



RESEARCH PAPER

Source Odor, Intensity, and Exposure Pattern Affect Antipredatory Responses in the Subterranean Rodent *Ctenomys talarum*

Valentina Brachetta, Cristian E. Schleich & Roxana R. Zenuto

Laboratorio de Ecofisiología, Instituto de Investigaciones Marinas y Costeras (IIMyC), Universidad Nacional de Mar del Plata – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mar del Plata, Argentina

Correspondence

Valentina Brachetta, Laboratorio de Ecofisiología, Instituto de Investigaciones Marinas y Costeras (IIMyC), Universidad Nacional de Mar del Plata – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Funes 3250, 7600 Mar del Plata, Argentina.
E-mail: vbrachetta@mdp.edu.ar

Received: April 20, 2016
Initial acceptance: May 30, 2016
Final acceptance: May 10, 2016
(L. Ebensperger)

doi: 10.1111/eth.12568

Keywords: *Ctenomys talarum*, anxiety, predation, intensity, habituation

Abstract

Predation is a strong selective force, and prey species may show specific adaptations that allow recognition, avoidance, and defense against predators. Facing a situation of predatory risk, anxiety constitutes a reaction of adaptive value, allowing to evaluate the potential risk of this encounter as well as to generate a physiological and behavioral response. Previous studies in the subterranean rodent *Ctenomys talarum* revealed that exposure to predator odors (urine or fur) generates an anxiety state and induces behavioral changes. However, no differences between the responses generated by both odor sources were observed, although fur odors may indicate a higher level of predatory immanence. Therefore, the aim of this study was to evaluate the behavioral and physiological responses of *C. talarum* to different intensities of predator odors (urine and fur) and to the repeated exposition to the same odorous stimulus. When comparing the highest behavioral effects elicited by both predatory odors on *C. talarum*, our study supports the assumption that fur odors are more anxiogenic than urine, while the former provoked significant changes in the distance traveled, the number of arm entries and time in transparent arms in the elevated plus maze; cat urine only caused slight changes on those behavioral parameters. Furthermore, we also found that the intensity of natural predator odor presented to tuco-tucos has a role on the appearance of defensive behaviors, although an amount-dependent relationship between predator odor and anxiety levels was not observed. Finally, while individuals exposed for 1 day to fur odor displayed an evident anxiety state, those exposed repeatedly for 5 consecutive days did not differ with the control group in their behavioral response, indicating a clear habituation to the predatory cue. In our intensity and habituation experiments, we did not find differences in the measured physiological parameters among control individuals, exposed to different cues intensity (urine and fur odor) and exposed only once or for 5 days to fur odor. These results provide valuable evidence that the types of predatory odor, along with the frequency of exposition, are important determinants of the appearance, strength, and extinction of defensive behaviors in the subterranean rodent *C. talarum*.

Introduction

In nature, animal species usually confront with diverse risks and threats, covering from bacteria and

parasites to conspecifics and predators (Kavaliers & Choleris 2001). Dealing with predators is a key problem for virtually all prey, and predators themselves may become prey. Predators affect prey both directly

by killing them and indirectly by affecting their behavior, foraging patterns, reproduction, and stress physiology and thus affecting their fitness (Clinchy et al. 2013). Thus, predation is a major evolutionary force shaping the adaptations of the prey, and many mammalian species show specific adaptations that allow recognition, avoidance, and defense against predators (Apfelbach et al. 2005; Hegab et al. 2015). These behavioral adaptations, like predator avoidance and the development of antipredator behaviors, are fundamental for survival (Hegab et al. 2014). In many cases, antipredator defense involves the detection and response to specific chemical cues that predators produce. Antipredator behaviors generated in the presence of predator scents have been investigated in many mammals and include direct avoidance of the odor source, variations in space use, increases in vigilance, and decrease or inhibition of activities such as grooming and feeding (Dielenberg & McGregor 2001; Apfelbach et al. 2005; Staples 2010).

Exposure of rodents to natural predators or their odors has been shown to induce anxiety-like states (Zangrossi & File 1992; Adamec & Shallow 1993; Apfelbach et al. 2005). Facing a predatory risk situation, anxiety is a reaction of adaptive value, assessing the potential risk of this encounter as well as generating a physiological (activation of the hypothalamic–pituitary–adrenal axis) response and behavioral (suppression of non-defensive behaviors, decreased activity, and avoidance of the source of danger) response. However, not all types of predator odors generate a common range of fear-related responses and even the same odorous cue may evoke different responses according to its intensity and spatial distribution frequency in the prey's habitat (Takahashi et al. 2005).

Recently, some studies focused their interest on the relative efficacy of the diverse predatory odor sources on the appearance of antipredator behaviors. An interesting study by McGregor et al. (2002) compared the effect of cat fur-skin odor and 2,4,5 trimethylthiazoline (TMT), an extract of fox feces commonly used in studies of predator odor effects. The results showed that both odorous cues clearly differed in their effects, with cat fur odor generating defensive behaviors in rats and TMT eliciting effects similar to the ones produced by aversive odors. A similar result was obtained by Blanchard et al. (2003) who failed to produce conditioning at different concentrations of TMT. Also, these authors tested the effects of cat urine, feces, and fur-skin odors on rats, finding that while urine did not produce behavioral changes, feces and fur-skin odors elicited similar changes in defensive behaviors during

exposure, although only fur-skin odors generate long-lasting effects. Likewise, a recent study by Masini et al. (2005) showed that ferret fur (but not feces, urine, or anal gland secretions) produced behavioral and physiological changes (increments in corticosterone and adrenocorticotropin levels and induction of *c-fos* mRNA expression) in laboratory rats, suggesting again that the information carried by these diverse odorous cues caused different reactions in the preys.

Apart from the type of predatory odor, the cue intensity encountered by the prey is also an important factor that could modify the appearance and development of antipredatory behaviors. Prey species are usually able to assess the different intensities of a threat and flexibly respond to it (Hegab et al. 2015). Nonetheless, some studies indicate that rodents respond differently to different amounts of predator cues. Wallace & Rosen (2000) found that in rats exposed to increasing amounts of TMT, 300 and 600 nmol elicited higher freezing levels than 1, 10, and 100 nmol. Using predator fur odor, Takahashi et al. (2005) demonstrated that rats exposed to 3× and 10× size cat odor cloths exhibited more freezing and made fewer contacts with the odor source than rats exposed to no predator odor. Also they found that the cloth 10× provoked more freezing and fewer contacts than the 1× cloth. These studies support the idea that the intensity of predator odor is an important factor underlying the level of expression of defensive behaviors, indicating that the exposure does not trigger an unconditioned response similar to magnitude across varying odor intensities (Takahashi et al. 2005).

Lastly, two different behavioral and physiological responses can emerge after repeated exposure to predators and their odors: habituation or sensitization (Takahashi et al. 2005; Hegab et al. 2015). In the field of stress neurobiology, the term 'habituation' refers to the reduction in physiological responses elicited by an exposure to a repeated homotypic (same) stressor in comparison with the responses elicited by acute exposure to that stimulus (Grissom & Bhatnagar 2009). This process is important in an antipredator context because it helps prey organisms to filter biologically less relevant input and devote more attention and processing energy toward more relevant stimuli (Wilson 2009). On the other hand, sensitization is characterized by an increased responsiveness to the repeated stimulus and often to other stimuli as well (Hegab et al. 2015). Studies involving repeated exposures to predator odors have yielded mixed results (Zangrossi & File 1994; Dielenberg & McGregor 1999), a situation that may be connected to the cue intensities used in the different studies (Takahashi et al. 2005).

Ctenomys talarum (Thomas 1898) is a small subterranean rodent found in coastal sandy soils of Buenos Aires Province (Argentina). Both sexes at all ages (except the offspring until dispersal) are sedentary and maintain exclusive territories. The burrow system of this species is a complex structure of closed galleries parallel to the soil surface, usually characterized by a main axial tunnel and a variable number of lateral branches and feeding tunnels, all of them plugged (Busch et al. 1989, 2000). The home-range size differs between sexes, with males occupying larger areas than females. This difference has been attributed to variations in energetic demands between males and females, with males being heavier than females and thus requiring larger foraging areas. Additionally, it has been related with the mating system of this species, a resource-defense polygyny (Cutrera et al. 2006). The subterranean system allows tuco-tucos to effectively avoid predators; however, this species perform excursions on the surface while dispersing or foraging. Although *C. talarum* has the ability to use olfaction to orient their digging while searching for food patches, most of the food collection occurs above ground, when animals emerge short distances from burrow openings to cut vegetation growing in the soil and run backwards into the burrows where they later consume the leaves and stems (Busch et al. 2000). During these brief periods at the surface, tuco-tucos become vulnerable to aerial and terrestrial predators. Previous studies on *C. talarum* showed that this species is frequently preyed by owls, foxes, and wild cats (Vassallo et al. 1994; Busch et al. 2000; Canepuccia 2005). Moreover, in suburban areas, predatory events by dogs and domestic cats have also been detected (C. E. Schleich, personal observation).

In many prey species, it has been shown that, besides the obvious direct and detrimental effects of a predatory event, a failed attack has also strong consequences for their fitness. For example, previous works on *C. talarum* showed that acute or chronic exposure to predatory cues provoked a stress response that negatively affects its spatial learning and memory capacities (Mastrangelo et al. 2009; Brachetta et al. 2014). Furthermore, in a previous study, we found that exposure of tuco-tucos to predator odors (from urine or fur) generates a state of anxiety and induces behavioral changes associated with decreased locomotor activity and appearance of avoidance behaviors (Brachetta et al. 2015). These behavioral responses when tuco-tucos fear predators may lead to an increase in the time spend being cautious, leaving less time for foraging aboveground and resulting in a negative impact on body condition, which can finally cause

survival or reproductive costs. Besides this impact of fear on fitness, interestingly we found that males and females of *C. talarum* responded in a similar manner to both odor sources (Brachetta et al. 2015), challenging the general view that odors derived from the fur or skin induce stronger antipredator responses than those originated from feces due to their differential degree of predictive information (Kavaliers & Choleris 2001; Staples 2010; Hegab et al. 2015). However, as explained before, this interesting outcome could be in part a result of the odor intensities and exposure frequency utilized in the experiment. Therefore, the general aim of this article was to assess the behavioral response (antipredatory) and physiological (activation of pituitary–adrenal axis) response of individuals of *C. talarum* exposed (1) to different intensities of urine and fur odor from a predator (cat) presented in a single exposition manner and (2) the repetition of the exposure to fur odor in order to assess the generation and extinction of responses.

We predict that higher intensities of odors from a predator would generate in individuals of *C. talarum* more elevated levels of anxiety, in the form of decreased locomotor and exploratory activity, and appearance of defensive behaviors, which would be accompanied by variations in the physiological parameters related to stress such as higher levels of plasmatic glucose, cortisol and neutrophil: lymphocyte ratio (N/L). This effect would be higher with the presentation of odor of the fur than with exposure to predator urine due to the immediacy of a potential attack. Furthermore, tuco-tucos would show behavioral habituation to a repeated presentation of odor derived from predator fur.

Materials and Methods

Animal Capture and Housing Conditions

Adult, sexually mature, tuco-tucos of both sexes (53 males – mean weight 160 g – and 64 females – mean weight 117 g) were captured during the breeding (June–February) and no breeding (March–May) seasons of 2014 and 2015 years in Mar de Cobo (37°46'S, 57°26'W, Buenos Aires province, Argentina) using wire mesh live traps (diameter 10 cm) located at burrow entrances. Ninety-four tuco-tucos were used for the intensity experiment (43 males and 51 females), of which seventy were captured in 2014 and 24 in 2015. All individuals of the habituation experiment ($n = 23$; 12 males and 11 females) were captured in 2015. The traps were checked every 15- to 20-min period until the animal's capture. Pregnant

or lactating females were discarded for the experiments and returned immediately to their burrows. Adult males were used without distinction because, after attaining reproductive maturity, they do not undergo regression of their testes and contain sperm in their epididymis year round. Then, animals were taken to the laboratory and placed in individual plastic cages (25 × 32 × 42 cm) provided with wood shavings. Food was supplied daily and consisted of sweet potatoes, lettuce, corn, mixed grass, and sunflower seeds. Temperature in the laboratory remained constant (25 ± 1°C) with a light–dark cycle of 12:12 h. After 7 days of acclimatization to the laboratory, the animals were randomly assigned to the different experiments. Once experiments finished, animals were returned to the capture site.

Exposition to Predatory Cues

Animals were exposed to olfactory cues indicative of the presence of a predator: wood shavings soiled with urine from an adult male cat (obtained 24 h before the experiments) or a piece of cloth impregnated with cat fur odor (also obtained 24 h before the experiments and after allowing a cat to use the cloth to rest on it for a 7 days period). The samples were frozen at –20°C in sealed plastic bags until use. The day of the experiments, the bags were placed at ambient temperature to unfreeze odor samples.

To compare the effects of exposure of tuco-tucos to odors from a predator, control individuals were exposed to odorless cloth or clean wood shavings.

Procedure

Effects of predator odor intensity on defensive behaviors

To analyze the effect of different intensities of a predator cue (urine or fur odor) on the behavioral and physiological response of *C. talarum*, animals were randomly assigned to different treatments which differed in the type and intensity of predatory cue used:

G1: no-odor (control group; n = 18); G2-1/3×: urine low intensity (n = 11); G2-1×: urine medium intensity (n = 16); G2-3×: urine high intensity (n = 12) that corresponded to 3.33, 10, and 30 g of wood shavings with urine, respectively; G3-1/3×: fur low intensity (n = 10); G3-1×: fur medium intensity (n = 15); and G3-3×: fur high intensity (n = 12) that corresponded to cloth pieces of 2 × 2 cm, 6 × 6 cm, and 18 × 18 cm, respectively. Prior to the start of the experiments, the animals were exposed in individual boxes to the predatory odor sources or control samples for a 60-min period (Fig. 1a). The different odor samples were presented to the individuals in plastic pots covered with a wire mesh, lowering the incidence of visual cues associated with the amounts of cue used (quantity of shavings impregnated with urine or size of the cloth). Subsequently, the performance of the animals was evaluated during 5 min on the elevated plus maze, a test commonly used to assess anxiety in rodents (Pellow et al. 1985; Rodgers & Dalvi 1997).

Effects of habituation to predator odor on defensive behaviors

To evaluate the effect of repeated exposures to a cue indicative of the presence of a predator, tuco-tucos (n = 12) were exposed for 60 min on 5 consecutive days to cat fur odor presented in a plastic pot (T5: cloth 6 × 6 cm; Fig. 1b). A control group (C5, n = 11) followed the same exposition protocol but to odorless cloths. After the last exposure event, the performance of each individual was evaluated in the elevated plus maze in order to analyze whether repeated contact with a predatory cue leads to variation or even the extinction of the behavioral response. The results obtained from this experiment were also compared with the ones obtained from groups exposed one time to cat fur odor (control: C1 and G3-1×: T1, see results).

All experimental procedures were always performed during late morning. The behavioral response

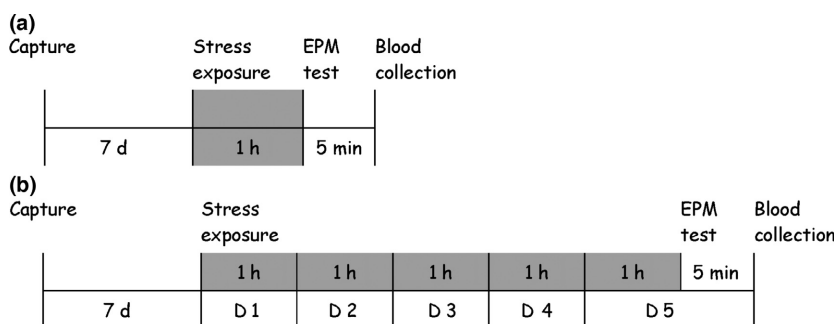


Fig. 1: Testing schedule for the (a) intensity and (b) habituation treatments.

of each tuco-tuco was also recorded in the elevated plus maze.

Elevated Plus Maze

The elevated plus maze (EPM) consisted of an acrylic cross with two opposite arms made with transparent walls and two with dark walls (25 cm high). The length of the arms was 45 cm and extended from a central platform of 10 × 10 cm. After exposition to predatory odors, the animals were taken to the labyrinth and placed in the central platform to begin the experiment. Tuco-tucos' behavior was registered using a video camera for 5 min, and the following parameters were determined from recordings: the total distance (cm), total number of arm entries, number of entries to transparent arms (% total), time of permanence in transparent arms (% total time), and time scratching walls in transparent arms (% total time) in the maze. After each experiment, the individuals were returned to their cages and the apparatus was carefully washed with water and odorless detergent, cleaned with ethanol, and then allowed to air dry to guarantee that the odors from the previous treatments did not remain. Latex gloves were used for handling the animals and the apparatus to avoid transfer of human odor.

Physiological Stress Measures

After the end of each trial of the intensity experiments or at the end of the trials of the habituation experiments, a blood sample from the retro-orbital sinus was taken from each animal. Blood sampling did not take more than 3 min to guarantee that glucocorticoid levels were not affected by the extraction procedure (Vera et al. 2011). Then, the following physiological parameters were determined from blood samples: hematocrit, blood glucose levels, plasma cortisol levels, and neutrophil/lymphocyte ratio, being the latter three parameters that typically change during the stress response (Armario 2006; Johnstone et al. 2012), and were used in previous studies in *C. talarum* (Vera et al. 2008, 2011; Cutrera et al. 2010; Brachetta et al. 2014, 2015; Schleich et al. 2015). Hematocrit levels are considered informative of the physiological condition of an organism (Johnstone et al. 2012), and these values were obtained by capillary centrifugation during 15 min at 14000 rpm. Blood glucose levels, which vary in response to stressors of intermediate level (Armario et al. 1986), were determined using a glucometer

(Accu-Chek Active Roche Diagnostics, measuring range of system: 10–600 mg/dL). Since *C. talarum* cortisol, and not corticosterone, was found to vary in response to exposition to stress factors (Vera et al. 2011), values of this hormone were obtained using a DRG™ kit (Cortisol ELISA EIA-1887, solid-phase enzyme-linked immunosorbent assay). The N/L ratio is a chronic stress indicator (Davis et al. 2008) and allows monitoring of stress of captivity (Vera et al. 2008) and also response to acute stressors (Vera, unpublished data). The number of lymphocytes, neutrophils, eosinophils, basophils, and monocytes were determined using a microscope at 450×, from blood smears fixed in 70% methanol for 10 min and stained with May–Grunwald–Giemsa. We counted all cell types in a total of 200 cells and then calculated the N/L ratio.

Statistical Analysis

One-way ANOVA tests were performed to evaluate whether the different behavioral and physiological stress parameters differed between tuco-tucos' exposed or not to predatory odors (urine and cloth impregnated with cat fur odor). When statistical significant effects of factors were observed, Tukey's tests were performed to identify differences between groups.

Results

Effects of Predator Odor Intensity on Defensive Behaviors and Physiological Parameters

Cat urine odor

Individuals exposed to the three different cue intensities showed a similar decrease in the distance traveled (Fig. 2a), number of arm entries (Fig. 2c), and number of entries to transparent arms (Fig. 2g) in the elevated plus maze, although the overall effect of the treatment was statistically similar or marginally significant at the different concentrations of urine (ANOVA, $n = 58$, distance: $F = 2.21$, $p = 0.09$; arm entries: $F = 2.53$, $p = 0.06$; transparent arm entries: $F = 2.75$, $p = 0.051$, respectively). A clear effect of the treatment was observed in the time spent in the transparent walls (ANOVA, $n = 58$; $F = 3.23$, $p = 0.029$). Individuals exposed to 1/3×, 1×, and 3× concentrations exhibited significant or marginally significant decreases in the time spent in transparent arms (ANOVA, $n = 58$, control vs. G2-1×: $p = 0.014$, control vs. G2-3×: $p = 0.086$, control vs. G2-1/3×: $p = 0.05$; Fig. 2e), although the response was similar

among concentrations. Time scratching walls in the transparent arms did not differ between groups (Kruskal–Wallis, $n = 58$; $H = 4.65$, $p = 0.19$; Fig. 2i).

Physiological stress measures

Values of hematocrit did not differ significantly between control groups and groups exposed to cat urine (ANOVA, $n = 58$, $df = 3$, $F = 0.463$, $p = 0.71$; Table 1). In a similar manner, the exposition to the different concentrations of cat urine did not affect N/L ratio, blood glucose, or cortisol levels (N/L: Kruskal–Wallis, $n = 58$, $p = 0.118$; glucose: ANOVA, $n = 58$, $F = 2.375$, $p = 0.083$; cortisol: Kruskal–Wallis, $n = 58$, $df = 3$, $p = 0.161$; Table 1).

Cat fur odor

In relation with the exposure to different intensities of fur predator odor, an overall effect of the treatment was observed in all the parameters analyzed (ANOVA, $n = 57$, distance: $F = 6.32$, $p < 0.001$; arm entries: $F = 4.02$, $p = 0.01$; time in transparent walls: $F = 7.63$, $p < 0.01$; transparent arms entries: $F = 3.67$, $p = 0.018$; time scratching in transparent arms: $H = 20.97$, $p < 0.01$). However, the behavioral response was different according to the intensity of the odor source. While animals subjected to the lowest cue intensity traveled similar distances than the controls (ANOVA, $n = 57$, $G3-1/3 \times$ vs. control: $p = 0.89$), those exposed to higher intensities traveled less (ANOVA, $n = 57$, $G3-1 \times$ vs. control: $p = 0.02$; $G3-3 \times$ vs. control: $p = 0.03$; Fig. 2b). A similar situation was observed in the number of entries to the arms (ANOVA, $n = 57$, $G3-1/3 \times$ vs. control: $p = 0.99$; $G3-1 \times$ vs. control: $p = 0.05$; $G3-3 \times$ vs. control: $p = 0.05$; Fig. 2d) and in the time spent in transparent arms (ANOVA, $n = 57$, $G3-1/3 \times$ vs. control: $p = 1$; $G3-1 \times$ vs. control: $p = 0.005$; $G3-3 \times$ vs. control: $p = 0.005$; Fig. 2f). Regarding the number of entries to transparent arms, individuals exposed to the low and high intensities behaved similarly than the control (ANOVA, $n = 57$, $G3-1/3 \times$ vs. control: $G3-3 \times$ vs. control: $p = 0.4$), while only individuals of the group $1 \times$ entered less to the transparent arms than the controls (ANOVA, $n = 57$, $G3-1 \times$ vs. control, $p = 0.01$; Fig. 2h). Finally, the time spent scratching the walls of the transparent arms was similar among individuals exposed to the lowest concentration and the control (Kruskal–Wallis, $n = 57$, $G3-1/3 \times$ vs. control: $p > 0.05$), although higher than those exposed to $1 \times$ and $3 \times$ (Kruskal–Wallis, $n = 57$, $G3-1 \times$ vs. control: $p < 0.05$, $G3-3 \times$ vs. control: $p < 0.05$; Fig. 2j).

Physiological stress measures

Values of hematocrit did not differ significantly between control group and groups exposed to cat fur odor (ANOVA, $n = 57$, $df = 3$, $F = 1.61$, $p = 0.198$; Table 1). A decrease in blood glucose levels was found in the group exposed to the lowest odor intensity compared to the one observed in the control group (Tukey's test, $p = 0.034$; Table 1). Values of N/L ratio and cortisol were not affected by the exposition to cat fur odor (Kruskal–Wallis, $n = 57$, $p = 0.217$; ANOVA, $n = 57$, $F = 1.456$, $p = 0.238$, respectively; Table 1).

Effects of habituation to predator odor on defensive behaviors and physiological parameters

While individuals exposed only once to cat fur odor traveled less than its respective control (Tukey's test, T1 vs. C1: $p = 0.03$; Fig. 3a), tuco-tucos exposed repeatedly for 5 days to the predator cue caused individuals to travel similar distances than its respective control group (Tukey's test, T5 vs. C5: $p = 0.718$; Fig. 3a). This behavioral response was also observed in the number of arm entries (Tukey's test, T1 vs. C1: $p = 0.05$, T5 vs. C5: $p = 0.80$; Fig. 3b), number of entries to transparent arms (Dunn's test, T1 vs. C1: $p < 0.05$, T5 vs. C5: $p > 0.05$; Fig. 3c), time spent in transparent arms (Tukey's test, T1 vs. C1: $p = 0.01$, T5 vs. C5: $p = 0.98$; Fig. 3d), and time scratching in transparent arms (Dunn's test, T1 vs. C1: $p < 0.05$, T5 vs. C5: $p > 0.05$; Fig. 3e). In addition, tuco-tucos exposed for 5 days to cat fur odor traveled more, displayed more arm entries, and spent more time in transparent arms and scratching walls than individuals exposed only once to the predator cue (distance traveled: Tukey's test, T1 vs. T5: $p < 0.001$; arm entries: Tukey's test, T1 vs. T5: $p = 0.004$; time in transparent arms: Tukey's test, T1 vs. T5: $p = 0.012$; time scratching in transparent arms: Dunn's test: T1 vs. T5: $p < 0.05$). Only in the number of entries to transparent arms, individuals exposed only once or for 5 days to cat fur odor did not display statistical differences (Dunn's test: T1 vs. T5: $p > 0.05$).

Physiological stress measures

No effect of the exposition procedure on hematocrit (Kruskal–Wallis, $n = 53$, $df = 3$, $p = 0.248$; Table 2), N/L ratio (Kruskal–Wallis, $n = 53$, $p = 0.860$; Table 2), blood glucose (Kruskal–Wallis, $n = 53$, $df = 3$, $p = 0.384$; Table 2), or cortisol levels (Kruskal–Wallis, $n = 53$, $df = 3$, $p = 0.654$; Table 2) was observed.

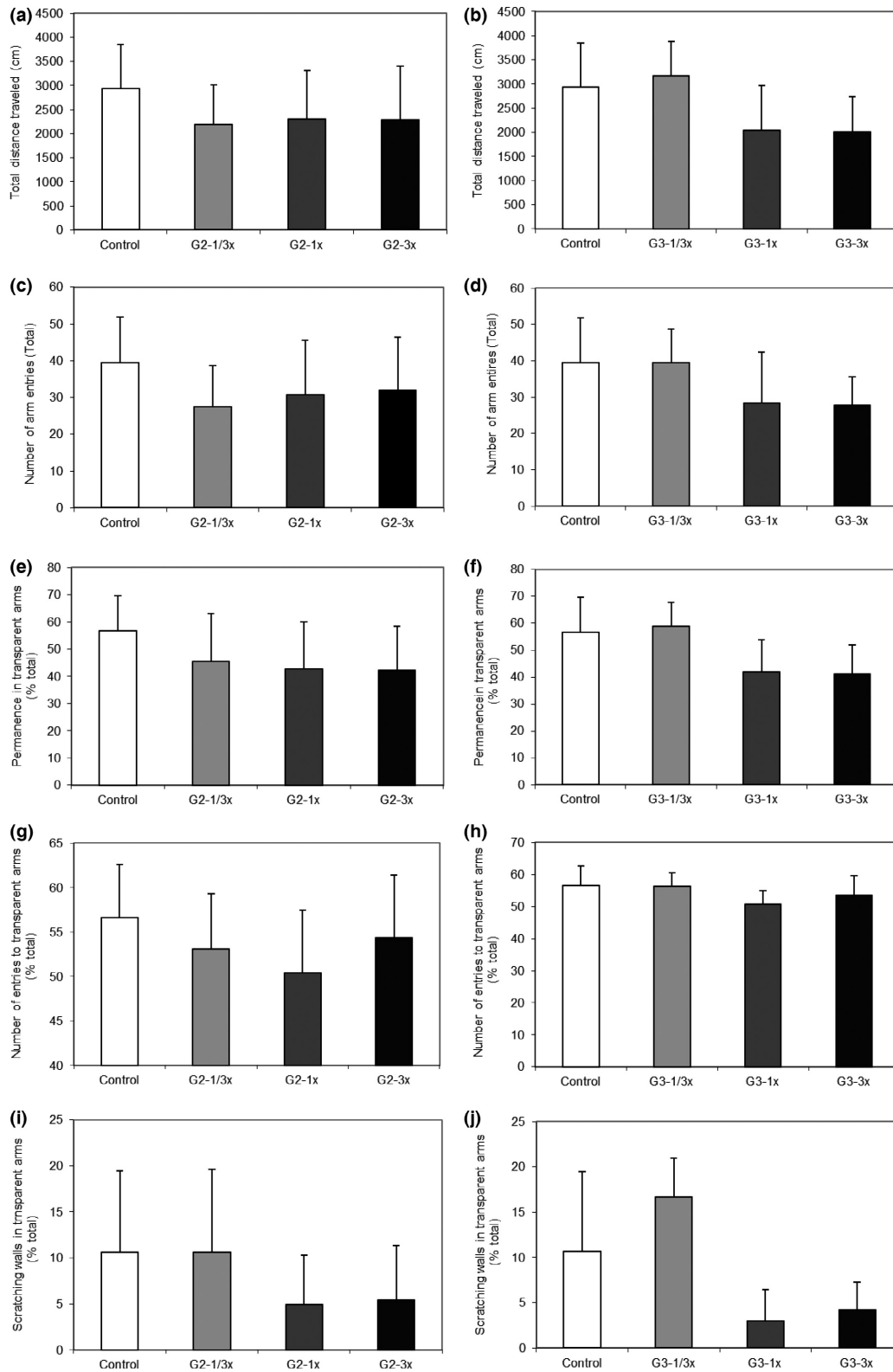


Fig. 2: Total distance traveled (cm; Mean \pm SD) (a) and (b); total number of entries into the arms (Mean \pm SD) (c) and (d); time spent in transparent arms (Mean \pm SD) (e) and (f); number of entries (Mean \pm SD) (g) and (h); and time scratching the walls in transparent arms (Mean \pm SD) (i) and (j) of individuals exposed to different intensities (1/3 \times , 1 \times and 3 \times) of urine (G2) or odor of the fur (G3) in the elevated plus maze.

Table 1: Hematocrit (Mean ± SD), blood glucose levels (mg/ml; Mean ± SD), neutrophil/lymphocyte ratio (Mean ± SD), and plasma cortisol levels (ng/ml; Mean ± SD) in individuals of *Ctenomys talarum* in control and exposed to different intensities (G3-1/3×, G3-1× and G3-3×) of cat urine odor or cat fur odor in the elevated maze test

	Control	G2-1/3×	G2-1×	G2-3×	G3-1/3×	G3-1×	G3-3×
Hematocrit	45.33 ± 2.19	44.5 ± 2.71	45.43 ± 2.33	45.58 ± 2.87	45.27 ± 4.12	47 ± 2.41	44.25 ± 4.57
Blood glucose levels (mg/ml)	88.87 ± 26.27	84 ± 31.17	84 ± 33.08	64 ± 24.05	64.44 ± 20.39	82 ± 29.39	90.75 ± 20.43
Neutrophil/Lymphocyte ratio	1.46 ± 1	0.8 ± 0.67	1.53 ± 1.67	1.36 ± 0.97	0.93 ± 0.99	2.29 ± 2.43	1.09 ± 0.98
Plasma cortisol levels (ng/ml)	23.34 ± 25.58	21.57 ± 13.17	20.31 ± 33.27	37.78 ± 55.29	27.57 ± 25.43	12.13 ± 6.9	26.87 ± 26.42

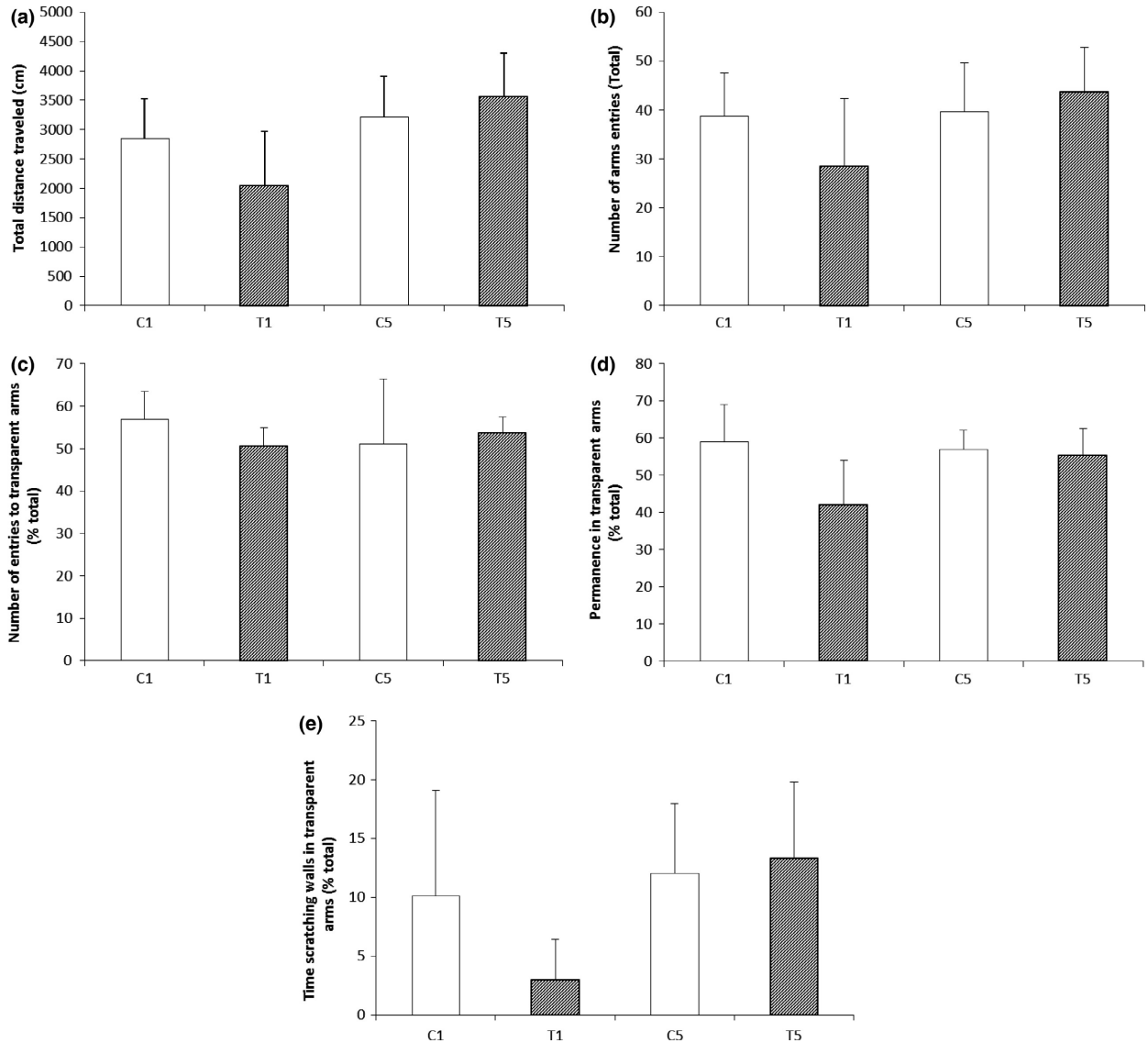


Fig. 3: Total distance traveled (cm; Mean ± SD) (a), total number of entries into the arms (Mean ± SD) (b), number of entries in transparent arms (Mean ± SD) (c), time spent in transparent arms (Mean ± SD) (d), and time scratching the walls in transparent arms (Mean ± SD) (e) of control groups (C1 and C5) and individuals exposed to fur odor once (T1) or five times (T5) in the elevated plus maze.

Discussion

Predator–prey interactions are a major evolutionary driving force, mediating the behavior of both predator and prey (Boeing 2010). Individuals may often have to deal with predators during their lifetime, and being unsuccessful in this task would inevitably mean death (Hegab & Wei 2014). Therefore, avoiding an encounter with a predator is the single most effective way to guarantee survival and increase fitness. To achieve this task, the proper identification of predators by prey is vital for survival, and, in some species, natural selection has engendered the evolution of different sensory modalities to properly recognize predators and generate behavioral and physiological responses in order to reduce and avoid the predation pressure (Coss 2010). In the last decades, predator odors have come to be recognized as an important elicitor of defensive behaviors, fear, and anxiety in animals (Blanchard et al. 2003). However, not all types of predatory odors induce significant and equivalent avoidance behaviors in the preys. A general view considers that fur-derived odors are the most relevant to induce anxiety and fear conditioning, while urine or feces-associated odors may be aversive but not anxiogenic (Apfelbach et al. 2005; Takahashi et al. 2005; Muñoz-Abellán et al. 2010). However, only a few studies have simultaneously compared the effect of both odor sources on eliciting defensive behaviors (Blanchard et al. 2003; Masini et al. 2005), leaving the question of which type of odor is more relevant to induce changes in anxiety still unresolved. When comparing the highest behavioral effects elicited by both predatory odors on *C. talarum*, our study supports the assumption that fur odors are more anxiogenic than urine, while the former provoked significant changes in the distance traveled, the number of arm entries and time in transparent arms in the elevated plus maze, cat urine only caused slight changes on those behavioral parameters. This result is in accordance with the proximity assumption which suggests that predator fur odors may indicate a higher

level of predatory immanence than feces or urine, generating therefore stronger increments in anxiety-like behaviors (Blanchard et al. 2003; Apfelbach et al. 2005; Takahashi et al. 2005). Also, and coincident with a general view that considers that predator detection abilities might be tightly correlated with other chemosensory abilities (Kats & Dill 1998), individuals of *C. talarum* that rely on chemical signals for individual, sexual and reproductive-state recognition and food detection (Zenuto et al. 2004; Schleich & Zenuto 2007; Fanjul & Zenuto 2008; Zenuto 2010) are also good at detecting and discriminating predator odors.

To respond adaptively, it is important that preys can accurately assess the predation risk associated with the intensity of perceived cues. Also, better spatial resolution of predator location might be possible if organisms are able to recognize and differentiate chemical gradients present in the field (Kats & Dill 1998). Therefore, odor intensity could be a key factor that would allow prey species to determine the 'optimal' behavioral response (Hegab et al. 2015). Having found evidence that tuco-tucos utilize fur and urine odors as cues of possible predator activity, it would be expected that more concentrated predator odors might be interpreted as higher risks of predation (Powell & Banks 2004), eliciting consequently higher levels of anxiety. Although individuals of *C. talarum* exposed to three different urine odor intensities showed a tendency to decrease the distance traveled, the number of arm entries, and entries to the transparent arms, this effect was similar at the different concentrations of predator odor. On the other hand, exposure to various intensities of predator fur odor caused variations in the behavioral response of tuco-tucos. While animals in the group exposed to the lower cue intensity behaved similar than the control group, those exposed to the higher fur odor intensities displayed clear anxiety states, with decreased locomotor activity and clear avoidance behavior. Interestingly, no differences were observed among individuals confronted with fur odor intensities of $1 \times$

Table 2: Hematocrit (Mean \pm SD), blood glucose levels (mg/ml; Mean \pm SD), neutrophil/lymphocyte ratio (Mean \pm SD), and plasma cortisol levels (ng/ml; Mean \pm SD) in individuals of *Ctenomys talarum* in control groups (C1 and C5) and exposed to cat fur odor groups (T1 and T5) in the elevated plus maze test

	C1	T1	C5	T5
Hematocrit	45.33 \pm 2.19	47 \pm 4.12	43.5 \pm 5.76	44.17 \pm 3.41
Blood glucose levels (mg/ml)	88.87 \pm 26.27	82 \pm 23.32	111 \pm 67.54	89.92 \pm 72.87
Neutrophil/Lymphocyte ratio	1.46 \pm 1	2.29 \pm 2.43	1.58 \pm 1.06	1.63 \pm 0.78
Plasma cortisol levels (ng/ml)	23.34 \pm 25.58	12.13 \pm 6.9	22.069 \pm 28.15	19.99 \pm 24.19

and 3×. Although Takahashi et al. (2005) considered that the behavioral and physiological effects are dependent on the magnitude of the predator odor delivered to the animal, their results were similar to those obtained in this work, with rats exposed to 3× and 10× sizes of cat odor cloths exhibiting more freezing and fewer contacts with odor sources than rats exposed to no predator odor, with no statistical differences among 3× and 10× groups in these behavioral parameters. Therefore, and according to these results, the intensity of natural predator odor presented to tuco-tucos has a role on the appearance of defensive behaviors, although an amount-dependent relationship between predator odor and anxiety levels was not observed, at least at the stimulus intensities used in this study. Considering that selection for chemical detection of predators by prey may be dependent not only on life-history characteristics of the prey but also upon diverse features of the predator (e.g. mobility, density), it would be also interesting to see whether abilities to detect gradients of predatory signals differ in populations of tuco-tucos under diverse predatory pressures.

Several studies in rodents have shown that the behavioral effects of exposition to predator odors may be accompanied by a physiological response (File et al. 1993; Figueiredo et al. 2003). The stress axis, that plays a central role in evolutionary adaptations to particular ecological pressures (Boonstra et al. 2007), constitutes a major pathway of the neuroendocrine system that integrates environmental change and through which life-history decisions (reproduction, growth, feeding) are implemented (Ricklefs & Wikelski 2002). In the present study, we measured physiological parameters that allow the estimation of the physiological condition of the tuco-tucos (hematocrit) or change during the stress response and can be used as stress indicators: blood glucose levels, N/L ratio, and plasma cortisol levels (Vera et al. 2008, 2011). In this study, the values of hematocrit were similar among treatment groups, indicating that the physical condition of the tuco-tucos exposed to the different intensities and frequencies of both predator cues was equivalent. Regarding N/L ratio, a parameter found to increase in response to immobilization or chronic stress caused by captivity or food restriction (Vera et al. 2011), and it did not differ between the control groups and those exposed to the different intensities of both odor sources. There is evidence that glucose levels are responsive to stressors of intermediate level (Armario 2006). In the present work, we did not observe any effect of the exposition to cues of a predator on blood glucose levels, with the exception of the

group exposed to the lowest fur odor intensity. Besides this difference, no particular trend in the response of blood glucose could be identified. Finally, we did not find differences in plasma cortisol levels between animals exposed to the different intensities of cat odors. Although the detection of the response associated with increased levels of glucocorticoids can be variable and dependent of the methods used (Tidhar et al. 2007), the lack of variation in this and the other physiological parameters suggests for a poor association between the physiological and behavioral responses to predator odors in *C. talarum*. The absence of an endocrine correlate of the behavioral response of the prey was also observed in other species of rodents (File et al. 1993; Perrot-Sinal et al. 1999; Dias Soares et al. 2003), and more interesting, in a previous study on this species of subterranean mammal (Brachetta et al. 2015). On the other hand, the relationship between HPA axis and anxiety is more evident for immobilization, another component of predatory stress. For example, rats exposed to restraint exhibited anxiety-like behaviors in the EPM and increments in ACTH and corticosterone (Muñoz-Abellan et al. 2008). Furthermore, these effects were reversed by inhibition of corticosterone, and both mineralocorticoid and glucocorticoid receptors were identified involved in the modulation of anxiety response (Calvo & Volosin 2001). Overall, the need for studying deeply the relationships between the endocrine status and the behavioral outputs is reinforced.

Several works with rodents indicate that repeated exposures to predator odors can result in either sensitization or habituation in response to these cues (Endres & Fendt 2007; Hegab et al. 2015). Although there are few clear examples of sensitization (Staples 2010), a recent study on rats showed that repeated exposure to ferret odor leads to an increasing sensitization of the neural response (Weinberg et al. 2009). More common are studies which failed into finding habituation responses to repeated exposures. For example, File et al. (1993) found that after five exposures to cat odor, the rats continued to avoid the odor cloth, although corticosterone levels stopped rising. Similarly, Wallace & Rosen (2000) showed that rats exposed repeatedly over a 5-day period to TMT failed to show habituation in freezing, although this result should be taken cautiously due to the lack of difference with the no-odor control. On the other hand, several works have shown that animals have the ability to habituate to repeated stressful stimulus (Grissom & Bhatnagar 2009). For example, File et al. (1993)

found that rats spent most of their time hiding on exposure to cat odor, but this response gradually habituated over repeated daily exposures. Staples et al. (2009) found that Albino Wistar rats exposed to cat odor initially showed strong defensive responses, although these responses habituated by the final exposure. In a complete experiment, Takahashi et al. (2005) also showed that rats displayed habituation to cat fur odor, being this behavioral response dependent of the concentration of fur odor utilized.

In our experiments, we found that while individuals exposed for 1 day to cat fur odor displayed an evident anxiety state, those exposed repeatedly for 5 consecutive days did not differ with the control group in their behavioral response, indicating a clear habituation to the predatory cue. As explained before, this process allows animals to filter biologically less relevant input and in turn devote more attention and processing energy toward more relevant or dynamic stimuli (Leussis & Bolivar 2006; Wilson 2009). In the particular case of *C. talarum*, individuals of this species usually encounter predatory cues while foraging above-ground. By reducing the time devoted to evaluate the potential risk of a predator presence after facing the same odor repeatedly, tuco-tucos can spend more time and energy in their foraging effort, obtaining an important metabolic reward for the waning of the antipredator behavioral response. At this point, it must be taken into account that different rates of habituation are likely to be influenced by the intensity of the predatory cue and the adaptive value of resuming each behavior (Staples 2010).

Variations in the endocrine response are also expected concomitant with the habituation process in the behavioral response (Apfelbach et al. 2005). In particular, the magnitude of HPA activation triggered in response to the presence of cues indicative of the presence of a predator may decline with repeated exposure to this stimulus, although this physiological habituation may be also dependent on the degree of stressor predictability (Smith et al. 2013). In our habituation experiments, and similar to our previous endocrine results, we did not find differences in the measured physiological parameters among control individuals and those exposed only once or for 5 days to cat fur odor, even when a clear anxiety response was observed in tuco-tucos exposed one time to the predatory cue. These particular outcomes in the physiological response of *C. talarum* enlighten the need for

more work to disentangle the endocrine-behavioral relationship activated by predator odors.

Conclusion

The innate defensive behaviors that prey species display when exposed to predator odors have provided valuable means of studying how brain and endocrine systems of mammals respond during stressful situations. Inside this scheme, the results of the present study provide valuable evidence that the type of predatory odor, along with the frequency of exposition, is important determinants of the appearance, strength, and extinction of defensive behaviors in the subterranean rodent *C. talarum*. Concerning the odor intensity, although a threshold level that triggered the development of defensive behaviors was observed, no further associations among predator odor intensity and prey response could be found. From these results, it is evident the need for studying predator risk effects in wild species to complement the vast arrange of physiological and biomedical works with laboratory animals carried out the last decades (Boonstra 2013; Clinchy et al. 2013). While the latter has provided an important framework to understand the diverse effects of stress on the neurophysiology of animals, only the extension of studies to wild species under more naturalistic conditions will offer a clear advance on its ecological validity. The main findings obtained from the present study also revealed that *C. talarum* is not the exception, with some behavioral responses similar to what observed in previous works on predatory stress but others clearly different, emphasizing the importance of the life-history characteristics of each species in shaping the diverse solutions encountered in nature.

Future studies utilizing diverse anxiety-eliciting factors to see whether these stressors are connected or not to a neuroendocrine response, or using activators and inhibitors to affect different steps of the HPA axis to see whether they can elicit an anxiety state without the presence of a stressor, are needed to fully understand the connection between the behavioral and physiological response in this species of subterranean rodent.

Literature Cited

- Adamec, R. E. & Shallow, T. 1993: Lasting effects on rodent anxiety of a single exposure to a cat. *Physiol. Behav.* **54**, 101–109.

- Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A. & McGregor, I. S. 2005: The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neurosc. Biobehav. Rev.* **29**, 1123–1144.
- Armario, A. 2006: The hypothalamic–pituitary–adrenal axis: what can it tell us about stressors? *CNS Neurol. Disord. Drug Targets* **5**, 485–501.
- Armario, A., Montero, J. L. & Balash, J. 1986: Sensitivity of corticosterone and some metabolic variables to graded levels of low intensity stresses in adult male rats. *Physiol. Behav.* **37**, 559–561.
- Blanchard, D. C., Markham, C., Yang, M., Hubbard, D., Madarang, E. & Blanchard, R. J. 2003: Failure to produce conditioning with low-dose trimethylthiazoline or cat feces as unconditioned stimuli. *Behav. Neurosci.* **117**, 360–368.
- Boeing, W. J. 2010: Defensive avoidance. In: *Encyclopedia of Animal Behavior*. (Breed, M. D., Moore, J., eds). Academic Press, Oxford, UK, pp. 476–480.
- Boonstra, R. 2013: Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Funct. Ecol.* **27**, 11–23.
- Boonstra, R., Barker, J. M., Castillo, J. & Fletcher, Q. E. 2007: The role of the stress axis in life-history adaptations of rodents. In: *Rodent Societies: An Ecological and Evolutionary Perspective*. (Wolff, J. O., Sherman, P. W., eds). University of Chicago Press, Chicago, pp. 139–149.
- Brachetta, V., Schleich, C. E. & Zenuto, R. R. 2014: Effects of acute and chronic exposure to predatory cues on spatial learning capabilities in the subterranean rodent *Ctenomys talarum* (Rodentia: Ctenomyidae). *Ethology* **120**, 563–576.
- Brachetta, V., Schleich, C. E. & Zenuto, R. R. 2015: Short-term anxiety response of the subterranean rodent *Ctenomys talarum* to odors from a predator. *Physiol. Behav.* **151**, 596–603.
- Busch, C., Malizia, A. I., Scaglia, O. A. & Reig, O. A. 1989: Spatial distribution and attributes of a population of *Ctenomys talarum* (Rodentia: Octodontidae). *J. Mammal.* **70**, 204–208.
- Busch, C., Antinuchi, D., Del Valle, J., Kittlein, M., Malizia, A., Vassallo, A. & Zenuto, R. 2000: Population ecology of subterranean rodents. In: *Life Underground: The Biology of Subterranean Rodents*. (Lacey, E. A., Patton, J. L., Cameron, G. N., eds). The Univ. of Chicago Press, Chicago, IL, pp. 183–226.
- Calvo, N. & Volosin, M. 2001: Glucocorticoid and mineralocorticoid receptors are involved in the facilitation of anxiety-like response induced by restraint. *Neuroendocrinology* **73**, 261–271.
- Canepuccia, A. 2005: Efectos del incremento de las precipitaciones sobre la estructura comunitaria de un pastizal del Sudeste de la región Pampeana, Argentina. Doctoral thesis. Univ. Nacional de Mar del Plata.
- Clinchy, M., Sheriff, M. J. & Zanette, L. Y. 2013: The ecology of stress: predator-induced stress and the ecology of fear. *Funct. Ecol.* **27**, 56–65.
- Coss, R. G. 2010: Predator avoidance: mechanisms. In: *Encyclopedia of Animal Behavior*. (Breed, M. D., Moore, J., eds). Academic Press, Oxford, UK, pp. 757–764.
- Cutrera, A. P., Antinuchi, C. D., Mora, M. S. & Vassallo, A. I. 2006: Home-range size and activity patterns of the South American subterranean rodent *Ctenomys talarum*. *J. Mammal.* **87**, 1183–1191.
- Cutrera, A. P., Zenuto, R. R., Luna, F. & Antenucci, C. D. 2010: Mounting a specific immune response increases energy expenditure of the subterranean rodent *Ctenomys talarum* (tuco-tuco): implications for intra and inter-specific variation in immunological traits. *J. Exp. Biol.* **213**, 715–724.
- Davis, A. K., Maney, D. L. & Maerz, J. C. 2008: The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Funct. Ecol.* **22**, 760–772.
- Dias Soares, D., Fernandez, F., Aguerre, S., Foury, A., Mormede, P. & Chaouloff, F. 2003: Fox odour affects corticosterone release but not hippocampal serotonin reuptake and open field behaviour in rats. *Brain Res.* **961**, 166–170.
- Dielenberg, R. A. & McGregor, I. S. 1999: Habituation of the hiding response to cat odor in rats (*Rattus norvegicus*). *J. Comp. Psychol.* **113**, 376–387.
- Dielenberg, R. A. & McGregor, I. S. 2001: Defensive behavior in rats towards predatory odors: a review. *Neurosc. Biobehav. Rev.* **25**, 597–609.
- Endres, T. & Fendt, M. 2007: Conditioned behavioral responses to a context paired with the predator odor trimethylthiazoline. *Behav. Neurosci.* **121**, 594–601.
- Fanjul, M. S. & Zenuto, R. 2008: Female reproductive responses to photoperiod and male odours in the subterranean rodent *Ctenomys talarum*. *Acta Theriol.* **53**, 73–85.
- Figueiredo, H. F., Bodie, B. L., Tauchi, M., Dolgas, C. M. & Herman, J. P. 2003: Stress integration after acute and chronic predator stress: differential activation of central stress circuitry and sensitization of the hypothalamo-pituitary-adrenocortical axis. *Endocrinology* **144**, 5249–5258.
- File, S. E., Zangrossi, H. Jr, Sanders, F. L. & Mabbutt, P. S. 1993: Dissociation between behavioral and corticosterone responses on repeated exposures to cat odor. *Physiol. Behav.* **54**, 1109–1111.
- Grissom, N. & Bhatnagar, S. 2009: Habituation to repeated stress: get used to it. *Neurobiol. Learn. Mem.* **92**, 215–224.
- Hegab, I. M. & Wei, W. 2014: Neuroendocrine changes upon exposure to predator odors. *Physiol. Behav.* **131**, 149–155.

- Hegab, I. M., Shang, G., Ye, M., Jin, Y., Wang, A., Yin, B., Yang, S. & Wei, W. 2014: Defensive responses of Brandt's voles (*Lasiopodomys brandtii*) to chronic predatory stress. *Physiol. Behav.* **126**, 1—7.
- Hegab, I. M., Kong, S., Yang, S., Mohamaden, W. I. & Wei, W. 2015: The ethological relevance of predator odors to induce changes in prey species. *Acta Ethol.* **18**, 1—9.
- Johnstone, C. P., Reina, R. D. & Lill, A. 2012: Interpreting indices of physiological stress in free-living vertebrates: a review. *J. Comp. Physiol.* **182**, 861—879.
- Kats, L. B. & Dill, L. M. 1998: The scent of death: chemosensory assessment of predation risk by prey animal. *Ecoscience* **5**, 361—394.
- Kavaliers, M. & Choleris, E. 2001: Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neurosci. Biobehav. Rev.* **25**, 577—586.
- Leussis, M. P. & Bolivar, V. J. 2006: Habituation in rodents: a review of behavior, neurobiology, and genetics. *Neurosci. Biobehav. Rev.* **30**, 1045—1064.
- Masini, C. V., Sauer, S. & Campeau, S. 2005: Ferret odor as a processive stress model in rats: neurochemical, behavioral, and endocrine evidence. *Behav. Neurosci.* **119**, 280—292.
- Mastrangelo, M. E., Schleich, C. E. & Zenuto, R. R. 2009: Short-term effects of an acute exposure to predatory cues on the spatial working and reference memory performance in a subterranean rodent. *Anim. Behav.* **77**, 685—692.
- McGregor, I., Schrama, L., Ambermoon, P. & Dielenberg, R. 2002: Not all "predator odours" are equal: cat odour but not 2,4,5 trimethylthiazoline (TMT; fox odour) elicits specific defensive behaviours in rats. *Behav. Brain Res.* **129**, 1—16.
- Muñoz-Abellan, C., Andero, R., Nadal, R. & Armario, A. 2008: Marked dissociation between hypothalamic-pituitary-adrenal activation and long-term behavioral effects in rats exposed to immobilization or cat odor. *Psychoneuroendocrinology* **33**, 1139—1150.
- Muñoz-Abellán, C., Armario, A. & Nadal, R. 2010: Do odors from different cats induce equivalent unconditioned and conditioned responses in rats? *Physiol. Behav.* **99**, 388—394.
- Pellow, S., Chopin, P., File, S. & Briley, M. 1985: Validation of open: closed arm entries in an elevated plus maze as a measure of anxiety in the rat. *J. Neurosci. Meth.* **14**, 149—167.
- Perrot-Sinal, T. S., Ossenkopp, K. P. & Kavaliers, M. 1999: Brief predator odour exposure activates the HPA axis independent of locomotor changes. *NeuroReport* **10**, 775—780.
- Powell, F. & Banks, P. B. 2004: Do house mice modify their foraging behaviour in response to predator odours and habitat? *Anim. Behav.* **67**, 753—759.
- Ricklefs, R. E. & Wikelski, M. 2002: The physiology-life history nexus. *Trends Ecol. Evol.* **17**, 462—468.
- Rodgers, R. J. & Dalvi, A. 1997: Anxiety, defence and the elevated plus-maze. *Neurosci. Biobehav. Rev.* **21**, 801—810.
- Schleich, C. & Zenuto, R. 2007: Use of vegetation chemical signals for digging orientation in the subterranean rodent *Ctenomys talarum* (Rodentia: Ctenomyidae). *Ethology* **113**, 573—578.
- Schleich, C. E., Zenuto, R. R. & Cutrera, A. P. 2015: Immune challenge but not dietary restriction affects spatial learning in the wild subterranean rodent *Ctenomys talarum*. *Physiol. Behav.* **139**, 150—156.
- Smith, A. S., Lieberwirth, C. & Wang, Z. 2013: Behavioral and physiological responses of female prairie voles (*Microtus ochrogaster*) to various stressful conditions. *Stress* **16**, 531—539.
- Staples, L. G. 2010: Predator odor avoidance as a rodent model of anxiety: learning-mediated consequences beyond the initial exposure. *Neurobiol. Learn. Mem.* **94**, 435—445.
- Staples, L. G., McGregor, I. S. & Hunt, G. E. 2009: Long-lasting FosB/ Δ FosB immunoreactivity in the rat brain after repeated cat odor exposure. *Neurosci. Lett.* **462**, 157—161.
- Takahashi, L. K., Nakashima, B. R., Hong, H. & Watanabe, K. 2005: The smell of danger: a behavioral and neural analysis of predator odor-induced fear. *Neurosci. Biobehav. Rev.* **29**, 1157—1167.
- Tidhar, W. L., Bonier, F. & Speakman, J. R. 2007: Sex- and concentration-dependent effects of predator feces on seasonal regulation of body mass in the bank vole *Clethrionomys glareolus*. *Horm. Behav.* **52**, 436—444.
- Vassallo, A., Kittlein, M. & Busch, C. 1994: Owl predation on two sympatric species of tuco-tucos (Rodentia: Octodontidae). *J. Mamm.* **75**, 725—732.
- Vera, F., Zenuto, R. & Antenucci, D. 2008: Decreased glucose tolerance but normal blood glucose levels in the field in the caviomorph rodent *Ctenomys talarum*: the role of stress and physical activity. *Comp. Biochem. Physiol. A* **151**, 232—238.
- Vera, F., Antenucci, C. D. & Zenuto, R. R. 2011: Cortisol and corticosterone exhibit different seasonal variation and responses to acute stress and captivity in tuco-tucos (*Ctenomys talarum*). *Gen. Comp. Endocrinol.* **170**, 550—557.
- Wallace, K. J. & Rosen, J. B. 2000: Predator odor as an unconditioned fear stimulus in rats: elicitation of freezing by trimethylthiazoline, a component of fox feces. *Behav. Neurosci.* **114**, 912—922.
- Weinberg, M. S., Bhatt, A. P., Girotti, M., Masini, C. V., Day, H. E., Campeau, S. & Spencer, R. L. 2009: Repeated ferret odor exposure induces different

- temporal patterns of same-stressor habituation and novel-stressor sensitization in both hypothalamic-pituitary-adrenal axis activity and forebrain *c-fos* expression in the rat. *Endocrinology* **150**, 749–761.
- Wilson, D. A. 2009: Olfaction as a model system for the neurobiology of mammalian short-term habituation. *Neurobiol. Learn. Mem.* **92**, 199–205.
- Zangrossi, H. & File, S. E. 1992: Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. *Brain Res. Bull.* **29**, 381–388.
- Zangrossi, H. Jr & File, S. E. 1994: Habituation and generalization of phobic responses to cat odor. *Physiol. Behav.* **33**, 189–194.
- Zenuto, R. 2010: Dear enemy relationships in the subterranean rodent *Ctenomys talarum*: the role of memory of individual odours. *Anim. Behav.* **79**, 1247–1255.
- Zenuto, R., Fanjul, M. S. & Busch, C. 2004: Use of chemical communication by subterranean rodent *Ctenomys talarum* during the reproductive season. *J. Chem. Ecol.* **30**, 2111–2126.