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Brief communication

Effects of weasel odor on behavior and physiology of two hamster species

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Abstract

This study examined the behavioral and physiological effects of long-term exposure to overdose of aversive odor (predator odor) in two species of hamsters. About 0.05 mg of anal gland secretions of Siberian weasels (*Mustela sibirica*) was smeared at the oronasal groove of wild male ratlike hamsters (*Cricetulus triton*) (natural prey) and laboratory golden hamsters (*Mesocricetus auratus*) once every day for 4 weeks. After 28 days, the experimental groups of both hamster species displayed higher cortisol level, larger adrenal gland (in ratlike hamsters only), smaller thymus and flank gland, and lower aggression level than the conspecific control group (presented with water). Thus, the long-term presence of overdose of the anal gland secretion of the Siberian weasel could lower the aggression and social rank and suppress the immunity in the hamsters. The reproductive conditions of these prey species, however, seemed not to be affected. In addition, the similarities in the behavioral and physiological responses to the predator odor between the two species of hamsters showed that the responses to predator odor might be innate.

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1. Introduction

Numerous studies have revealed that rodents' avoidance of predator odors as aversive stimuli or predator cues seems to be innate and could be reinforced by encounters with the predator [10,11]. Exposures of rodents to predator odors can cause the elevation in the glucocorticoid level and/or the enlargement of the adrenal gland [4,5]. Nevertheless, previous findings on the effects of a predator or its odor on reproductive conditions of prey are inconsistent [1,9,13]. The disagreement might be partially due to the methodological aspects in laboratory and field studies. Specifically, few studies have investigated behavioral and physiological stress in the voles induced by chronic presence of predator odors [4,13]. No attempt has so far been available to examine the effect caused by excessive exposure of predator odors.

The Siberian weasel (*Mustela sibirica*) is mainly distributed in China and the neighboring countries [6]. In North China, the Siberian weasel is a key natural predator of the ratlike hamster (*Cricetulus triton*) and they are sympatric in distribution. The golden hamster (*Mesocricetus auratus*) was originally distributed in the Middle East and Eastern Europe and is now a common laboratory animal without natural ecological relevancy with Siberian weasels. These two hamsters are both solitary and share numerous similarities such as body size, having the flank gland, and behavioral patterns [7,15]. In this study, we attempted to examine how predator odor exposure affects the level of aggression and physiological conditions in the two species of hamsters through a long-term presentation of overdose of anal gland secretion of the weasels, the malodorous sulfur-containing compounds.

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2. Methods and materials

Twenty male adult ratlike hamsters and 20 male adult golden hamsters were used as the test subjects. The ratlike hamsters were captured by live traps in the farmland in central Hebei Province, North China, in April (breeding season), 2000. The golden hamsters (90–120 days old) were bred at the Laboratory of Animal Services, Chinese Academy of Preventative Medical Science. Animals in the control and experimental groups were housed separately and caged individually in plastic cages ($40 \times 25 \times 15$ cm) containing wood shavings and cotton nesting materials. All were fed with rat chow and water ad libitum. The colony was maintained on a 14:10 light/dark cycle (lights on at 1700 h) at approximately 20 °C.

Anal gland secretions of Siberian weasels were obtained through same procedures with our previous studies [14]. About 0.05 mg anal secretions (experimental groups) or water (control groups) was smeared at the oronasal groove of the hamsters once every day for 28 consecutive days. Water has been also commonly used in similar studies [2,13].

On Day 29, the first day after completing the presentation of the predator odor to the test subjects, we studied staged dyadic interactions between experimental and control groups. Definition of behavioral patterns and behavioral observation was carried out as those described by Johnston [7] and Zhang et al. [15].

On Day 30, all subjects were sacrificed by decapitation and blood samples were collected individually. Organs (spleen, adrenal gland, thymus, scent gland, testis, and epididymis) were removed and weighed (±0.1 mg) immediately. The blood samples were centrifuged at 4000 rpm and the serum was collected and stored at −20 °C for radioimmunoassay to measure testosterone and cortisol levels.

Radioimmunoassays were performed using the methods developed by Li [8]. Specifically, we used commercially available testing kits (Beijing Institute of Radio-immunoassay, China) for ^{125}I-cortisol and ^{125}I-testosterone 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. The intra- and interassay coefficients of variation were less than 5% and 10%, respectively. For testosterone, the detectable range of the assay was 0–20 ng/ml and the sensitivity was higher than 0.02 ng/ml. The intra- and interassay coefficients of variation were 5.4–7.4% and 3.1–6.1%, respectively.

Significance in differences in behavior and physiology was statistically analyzed by the Wilcoxon matched-pairs signed ranks test and two-tailed independent *t* test, respectively. The level of significance was set at α=.05 for all tests.

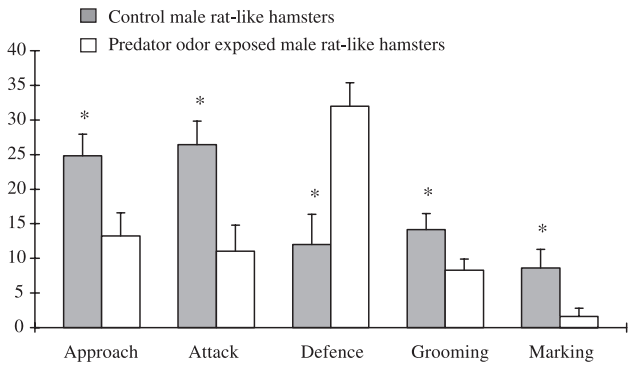

Fig. 1. Behavior in staged dyadic encounters between the experimental and control groups of male ratlike hamsters (* P<.05, Wilcoxon matched-pairs test).

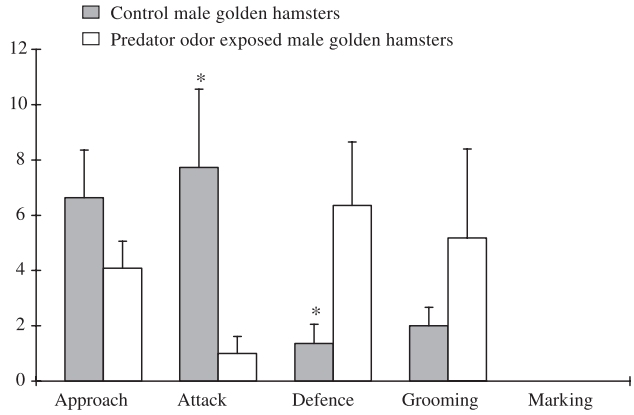

Fig. 2. Behavior in staged dyadic encounters between the experimental and control groups of male golden hamsters (* P<.05, Wilcoxon matched-pairs tests).

3. Results

For both ratlike and golden hamsters, individuals in the control group consistently defeated individuals in the experimental group for conspecifics. The hamsters in the control groups initiated attacks more frequently (male ratlike hamster: Z=2.199, P<.05; golden hamsters: Z=2.293, P<.05) and displayed defensive behavior less often (Male ratlike hamsters: Z=2.192, P<.05; male golden hamsters: Z=2.311, P<.05, see Figs. 1 and 2).

In ratlike hamsters, the experimental group displayed less flank marking (Z=2.215, P<.05), investigation (Z=2.156, P<.05), and grooming (Z=2.439, P<.05) than did the control group. In golden hamsters, however, flank gland marking was not observed in either group. Also, no significant difference was found in grooming and investigation between the experimental and control group (Z=0.306 and Z=1.478, respectively, P>.05).

In ratlike hamsters, the experimental group had larger adrenal glands (t_{18}=2.29, P<.05), a higher cortisol level (t_{18}=2.16, P<.05), a smaller thymus (t_{18}=2.87, P<.01), and smaller flank glands (t_{18}=3.51, P<.01) than the control. There were no significant differences in body mass

Table 1
The mass of the body adrenal gland, thymus, spleen, scent glands, and reproductive organs measured in ratlike hamsters (*C. triton*) and golden hamsters (*M. auratus*)

		Initial body weight (g)	Final body weight (g)	Spleen (mg)	Adrenal (mg)	Thymus (mg)	Flank gland (mg)	Midventral gland (mg)	Testis (g)	Epididymis (g)
Ratlike	Control	149.17 ± 18.56	155.6 ± 20.5	149.7 ± 47.6	15.8 ± 4.5 *	247.0 ± 125.0 * *	233.0 ± 57.7 * *	56.9 ± 22.0	3.49 ± 0.37	1.2 ± 0.6
hamsters:	Treatment	143.09 ± 14.71	149.6 ± 19.3	141.4 ± 32.1	18.5 ± 6.7	162.0 ± 43.8	146.5 ± 49.8	57.0 ± 22.7	3.44 ± 0.34	1.2 ± 0.3
Golden	Control	107.23 ± 19.03	110.2 ± 20.5	110.5 ± 28.6	23.4 ± 3.06	210.4 ± 70.4 *	81.1 ± 21.5 *	–	2.5 ± 0.3	1.02 ± 0.11
hamsters:	Treatment	102.55 ± 20.71	104.1 ± 17.0	115.3 ± 41.2	23.3 ± 4.5	178.80 ± 19.8	61.4 ± 17.6	–	2.5 ± 0.3	0.98 ± 0.12

t test, $df = 18$.
* $P < .05$.
** $P < .01$.

($t_{18} = 1.75$, $P > .05$), spleen mass ($t_{18} = 0.45$, $P > .05$), midventral gland mass ($t_{18} = 0.01$, $P > .05$), testis mass ($t_{18} = 0.10$, $P > .05$), epididymides mass ($t_{18} = 0.01$, $P > .05$), and testosterone level ($t_{18} = 0.02$, $P > .05$, see Tables 1 and 2).

Most results obtained in golden hamsters were similar to those in ratlike hamsters: experimental golden hamsters had a higher cortisol level ($t_{18} = 2.06$, $P < 0.05$), a smaller thymus ($t_{18} = 2.73$, $P < .05$), and smaller flank glands ($t_{18} = 2.20$, $P < .05$) than the control. However, there were no differences in body mass ($t_{18} = 0.68$, $P > .05$), spleen mass ($t_{18} = 0.29$, $P > .05$), testis mass (male: $t_{18} = 0.34$, $P > .05$), epididymides mass (male: $t_{18} = 0.64$, $P > .05$), and testosterone level ($t_{18} = 0.13$, $P > .05$) between the experimental group and the control group. Unlike ratlike hamsters, there was no difference in the size of the adrenal gland between experimental and control group for the golden hamster ($t_{18} = 0.08$, $P > .05$, see Tables 1 and 2).

4. Discussion

Our results confirmed that psychological stressors have negative effects on some aspects of the rodents' behavior and physiology [1,4,5]. Specifically, we found that the aggression level of both hamsters in the experimental groups was lowered after long-term exposure to the weasel odor. This negates the previous finding that acute exposure of rodents to predator odors would increase the aggression [4].

Exposures to psychological stressors are likely to cause glucocorticoid level elevation and/or adrenal gland enlargement as indicators for immunosuppression [1,4,5]. Basically, our results followed this pattern. This suggested that long-term exposure to predator odor could persistently suppress rodents' immunity without habituation. This notion of immunosuppression due to chronic exposure to predator odor was further supported by the reduction in the thymus gland of both species and increase in the adrenal gland of the ratlike hamster after a long-term exposure to the predator odor. In golden hamsters, further enlargement of the adrenal gland was not detected possibly due to prior stressful group-housing conditions by the supplier.

Table 2
Testosterone and cortisol level in male ratlike hamsters (*C. triton*) and golden hamsters (*M. auratus*)

	Ratlike hamsters		Golden hamsters	
	Testosterone level (mg/dl)	Cortisol level (ng/ml)	Testosterone level (mg/dl)	Cortisol level (ng/ml)
Control	141.2 ± 77.9	10.3 ± 8.0 *	166.63 ± 62.72	12.71 ± 4.47 *
Treatment	142.0 ± 86.5	20.52 ± 5.58	174.98 ± 34.99	20.56 ± 6.01

* * $P < .01$, *t* test, $df = 18$.
* $P < .05$.

Unlike immunosuppression, there is controversy over suppression of reproductive physiology by various predator stressors. For example, in juvenile male Campbell's hamster (*Phodopus campbelli*), a 20-day exposure to cat urine can delay sexual maturation with a smaller epididymis and a larger adrenal gland, but testis development is not affected [13]. In laboratory Long–Evans rats, a 20-day exposure to cats is enough to significantly increase adrenal gland size and plasma corticosterone level, but testis size and testosterone levels are not affected [1]. In our study, no significant differences were found between the experimental and control groups in the measured reproductive organs and gonad hormone levels for both species after long-term exposure to an overdose of predator odor. However, we recently found that reproductive organs (e.g., epididymis, uterine) of juvenile ratlike hamsters were reduced after a 4-week exposure to weasel odor (unpublished data). Thus, suppression of the development of reproductive organs by the predator odor may be effective in juvenile rodents.

Social odors can influence the size of specialized skin glands [12]. Males with larger flank glands and more active flank marking always have higher social ranks and aggressiveness and are preferred by females [7,15]. Our results showed that weasel odors inhibited the flank gland size and flank marking (only in ratlike hamsters), and consequently might suppress the social rank and the mating [4,5]. That golden hamsters did not exhibit flank marking in other conditions might be ascribed to their weak aggression level that was too low to be detected in this study.

In addition, the two species of hamsters shared similar patterns of behavioral and physiological responses to the

predator odor. This demonstrated that these effects of predator odor on prey might not depend on whether they are sympatric or allopatric in distribution. This complies with the general belief that rodents' responses to predator odors seem to be innate, although their avoidance of predator odors as stress factors can be reinforced by an encounter with the predator itself [11]. Some sulfur compounds shared by the species in the *Mustela* genus might account for such innate avoidance [9]. In seven *Mustela* species (including Siberian weasels), major volatiles in their anal gland secretions were composed of several similar sulfur-containing compounds [3,14]. Thus, it was possible for the golden hamster to use these compounds in their natural response to other sympatric weasels to avoid the odors from the allopatric Siberian weasel.

In conclusion, our results showed the excessive stimulus of predator cues could lower the aggression and social rank and suppress the immunity in the hamsters. However, reproductive physiology was not affected similarly to the findings from field experiments [9]. The similarities between these two species of hamsters in the behavioral and physiological responses to the predator odor suggested the responses to predator odor might be innate. Further studies are needed to compare the differences in rodents' responses to different dosages of predator odors and non-predator novel odors.

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