92^{c}
	Single	12	83.24 ± 52.97^{b}	50.83 ± 24.84^{c}
Spleen	Paired	24	131.20 ± 44.22	79.28 ± 22.01
	Single	12	124.77 ± 51.02	77.54 ± 22.75
Adrenal Gland	Paired	24	19.68 ± 5.62	12.12 ± 3.72
	Single	12	19.13 ± 3.77	12.08 ± 1.53

Table 4. Differences in organ weights of subadult females ($df = 25$). Values given are mean ± SD. The means in a column marked by the same superscript letter are significantly different at 0.05 (single letter) or 0.01 (double letters) using dependent *t*-test

Organ	Group	*N*	Absolute weight of organs (mg)	Relative organ weight in 100 g body weight (mg)
Ovary	Paired	18	41.49 ± 10.40^{a}	48.13 ± 9.23^{a}
	Single	9	32.20 ± 10.46^{a}	38.64 ± 12.78^{a}
Uterus	Paired	18	161.28 ± 40.64^{bb}	190.32 ± 56.09^{b}
	Single	9	94.69 ± 75.39^{bb}	113.95 ± 95.28^{b}
Flank gland	Paired	18	26.42 ± 19.09	31.10 ± 22.01
	Single	9	21.67 ± 11.71	25.91 ± 14.14
Spleen	Paired	18	64.18 ± 12.22^{c}	75.79 ± 17.69
	Single	9	52.37 ± 13.35^{c}	64.08 ± 23.38
Adrenal Gland	Paired	18	9.58 ± 1.20^{d}	11.34 ± 2.18
	Single	9	10.96 ± 1.89^{d}	13.10 ± 2.12

Table 5. Differences in serum cortisol and progesterone leves in adult and subadult female rat-like hamsters. The means in a column marked by the same superscript letter are significantly different at 0.05 using dependent *t*-test

Group	Cortisol (ng/ml)	Progesterone (ng/ml)	Estradiol (pg/ml)
Adults df = 34			
Paired	45.517 ± 18.100[a]	1.133 ± 1.034	6.882 ± 3.661
Single	27.725 ± 17.491[a]	1.012 ± 1.261	5.242 ± 2.940
Subadult (df = 25)			
Paired	62.360 ± 33.924	2.124 ± 1.797[a]	11.148 ± 4.792
Single	77.770 ± 46.544	1.077 ± 0.720[a]	11.158 ± 2.626

Discussion

In solitary species, such as golden hamsters, increases in body weights caused by group housing are used as an indicator of social stress (Borer et al. 1988; Meisel et al. 1990; Fritzsche et al. 2000). This body weight gain is primarily due increased body fat (Borer et al. 1988). Our results show that body weight of paired caged adult rat-like hamsters increased significantly faster than those of singly caged adults, but in subadults there was no significant difference in body weights between paired and singly caged animals. This suggests that paired housing was a social stressor on adults, but not on subadult female rat-like hamsters.

Adrenal gland activity and glucocorticoid levels are sensitive to social stress (Creel 2001). Although the size of the adrenal gland of adult rat-like hamsters did not differ in animals kept in pairs and those housed alone, the activity of adrenal glands was stimulated by paired caging, as shown by elevated cortisol levels. In contrast, separation may be a stressor in subadults, since singly caged subadults had larger adrenal glands and smaller spleens than paired caged subadults, which indicates the impairment of immunity. In contrast to the hamsters, separation was a stressor in female adult mice (Bronson and Champman 1968; Palanza et al. 2001).

When rat-like hamsters are stressed by predator odor, the flank gland becomes significantly smaller in males and larger in females (Zhang et al. 2003, 2004). The flank gland inflation in paired caged adults may also indicate that paired caging is a stressful social condition. Since castration can decrease the flank gland size and ovariectomy can stimulate the flank gland in rat-like hamsters (Zhang et al. 1999a–c), physiologically, the enlargement of flank glands of paired caged adult females may be partially caused by atrophy of the ovary induced by caging them in pairs. Other hormones, including pituitary hormones, might be affected by social stress (Ebling 1977; Human et al. 1995) and, consequently, could stimulate or inhibit flank glands.

It is generally believed that group-housing suppresses reproduction by prolonging the diestrous phase, decreased ovarian weight, increased uteri weight and corpora lutea size, elevated progesterone level, and heightened incidence of pseudopregnancy. For example, in the female house mice (*Mus domestic*), group housing suppresses reproduction by prolonging their diestrous phase, decreasing the frequency of estrus, and lowering the ovarian weight (Bronson and Champman 1968). Also, the incidence of pseudopregnancy increases significantly in group-housed mice (Marchlewska-Koj et al. 1994). In solitary golden hamsters, body weight, plasma progesterone levels and the absolute mass of ovaries of group-caged females are higher than those of singly caged females, but the estrous cycles are not affected. The increases in the number and size of the corpora lutea seem to be responsible for elevation of progesterone levels (Fritzsche et al. 2000). In female rat-like hamsters, we found that keeping females in pairs suppressed the ovaries and uteri of the adult, although it was not enough to cause a significant difference in estradiol or progesterone levels between paired and singly caged females; in subadults, keeping females in pairs accelerated growth of the ovaries and uteri and elevated progesterone level.

In summary, housing females in pairs had negative effects on the physiological status in adults and positive effects in subadults. This might be an adaptive mechanism for female rat-like hamsters. Adult female rat-like hamsters reside in a small, fixed territory and live a long solitary life (Wang et al. 1996; Zhang et al. 2001a). Low social tolerance might be a proximate mechanism to maintain such a fixed territory. Dispersal subadults may be stressed by the sudden transition of their life from social to solitary, although the dispersal distances of females may be shorter than that of males (Wang et al. 1996; Zhang et al. 2001a). Moreover, recruitment of subadults is fast in natural populations of rat-like hamsters (Zhang et al. 1992). This may lead subadults to compete for establishing themselves quickly and thus there was the observed facilitative effect when they were housed together. It is also likely that a high social tolerance exhibited at the time of new establishment might allow many subadults to temporarily cooperate by sharing some critical resources such as food and space in case they failed to establish a fixed territory immediately. In established adult females, however, any encroachment by outsiders would pose a threat to their reproductive success via challenges to the ownership of their territories or direct injurious or infanticidal behaviors by invaders toward the owner's offspring.

Although the experimental subadults were born and raised under laboratory conditions, we believed it was reasonable to compare them with adult females caught in the wild, because the social environments in the laboratory of the subadult females should be very similar to those in the field before they left their family and, moreover, the laboratory subadults were born to pregnant females caught in the wild.

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