



Short-term anxiety response of the subterranean rodent *Ctenomys talarum* to odors from a predator



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HIGHLIGHTS

- We evaluated the behavioral and physiological response to predator odors in *C. talarum*.
- Exposure to predatory cues generates a state of anxiety.
- Odors induce behavioral changes associated with decreased locomotor and exploratory activities.
- No differences between the behavioral responses to urine or fur cat odor were found.

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ABSTRACT

Prey organisms exhibit primary adaptations that contribute to avoid predators and secondary mechanisms that allow them to defend themselves. Particularly behavioral adaptations allow them to recognize, avoid and defend against predators. Facing a predatory risk, anxiety is a reaction of adaptive value, assessing the potential risk of this encounter as well as generating an autonomic and behavioral response that would help resolve that situation. Concomitantly, a stressful condition could result according to intensity and length of exposure. Previous studies in the subterranean rodent *Ctenomys talarum* revealed that exposure to direct cues of the presence of a predator has negative effects on learning and spatial memory. These impairments in their cognitive abilities could be avoided by the development of defensive anti-predatory behaviors. Thus, in this study we evaluated the behavioral and physiological responses of *C. talarum* to odors derived from predators (urine and fur of domestic cat) used as indicators of potential risk of predation. In the open field, exposure to odors from a predator induced a decrease in time moving with respect to control (not exposed) individuals, as well as an increase in the time scratching the walls near and far from the odor source. In the elevated plus maze, exposed individuals walked shorter distances, entered less frequently and remained less time scratching the walls in transparent arms. Physiological parameters did not show differential variations among treatments in both tests utilized. The results shows that exposure of the tuco-tuco to odors of a predator generates a state of anxiety and induces behavioral changes associated with decreased locomotor activity and avoidance behavior generation.

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1. Introduction

In nature, organisms are exposed to a number of risks or threatening stimulus, such as the presence of predators, conspecifics, parasites and toxins associated with food [1]. One of the most studied factors in relation to these risks is predation. Throughout generations, prey organisms have developed specific behaviors to facilitate recognition, avoidance and defense against predators [2]. These behavioral adaptations, that

may comprise a decrease or inhibition of activity, suppression of foraging, feeding or/and grooming, as well as movements to places where predatory cues are not present, reduce the probability of encounter between predators and preys [2,3].

Many species of mammals show behavioral and physiological responses to cues from predators (e.g. fur odor, urine, feces, saliva and secretions from the anal gland; [2,4]). In rodents, exposure to natural predators or their odors usually induces anxiety-like states [5–11]. Belzung and Griebel [12] define anxiety as the response of an individual to real or potential threats that can endanger their homeostasis. This response may be physiological (mainly associated to the activation of the HPA – hypothalamic–pituitary–adrenal-axis), and/or behavioral (decreased locomotor activity or immobilization, suppression of non-

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defensive behaviors, scanning, avoidance of the source of danger). On the neural plane, different exposures to predator odors induce the activation of early genes, like *c-fos* and/or *ΔFosB* genes, which participate in changes in the neural activity of the CNS [13]. The behavioral expression of anxiety, when not controlled, might easily consume a disproportionate portion of the energy and time budgets of individual animals, in detriment of the other crucial behaviors previously mentioned [14]. Recently, an increased interest is focused on the non-lethal effects of predation upon prey demography; in this sense, predation risk has been proposed as an important factor affecting not only cognition but also a suite of measures strongly associated to survival and reproduction such as body condition, parental behavior, foraging efficiency, and stress condition until chromosomal damage, overall comprising the “ecology of fear” [15].

Even if prey individuals, like rodents, display innate defensive responses when exposed to the odor of a predator, they vary according to the odor source detected. Odors derived from urine or feces usually last long after the predator departed (besides the predator tendency for selectively defecate in places where they do not hunt) and are consequently less predictive of a predatory threat, eliciting therefore a weaker behavioral response. On the contrary, odors derived from the predator fur or skin indicate its proximity and immediate risk, generating a more robust defensive behavior [16,17].

Although mammals respond to odors from predators in a diversity of forms, the available evidence in relation to wild rodents is restricted to a few species and includes reports on modifications in locomotor activity, feeding and space use in *Microtus oeconomus*, *Microtus agrestis*, *Microtus arvalis*, and *Clethrionomys glareolus* [18–21], and a suite of effects on reproduction and development of offspring in *Microtus unguiculatus*, *M. auratus* and *Phodopus campbelli* [22,23]. Sullivan and colleagues [24] reported for *Thomomys talpoides* that the gophers avoided the TMT – a synthetic fox fecal odor – in the laboratory but not the urine of various predators. However, the evidence presented emphasizes that there is little information available as evidence of behavioral responses to these threatening situations in wild rodents.

Ctenomys talarum (tuco-tucos) is a wild solitary rodent, which inhabits closed systems of galleries parallel to the soil surface [25]. This subterranean ecotope has been proposed as buffered against severe predation since the burrow itself represents an adaptation that allows animals to avoid predators [26,27]. However, and although spending most of their day-time underground, tuco-tucos emerge short distances from burrow openings to cut grasses and perennial forbs growing in the soil and run backwards into the burrows where they later consume the leaves and stems [28–30]. During these brief periods on the surface, tuco-tucos become exposed to aerial and terrestrial predators. Previous studies showed that this species is often predated by owls, foxes and wild cats [28,31,32]. It has also been observed in peri-urban areas predatory events by dogs and domestic cats (C.E. Schleich, pers. obs.).

Apart from the obvious direct and detrimental effects of a predatory event, a failed attack has also strong consequences for the prey fitness. For example, previous work on *C. talarum* showed that an acute exposure to direct cues (immobilization) from a natural predator (e.g. cat) produced a short-term stress response that negatively affects its spatial reference memory [33]. Also, it has been observed that spatial learning is largely affected by an acute or a chronic presentation of direct signals (immobilization + cat fur odor) of the presence of predators [34]. As a consequence, it is expected that when confronted with a situation of predatory risk, preys will experience a state of anxiety that would allow them to assess the potential risk of this encounter and generate a behavioral response that would let solve this stressful situation successfully. In the particular case of *C. talarum*, the affectation of cognitive abilities caused by the contact with a predator could be avoided by the development of an anti-predatory defensive behavior. Consequently, the general aim of this paper consists of assessing the behavioral and physiological response of individuals of *C. talarum* exposed to predator

odors used as indicators of potential risk of predation. Besides adding knowledge on the evolution of antipredator behavioral adaptations in rodents, the incorporation of a wild species of subterranean rodent to this kind of studies provides new and valuable information on a group barely included in these analyses and completely different in its lifestyle to those species commonly used to investigate prey–predator relationships.

In particular, we will assess whether:

- 1) the odor of a predator (urine or fur odor from a cat) induces changes in the behavioral response;
- 2) as well as the activation of pituitary–adrenal axis.

We predict that the stress caused by the presence of cues from predators would cause in individuals of *C. talarum* increases in their 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 skin and fur than with exposure to predator urine due to the immediacy of the potential attack.

2. Material and methods

2.1. Animals and laboratory conditions

We captured adults of both sexes (41 males and 63 females) between June 2013 and September 2014. Animals were captured in Mar de Cobo (37°46'S, 57°26'W, Buenos Aires province, Argentina) using plastic live traps set at fresh surface mounds. Then the individuals were transported to the laboratory and housed in individual plastic cages (30 × 40 × 25 cm) provided with wood shavings. The temperature in the animal room was maintained constant at 25 ± 1 °C and a photoperiod of LD 12:12. Food was supplied daily and consisted of sweet potatoes, lettuce, corn, mixed grass and sunflower seeds. After 7 days of acclimatization to the laboratory, the animals were used in the different experiments. At the end of the experiments all animals were returned to the capture site in good health conditions.

2.2. Exposition to predatory cues

Tuco-tucos were exposed to two different olfactory cues indicative of the presence of a predator: wood shavings with urine from an adult male cat (obtained 24 h before the experiments) and a piece of cloth (6 cm × 6 cm) impregnated with cat fur odor (obtained after allowing a cat to use the cloth to rest on it for a 7 day period before the experiment). The different samples were stored at 4–6 °C in sealed plastic bags until use.

To compare the effects of exposure of individuals to odors from a predator, control individuals were exposed to odorless water or to concentrated citronella oil, which is commonly used as a control odor in many studies since it has been shown not to present any emotional or hedonic value for the rats [35,36]. For this, 1 μl of citronella was placed in an adsorbent paper which was mixed for 1 min with 10 g of wood shavings and later conserved in sealed plastic bags until use. Since no statistical difference was observed between water or citronella (see results), data from both control conditions were pooled together.

2.3. Procedure

All experimental procedures were performed during late morning. The exploratory behavior of tuco-tucos was recorded in two tests commonly used to assess anxiety in rodents: the open field [37,38] and the elevated plus maze [39–41]. Individuals were used only one time in one of the tests along the study.

2.3.1. Open field

Open field consisted of a dark acrylic box (100 cm × 100 cm × 35 cm height) divided into 25 identical squares marked by lines on the floor. The squares were consecutively labeled starting from the left inferior one (named A1) until the superior right square (named E5). Individuals entered the open field by a hole next to the A3 square while the odor source was placed in the middle of the E3 square. Predatory odors and control samples were presented to the individuals in plastic pots (diameter: 11 cm) covered with a wire mesh. The animals were previously randomly assigned to 4 groups according to the type of predatory odor used: G1 control (7 males and 9 females), G2 urine (9 males and 11 females), G3 cloth impregnated with cat fur odor (6 males and 9 females) and G4 control citronella (2 males and 4 females). Tuco-tucos' behavior was registered using a video camera for a 10 min period and the following parameters were determined from recordings: total distance walked (cm), time moving (% total time), latency to reach and time in the area near the odor source (% time total in squares E2 to E4 and D2 to D4), number of contacts with the odor source, time scratching walls in the proximity or distant from the odor source (s), and number of times individuals urinate in the open field. After each experiment, the apparatus was 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.

2.3.2. Elevated plus maze

Elevated plus maze consisted of an acrylic cross with two opposite arms made with transparent walls (20 cm high) and two with dark walls (25 cm high). The length of the arms was 45 cm and extended from a central platform of 10 cm × 10 cm. The maze was raised 70 cm above floor level with a wooden stand. Prior to the start of the experiments the animals were exposed in their boxes to the different predatory odor sources or control samples for a 60 min period: G1 controls (7 males and 7 females), G2 urine (6 males and 10 females), G3 cloth impregnated with the odor of the fur (6 males and 9 females) and G4 control citronella (2 males and 4 females). Similar to the previous experiment, the different samples were presented to the individuals in plastic pots covered with a wire mesh for a one hour period before the experiments. Then, 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: total distance (cm), 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) and number of times individuals urinate in the maze. After each experiment, the labyrinth was cleaned as explained before.

2.4. Physiological stress measures

After the end of each experiment, 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 [42]. Then the following physiological parameters were determined from blood samples: hematocrit, blood glucose, plasma cortisol and N/L ratio; being the latter three parameters that typically change during the stress response [43,44] and were used in previous studies in *C. talarum* [34,42,45,46]. Hematocrit levels, informative of the physiological condition of an organism [44], were obtained by capillary centrifugation during 15 min at 14,000 rpm. Blood glucose levels, which vary in response to stressors of intermediate level [47], were determined using a glucometer (Accu-Chek Active Roche Diagnostics). Since in *C. talarum* cortisol, and not corticosterone, was found to vary in response to exposition to stress factors [42], values of this hormone were obtained using an DRG™ EIA-1887 kit. 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.

2.5. Statistical analysis

Two-way ANOVA tests were performed to evaluate if the different behavioral and physiological stress parameters differed between male and female 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 tests were performed to identify differences between groups.

3. Results

3.1. Control: water vs citronella

No statistical differences between water or citronella groups were found: open field test; total distance traveled ($p = 0.533$), time moving ($p = 0.719$), latency to reach the odor source ($p = 0.876$), time in the area near the odor source ($p = 0.212$), number of contacts with the odor source ($p = 0.265$), time scratching walls near ($p = 0.100$) or far ($p = 0.978$) the zone of the odor source; physiological stress measures: hematocrit ($p = 0.537$), cortisol ($p = 0.707$), glucose ($p = 0.101$) and N/L ratio ($p = 0.558$). Elevated plus maze test: total distance traveled ($p = 0.963$), number of arm entries ($p = 0.963$), number of entries to transparent arms ($p = 0.952$), time of permanence in transparent arms ($p = 0.116$), time scratching walls in transparent arms ($p = 0.388$); physiological stress measures: hematocrit ($p = 0.786$), cortisol ($p = 0.963$), glucose ($p = 0.720$) and N/L ratio ($p = 0.673$).

3.2. Open field test

- *Total distance traveled (cm)*: total distance traveled did not differ between the treatment groups (ANOVA, $n = 52$; $df = 2$, $F = 1.611$, $p = 0.211$) nor between sexes (ANOVA, $n = 52$; $df = 1$, $F = 0.0130$, $p = 0.910$).
- *Time moving (% total time)*: the presence of predatory odors decreased the percentage of time moving in exposed individuals (ANOVA, $n = 52$; $df = 2$, $F = 24.301$, $p < 0.001$, Fig. 1a) although no differences between sexes were found (ANOVA, $n = 52$; $df = 1$, $F = 0.786$, $p = 0.380$). Tukey tests revealed that exposed groups were different than controls (control vs G2: $p < 0.001$; control vs G3: $p < 0.001$), but no differences between G2 and G3 were found ($p = 0.599$).
- *Latency to reach the odor source*: time spent until making contact with the odor source did not differ between control and exposed groups (ANOVA, $n = 52$; $df = 2$, $F = 0.918$, $p = 0.407$) nor between sexes (ANOVA, $n = 52$; $df = 1$, $F = 0.854$, $p = 0.360$).
- *Time in the area near the odor source (% time total)*: no differences between treatments groups (ANOVA, $n = 52$; $df = 2$, $F = 0.230$, $p = 0.796$) nor between sexes were found (ANOVA, $n = 52$; $df = 1$, $F = 1.245$, $p = 0.270$).
- *Number of contacts with the odor source*: number of contacts did not differ significantly between control and groups exposed to the different odor sources (ANOVA, $n = 52$; $df = 2$, $F = 0.516$, $p = 0.600$) nor between sexes (ANOVA, $n = 52$; $df = 1$, $F = 1.741$, $p = 0.194$).
- *Time scratching walls near the zone of the odor source (s)*: time scratching walls near the zone of the odor source differed significantly between control and treatments groups (ANOVA, $n = 52$; $df = 2$, $F = 3.853$, $p = 0.028$; Fig. 1b). Tukey test revealed that values of control groups were higher than those observed in group G2 ($p = 0.028$). No differences between sexes were found (ANOVA, $n = 52$; $df = 1$, $F = 0.209$, $p = 0.650$).

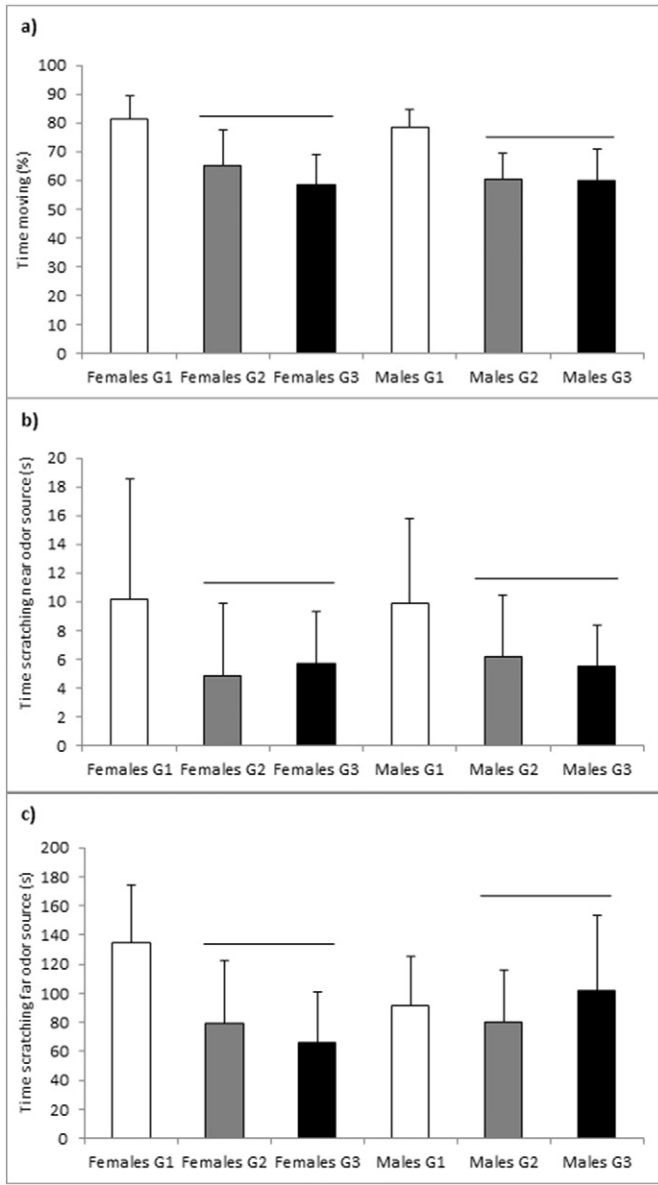


Fig. 1. Time moving (% total) (Mean ± SD) (a), time scratching walls near (b) and far (c) zone the odor source (s) (Mean ± SD) in the open field (females and males, controls and exposed to stress). The bars indicate significant differences ($p < 0.05$) between control and exposed groups.

- *Time scratching walls in the far zone from the odor source (s)*: similarly, time scratching walls distant of odor source differed between control and treatments groups (ANOVA, $n = 52$; $df = 2$, $F = 6.571$, $p = 0.003$; Fig. 1c). Tukey tests revealed that values of control groups were higher than those observed in group G2 ($p < 0.001$) and G3 ($p < 0.001$). No differences between sexes was observed (ANOVA, $n = 52$; $df = 1$, $F = 0.442$, $p = 0.510$).
- *Urination and defecation*: due to only 12 females and 8 males urinated, while 17 females and 15 males defecated in the open field, this parameter was not analyzed.

3.3. Physiological stress measures

- *Hematocrit*: hematocrit did not differ between treatment and control groups (ANOVA, $n = 52$; $df = 2$, $F = 0.168$, $p = 0.846$). Higher hematocrit values were detected in males than in females (ANOVA, $n = 52$; $df = 1$, $F = 20.651$, $p < 0.001$, Table 1).

Table 1 Hematocrit (Mean ± SD), blood glucose levels (mg/ml) (Mean ± SD), neutrophil/lymphocyte ratio (Mean ± SD) and plasma cortisol levels (ng/ml) in males and females of *Ctenomys talarum* in control (G1) and exposed to predatory odors groups (G2: urine and G3: cat fur odor) in the open field and the elevated plus maze tests.

	Open field						Elevated plus maze					
	G1		G2		G3		G1		G2		G3	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
Hematocrit	44.2 ± 2	48.14 ± 4	42.09 ± 3	49.55 ± 5	44.67 ± 5	48.5 ± 3	44 ± 1	46.86 ± 2	44.6 ± 2	46.83 ± 3	44.67 ± 3	50.5 ± 3
Blood glucose levels (mg/ml)	75 ± 12	94 ± 32	93.6 ± 23	80.71 ± 26	104.67 ± 32	85.2 ± 25	90.12 ± 29	87.43 ± 24	95.3 ± 47	80 ± 18	90.22 ± 25	69.67 ± 14
Neutrophil/Lymphocyte ratio	1.15 ± 1	1.17 ± 1	1.02 ± 1	1.9 ± 1	2.56 ± 5	4.87 ± 3	1.72 ± 1	1.17 ± 1	1.12 ± 1	2.22 ± 2	1.52 ± 1	3.45 ± 3
Plasma cortisol levels (ng/ml)	33.91 ± 22	9.80 ± 4	31.94 ± 30	14.01 ± 9	24.18 ± 17	13.88 ± 5	30.07 ± 34	15.65 ± 9	27.67 ± 41	8.05 ± 3	14.32 ± 8	8.84 ± 3

- **Blood glucose levels:** levels of blood glucose did not differ between the treatment and control groups (ANOVA, $n = 52$; $df = 2$, $F = 0.410$, $p = 0.667$), whereas no differences were observed between sexes (ANOVA, $n = 52$; $df = 1$, $F = 1.692$, $p = 0.199$, Table 1).
- **Neutrophil/lymphocyte (N/L) ratio:** Although a tendency for higher ratios is observed for group 3 in both sexes, no differences were observed in the N/L ratio between the control and treated groups (ANOVA, $n = 52$; $df = 2$, $F = 2.425$, $p = 0.100$), while differ between sexes, with higher values observed in males (ANOVA, $n = 52$; $df = 1$, $F = 7.494$, $p = 0.009$, Table 1).
- **Plasma cortisol levels:** cortisol levels did not differ between treatments and control groups (ANOVA, $n = 52$; $df = 2$, $F = 0.0893$, $p = 0.915$), but significant differences were observed in cortisol levels between sexes, with higher values observed in females (ANOVA, $n = 52$; $df = 1$, $F = 13.309$, $p < 0.001$, Table 1).

3.4. Elevated plus maze test

- **Total distance traveled (cm):** total distance traveled was lower in individuals exposed to predatory odors, although this result was only marginally significant (ANOVA, $n = 46$; $df = 2$, $F = 2.800$, $p = 0.079$, Fig. 2a). No differences between sexes were observed (ANOVA, $n = 46$; $df = 1$, $F = 0.884$, $p = 0.353$).
- **Number of arm entries:** no differences between control and exposed groups were observed (ANOVA, $n = 46$; $df = 2$, $F = 2.313$, $p = 0.112$) nor between males and females (ANOVA, $n = 46$; $df = 1$, $F = 1.071$, $p = 0.307$).
- **Number of entries to transparent arms (% total):** individuals exposed to predatory odors entered less often to transparent arms than control ones (ANOVA, $n = 46$; $df = 2$, $F = 4.753$, $p = 0.014$, Fig. 2b). Tukey test revealed differences between groups G1 vs G2 ($p = 0.029$) and G1 vs G3 ($p = 0.030$), but no differences between G2

and G3 were found ($p = 1.000$). Number of entries did not differ between sexes (ANOVA, $n = 46$; $df = 1$, $F = 1.167$, $p = 0.286$).

- **Time of permanence in transparent arms (% total time):** significant effect of exposing individuals to predatory odors was observed (ANOVA, $n = 46$; $df = 2$, $F = 7.226$, $p = 0.002$), with exposed tuco-tucos staying less time in transparent arms than control ones (Tukey test, G1 vs G2: $p = 0.013$; G1 vs G3: $p = 0.003$), but no differences between G2 and G3 were found ($p = 0.844$). No differences between males and females were observed in this parameter (ANOVA, $n = 46$; $df = 1$, $F = 2.648$, $p = 0.112$, Fig. 2c).
- **Time scratching walls in transparent arms (% total time):** significant overall effect of exposing individuals to predatory odors was observed (ANOVA, $n = 46$; $df = 2$, $F = 4.204$, $p = 0.022$, Fig. 2d), Tukey test revealed that tuco-tucos exposed to cat fur odor scratch less time than control individuals while being at the transparent arms ($p = 0.017$), but no differences between G2 and G3 were found ($p = 0.410$). Again, no differences were detected between males and females (ANOVA, $n = 46$; $df = 1$, $F = 0.852$, $p = 0.362$).

3.5. Physiological stress measures

- **Hematocrit:** higher hematocrit values were also detected in treatment groups than in controls (ANOVA, $n = 46$; $df = 2$, $F = 3.703$, $p = 0.033$). Post hoc analysis revealed significant differences between G1 and G3 ($p = 0.042$), but no differences between G1 and G2 ($p = 0.939$) and between G2 and G3 ($p = 0.087$) were found. Differences between the sexes were observed, with higher values in males (ANOVA, $n = 46$; $df = 1$, $F = 27.256$, $p < 0.001$, Table 1).
- **Blood glucose levels:** no differences were observed between treated and control groups (ANOVA, $n = 46$; $df = 2$, $F = 0.359$, $p = 0.701$) or between sexes (ANOVA, $n = 46$; $df = 1$, $F = 1.946$, $p = 0.171$, Table 1).

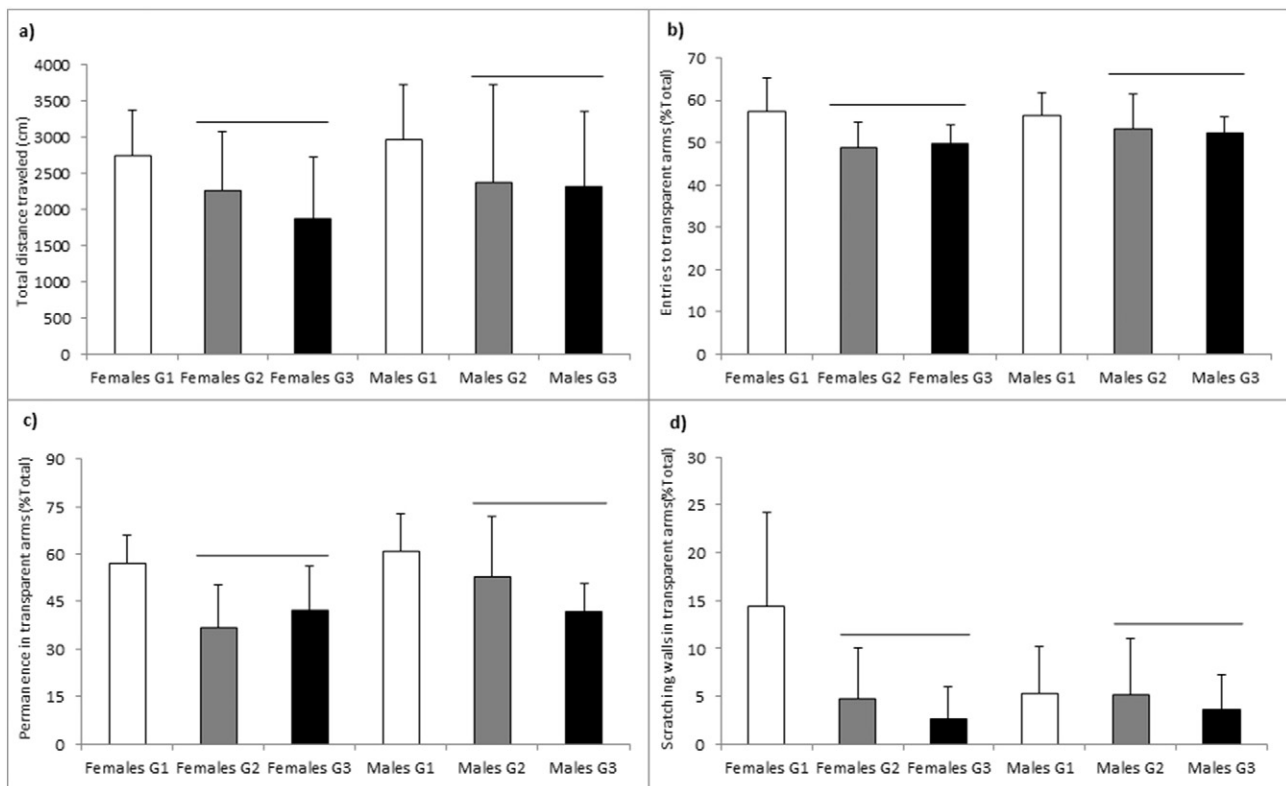


Fig. 2. Total distance traveled (cm) (Mean \pm SD) (a) and number of entries to transparent arms (%) (Mean \pm SD) (b), time in transparent arms (% total) (Mean \pm SD) (c) and time scratching walls in transparent arms (% total) (Mean \pm SD) (d) in elevated plus maze (females and males, controls and exposed to stress). The bars indicate significant differences ($p < 0.05$) between control and exposed groups.

- *Neutrophil/lymphocyte (N/L) ratio*: in the maze test no differences were observed between control and treatment groups (ANOVA, $n = 46$; $df = 2$, $F = 0.123$, $p = 0.884$), or between sexes (ANOVA, $n = 46$; $df = 1$, $F = 2.374$, $p = 0.131$, Table 1).
- *Plasma cortisol levels*: cortisol levels did not differ between treatments and control groups (ANOVA, $n = 46$; $df = 2$, $F = 1.113$, $p = 0.339$), however higher values were observed in females (ANOVA, $n = 46$; $df = 1$, $F = 4.733$, $p = 0.036$, Table 1).

4. Discussion

Predation carries a much higher risk than temporarily losing food, water or a mate, since a single prey–predator encounter can lead to the prey death or produce a significant effect on its fitness [1]. Therefore, it is expected that preys would display developed abilities to distinguish predator cues that indicate a life-threatening risk and generate proper behavioral reactions in response to this menace. These “anti-predator” behaviors are crucial to survival. In many cases, anti-predator defense involves the detection and response to specific chemical cues that predators produce [2].

Several works have shown that odors of predators trigger a range of unconditioned behavioral and physiological responses in preys. In field studies, predator odors were shown to have a direct impact on reproduction, space use, locomotor activity or intraspecific competence [48, 49, 21]. In laboratory works, higher levels of anxiety and appearance of defensive behaviors were observed after exposure to predator odors, although some discrepancies were also detected [50, 51]. For instance, De Paula and colleagues [52] proved that male Wistar albino rats displayed anxiety-like behaviors after predator odor exposure, while Dielenberg and colleagues [8] showed that *Rattus norvegicus* exhibited increased anxiety on the elevated plus-maze after exposure to a cat collar. With respect to the behavioral changes observed after predator odor exposure, decreases in locomotor activity and non-defensive behaviors -like grooming and reproduction-, and retreat to places far from the odor site are considered as the most common ones [53, 54].

In the present work we exposed adult individuals of *C. talarum* to two different predator odors and tested their response in the open field and the elevated plus maze tests. In the first test we found that the presence of predator odors decreased the percentage of time moving in exposed individuals to either urine or fur/skin odors, a response also observed, for example, in male Sprague–Dawley rats that displayed hypo-activity in response to cat odor [55]. On the other hand, we found that time spent until making contact, number of contacts with the odor source, and time in the area near the odor source did not differ between control and exposed groups. However, time scratching walls near and far the odor source in exposed groups was lower than in control groups, indicating again lower levels of activity in individuals confronted with predator cues.

Results of the elevated plus maze, a classical test for anxiety in rodents, also revealed a decrease in the total distance traveled in individuals exposed to predator odors, although this result was only marginally significant. No differences in the total number of arm entries between control and exposed groups were found. However, individuals exposed to predator odors entered less often and stayed less in transparent arms than in control ones. Hence, tuco-tucos exposed to a threatening situation tend to avoid the open arms and show a clear preference for protected areas, a result consistent with an anxiogenic response. Similar results were observed in diverse laboratory rats, like in male Wistar rats [54], which displayed fewer entries and spent less time in the open arms of the elevated plus maze when exposed to cat odor. Furthermore, and concomitant to what observed in the open field, individuals exposed to cat fur odor scratch less than control individuals while being at the transparent arms. Taken the results of both tests together, it becomes clear that the exposition of individuals of *C. talarum* to predator odors triggers a state of anxiety and the appearance of anti-predator

behaviors, mainly characterized by a decrement in the locomotor and exploratory activities. Such changes in their behavior represent a clear antipredatory benefit since stealthy animals would be more attentive to the presence of a predator in the immediacy. Also, the encounter rate and detection by a predator are expected to be lowered and consequently, the probability of capture is potentially reduced [56].

Although the majority of studies investigating anxiety in rodent were focused on one sex (mainly males; [57, 58]), some works aimed to examine differences in the anxiety response between males and females revealing their results a complex picture. Although sex differences in levels of anxiety before or after exposure to predatory odors were described [58–62], these results showed the influence of several factors, such as the species or type of rat strain utilized, the age and/or reproductive condition of the individuals, and the kind of behavioral test performed, highlighting the complexity of establishing a general trend for the existence of a sexual divergence in anxiety levels in rodents. In the present study we found that behavioral parameters obtained in the open field and the elevated plus maze did not differ between males and females, either with or without exposure to predator odors. This result is congruent with the behavioral characteristics of this species of solitary rodent, in which both sexes are equally exposed to predation during their aboveground foraging bouts, and also with previous studies revealing that exposition to predator cues affected the spatial abilities of both sexes in a similar way [33, 34].

Other remarkable outcome derived from this work is the absence of difference between the behavioral responses of individuals exposed to urine or odor from cat fur. In rodents, the behavioral effects of feces/anal gland odorants (e.g. TMT) were considerably less clear than those obtained by fur/skin contact with a live cat or natural odors of a cat [17, 54]. In this line, Muñoz-Abellán and colleagues [63] found that exposure of rats to fur odor elicited more behavioral inhibition in comparison with others exposed to urine odors. This dissimilar effect may be ascribed to the different degrees of predictive information carried by these signals, with cat hair/skin odor indicating that a cat is nearby now and feces or anal gland odors indicating that a cat has been present [17]. Therefore, in light of this hypothesis, the lack of differences among the behavioral response of *C. talarum* to cat urine or fur is intriguing. One possible explanation could be attributed to the odor intensities used in this study. It is known that the amount of cat odor is an important factor underlying the level of expression of unconditioned fear behavior [4]. Therefore, it would be interesting to check if this lack of difference in the behavioral response between both odor sources is also expressed at various urine or fur cat odor intensities. Other reports support the notion that odors from litter soiled by a cat and fur odors resulted in similar long-lasting behavioral changes [64, 65]. It was proposed that the accidental inclusion of fur in the sample odor may be responsible for that finding [63]. In our study we collected urine samples just after the cat urinated. However we cannot fully discard a contamination of the sample with fur.

Apart from the analysis of the behavioral response, several studies have investigated the physiological effects of exposition to predator odors in rodents [2]. In the present study we measured physiological parameters that estimate the physiological condition of the individuals (hematocrit) or change during the stress response and can be used as stress indicators (glucose levels, N/L ratio and plasma cortisol levels). Higher hematocrit values were detected in groups exposed to skin/fur odor in the elevated plus maze. Individuals of both sexes were fed in the same manner; therefore the differences observed cannot be attributed to variations in the hydration. Furthermore, in both tests higher hematocrit values were detected in males than in females. Similar results were also obtained in a previous study [34], and could be attributed to sexual size dimorphism in this species, where males show higher muscle mass and robustness.

In this study, we also found that blood glucose, responsive to stressors of intermediate level [43], did not differ between control and groups exposed to predator odor in the open field and elevated plus maze tests.

Regarding N/L ratio, Vera and colleagues [42] found that *C. talarum* displayed increments in these values after a situation of chronic stress caused by captivity and food restrictions and also in response to immobilization. In this study, no differences were detected in the N/L ratio between the control and exposed groups, even though N/L values in the latter group were clearly higher than those expected for captive condition [42], which may be attributed – at least in part – to a high variance in this parameter. The detection of increased levels of glucocorticoids in rodents exposed to predator risk is highly variable and accompanied the wide variation in duration (few minutes to weeks); type of exposure (different odor sources, cat presence, sounds) and hormone sampling method (plasma, feces) ([66,67] and cites therein; [63]). Such differences contribute to obscure the relationship between predator odor exposition and the level of physiological response triggered. Remarkably, we found no differences in plasma cortisol levels between control and exposed groups after both tests. This result, together with the observed lack of variation in blood glucose levels and the slight increase in the N/L ratio, suggests for a poor association between the physiological and behavioral responses to predator odors in *C. talarum*. Lack of association between both responses was reported, for example, in female prairie voles, exhibiting rises in corticosterone levels but not anxiety-like behaviors on the EPM test after a brief social stress exposition [68]. On the other hand, it has been recently demonstrated that TMT, which produces a state of increased vigilance and anxiety in rodents and was used as a predator odor, causes its effect through neural systems different from those obtained with predator odor [69], indicating that similar behavioral responses could be related to different physiological reactions. It would be interesting to explore if different stressors, apart from predator odor, elicit an anxiety state in *C. talarum*, and whether this response is correlated with an activation of the HPA axis.

In conclusion, this paper presented information on anxiety behavior induced by predator odors with different degrees of predictive risk information in the wild subterranean rodent *C. talarum*. The results showed that exposure of tuco-tucos to predator odors generates a state of anxiety and induce behavioral changes associated with decreased locomotor activity and generation of avoidance behavior. The development of these antipredatory defensive behaviors would contribute to the avoidance of the deleterious consequences of a direct predatory attack, which were shown to produce clear negative effects on cognitive processes in this subterranean rodent [33,34].

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