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Prenatal predatory stress in a wild species of subterranean rodent: Do ecological stressors always have a negative effect on the offspring?

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Abstract

In pregnant females, a failed predatory event not only induces individual responses but also represents a significant change in the developmental environment of the offspring, which may lead to modifications in their phenotype that may persist at different stages of life. We evaluate whether prenatal exposure to predatory cues affects anxiety behavior, behavioral response to predator cues, stress response to immobilization, and immune response to sheep red blood cells (SRBC) and phytohemagglutinin (PHA) in juveniles of the subterranean rodent *Ctenomys talarum*. We found that prenatal predator stress (PPS) (1) increased juvenile anxiety-like behaviors and the appearance of antipredator behaviors, (2) did not affect the response of offspring to predatory stressors, and (3) did not influence the physiological response of juveniles to stressors (immobilization) nor the immunological responses to SRBC and PHA challenges. This work shows the influence of PPS on the development of behavioral responses in the offspring, whom displayed a state of anxiety and behavioral changes associated with decreased locomotor activity and avoidance behaviors. Thus, these individuals prenatally exposed to predatory cues show behavioral adaptations that may contribute to avoid predators in the adult life.

KEYWORDS
Ctenomys talarum, predation, prenatal stress, subterranean rodent

1 | INTRODUCTION

In mammal species, the exposure to ecological and environmental stressors, such as the presence of predators or their odors, can cause severe changes in the physiology and behavior of individuals (Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005; Hegab et al., 2014; Hegab, Kong, Yang, Mohamaden, & Wei, 2015; Kavaliers & Choleris, 2001; Takahashi, Nakashima, Hong, & Watanabe, 2005). Prey species exposed to predator-derived odors have shown alterations in foraging, feeding, general activity, and, mainly in females, reproduction (Apfelbach et al., 2005), although negative results were also described depending upon prey life history (Orrock, 2010)

These changes may be accompanied by an impact on the regulation of the hypothalamic–pituitary–adrenal (HPA) axis, with increased levels of stress hormones such as glucocorticoids (GCs) and adrenocorticotrophic hormone (ACTH) (Apfelbach et al., 2005; Figueiredo, Bodie, Tauchi, Dolgas, & Herman, 2003; File, Zangrossi, Sanders, & Mabbutt, 1993). Therefore, predators or the presence of their cues can have nonconsumptive effects that are sufficiently long-lasting to affect demographic processes (Clinchy, Sheriff, & Zanette, 2013). These effects, associated with predation risk situations (e.g., changes in body condition, foraging activity, parental behavior), may finally affect the survival and reproduction of prey species (McLeod, Krebs, Boonstra, & Sheriff, 2017; Sheriff, Krebs, & Boonstra, 2009).

In the last years, many authors described these changes in prey species under the term "ecology of fear" (Brown, Laundré, & Gurung, 1999; Clinchy et al., 2013), highlighting that the contact with cues of the presence of a natural predator represents a risky situation to prey organisms and may have strong consequences on their life.

In addition to these changes at the individual level, in several species, offspring are influenced by the environmental conditions experienced by their mother, who can affect their progeny not only during gestation but also after birth by altering the quality and quantity of parental care (Bauer et al., 2015; Mashoodh, Sinal, & Perrot-Sinal, 2009; Sheriff, McMahon, Krebs, & Boonstra, 2015). Focusing on prenatal exposure to predator odors, it has been known that maternal exposure to such odors during pregnancy increases plasma levels of GCs in the mother which in turn may have strong effects on fetal development, litter size, body weight development, and time of sexual maturation of offspring (Apfelbach et al., 2005; Love, McGowan, & Sheriff, 2013; Monclús, Tiulim, & Blumstein, 2011; Vasilieva Parfenova, & Apfelbach, 2001; Weinstock, 2008). Recently, studies have shown that exposure to maternal stress hormones can alter offspring phenotype and potential prepare offspring to cope with the environment experienced by the mother. For example, elevated maternal stress hormones may provide a good indication of a high risk environment, and therefore, prenatal exposure to such hormones, and the subsequent effects on offspring phenotype, may be beneficial to offspring (Boonstra, 2013; Sheriff & Love, 2013). This programming process results in the organization of target tissues and could affect gene expression in the offspring, affecting function throughout life (Meaney, Szyf, & Seckl, 2007). For example, in several species, the offspring of mothers exposed to cues of predators are better-defended against predators when compared to the offspring of unexposed mothers (McGhee, Pintor, Suhr, & Bell, 2012; Monclús et al., 2011; Shine & Downes, 1999). Likewise, there is increasing evidence that variations in prenatal environment can influence the responses of the offspring in both short- and long term (Barbazanges, Piazza, Le Moal, & Maccari, 1996; Boersma & Tamashiro, 2015; Vallee et al., 1997; Zimmer, Larriva, Boogert, & Spencer, 2017).

A number of studies performed in rodents have shown a relationship between stressful situations during pregnancy and changes in the behavior, physiology, or immunology of the offspring (Götz & Stefanski, 2007; Götz, Wittlinger, & Stefanski, 2007; Guo et al., 1993; Lordi, Patin, Protais, Mellier, & Caston, 2000; Lordi, Protais, Mellier, & Caston, 1997; Wilson, Vazdarjanova, & Terry, 2013). For example, behavioral experiments demonstrated that prenatally stressed rats show higher levels of anxiety (Abe et al., 2007; Estanislau & Morato, 2005), variations in the motivation to explore mazes (Patin, Lordi, Vincent, & Caston, 2005; Valleé et al., 1997), and several abnormalities in behavior, including decreased innate fear in response to the odor of predators, impaired extinction of fear, increased locomotor activity, and appearance of stereotypic behaviors (Wilson et al., 2013). Concomitantly, changes in behavior induced by prenatal stress can be accompanied with alterations in the function of the HPA axis (Maccari Piazza et al., 1995; Barbazanges et al., 1996; Abe

et al., 2007). For example, Schöpper Palme Ruf and Huber (2012) found that stress exposure of pregnant guinea pigs resulted in lower basal GC levels in pups during the prepubertal phase, although pups were still capable of increasing plasma cortisol levels after stress exposure. On the other hand, Bian et al. (2015) demonstrated that density-induced maternal stress in root voles (*Microtus oeconomus*) alters the offspring stress axis, with pups born from stressed mothers (high-density) presenting greater fecal corticosterone metabolites (FCM) levels. Interestingly, these physiological changes may depend on the timing of the maternal stress, its intensity and duration (Weinstock, 2008).

Aside from the effects on the maternal and offspring HPA axes, recent studies suggest that stress experienced by the mother during pregnancy can also affect the immune system in the developing progeny (Bakker et al., 1998; Kay, Tarcic, Poltyrev, & Weinstock, 1998; Merlot, Couret, & Otten, 2008). Experimental works that evaluated the consequences of stress during the gestational period indicated detrimental effects in the offspring's immune cell numbers and responsiveness. Götz and Stefanski (2007) investigated the effect of a maternal social stressor in Long–Evans rats and found that prenatally stressed males had a lower basal number of total leukocytes. Also, a reduced lymphocyte proliferative response to pokeweed mitogen stimulation was observed. However, there were not differences in the proliferative index of control and prenatally stressed males in response to the mitogen concanavalin A (Götz & Stefanski, 2007). Besides affecting antibody responses (Sobrian et al., 1997), prenatal stress may also influence the mechanisms of regulation of inflammation (Vanbesien-Mailliot et al., 2007).

While these effects of prenatal stress in offspring have been extensively investigated in laboratory rats using usually conventional laboratory stressors (e.g., physical restraint, Maccari et al., 1995; electric foot shock, Estanislau & Morato, 2005; psychological stress, Abe et al., 2007; and psychosocial stress, Götz & Stefanki, 2007), comparatively fewer studies have involve wild species and ecologically relevant stressors (Sheriff et al., 2017). Examples of this last situation in rodents were carried out by Bian and colleagues (2015), who studied the effects of maternal population density on offspring physiology, reproduction and population dynamics in root voles (*M. oeconomus*), and Dantzer et al. (2013) who found that density-induced maternal stress in red squirrels (*Tamiasciurus hudsonicus*) results in faster growing offspring through an adaptive hormone-mediated maternal effect. Natural stressors, like the presence of a predator or cues indicative of its presence, which certainly represent an ecologically more relevant stimulus, are comparatively less used as stressors in experimental laboratory conditions. Therefore, in nontraditional model organisms, the link between expositions to natural stressors in the gestational period and the effects on the offspring has been poorly explored (Boonstra, 2013; but see Adamec, Blundell, & Burton, 2005). Nevertheless, this topic needs to be considered to deepen our understanding about potential adaptive role of ecological and environmental stressors for adult offspring.

Ctenomys talarum (Talas tuco-tuco; Thomas, 1898) is a subterranean rodent that is distributed in South America. This species, a

small size rodent (120 and 160 g body mass for females and males, respectively), inhabits closed systems of galleries parallel to the soil surface (Antinuchi & Busch, 1992). Both sexes are sedentary and maintain exclusive territories (with the exception of mothers that share the burrow with offspring until they disperse; Busch, Malizia, Scaglia, & Reig, 1989). These rodents have a polygynous mating system in which males never exert preparental or parental care (Zenuto, Antinuchi, & Busch, 2002; Zenuto, Vassallo, & Busch, 2001). The gestation period is 95 days from copulation (Zenuto et al., 2001). Pups become behaviorally and physiologically independent from their mother at about 60 days after birth (Cutrera, Antinuchi, & Busch, 2003; Zenuto et al., 2002). Therefore, the weaning period starts at this age, when pups start to display active exploratory behavior (Schleich, 2010). Sexual maturation age of this species is 6 months for females and 9 months for males (Malizia & Busch, 1991).

Tuco-tucos are herbivores and they 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 (Busch et al., 2000; del Valle, Lohfelt, Comparatore, Cid, & Busch, 2001; Schleich & Zenuto, 2007). During these brief periods on the surface, and also when individuals disperse (dispersal age of pups: approximately 2 months old; Malizia, Zenuto, & Busch, 1995), tuco-tucos become exposed to aerial and terrestrial predators. This species is often depredated by owls, foxes, and wild cats (Busch et al., 2000; Canepuccia, 2005; Vassallo, Kittlein, & Busch, 1994). Predatory events by dogs and domestic cats have also been observed in periurban areas (C.E. Schleich, pers. obs.).

An acute or chronic exposure to direct and indirect predatory cues has been shown to produce a stress response (behavioral and physiological) in adult individuals of C. talarum that negatively affects spatial learning and memory capacities (Brachetta, Schleich, & Zenuto, 2014; Mastrángelo, Schleich, & Zenuto, 2009). Additionally, the exposure of adult tuco-tucos to predator odors (fur or urine) generates a state of anxiety and induces behavioral changes associated with decreased locomotor activity and avoidance of open spaces (Brachetta, Schleich, & Zenuto, 2015). Recently, we found that the intensity of 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 (Brachetta, Schleich, & Zenuto, 2016). Moreover, cat fur odors are more anxiogenic than cat urine for this subterranean species; 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, urine only caused slight changes on those behavioral parameters (Brachetta et al., 2016).

Previous studies have also explored the immune system of adults and pups of C. talarum. For example, Cutrera, Luna, Merlo, Baldo, and Zenuto (2014) found that young tuco-tucos (60 days old) mounted a significantly higher inflammatory response to phytohemagglutinin (PHA) than adults. Interestingly, at this age, wild young tuco-tucos start to leave the maternal burrow, a situation that could increase the frequency of injuries caused by interactions with predators (Zenuto et al., 2002). Also, Cutrera, Zenuto, Luna, and Antenucci

(2010) found that adults of this subterranean species are capable of mounting a humoral immune response against a novel antigen (sheep red blood cells; SRBC) and that response represents a significant energetic cost for them. However, the humoral response remains unexplored in juvenile C. talarum.

Until now, the generational effects of a threatening situation, like the presence of predator cues, during the gestational period and their consequences in the offspring of C. talarum have been unexplored. Therefore, the aim of this study is to evaluate whether prenatal exposure to cues indicative of the presence of a predator—cat—(urine, fur odor, and immobilization) affects the physiology and behavior of C. talarum pups at the juvenile stage, when natal dispersal occurs. In particular, we assessed whether maternal exposure to predator cues induced variation in offspring: (1) levels of anxiety, locomotor activity, exploration, defensive behaviors, (2) behavioral response to direct cues indicative of the presence of a predator, (3) immune response, and (4) activation of the HPA axis.

We predicted that the prenatal stress caused by the presence of cues from a predator will cause juveniles of C. talarum to increase their levels of anxiety, in the form of decreased locomotor activity and avoidance of open spaces. These behavioral responses will be higher in individuals from stressed mothers after exposition to direct predatory cues. Also, we expect that the ability to mount both inflammatory and antibody responses will be negatively affected in prenatally stressed individuals. Finally, increments in baseline levels of GCs and their reactivity to immobilization are expected.

2 | MATERIAL AND METHODS

2.1 | Animals and laboratory conditions

Pregnant females of C. talarum (n = 15) were captured using plastic live traps in the sand dunes of Villa Gesell (37°15′19″ S, 56°58′04″ W, Buenos Aires Province, Argentina) between May and December 2015 (reproductive season). Captured females were immediately carried to the laboratory and housed individually in plastic boxes (0.30 × 0.40 × 0.25 m), which contained a half terracotta pot as a refuge and wood shavings as a nesting material. Animal room temperature was maintained at 24 ± 1°C, and photoperiod was light/dark 12:12. Animals were fed with mixed grasses, lettuce, corn, and sunflower seeds ad libitum. After 7 days of acclimatization to the laboratory conditions, females were assigned to the prenatal predator stress (PPS) or control (C) groups. At the end of the experiments, the mothers and their offspring were returned to the capture site in good health conditions. Low reproductive activity in captive conditions—even allowing the females to mate with preferred males—(Zenuto, Estavillo, & Fanjul, 2007) and long gestation period in C. talarum (Zenuto et al., 2001) would make it difficult to obtain the required pool of pups to conduct this kind of study from captive mating pairs.

In this study, the capture, handling, and maintenance in captivity of all animals were conducted in accordance with the guidelines approved by Sikes & The Animal Care and Use Committee of

the American Society of Mammalogists (2016) and current laws of Argentina.

2.2 | Predator prenatal stress procedure

Pregnant females were randomly divided into two groups. One group served as a C (without exposure to predator cues, *n* = 6) and the other group was subjected to PPS (*n* = 9). Individuals of the PPS group were exposed to predator cues (urine odor + fur/immobilization) once a week until the day of birth. Following this schedule, pregnant females were exposed on average two to three times to predator cues, according to the duration of pregnancy. Therefore, in this study, prenatal stress corresponds to the last third of fetus development. The stress exposure method was previously described in Brachetta and colleagues (2014). Briefly, exposure to cat urine consisted in leaving the animal in a plastic cage (0.30 × 0.40 × 0.25 m) with a plastic pot containing wood shavings impregnated with domestic cat urine (obtained 24 hr before starting the experiments and conserved in sealed bags at –20°C) for a 5-min period or clean wood shavings for control individuals. The immobilization consisted was placing each individual in the same plastic cage, holding it firmly by hand with a cloth impregnated with cat fur odor to restrict its movement and intermittently releasing it for a period of 2 min. The odor-impregnated cloth was obtained after allowing a cat to rest on it for a period of 7 days before the experiment. Animals of the C group were left in the plastic cage for the same period of time but without exposure to predator cues. The behavioral and physiological effect of predatory exposure were not assessed in mothers in order to diminish the probability of embryo reabsorption, fetus abortion, or pup mortality due to cannibalism or even infanticide, two common behaviors in severely stressed mothers.

2.3 | Litters and housing conditions

The date of birth was determined by daily observing plastic boxes containing each pregnant female. All the mothers used in this study were in the last month of gestation. Litter size of control and exposed mothers was similar (control = 4 ± 0.8; exposed = 3.5 ± 1.6, *t*-test, *df* = 13, *p* = .64). Also, the number of pups per litter that survived to the age of 60 days was similar in prenatally stressed (27 pups survived of a total of 33) and control individuals (19 pups survived of a total of 22). The weight at birth in pups of the C (10.14 g ± 0.77) and PPS (10.67 g ± 1.75) groups was similar. For the experiments, 25 pups from a total of 15 litters were used: 6 controls (C; total of 4 males and 5 females) and 9 PPS (total of 6 males and 10 females). All the pups were marked with ink to allow individual recognition. All the litters were sexed in the first month. For the experiments, two individuals from each litter were randomly picked (with the exception of litters of one individual). Finally, when juveniles were 60 days old (age at which dispersal begins), they were separated from the mother and placed in individual boxes as described before.

2.4 | Experiments

2.4.1 | Behavioral response

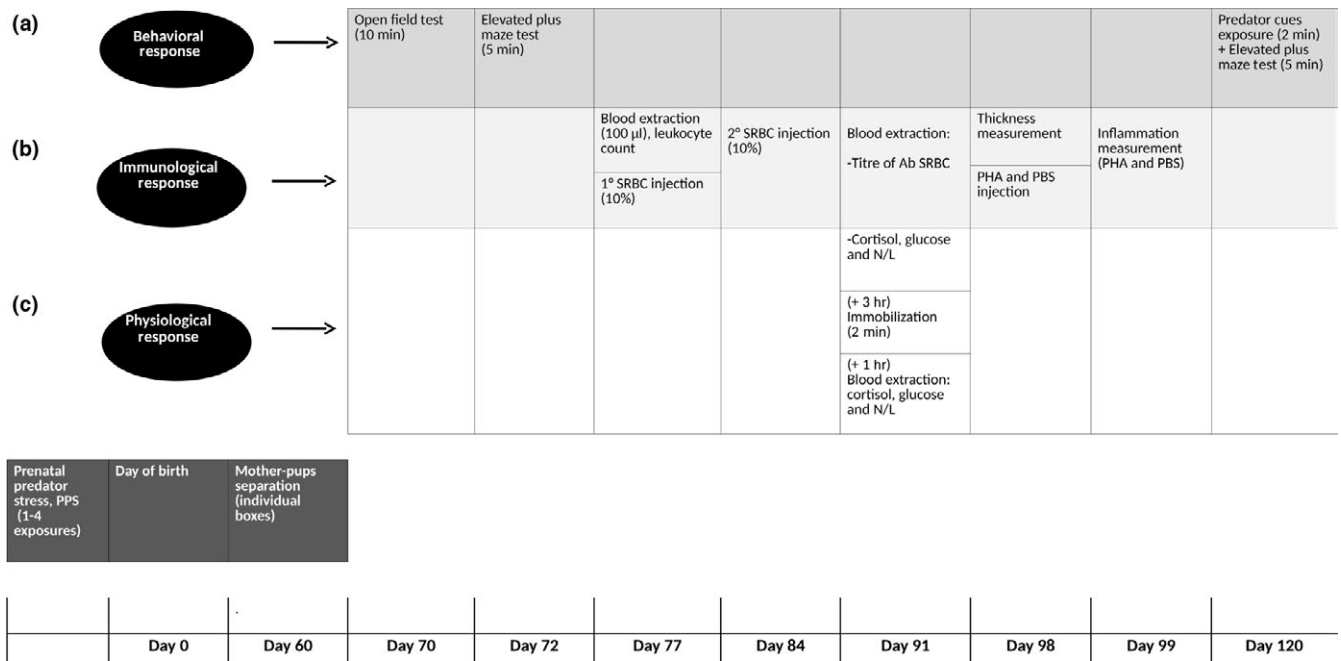**Prenatal predator stress and anxiety tests**
The performance of C and PPS pups was evaluated in two tests commonly used to assess exploratory behavior and anxiety state in prenatally stressed rodents: the open field (Abe et al., 2007; Baker et al., 2008; Götz & Stefanski, 2007; Mashoodh et al., 2009) and the elevated plus maze (Baker et al., 2008; Estanislau & Morato, 2005; Götz & Stefanski, 2007; Laloux et al., 2012). Experiments were carried out when individuals were 70 and 72 days old, respectively.

Open field: It consisted of a dark acrylic box (100 × 100 × 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). Pups entered the open field by a hole next to the A3 square. Tuco-tucos' behavior was registered using a video camera for a 10-min period and the following parameters were determined from recordings: total distance traveled (cm), time moving (% total time), time spent in the center (% time total in squares B2 to B4, C2 to C4, and D2 to D4), time scratching walls (s), and number of rearing postures in the open field. After each experiment, the individual was returned to its home cage and 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 scent (Figure 1a).

Elevated plus maze: It consisted of an acrylic cross with two opposite arms made with dark walls (25 cm high) and two with transparent walls (25 cm high) to avoid falls. The length of the arms was 45 cm and extended from a central platform of 10 × 10 cm. The maze was raised 70 cm above floor level with a wooden stand. Each animal was taken to the labyrinth and placed in the central platform to begin the experiment. Juveniles' 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). One person did all the behavioral scoring (VB). After each experiment, the labyrinth odor was cleaned as explained before (Figure 1a).

Predator cues and anxiety test
At the age of 120 days, all tuco-tucos (C and PPS groups) were exposed to the predator stress factor (2 min of immobilization with a cloth impregnated with cat fur odor) to evaluate if the behavioral response of individuals exposed or not to predator stress during maternal gestation is similar when subjected to stress at a juvenile age. After 30 min of the exposition, the behavioral performance of animals was evaluated in the elevated plus maze (methodology explained above) (Figure 1a).

		Day 60	Day 70	Day 77	Day 84	Day 91	Day 98	Day 99	Day 120
(a) Behavioral response	→	Open field test (10 min)	Elevated plus maze test (5 min)						Predator cues exposure (2 min) + Elevated plus maze test (5 min)
(b) Immunological response	→			Blood extraction (100 µl), leukocyte count; 1° SRBC injection (10%)	2° SRBC injection (10%)	Blood extraction: -Titre of Ab SRBC	Thickness measurement; PHA and PBS injection	Inflammation measurement (PHA and PBS)	
(c) Physiological response	→					-Cortisol, glucose and N/L; (+ 3 hr) Immobilization (2 min); (+ 1 hr) Blood extraction: cortisol, glucose and N/L			

Prenatal predator stress, PPS (1-4 exposures)	Day of birth	Mother-pups separation (individual boxes)
	Day 0	Day 60 ... Day 70 ... Day 72 ... Day 77 ... Day 84 ... Day 91 ... Day 98 ... Day 99 ... Day 120

FIGURE 1 Timeline of experimental procedures to evaluate (a) behavioral, (b) immunological, and (c) physiological responses

2.4.2 | Immune response

SRBC (sheep red blood cells) challenge

To assess the adaptive immune function in young tuco-tucos (77 days old), each animal of the C and PPS groups was injected intraperitoneally with the nonpathogenic antigen SRBC (Fitzgerald, 10% suspension, 1.5 µl/g of animal mass). Seven days later, they were injected again with SRBC, following a protocol previously developed for *C. talarum* by Cutrera et al. (2010). Seven days after last injection, the immune response was estimated by the mean antibody titers against this antigen detected in hemagglutination assays using 96-well microplates. Blood samples from the retroorbital sinus were taken from each animal. Blood sampling did not take more than 3 min to guarantee that GC levels were not affected by the extraction procedure (Vera, 2011). Immediately after collection, blood was kept at 4°C until it was centrifuged at 660 *g* for 15 min, after which plasma was separated and heated at 56°C for 30 min to inactivate the complement. Plasma was stored at −20°C until used in the hemagglutination assay. A quantity of 20 µl of heat-inactivated plasma was added to 20 µl of phosphate-buffered saline (PBS) in the first well of the plate; serial dilutions in PBS (1:2–1:256) were then carried out followed by the addition of 20 µl of a 1% suspension of SRBC to each well. The plates were gently agitated for 1 min and then incubated at 37°C for 1 hr. After that, plates were kept still at 4°C for 2 hr before macroscopic examination for agglutination was performed. For comparative purposes, antibody titers were expressed as the negative log2 (−log2) of the minimum plasma concentration that contained enough antibody to agglutinate the antigenic SRBC. In addition, analysis of an extra control group (n = 6) composed of other individuals of the same litters injected intraperitoneally with sterile PBS (1.5 µl/g of animal mass) confirmed that the juveniles do not exhibit antibodies without having been previously exposed to the antigen (Figure 1b).

Phytohemagglutinin challenge

To investigate the inflammatory response of juveniles, we used one of the most common techniques utilized by ecologists: PHA skin test. PHA is a vegetal lectin and it is expected to trigger a local inflammatory response that in tuco-tucos is mediated both by components of the adaptative as well as the innate immunity (Merlo, Cutrera, & Zenuto, 2014). Prior to injection with PHA, the thickness of each hind foot was measured with a micrometer. Then, each individual (91 days old) was injected subcutaneously in the instep of the left hind foot with sterile PBS (0.3 µl/g of mass) and on the right hind foot with PHA (*Phaseolus vulgaris* PHA Sigma L-8754 solution dissolved in PBS, 3 mg/ml; 0.3 µl/g of mass) using a 30G needle, methodology described previously by Merlo et al. (2014). The degree of swelling was measured 24 hr postinjection. Finally, the PHA or PBS response was determined as the difference between pre- and postinjection thickness divided by initial foot thickness (response = (post–pre)/pre) (Figure 1b).

Determination of leukocyte profile

The leukocyte profile was used to evaluate the innate immune defenses in the juveniles (Figure 1b), in a similar way to that previously performed in adults of *C. talarum* (Cutrera et al., 2010; Merlo et al., 2014; Merlo, Cutrera, & Zenuto, 2016a; Merlo, Cutrera, & Zenuto, 2016b; Schleich, Zenuto, & Cutrera, 2015). Relative counts of each leukocyte type in blood provide information about different processes that could be occurring in the individual, such as stress (increased neutrophils: lymphocytes ratio), virus (increased lymphocyte count), bacteria and microparasites (increased neutrophil counts), allergies and parasitism (eosinophilia), infection or endocrine disorders (increased

basophil count), chronic infection or inflammation (increased monocyte count; Voigt, 2000). Thus, leukocyte diversity and abundance of blood smears performed on day 14 were quantified following standard protocols (Voigt, 2000). After fixation with methanol, the slides were stained with May–Grunwald Giemsa solution and then examined under oil immersion at 1,000× magnification (Olympus CX 31, Tokyo, Japan). The "wandering technique" (Voigt, 2000) was used to record the abundance of lymphocytes, neutrophils, eosinophils, monocytes, and basophils (identifications based on cell morphology described by Voigt (2000) in vertebrates; Vera, Zenuto, and Antenucci (2008) and Cutrera et al. (2010) for *C. talarum*) until a total of 200 leukocytes had been examined. The wandering technique allows a random sampling of cells, avoiding the recount of the same field. The smear is examined from one edge, across the entire monolayer, up to the other edge, moving inward a short distance and parallel to the edge for 3–5 fields, and then moving back to the first edge, repeating this procedure as often as necessary to identify the required number of cells (Voigt, 2000). To calculate the total leukocyte abundance, the number of leukocytes encountered in 30 fields in which there was a single layer of erythrocytes (~20,000 erythrocytes, R. R. Zenuto, unpublished) was recorded and then standardized to 100,000 erythrocytes for comparative purposes.

2.4.3 | Physiological response to stress

To assess the effect of prenatal stress on physiological parameters, blood samples were obtained from each juvenile of both groups (PPS and C) for the determination of cortisol concentrations, blood glucose levels, and neutrophils/lymphocyte ratio (N/L) at the age of 91 days (Figure 1c). Blood samples (100–200 µl) were obtained from the suborbital sinus using a syringe fitted with a flexible plastic tube, which was connected to a microcapillary tube. Blood sampling did not take more than 3 min to guarantee that cortisol levels were not affected by manipulation and extraction procedure (Romero, 2002; Vera, Antenucci, & Zenuto, 2011). Then, they were exposed to an acute stress factor to evaluate the response of both groups to stress. To accomplish this, tuco-tucos were subjected to immobilization with the methodology explained in Vera and colleagues (2008): each individual was firmly held for a 2-min period in a restraint apparatus consisting of two parallel wire grids (spaced 3.5 cm) that precluded them from making almost any kind of movement. Then, blood samples were obtained to measure cortisol concentrations, blood glucose levels, and N/L ratio from all individuals after the immobilization (1 hr) since cortisol levels significantly increase at 30–60 min in tuco-tucos subjected to this acute stressor (Vera et al., 2011). In *C. talarum*, cortisol is responsive to the factors that typically regulate cortisol and corticosterone concentrations (acute stressors and adrenocorticotropic hormone, ACTH), but corticosterone is not, suggesting differentiated physiological roles for both hormones (Vera et al., 2011; Vera et al., 2008). Furthermore, both cortisol and corticosterone levels diminished under the chronic stress condition imposed by captivity; corticosterone was below detectable levels in both sexes while for cortisol, plasma concentration diminished in relation to field condition in both sexes, but especially in females (Vera, 2011; Vera et al., 2011). However, and besides the lower values found during captivity, cortisol values still increased after stress exposure (Vera et al., 2011).

In two extra control groups (not subjected to immobilization), we found that individuals did not show increased plasma cortisol levels after 3 hr (n = 7, p = .250) and 4 hr (n = 6, p = .959) due to the blood extraction procedure. Hence, cortisol levels sampled 4 hr later (but 1 hr postimmobilization) revealed the response to immobilization and not to the blood sampling pretreatment.

Values of cortisol were obtained using a DRG[TM] kit (Cortisol ELISA EIA-1887, solid-phase enzyme-linked immunosorbent assay). The cortisol assay is capable of measuring cortisol levels up to 800 ng/ml, the limit of detection is 2.5 ng/ml, and intra- and interassay CVs were 7.3% and 12% (Vera et al. unpublished data). Furthermore, blood glucose levels were determined using a glucometer (Accu-Chek Active Roche Diagnostics, measuring range of system: 10–600 mg/dl) immediately after extraction. Finally, the number of lymphocytes, neutrophils, eosinophils, basophils, and monocytes were determined as previously detailed. We counted all cell types in a total of 200 cells to calculate the N/L ratio (Figure 1c) which is a known stress parameter that increases with GC secretion particularly associated to chronic stress (Davis, Maney, & Maerz, 2008) but more recently Müller and colleagues (2011) proposed that N/L ratios and GCs would indicate different stress conditions. In *C. talarum*, increases in N/L have been verified during acute (brief immobilization; Vera et al., 2011) and chronic stressful situations (e.g., captivity, Vera et al., 2008, and food restriction, Schleich et al., 2015).

2.5 | Statistical analysis

All statistical comparisons involved a nested structure in which measurements were performed on young individuals that developed in litters bore by a female tuco-tuco. In addition, comparisons that involved the measurement of the same individuals at different occasions added a repeated-measures structure to the analysis. Differences in the responses to experimental treatments were assessed through nested and repeated measures ANOVAs using the "nlme" library (Pinheiro, Bates, DebRoy, Sarkar, and R Core Team, (2014) in R (R Core Team, 2017). Normality and homoscedasticity of residuals were tested with Shapiro's and Levene's tests, respectively. In the few instances where these assumptions were not met, data were square-root transformed (*prenatal predator stress and anxiety test*—open field test: time moving, time spent in the center, time scratching walls; *prenatal predator stress and anxiety tests*—elevated plus maze: time scratching walls in transparent arms; *predator cues and anxiety test*—elevated plus maze: time scratching walls in transparent arms [control vs. PPS, control pre- vs. control postexposure, PPS pre- vs. PPS postexposure]; *physiological response to stress*—glucose: control vs. PPS [postexposition], cortisol: control vs. PPS (post exposition)—N/L: control vs. PPS (postexposition), control pre- vs. control postexposure, PPS pre- vs. PPS postexposure; *immune response*—none). Sex of juveniles was not considered as a factor in the analysis due to a low and uneven number of males and females in each group (control and treatment). Besides this, previous works in *C. talarum* showed that behavioral and

physiological parameters did not differ between males and females after exposure to predator odors (Brachetta et al., 2015).

3 | RESULTS

3.1 | Behavioral response

3.1.1 | Prenatal predator stress and anxiety tests

Open field: Prenatal exposition to predatory odors decreased the percentage of time moving (nested ANOVA, df = 13, t = −2.30, p = .038; Figure 2a) and the total distance traveled in exposed juveniles (df = 13, t = −2.77, p = .016; Figure 2b). Time spent in the center (df = 13, t = −0.89, p = .386; Figure 2c), time scratching walls (df = 13, t = 0.83, p = .418; Figure 2d), and number of rearing postures (df = 13, t = −1.40, p = .184; Figure 2e) in the open field did not significantly differ between C and PPS groups.

Elevated plus maze: Prenatally stressed juveniles showed a clear decrease in time spent (nested ANOVA, df = 13, t = −4.43, p < .001; Figure 3a) and the number of entries (nested ANOVA, df = 13, t = −2.46, p = .028; Figure 3b) to the transparent arms in the elevated plus maze. Distance traveled (nested ANOVA, df = 13, t = −1.03, p = .319; Figure 3c), number of total arm entries (df = 13, t = 13 −0.04, p = .965; Figure 3d), and time scratching walls in the transparent arms (df = 13, t = −0.71, p = .486; Figure 3e) did not differ between PPS and C groups.

3.1.2 | Predator cues and anxiety test

The comparison of C against PPS groups after the exposition to predator stress revealed that juveniles from the PPS group spent less time (nested ANOVA, df = 13, t = −3.33, p = .005; Figure 4a) and entered less (df = 13, t = −2.33, p = .036; Figure 4b) in the transparent arms of the elevated plus maze compared to the control (C) group. Distance traveled (nested ANOVA, df = 13, t = − 0.96, p = .350; Figure 4c), number of total arm entries (df = 13, t = 0.35, p = .729; Figure 4d), and time scratching walls (df = 13, t = −1.41, p = .181; Figure 4e) in the transparent arms did not differ between both groups.

Compared to the values obtained previously in the same group (juveniles at 72 days old), individuals from the control group displayed a decrease in the time spent (RM ANOVA, df = 10, t = −3.25, p = .008; Figure 4a) and in the number of entries to transparent arms (RM ANOVA, df = 10, t = −2.31, p = .043; Figure 4b) after being subjected to the stress factor. No differences were observed in the distance traveled (RM ANOVA, df = 10, t = −1.64, p = .13; Figure 4c), number of total arm entries (RM ANOVA, df = 10, t = −0.46, p = .65; Figure 4d), and time scratching walls (RM ANOVA, df = 10, t = −1.80, p = .1, Figure 3e). Similarly, individuals of the PPS group stayed (RM ANOVA, df = 13, t = −1.97, p = .06; Figure 4a) and enter less (RM ANOVA, df = 13, t = −3.48, p = .043; Figure 4b) to the transparent arms after exposition to predator stress compared to their earlier behavioral response recorded at the elevated plus maze, while no differences were observed in the distance traveled (RM ANOVA, df = 13, t = −1.31, p = .21; Figure 4c), number of total arm entries (RM ANOVA, df = 13, t = −0.97, p = .34; Figure 4d), and time scratching walls (RM ANOVA, df = 13, t = −2.45 p = .12; Figure 4e).

## 3.2 | Immune response

3.2.1 | Sheep red blood cells challenge

Titers of antibodies against SRBCs did not differ significantly between PPS and C animals (nested ANOVA, df = 13, t = −0.83, p = .420; Figure 5a).
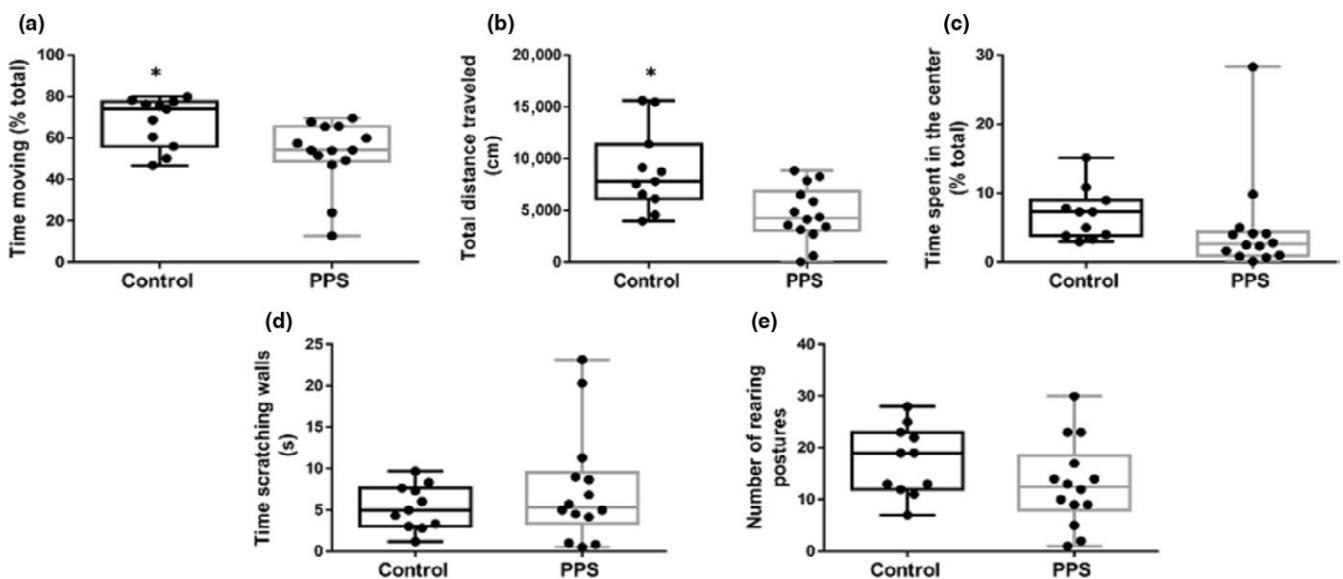
FIGURE 2 Box plot representing time moving (% total time) (a), total distance traveled (cm) (b), time spent in the center (% total time) (c), time scratching walls (s) (d), and total number of rearing postures (e) of control (n = 11) and PPS (n = 14) groups in the open field test. Asterisk indicates significant differences (p < .05)
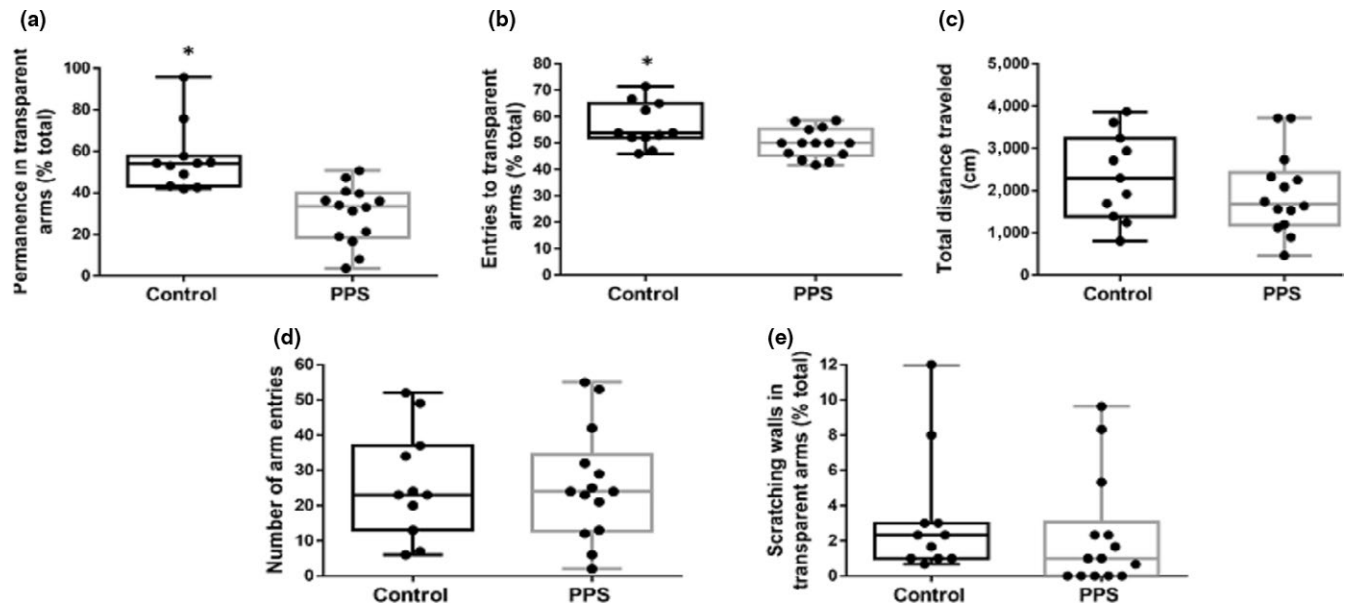
FIGURE 3 Box plot representing permanence in transparent arms (% total time) (a), number of entries to transparent arms (% total time) (b), total distance traveled (cm) (c), total number of arm entries (d), and time scratching walls in transparent arms (% total time) (e) of control (n = 11) and PPS (n = 14) groups in elevated plus maze. Asterisk indicates significant differences (p < .05)

3.2.2 | Phytohemagglutinin challenge

No significant difference between the inflammatory response 24 hr postinjection with PHA between juveniles of the C and PPS groups was observed (nested ANOVA, $df = 13$, $t = -0.68$, $p = .514$; Figure 5b).

3.2.3 | Determination of leukocyte profile

No differences in the amounts of lymphocytes and neutrophils between control and PPS groups were observed (nested ANOVA, $df = 13$, $t = -0.71$ $p = .759$, $df = 13$, $t = 0.55$, $p = .588$, respectively; Figure 5c and d).

3.3 | Physiological response to stress

N/L ratio: Before and after being exposed to immobilization, the N/L ratio was similar between individuals from the PPS and C groups (nested ANOVA, $df = 13$, $t = -1.17$, $p = .260$; $df = 13$, $t = 0.59$, $p = .559$, respectively). Comparing individuals of the control group before and after exposition to immobilization revealed an increase in the N/L ratio after the stressor (nested ANOVA, $df = 10$, $t = 2.91$, $p = .01$; Figure 6a). A similar trend was also observed in the PPS group (nested ANOVA, $df = 13$, $t = 2.57$, $p = .02$).

Glucose: Values of blood glucose were similar between individuals from the PPS and C groups both before and after the exposition to the stressor (nested ANOVA, $df = 13$, $t = 2.01$, $p = .065$; $df = 13$, $t = -0.22$, $p = .823$, respectively, Figure 6b). No differences were observed when comparing values of the control group before and after exposition (nested ANOVA, $df = 10$, $t = 0.5$, $p = .62$). Contrarily, the immobilization caused an increase in blood glucose

level in the PPS group (nested ANOVA, $df = 13$, $t = 3.75$, $p = .002$; Figure 6b).

Cortisol: Before and after being exposed to immobilization, cortisol levels were similar between individuals from the PPS and C groups (nested ANOVA, $df = 13$, $t = -0.66$, $p = .517$; $df = 13$, $t = 0.05$, $p = .958$; Figure 6c). Comparing individuals of the control group before and after exposition to immobilization revealed no differences in their cortisol levels (nested ANOVA, $df = 10$, $t = -0.98$, $p = .34$ Figure 6c). Similarly, individuals of the PPS group displayed comparable values of cortisol before and after being exposed to the stressor (nested ANOVA, $df = 13$, $t = 0.05$, $p = .958$; Figure 6c).

4 | DISCUSSION

In mammals, numerous studies have shown the diverse effects of prenatal stress on the behavioral and physiological development of the offspring (Kapoor & Matthews, 2005; Sheriff et al., 2009; Vallee et al., 1997). Stressful events during pregnancy can be associated with outcomes such as low birth weight, low survival rate (Brunton & Russell, 2010), and increased anxiety (Brunton & Russell, 2010; Meaney et al., 2007; Vallee et al., 1997). Recently, new ecological studies have proposed that the direction and magnitude of phenotypic responses are often species and context-specific (Sheriff et al., 2017). For example, while the increase of corticosterone in eggs of yellow-legged gulls (*Larus michahellis*) negatively affect the behavior and immunity of offspring (Rubolini et al., 2005), in wild house wrens caused an increase in maternal investment that resulted in offspring with greater prefledging body condition, a trait predictive of the probability of recruitment as breeding adults (Bowers, Bowden, Thompson, & Sakaluk, 2016). As
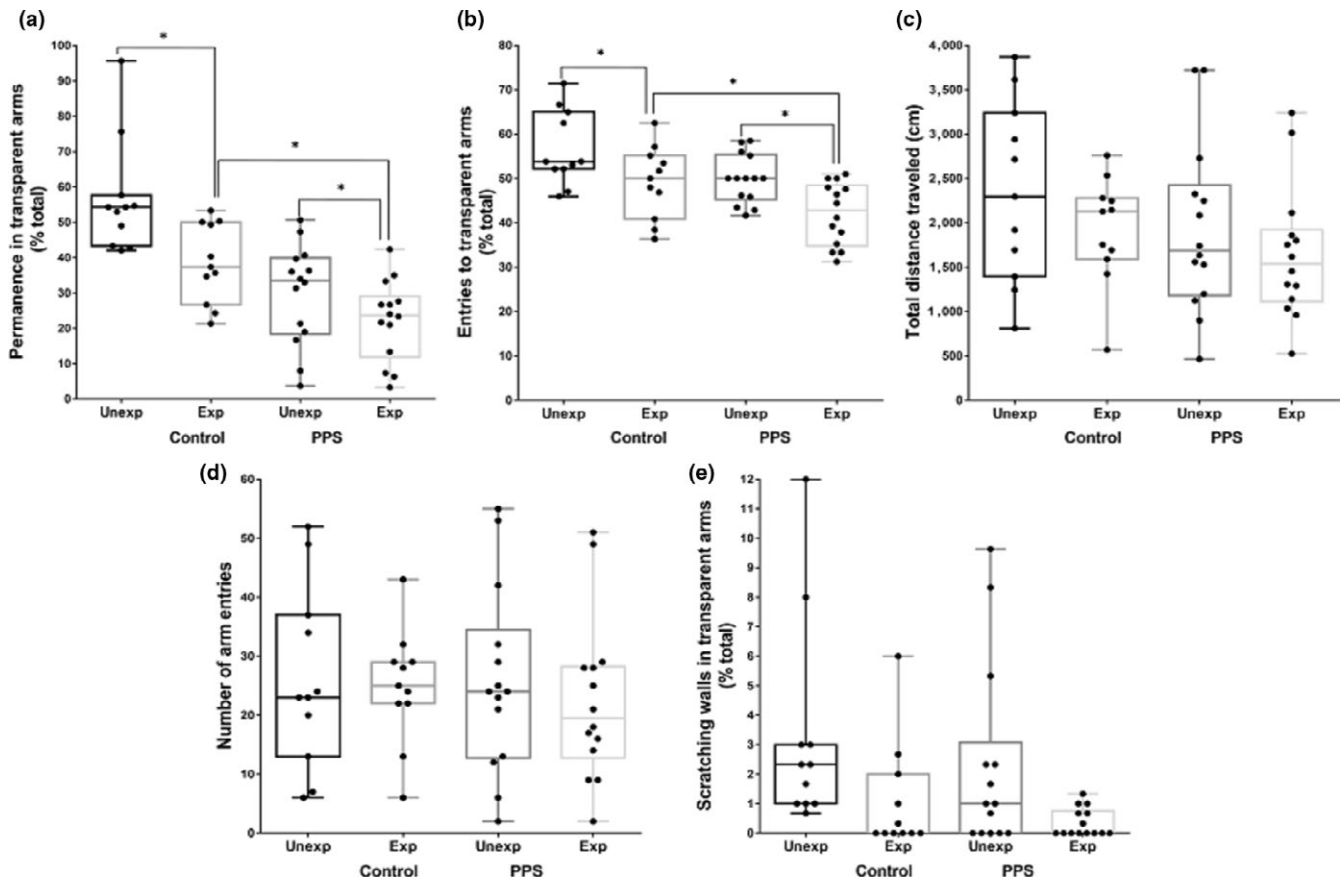
FIGURE 4 Box plot representing permanence in transparent arms (% total time) (a), number of entries to transparent arms (% total time) (b), total distance traveled (cm) (c), total number of arm entries (d), and time scratching walls in transparent arms (% total time) (e) of control (n = 11) and PPS (n = 14) groups (unexposed—72 days old—and exposed—120 days old—to predatory cues) in elevated plus maze. Asterisk indicates significant differences (p < .05)

clearly pointed by Sheriff et al. (2017), the diversity and complexity of natural stressors and how they interact with offspring phenotypes highlights the need for studies investigating maternal stress effects in free living systems. Although we could not study this species in its habitat because of its subterranean way of life, in the present work, we exposed pregnant females of *C. talarum* to predator cues (fur/skin odors and immobilization) and then tested the behavioral, physiological, and immunological responses of their offspring at the age of natal dispersal.

The results of our study showed that prenatal exposition to predatory cues clearly affect the behavioral response of juveniles in two different behavioral paradigms. Analysis of the open field test revealed that PPS tuco-tucos (70 days of age) decreased the percentage of time moving and the total distance traveled in the open field. This effect in juveniles is consistent with the one observed in adult tuco-tucos, which decreased the percentage of time moving in the open field in the presence of predatory cues (cat urine or fur/skin odors; Brachetta et al., 2015). Also, similar responses were observed in other rodent species. For example, male offspring (guinea pigs, *Cavia aperea* f.) of mothers exposed to a combination of stressors over the second half of gestation and from postnatal day until weaning showed a decrease in activity

compared to controls in an open field test (Emack, Kostaki, Walker, & Matthews, 2008). Concordantly, results of the elevated plus maze test revealed that prenatally stressed juveniles showed a clear decrease in time spent and the number of entries to transparent arms. Thus, young tuco-tucos exposed to a threatening situation during their gestational period tend to avoid the open arms, showing a clear preference for protected areas. Similar outcomes in the elevated plus maze were observed also in adult tuco-tucos (Brachetta et al., 2015) as well as in other rodent species prenatally exposed to diverse stressors. For example, Estanislau and Morato (2005) found that rats prenatally subjected to electric foot shocks showed reduced exploration of the open arms in the elevated plus-maze. In another study, pups from mothers exposed to a predator (cat) during pregnancy, displayed a lower number of total arm entries, entered significantly less often and spent less time in the open arms than the control group (Patin et al., 2005). Hence, taking together the results observed in both the elevated plus maze and open field tests, it becomes clear that the prenatal exposure of tuco-tucos to cues indicative of the presence of a natural predator triggers in the offspring a state of anxiety and the appearance of clear antipredator behaviors, like reductions in locomotor and exploratory activities.
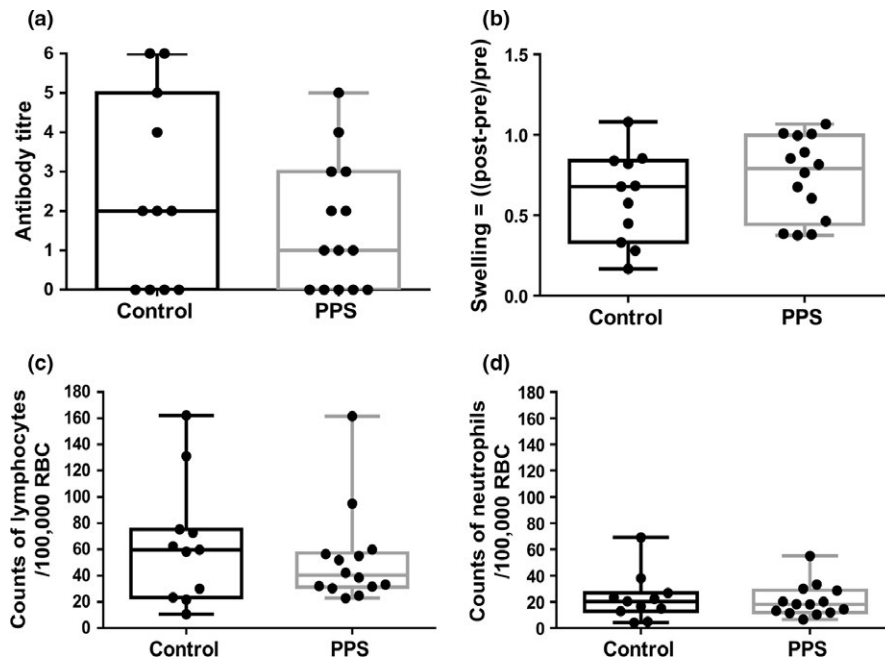
FIGURE 5 Box plot representing titers of antibodies against sheep red blood cells (SRBCs) (a), hind feet swelling after PHA injection (b), lymphocytes (c), and neutrophils (d) count in relation to 100.000 red blood cells in control (n = 11) and PPS (n = 14) juveniles of tuco-tucos
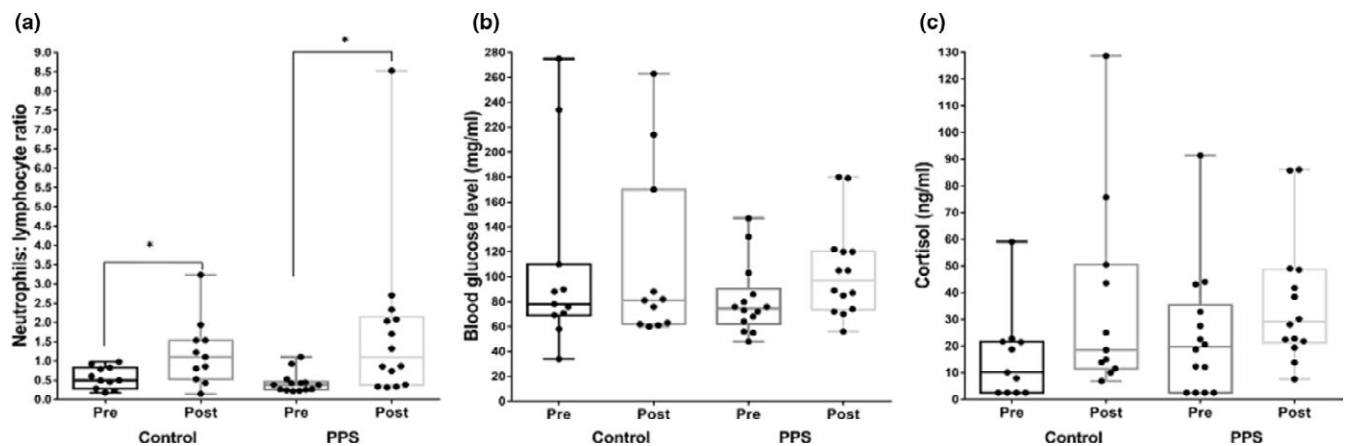
FIGURE 6 Box plot representing neutrophils/lymphocyte ratio (a), blood glucose levels (mg/ml) (b), and plasma cortisol levels (ng/ml) (c) before and after a stress period (immobilization) in control (n = 11) and prenatally stressed (PPS, n = 14) groups. Asterisk indicates significant differences (p < .05)

These offspring phenotypic responses to maternal stress that can be initially viewed as negative outcomes of maternal exposure to predatory stress, may adopt another meaning if the environment in which the offspring develop is taken into account (Sheriff & Love, 2013). For example, under high predation pressure, the increased anxiety levels and reduced locomotor activity observed in tuco-tucos' offspring can clearly enhance their survival. These maternal effects on offspring responses have also been observed in other species. For example, exposure to predation risk caused female fall field crickets (*Gryllus pennsylvanicus*) to produce offspring that exhibited more antipredator behaviors and exhibit greater survival in the presence of its natural predator (Storm & Lima, 2010). Similarly, the offspring of predator-exposed mothers of stickleback exhibited tighter shoaling behavior, an antipredator defense that may be highly

adaptive under high predation pressure (Giesing, Suski, Warner, & Bell, 2011). Therefore, to understand how maternal stress-induced phenotypic responses impact fitness, the environmental context in which they are generated and occur in must be considered (Sheriff & Love, 2013).

Many studies showed the impact of maternal stress during gestation on immune function in the offspring. A stressful experience at this period may disturb the fetal environment and, therefore, can affect the postnatal immune development. For example, experimental works that evaluate the consequences of stress during the gestational period, showed inhibitory effects in the offspring's immune processes by a decrease of blood lymphocytes. In a study of immunological responses in rats, Götz and colleagues (2007) demonstrated that males prenatally exposed to a psychosocial

stressor had a generally lower number of neutrophils, monocytes, T lymphocytes, and NK cells, and reduced lymphocyte proliferation than prenatally nonstressed individuals. Also, Toumi Merzoug Baudin and Tahraoui (2013) demonstrated that prenatally stressed pups of Wistar rats showed a decrease in total leukocytes and lymphocytes along with monocytes and granulocytes, although in this case the changes observed were sex-dependent. In the present study, we found that a stressful situation experienced by the mother during pregnancy did not modify the number of lymphocytes and neutrophils in juveniles of *C. talarum*. Also, the humoral response of juveniles was similar between PPS and C groups, as titers of SRBCs did not differ significantly among them. Interestingly, young tuco-tucos mounted a lower antibody response against SRBC compared with responses reported in adults of this species (Cutrera et al., 2010), a situation that may be related to the immaturity of pups' immune system. Regardless of this, prenatally stressed pups are clearly able to respond to an immunological challenge during the dispersal age, when young individuals are usually exposed to a diverse array of pathogens and injuries, which may cause severe health consequences to them.

Regarding the mechanisms involved in the regulation of inflammation, it is known that this physiological process is particularly susceptible to prenatal stress effects, with studies measuring proinflammatory cytokines yielding mixed results with an undefined response pattern (Veru, Laplante, Luheshi, & King, 2014). For example, Vanbesien-Mailliot and colleagues (2007) showed that prenatal stress caused by restraint increases proinflammatory status in rats, as in vitro stimulation with PHA resulted in an increased proliferation response of T lymphocytes and secretion of IFNγ. On the other hand, juveniles from prenatally stressed pregnancies had significantly lower cellular cytokine responses compared to nonstressed controls (Coe, Kramer, Kirschbaum, Netter, & Fuchs, 2002). In our case, we assessed the cell-mediated immune response using the PHA skin test. The results showed that the inflammatory response 24 hr postinjection with PHA between juveniles of the C and PPS groups was similar. Also considering SRBCs challenge, it became evident that juveniles' immunological processes may have not been affected by maternal exposure to predatory stress. It has been known that results obtained in maternal stressed animals can depend on the nature of the stressor, the duration of stress, the intensity and persistence of the stressor, the age (Tuchscherer, Kanitz, Otten, & Tuchscherer, 2002; Vanbesien-Mailliot et al., 2007), as well as sex of animals (Bakker et al., 1998). Also, the immune effects of prenatal stress may depend on the gestational timing of the stress (Coe, Lubach, & Karaszewski, 1999). Therefore, more studies, taking into account some of these above-mentioned factors, are required to fully comprehend the consequences of prenatal exposures to stressful situations on the immune system of this species of subterranean rodent.

With respect to the physiological status in juveniles of *C. talarum* and their variation during the stress response, offspring's of this species were exposed to an acute stress factor (immobilization). Before the exposition, we found that blood glucose, N/L ratio, and cortisol did not differ between C and PPS juveniles, indicating that prenatal stress did not affect physiological parameters associated with stress in the offspring. In first instance, these results could be attributed to the action of the placental enzyme 11-beta hydroxysteroid dehydrogenase 2 (11HSD2), which protects the developing fetus from high maternal levels of GCs (Drake, Tang, & Nyirenda, 2007). However, in adult tuco-tucos exposed to diverse stress factors, variations in cortisol levels are not so severe, even when clear behavioral changes were observed (Brachetta et al., 2014, 2015, 2016).

Immobilization affected the physiological status of both control and prenatally stressed juveniles. Control juveniles displayed an increase in the N/L ratio after the exposure to the acute stress factor, whereas juveniles stressed during gestation showed a similar raise in the N/L ratio and a slight increase in blood glucose level. None of the groups suffered detectable variations in their cortisol values after stress exposure. This result opposes to previous findings which indicated that prenatally stressed rodents display greater HPA axis responsiveness to acute stress during juvenile and adult stages (Brunton, 2013). Although it is known that the influence of maternal stress on the offspring HPA axis can be highly dependent on the stage of gestation when the contact with the stressors occurred (Kapoor & Matthews, 2005), preceding studies on adult tuco-tucos showed that physiological parameters did not vary after exposition to predator urine or fur odors presented at different intensities or frequencies (Brachetta et al., 2015, 2016). Therefore, it is more likely that this low HPA responsiveness is the result of a poor association between the physiological and behavioral responses to stress (predator cues exposures) in this species of subterranean rodent, than to an effect of the moment the stressor was applied.

Although various studies have provided evidence that stressors acting during pregnancy can have long-lasting effects on offspring, relatively few studies have analyzed if stress exposure during gestational period may also alter the ability of pups to respond to a postnatal exposition to ecologically relevant stressors or stressful situations such as predation risk. In this work, tuco-tucos of both PPS and C group were exposed to predator cues (fur odor and immobilization) at 120 days of age and their behavioral response evaluated in the elevated plus maze, comparing these results with the ones obtained in the same groups at 72 days old. The analysis of the behavioral parameters showed that exposure to predatory stress produced a similar and significant decrease in the time spent and in the number of entries to transparent arms of the elevated plus maze in both prenatally and nonstressed control groups, clearly indicating that offspring stress responses to stressors were not influenced by prenatal stress. At this point, it should be noted that although the overall response to predatory stress was similar in both groups, prenatally stressed individuals displayed higher levels of anxiety than control ones, characterized by an active open space avoidance (less entries and time in transparent arms). Thus, these individuals prenatally exposed to predatory cues show behavioral adaptations that contribute to avoid predators in the adult life. Therefore, maternal exposure to predation risk may result in improved offspring survival when adult offspring

encounters a natural predator. However, if the postnatal environment changes or predator risk is lower, this maternal programmed response can have negative effects in the offspring (Sheriff & Love, 2013). In the particular case of tuco-tucos, observed behavioral antipredator defenses (hiding and reduced activity) in prenatally stressed juveniles may be maladaptive under low predator densities, when sacrificing feeding rate for safety may lead to an overall fitness reduction. This mismatch between an offspring's behavioral response and its expected future environment, could ultimately guide to effects on population cycles and communities (Love et al., 2013).

In conclusion, this is the first study that investigated the consequences of prenatal stress caused by an ecological stressor (predator cues) over the behavior, physiology, and immune system of the offspring in a species of wild subterranean rodent. The present work clearly shows the important influence of prenatal stress on the development of behavioral responses in the offspring. Therefore, it is interesting that presumably negative outcomes derived of prenatal stress (e.g., increased anxiety) in wild animals may be adaptive and could increase offspring survival in an ecological context dependent manner (Sheriff & Love, 2013). On the other hand, we did not observe an effect of prenatal stress exposure on the immune and physiological response of the offspring. It is known that the consequences of maternal stress may depend on various factors, like the animal species, the nature of the stressor, the duration of stress, and the intensity and persistence of the stressor (Vanbesien-Mailliot et al., 2007). Therefore, studies exploring if the exposition to different stressors during the gestational period elicit a distinct behavioral response in juveniles of *C. talarum* and whether it is correlated with variations of the HPA axis or the immune system will improve our understanding of prenatal stress in this species of subterranean rodent.

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REFERENCES

Abe, H., Hidaka, N., Kawagoe, C., Odagiri, K., Watanabe, Y., Ikeda, T., ... Ishida, Y. (2007). Prenatal psychological stress causes higher emotionality, depression-like behavior, and elevated activity in the hypothalamo-pituitary adrenal axis. *Neuroscience Research*, *59*, 145–151.

Adamec, R. E., Blundell, J., & Burton, P. (2005). Neural circuit changes mediating lasting brain and behavioral response to predator stress. *Neuroscience and Biobehavioral Reviews*, *29*, 1225–1241.

Antinuchi, D., & Busch, C. (1992). Burrow structure in the subterranean rodent *Ctenomys talarum*. *Mammalian Biology*, *57*, 163–168.

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. *Neuroscience & Biobehavioral Reviews*, *29*, 1123–1144.

Baker, S., Chebli, M., Rees, S., LeMarec, N., Godbout, R., & Bielajew, C. (2008). Effects of gestational stress: 1. Evaluation of maternal and juvenile offspring behaviour. *Brain Research*, *1213*, 98–110.

Bakker, J. M., van den Dobbelsteen, G. P. J. M., Kroes, H., Kavelaars, A., Heijnen, C. J., Tilders, F. J. H., & van Rees, E. P. (1998). Long-term gender-specific effects of manipulation during pregnancy on immune and endocrine responsiveness in rat offspring. *Journal of Neuroimmunology*, *82*, 56–63.

Barbazanges, A., Piazza, P. V., Le Moal, M., & Maccari, S. (1996). Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *Journal of Neuroscience*, *16*, 3943–3949.

Bauer, C. M., Hayes, L. D., Ebensperger, L. A., Ramírez-Estrada, J., León, C., Davis, G. T., & Romero, L. M. (2015). Maternal stress and plural breeding with communal care affect development of the endocrine stress response in a wild rodent. *Hormones and Behavior*, *75*, 18–24.

Bian, J. H., Du, S. Y., Wu, Y., Cao, Y. F., Nie, X. H., He, H., & You, Z. B. (2015). Maternal effects and population regulation: Maternal density-induced reproduction suppression impairs offspring capacity in response to immediate environment in root voles *Microtus oeconomus*. *Journal of Animal Ecology*, *84*, 326–336.

Boersma, G. J., & Tamashiro, K. L. (2015). Individual differences in the effects of prenatal stress exposure in rodents. *Neurobiology of Stress*, *1*, 100–108.

Boonstra, R. (2013). Reality as the leading cause of stress: Rethinking the impact of chronic stress in nature. *Functional Ecology*, *27*, 11–23. https://doi.org/10.1111/1365-2435.12008

Bowers, E. K., Bowden, R. M., Thompson, C. F., & Sakaluk, S. K. (2016). Elevated corticosterone during egg production elicits increase maternal investment and promotes nestling growth in a wild songbird. *Hormones and Behavior*, *83*, 6–13.

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. *Physiology and Behavior*, *151*, 596–603.

Brachetta, V., Schleich, C. E., & Zenuto, R. R. (2016). Source odor, intensity, and exposure pattern affect antipredatory responses in the subterranean rodent *Ctenomys talarum*. *Ethology*, *122*, 923–936.

Brown, J. S., Laundré, J. W., & Gurung, M. (1999). The ecology of fear: Optimal foraging, game theory, and trophic interactions. *Journal of Mammalogy*, *80*, 385–399.

Brunton, P. J. (2013). Effects of maternal exposure to social stress during pregnancy: Consequences for mother and offspring. *Reproduction*, *146*, 175–189. https://doi.org/10.1530/REP-13-0258

Brunton, P. J., & Russell, J. A. (2010). Prenatal social stress in the rat programmes neuroendocrine and behavioural responses to stress in the adult offspring: Sex-specific effects. *Journal of Neuroendocrinology*, *22*, 258–271.

Busch, C., Antinuchi, D., Del Valle, J., Kittlein, M., Malizia, A., Vassallo, A., & Zenuto, R. (2000). Population ecology of subterranean rodents. In E. A. Lacey, J. L. Patton, & G. N. Cameron (Eds.), *Life underground: The biology of subterranean rodents* (pp. 183–226). Chicago, IL: The University of Chicago Press.

Busch, C., Malizia, A. I., Scaglia, O. A., & Reig, O. A. (1989). Spatial distribution and attributes of a population of *Ctenomys talarum* (Rodentia: Octodontidae). *Journal of Mammalogy*, *70*, 204–208.

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. University Nacional de Mar del Plata.

Clinchy, M., Sheriff, M. J., & Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. *Functional Ecology*, *27*, 56–65.

Coe, C. L., Kramer, M., Kirschbaum, C., Netter, P., & Fuchs, E. (2002). Prenatal stress diminishes the cytokine response of leukocytes to endotoxin stimulation in juvenile rhesus monkeys. *The Journal of Clinical Endocrinology & Metabolism*, *87*, 675–681.

Coe, C. L., Lubach, G. R., & Karaszewski, J. W. (1999). Prenatal stress and immune recognition of self and nonself in the primate neonate. *Biology of the Neonate*, 76, 301–310.

Cutrera, A. P., Antinuchi, C. D., & Busch, C. (2003). Thermoregulatory development in pups of the subterranean rodent *Ctenomys talarum*. *Physioology and Behavior*, 79, 321–330.

Cutrera, A. P., Luna, F., Merlo, J. L., Baldo, M. B., & Zenuto, R. R. (2014). Assessing the energetic costs and trade-offs of a PHA-induced inflammation in the subterranean rodent *Ctenomys talarum*: Immune response in growing tuco-tucos. *Comparative Biochemestry and Physiology, Part A*, 174, 23–28.

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 interspecific variation in immunological traits. *Journal of Experimental Biology*, 213, 715–724.

Dantzer, B., Newman, A. E. M., Boonstra, R., Palme, R., Boutin, S., Humphries, M. M., & Mcadam, A. G. (2013). Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. *Science*, 1215–1217.

Davis, A. K., Maney, D. L., & Maerz, J. C. (2008). The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. *Functional Ecology*, 22, 760–772.

del Valle, J. C., Lohfelt, M. I., Comparatore, V. M., Cid, M. S., & Busch, C. (2001). Feeding selectivity and food preference of *Ctenomys talarum* (tuco-tuco). *Mammalian Biology*, 66, 165–173.

Drake, A. J., Tang, J. I., & Nyirenda, M. J. (2007). Mechanisms underlying the role of glucocorticoids in the early life programming of adult disease. *Clinical Science*, 113(5), 219–232.

Emack, J., Kostaki, A., Walker, C. D., & Matthews, S. G. (2008). Chronic maternal stress affects growth, behavior and hypothalamo-pituitary- adrenal function in juvenile offspring. *Hormones and Behavior*, 54, 514–520.

Estanislau, C., & Morato, S. (2005). Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze. *Behavioral Brain Research*, 163, 70–77.

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. *Physiology and Behavior*, 54, 1109–1111.

Giesing, E. R., Suski, C. D., Warner, R. E., & Bell, A. M. (2011). Female sticklebacks transfer information via eggs: Effects of maternal experience with predators on offspring. *Proceedings of the Royal Society of London, Series B*, 278, 1753–1759.

Götz, A. A., & Stefanski, V. (2007). Psychosocial maternal stress during pregnancy affects serum corticosterone, blood immune parameters and anxiety behaviour in adult male rat offspring. *Physiology and Behavior*, 90, 108–115.

Götz, A. A., Wittlinger, S., & Stefanski, V. (2007). Maternal social stress during pregnancy alters immune function and immune cell numbers in adult male Long-Evans rat offspring during stressful life-events. *Journal of Neuroimmunology*, 185, 95–102.

Guo, A., Nappi, R. E., Criscuolo, M., Ficarra, G., Amram, A., Trentini, G. P., ... Genazzani, A. R. (1993). Effect of chronic intermittent stress on rat pregnancy and postnatal development. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 51, 41–55.

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 Ethologica*, 18, 1–9.

Hegab, I. M., Shang, G., Ye, M., Jin, Y., Wang, A., Yin, B., ... Wei, W. (2014). Defensive responses of Brandt's voles (*Lasiopodomys brandtii*) to chronic predatory stress. *Physiology and Behavior*, 126, 1–7.

Kapoor, A., & Matthews, S. G. (2005). Short periods of prenatal stress affect growth, behaviour and hypothalamo–pituitary–adrenal axis activity in male guinea pig offspring. *Journal of Physiology*, 566, 967–977.

Kavaliers, M., & Choleris, E. (2001). Antipredator responses and defensive behavior: Ecological and ethological approaches for the neurosciences. *Neuroscience and Biobehavioral Reviews*, 25, 577–586.

Kay, G., Tarcic, N., Poltyrev, T., & Weinstock, M. (1998). Prenatal stress depresses immune function in rats. *Physiology and Behavior*, 63, 397–402.

Laloux, C., Mairesse, J., Van Camp, G., Giovine, A., Branchi, I., Bouret, S., ... Maccari, S. (2012). Anxiety-like behaviour and associated neurochemical and endocrinological alterations in male pups exposed to prenatal stress. *Psychoneuroendocrinology*, 37, 1646–1658.

Lordi, B., Patin, V., Protais, P., Mellier, C., & Caston, J. (2000). Chronic stress in pregnant rats: Effects on growth rate, anxiety and memory capabilities of the offspring. *International Journal of Psychophysiology*, 37, 195–205.

Lordi, B., Protais, P., Mellier, D., & Caston, J. (1997). Acute stress in pregnant rats: Effects on growth rate, learning, and memory capabilities of the offspring. *Physiology and Behavior*, 62, 1087–1092.

Love, O. P., McGowan, P. O., & Sheriff, M. J. (2013). Maternal adversity and ecological stressors in natural populations: The role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology*, 27, 81–92.

Maccari, S., Piazza, P. V., Kabbaj, M., Barbazanges, A., Simon, H., & Le Moal, M. (1995). Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *Journal of Neuroscience*, 15, 110–116.

MacLeod, K. J., Krebs, C. J., Boonstra, R., & Sheriff, M. J. (2017). Fear and lethality in snowshoe hares: The deadly effects of non-consumptive predation risk. *Oikos*. https://doi.org/10.1111/oik.04890

Malizia, A. I., & Busch, C. (1991). Reproductive parameters and growth in the fossorial rodent *Ctenomys talarum* (Rodentia: Octodontidae). *Mammalia*, 55(2), 293–305.

Malizia, A. I., Zenuto, R. R., & Busch, C. (1995). Demographic and reproductive attributes of dispersers in two populations of the subterranean rodent *Ctenomys talarum* (tuco- tuco). *Canadian Journal of Zoology*, 73, 732–738.

Martin, L. B., Lewittes, J., Kuhlman, J. R., Klasing, K. C., & Wikelski, M. (2006). Phytohemagglutinin-induced skinswelling in birds: Histological support for a classic immunoecological technique. *Functional Ecology*, 20, 290–299.

Mashoodh, R., Sinal, C. J., & Perrot-Sinal, T. S. (2009). Predation threat exerts specific effects on rat maternal behaviour and anxiety-related behaviour of male and female offspring. *Physiology and Behavior*, 96, 693–702.

Mastrángelo, 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. *Animal Behavior*, 77, 685–692.

McGhee, K. E., Pintor, L. M., Suhr, E. L., & Bell, A. M. (2012). Maternal exposure to predation risk decreases offspring antipredator behaviour and survival in threespined stickleback. *Functional Ecology*, 26(4), 932–940.

Meaney, M., Szyf, M., & Seckl, J. R. (2007). Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends in Molecular Medicine*, 13, 269–277.

Merlo, J. L., Cutrera, A. P., & Zenuto, R. R. (2014). Inflammation in response to phytohemagglutinin injection in the Talas tuco-tuco (*Ctenomys talarum*): Implications for the estimation of immunocompetence in natural populations of wild rodents. *Canadian Journal of Zoology*, 92, 689–697.

Merlo, J., Cutrera, A. P., & Zenuto, R. (2016a). Parasite infection negatively affects PHA-triggered inflammation in the subterranean rodent *Ctenomys talarum*. *Journal of Experimental Zoology Part A-Ecological Genetics and Physiology*, 325, 132–141.

Merlo, J., Cutrera, A. P., & Zenuto, R. (2016b). Food restriction affects inflammatory response and nutritional state in Tuco-tucos (*Ctenomys talarum*). *Journal of Experimental Zoology Part A- Ecological Genetics and Physiology*, 325, 675–687.

Merlot, E., Couret, D., & Otten, W. (2008). Prenatal stress, fetal imprinting and immunity. *Brain, Behavior and Immunity*, 22, 42–51.

Monclús, R., Tiulim, J., & Blumstein, D. T. (2011). Older mothers follow conservative strategies under predator pressure: The adaptive role of maternal glucocorticoids in yellow-bellied marmots. *Hormones and Behavior*, 60, 660–665.

Müller, C., Jenni-Eiermann, S., & Jenni, L. (2011). Heterophils/lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian kestrel nestlings. *Functional Ecology*, 25, 566–576.

Orrock, J. (2010). When the ghost of predation has passed: Do rodents from islands with and without fox predators exhibit aversion to fox cues? *Ethology*, 116, 338–345. https://doi.org/10.1111/j.1439-0310.2010.01740.x

Patin, V., Lordi, B., Vincent, A., & Caston, J. (2005). Effects of prenatal stress on anxiety and social interactions in adult rats. *Developmental Brain Research*, 160(2), 265–274.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D.; R Core Team. (2014). nlme: Linear and nonlinear mixed effects models. R package version 3.1–117. Retrieved from http://CRAN.R-project.org/package=nlme.

R Core Team. (2017). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Romero, L. M. (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology*, 128, 1–24.

Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R., Martinelli, R., Galeotti, P., … Saino, N. (2005). Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. *Hormones and Behavior*, 47, 592–605.

Schleich, C. E. (2010). Ontogeny of spatial working memory in the subterranean rodent *Ctenomys talarum*. *Developmental Psychobiology*, 52, 592–597.

Schleich, C. E., & Zenuto, R. 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*. *Physiology and Behavior*, 139, 150–156.

Schöpper, H., Palme, R., Ruf, T., & Huber, S. (2012). Effects of prenatal stress on hypothalamic-pituitary-adrenal (HPA) axis function over two generations of guinea pigs (*Cavia aperea f. porcellus*). *General and Comparative Endocrinology*, 176(1), 18–27.

Sheriff, M. J., Bell, A., Boonstra, R., Dantzer, B., Lavergne, S., McGhee, K. E., … Love, O. P. (2017). Integrating ecological and evolutionary context in the study of maternal stress. *Integrative and Comparative Biology*, 57, 437–449.

Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2009). The sensitive hare: Sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology*, 78, 1249–1258.

Sheriff, M. J., & Love, O. P. (2013). Determining the adaptive potential of maternal stress. *Ecology Letters*, 16, 271–280.

Sheriff, M. J., McMahon, E. K., Krebs, C. J., & Boonstra, R. (2015). Predator-induced maternal stress and population demography in snowshoe hares: The more severe the risk, the longer the generational effect. *Journal of Zoology*, 296, 305–310.

Shine, R., & Downes, S. J. (1999). Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecology*, 119, 1–8.

Sikes & The Animal Care and Use Committee of the American Society of Mammalogists (2016). Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy*, 97(3), 663–688.

Sobrian, S. K., Vaughn, V. T., Ashe, W. K., Markovic, B., Djuric, V., & Jankovic, B. D. (1997). Gestational exposure to loud noise alters the development and postnatal responsiveness of humoral and cellular components of the immune system in offspring. *Environmental Research*, 73, 227–241.

Storm, J. J., & Lima, S. L. (2010). Mothers forewarn offspring about predators: A transgenerational maternal effect on behavior. *American Naturalist*, 175, 382–390.

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. *Neuroscience and Biobehavioral Reviews*, 29, 1157–1167.

Thomas, O. (1898). Description of two Argentine rodents. *Annu. Mag. Nat. Hist. Ser.*, 7(1), 283–286.

Toumi, M. L., Merzoug, S., Baudin, B., & Tahraoui, A. (2013). Quercetin alleviates predator stress-induced anxiety-like and brain oxidative signs in pregnant rats and immune count disturbance in their offspring. *Pharmacology, Biochemistry and Behavior*, 107, 1–10.

Tuchscherer, M., Kanitz, E., Otten, W., & Tuchscherer, A. (2002). Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. *Veterinary Immunology and Immunopathology*, 86, 195–203.

Vallee, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., & Maccari, S. (1997). Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. *Journal of Neuroscience*, 17, 2626–2636.

Vanbesien-Mailliot, C. C., Wolowczuk, I., Mairesse, J., Viltart, O., Delacre, M., Khalife, J., … Maccari, S. (2007). Prenatal stress has pro-inflammatory consequences on the immune system in adult rats. *Psychoneuroendocrinology*, 32, 114–124.

Vasilieva, N. Y., Parfenova, V., & Apfelbach, R. (2001). The effect of predator odour on reproduction success in three rodent species. In H.-J. Pelz, D. P. Cowan, & C. J. Feare (Eds.), *Advances in vertebrate pest management II*. Fürth: Filander.

Vassallo, A., Kittlein, M., & Busch, C. (1994). Owl predation on two sympatric species of tuco-tucos (Rodentia: Octodontidae). *Journal of Mammalogy*, 75, 725–732.

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*). *General and Comparative Endocrinology*, 170, 550–557.

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. *Comparative Biochemestry and Physiology*, 151, 232–238.

Vera, F., Zenuto, R. R., & Antenucci, C. D. (2012). Differential responses of cortisol and corticosterone to adrenocorticotropic hormone (ACTH) in a subterranean rodent (*Ctenomys talarum*). Journal of Experimental Zoology. *Part A: Ecological Genetics and Physiology*, 317, 173–184.

Vera, F. (2011). Caracterización funcional del eje hipotálamo-pituitaria-adrenal (HPA) en el roedor subterráneo *Ctenomys talarum*: Respuestas fisiológicas a factores ecológicos. Doctoral thesis. University Nacional de Mar del Plata.

Veru, F., Laplante, D. P., Luheshi, G., & King, S. (2014). Prenatal maternal stress exposure and immune function in the offspring. *Stress*, 17, 133–148.

Voigt, G. L. (2000). *Hematology techniques and concepts for veterinary technicians*. New York, NY: John Wiley & Sons.

Weinstock, M. (2008). The long-term behavioural consequences of prenatal stress. *Neuroscience and Biobehavioral Reviews*, *32*, 1073–1086.

Wilson, C. A., Vazdarjanova, A., & Terry, A. V. Jr (2013). Exposure to variable prenatal stress in rats: Effects on anxiety-related behaviors, innate and contextual fear, and fear extinction. *Behavioural Brain Research*, *238*, 279–288.

Zenuto, R. (2010). Dear enemy relationships in the subterranean rodent *Ctenomys talarum*: The role of memory of individual odours. *Animal Behavior*, *79*, 1247–1255.

Zenuto, R. R., Antinuchi, C. D., & Busch, C. (2002). Bioenergetics of reproduction and pup development in a subterranean rodent (*Ctenomys talarum*). *Physiological and Biochemical Zoology*, *75*(5), 469–478.

Zenuto, R. R., Estavillo, C., & Fanjul, M. S. (2007). Familiarity and mating behavior in the subterranean rodent *Ctenomys talarum* (tuco-tuco). *Canadian Journal of Zoology*, *85*, 944–955.

Zenuto, R. R., Vassallo, A. I., & Busch, C. (2001). A method for studying social and reproductive behavior of subterranean rodents in captivity. *Acta Theriologica*, *46*, 161–170.

Zimmer, C., Larriva, M., Boogert, N. J., & Spencer, K. A. (2017). Transgenerational transmission of a stress-coping phenotype programmed by early-life stress in the Japanese quail. *Science Reports*, *7*, 46125.