ORIGINAL ARTICLE

Behavioral and physiological responses of striped field mice (*Apodemus agrarius*) to predator odor

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Abstract

Predation risk is one of the most important selective forces in nature and has significant effects on the behavior and physiology of prey individuals. Prey species have evolved several different traits to reduce and avoid this predation pressure. This research aimed to determine the behavioral and physiological responses of striped field mice to predator risk. In the present study, we compared the agonistic behavior in male and female striped field mice (*Apodemus agrarius* Pallas, 1771) of the same sexes under the odor derived from a male Himalayan weasel (*Mustela sibirica*). Dyads were subjected to 5 min staged encounters in neutral arenas once a week for 3 weeks during which agonistic and social behaviors were recorded and fecal hormone concentrations were determined using pre-column derivatization and high performance liquid chromatography methods. Furthermore, we also tested the effects of weasel odor on the adrenal glands. The results showed that: (i) male striped field mice did not exhibit any change in body weight and physiological characteristics but their aggressive behavior changed over time, and (ii) females responded to predation risk by significantly decreasing body mass and through increases in fecal cortisol levels and adrenal gland indices. These data show significant sex differences in the body weight, adrenal gland indices and fecal cortisol levels of striped field mouse under predation risk.

Key words: attack behavior, cortisol, predator odor, striped field mouse (*Apodemus agrarius*).

INTRODUCTION

Predation is one of the most important selective forces in nature and has significant impacts on prey individuals, populations and communities. It can lead to physiological and behavioral adaptations in prey (Axelrod & Reisine 1984; Kats & Dill 1998; Lima 1998), which have evolved a variety of behavioral strategies to minimize or avoid predation risk (Lima & Dill 1990; Endler 1991; Kats & Dill 1998). Predator odor might provide information on predation risk, even when the predator is absent at the time of detection (Kleiman 1966; Ralls 1971; Johnson 1973; Halpin 1986).

The breeding suppression hypothesis suggests that some rodents respond adaptively to the odor of predators by suppressing reproduction (Mappes *et al.* 1998). This hypothesis has been widely discussed in relation to social behavior (Dickman & Doncaster 1984; Gorman 1984; Ylönen 1989; Ylönen *et al.* 1992) and physiological conditions (Ylönen 1989; Ylönen *et al.* 1992;

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Koskela & Ylönen 1995), but seldom when considering differences between males and females. The disruptive effects of stress on immune function, reproduction and behavior have been well documented (McEwen 2000; Sapolsky 2002; Dallman 2003). Previous published studies show that the glucocorticoids (GC) play an important role in responding to stressors such as predators, capture and handling, extreme weather and social insubordination (Saplosky *et al.* 2000; Romero 2002). Stressful situations usually evoke an increased glucocorticoid production, which is mediated by the release of adrenocorticotropic hormone from the pituitary gland (von Holst 1998; Buchanan 2000; Creel 2001; Sapolsky 2002). Assessing an animal's stress, physiology is therefore essential for the understanding and improvement of animal welfare, health and reproduction. Fortunately, the analysis of cortisol metabolites (Touma *et al.* 2003, 2004) excreted from urine and feces offer a non-invasive technique for assessing the adrenocortical function of mice. Reversed-phase high performance liquid chromatography (HPLC), has been adopted in material separation and detection (Turner *et al.* 2003). Pre-column derivatization with fluorescence detection in combination with reversed-phase liquid chromatography is a technique that has been effective for the determination of trace amounts of components from fecal sampling (You *et al.* 2005).

Striped field mouse (*Apodemus agrarius* Pallas, 1771) is a social, monogamous rodent species distributed throughout farmlands or forests in China, the Russian Far East and Korea (Sakka *et al.* 2010). We assessed behavioral and physiological changes of predator-naive striped field mice exposed to the odor of a predator and aimed to reveal differences in aggressive behavior and physiological parameters between males and females when exposed to predator odors.

MATERIALS AND METHODS

Experimental animals

Striped field mice were bred in a colony maintained in the laboratory, founded with animals that were live captured in 2003 from Qufu, Shandong Province, East China. Captured mice were individually raised in plastic cages (46 × 31 × 20 cm) containing cotton nesting materials in a predator-free environment. The housing room was maintained at 20 ± 2 °C and relative humidity 50 ± 10% with reverse light/dark cycle (14L:10D with light at 17:00) and food and water were provided *ad libitum*. All procedures involved in handling and care of animals were in accordance with the China Practice for the Care and Use of Laboratory Animals.

Source of predator odor

The anal glands from male Himalayan weasel (*Mustela sibirica*), instead of a real predator, were used as predation risk to avoid any superfluous stress to the experimental animals. The weasels were live-trapped from Boading (N38°49′N, 115°58′E) of Hebei, China. The anal gland samples were stored in aluminum foil at –30 °C until being used as the predator odor.

Experiment procedures

We performed 21-day experiments in Plexiglas neutral arenas (60 × 40 × 40 cm). One removable opaque partition divided the arena into 2 equal compartments and mice were placed into each compartment for an acclimatization period of 3 min. The opaque partition was then removed and mice were allowed to freely interact for 5 min. This was repeated once a week for 3 weeks. Encounters were recorded using digital video and all behavioral tests were conducted under dim red illumination during the first 4 h (07.00–11.00 hours) of the dark cycle. The arena was thoroughly cleaned between trials with water and 75% ethanol.

We used the body mass asymmetry method to establish the odor stress test, as described by Early *et al.* (2006) and Wang *et al.* (2007). The behavioral data between the same sexual subjects and fixed partner with smaller body mass (–2.7 ± 1.63 g) were collected. Aggressive behaviors during the 5 min encounters on days 1, 7, 14 and 21 were quantified using OBSERVER V5.0 (Noldus, NL). Attacking behavior included the following: sideways posture, biting and chasing (Siegel 1985; Huhman *et al.* 1990; Zhang *et al.* 2001; Wang *et al.* 2006).

In the present study, 16 males (33.06 ± 0.74 g) and 16 females (29.89 ± 0.63 g) were housed individually, and assigned into 4 groups. Each group consisted of 8 individuals. The odor treatment group (*n* = 16) and the control (*n* = 16) were all in a reproductive state based on morphological observations on testis size in males and vaginal opening in females. During the dark period, mice were exposed to predator odor (an anal gland of male weasels was hung above each of the covers of the individual cages from 11.00 to 17.00 hours; a wet tampon was used as a control in the other room). We had no null treatment with a novel odor because previous studies have shown that these have no effect on physiolog-

ical parameters and behavior of the field mice (Wang *et al.*, unpubl. data). Body weight was measured once a week during the experiments and adrenal glands were measured at the end of the experiments.

Hormonal analysis

Feces sampling

We collected feces of experimental subjects without handling the animal by using the methods described by Touma *et al.* (2003). After each mouse had been in the housing system for 3 days, feces were collected on days 0 (baseline), 1, 7, 14 and 21, placed into a vial, and frozen at –30 ºC until extraction.

Hormone extraction

Each collected fecal sample was well homogenized with a mortar and pestle, and fecal hormone metabolites were extracted following the method described by Touma *et al.* (2003) and Turner *et al.* (2003).

High performance liquid chromatography analysis

Pre-column derivatization and high performance liquid chromatography separation: Following You *et al.* (2005), pre-column derivatization and HPLC methods were used to determine the metabolites of fecal hormone.

Quantitative analysis: Quantitative conversion of steroids from the extracts of mice feces to their 1, 2-benzo-3, 4-dihydrocarbazole-9-ethoxy-carbonylhydrazine (BCEC)-hydrazones was accomplished by using a large excess of BCEC. All hormones in the fecal sampling were quantified using the external standard method with detection at 390 nm.

Data analysis

One-way repeated analyses of variance were used to determine differences in behavior and hormone levels within and between groups. Differences in behavior and hormone levels between treatment and control groups were analyzed using *t*-tests or the Mann–Whitney *U*-test (if data were not normally distributed). Data are presented where appropriate as mean ± SE in nanograms (ng) per gram of feces. *P*-values were 2-tailed and considered statistically significant if $P < 0.05$.

RESULTS

Variation in body weight

The weight of females in the experimental group differed significantly from those in the control group ($F_{1,14} = 7.65$, $P = 0.008$), but not in males. Experimental females were significantly smaller under weasel odor on day 7 ($P = 0.003$), day 14 ($P = 0.006$) and day 21 ($P = 0.010$) than those in the control group, respectively (Fig. 1a). The differences of weight in males were not significant between experimental and control groups, although the body weight decreased in 2 groups ($F_{4, 56} = 2.881$, $P = 0.036$) (Fig. 1b).
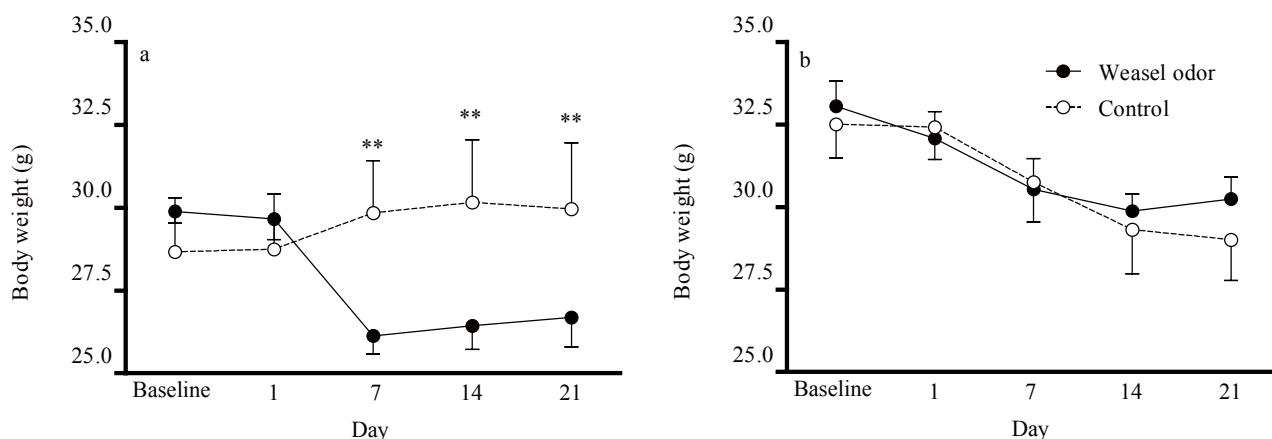
Figure 1 Changes of body weight in females (a) and males (b) of striped field mice during 21-day weasel odor exposing periods. (*n* = 8) **P < 0.01, indicating significant differences between weasel odor exposing (●) and control (○) groups.
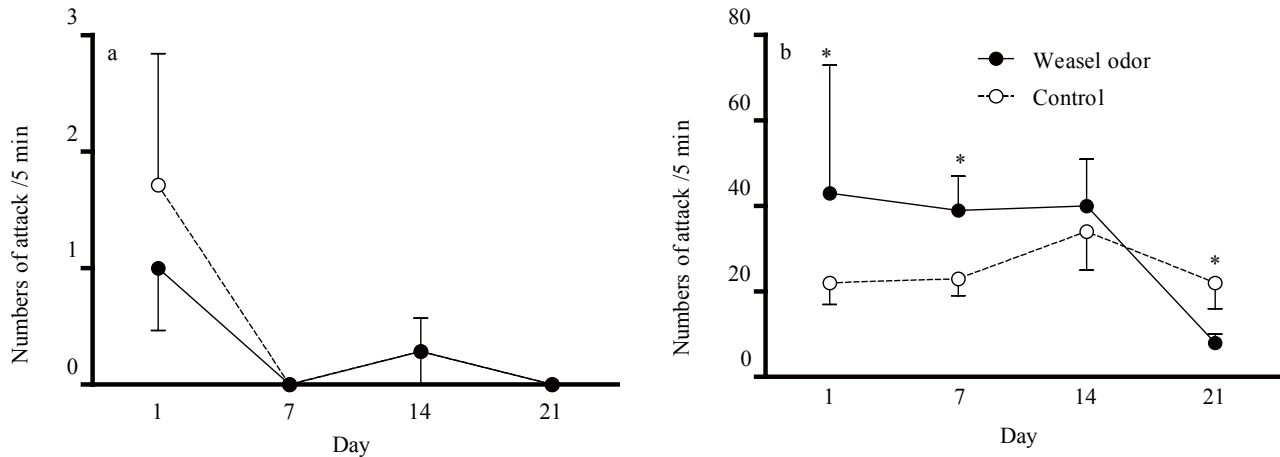
Figure 2 Numbers of attack in females (a) and males (b) during 21-day weasel odor exposing periods. **$P < 0.01$, indicating significant differences between weasel odor treatment (●) and control (○) mice.

Variation in attack behavior

The frequencies of attack behavior of male mice varied significantly both in experimental and control groups ($F_{1,14} = 3.84$, $P = 0.034$), but not in females (Fig. 2a). Males tended to show aggressive activity more frequently on day 1 ($Z = 2.21$, $P = 0.028$) and day 7 ($Z = 2.26$, $P = 0.023$) and less aggressive on day 21 ($Z = 2.1$, $P = 0.037$) under weasel odor (Fig. 2b).

Variation in adrenal gland

At the end of the experiments, adrenal gland indices of female mice were increased significantly more in the experimental group than those in the control group ($t_{14} = 2.37$, $P = 0.018$), but not in males (Fig. 3).
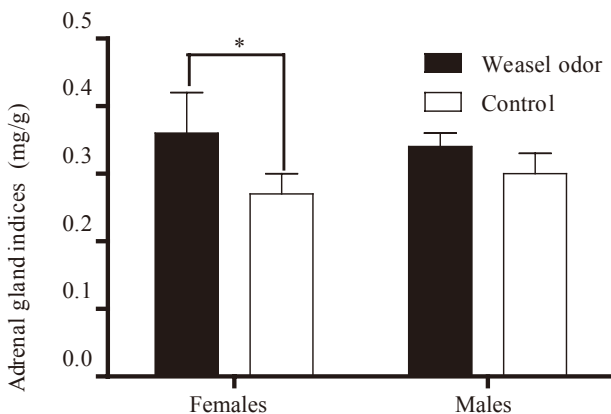
Figure 3 Adrenal gland indices of females and males under weasel odor exposing and control groups.
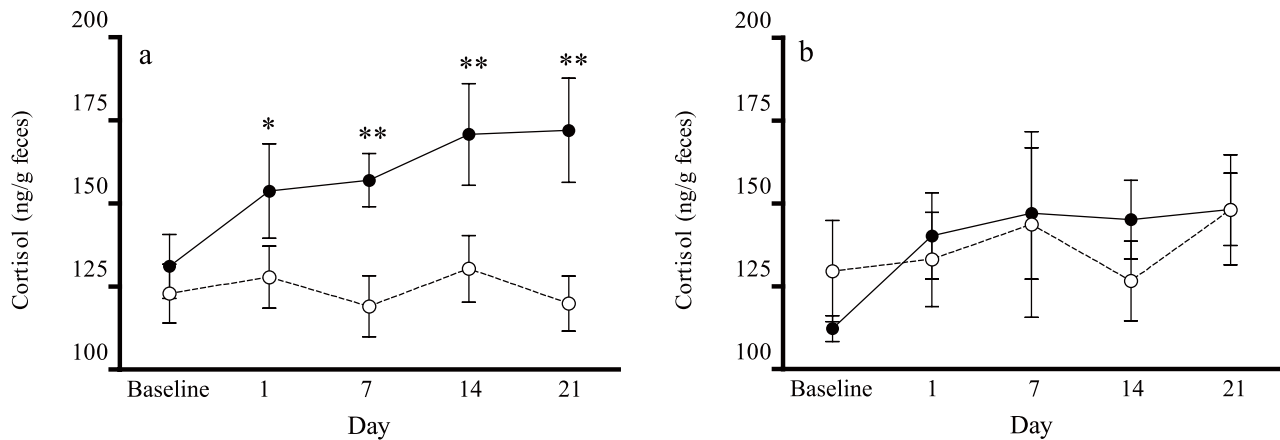
Variation in fecal cortisol level

The fecal cortisol level of females in the experimental group differed significantly from those in control groups during the experiment ($F_{1, 14} = 5.84$, $P = 0.021$), but not in males. Fecal cortisol level of tested females increased significantly under weasel odor compared to those in the control group on day 1 ($P = 0.033$), day 7 ($P = 0.009$), day 14 ($P = 0.010$) and day 21 ($P = 0.003$), respectively (Fig. 4a). For males, however, although fecal cortisol level seemed to slightly increase in both experimental and control groups, the difference was insignificant between the 2 groups ($F_{4,56} = 1.35$, $P = 0.27$) (Fig. 4b).

DISCUSSION

Our results revealed that small mustelid predation risk mainly stressed female striped field mice. Females exhibited variations in decreased body mass, increased fecal cortisol levels and adrenal gland indices when exposed to mustelid odor; however, no significant variations in the abovementioned aspects were observed in males. Nonetheless, the response of male mice to predation risk changed over time.

Predator odor decreased body mass of females

The results from this research indicated that body weight in female mice decreased after long-term exposure to predator odor. The body weight of the field mouse (*Microtus agrestis*) decreased under predation risk because of a decrease in foraging activity (Korpi-

Figure 4 Changes of fecal cortisol levels in females (a) and males (b) during 21-day weasel odor exposing periods. *$P < 0.05$ and **$P < 0.01$, indicating significant differences between treatment (●) and control (○) individuals.

maki *et al.* 1994). Therefore, decreased foraging could be the immediate cause of the observed breeding suppression (Batzli 1986). Animals respond to a stressor with a series of endocrine responses (Sapolsky 1992; Wingfield 1994). If the stressor is not eliminated and GC levels remain chronically high, a broad range of harmful consequences ensue including loss of body mass, immune suppression and reproductive suppression (Breuner & Wingfield 2000; Sapolsky 2002). Under such conditions, if there is not enough food to support energy usage, then animals either change their habits or they die (Dallman 2003).

Behavior and physiological characteristic interaction under predation risk

Chronic predation pressure increases hypothalamic–pituitary–adrenal axis activity and reduces hypothalamic–pituitary–gonadal axis activity, which consequently affect individual reproductive status (Blanchard *et al.* 2001). Both these axes have been proposed to underlie the inhibition of reproductive function due to stress (Selye 1946). Our results showed that females responded to predation risk by increasing cortisol levels over the tested periods and increased adrenal gland indices at the end of experiments. These results support the conclusions from previous studies (Blanchard *et al.* 2001; Moor & Jessop 2003; Soto-Gamboa *et al.* 2005).

According to the majority of published works concerning the effects of predators or their odors on ro-

dents, predation risk includes the suppression of rodents' behavior, physiology and reproduction, and chronic cat odor exposure imparts beneficial impacts on mice (Zhang *et al.* 2008; Eason *et al.* 2010). In the present research, females responded obviously to predator odor in terms of physiological characteristics, which supported Lima's statement that females, associated with locating mates, would be more sensitive under increased predation risk (Lima 1998). However, significant behavioral changes in females were not observed.

Male mice displayed more aggression under predator odor. Stronger males that survive high predation pressure might be more attractive to females (Zhang *et al.* 2008). This argument is in agreement with the indicator hypothesis developed from the well-known handicap hypothesis on sexual selection via female choice (Zahavi 1975). Our results show that male mice change their aggressive levels over time under predation risk, and the body mass and physiological characteristics did not change.

In conclusion, these data show significant sex differences in the body weight, adrenal gland indices and fecal cortisol levels of striped field mouse under predation risk.

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