Effects of maternal stress induced by predator cues on spatial learning and memory of offspring in the subterranean rodent Ctenomys talarum

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Abstract

One of the main selection pressures to which animals are exposed in nature is predation, which affects a wide variety of biological traits. When the mother experiences this stressor during pregnancy and/or lactation, behavioral and physiological responses may be triggered in the offspring as well. Thus, in order to broaden and deepen knowledge on the transgenerational effects of predation stress, we evaluated how maternal stress experienced during pregnancy and/or lactation affects the spatial abilities of progeny at the onset of adulthood in the subterranean rodent *Ctenomys talarum*. The results showed that, contrary to what was observed in other rodent species, maternal exposition to predator cues during pregnancy and lactation did not negatively affect the spatial abilities of the offspring, even registering some minor positive effects. Concomitantly, no effects of predatory cues on physiological parameters associated with stress were observed in the progeny. This difference in results between the present study and previous works on maternal stress highlights the importance of considering the species to be evaluated (strain, age and origin -wild or captive-) and the type of stressor used (artificial or natural, intensity of exposure) in the evaluation of the possible transgenerational effects of maternal stress.

Introduction

During daily experience, animals are exposed to physical, social and/or psychological stressors, which occur constantly and are a fundamental part of the ecological pressures that lead to evolutionary adaptations (Boonstra 2005). The resulting loss of homeostasis is tightly associated with the nature of the stressor and the magnitude of the response that is triggered, which can affect the survival of the individual (Armario 2006, Boonstra 2013).

In this sense, predation is recognized as one of the most important selection pressures operating in nature, affecting a multiplicity of biological characteristics, from effects on individual homeostasis to changes in patterns of environmental use and foraging, social organization, and at the ecosystem level, changes in its structure and functioning. Initially, only the lethal effects of predation were considered, although more recently the physiological and behavioral modifications that occur in organisms exposed to predator cues (non-lethal effects) have gained preponderance (Lima 1998). These non-lethal effects are considered to be of great importance, as it is a recurrent experience and affects a large number of individuals (Clinchy et al. 2013). This has recently become very important under the perspective of “fear ecology”, where predatory risk is recognized as a key factor affecting cognitive capacity, foraging efficiency, parental care and general condition of the prey (Clinchy et al. 2013).

In vertebrates, prey species are able to assess the potential risk of encountering a predator and generate physiological (activation of the hypothalamic-pituitary-adrenal axis -HPA- with increases in glucocorticoid and catecholamine levels; Sapolsky et al. 2000) and behavioral (suppression of non-defensive behaviors, decreased exploratory activity, avoidance of risky areas; Sievert et al. 2019) responses. Risk recognition by prey is a key feature within the evolutionary arms race between predator and prey (Sievert et al. 2019).
Recent studies in rodents showed that the non-lethal effects of predation may not be restricted only to the prey (Weinstock 2008; Schulz et al. 2011). Environmental adversity, and thus stress, experienced by a mother during pregnancy and/or lactation can significantly influence the phenotypes of her offspring, thus affecting their development and fitness (Champagne and Meaney, 2006). The mother and her offspring are connected prenatally by the placenta and postnatally by nursing, two different pathways for the transmission of the hormones released by the mother in response to predatory stress. During pregnancy, the increased cortisol in the intrauterine environment caused by stress induces behavioral, immunological, and neuroendocrine responses in the offspring throughout their lives, including adulthood (Bauer et al. 2016, Fowden et al. 2022). Behavioral experiments demonstrated that prenatally stressed rats show increased levels of anxiety (Estanislau and Morato 2005, Abe et al. 2007), variations in motivation to explore mazes (Valleé et al. 1997, Patin et al. 2005), and various behavioral abnormalities, including decreased innate fear to predator odor, impaired fear extinction, increased locomotor activity, and emergence of stereotypic behaviors (Wilson et al. 2013).

On the other hand, there is growing interest in the postnatal stage of offspring development, because the effects of maternal stress can impact offspring phenotype through variations in parental care, such as modifications in the pattern of suckling, grooming, nest building, and/or thermoregulation in altricial species (Patin et al. 2005, Champagne and Meaney 2006, Johnson et al. 2011; Kosten et al. 2012; Babb et al. 2014, Bauer et al. 2016; van Steenwyk et al. 2018, Stead et al. 2021). These interactions between mother and offspring in the early postnatal period play a fundamental role in shaping adult behavior (Cirulli, et al. 2003). Stress during early life can lead to behavioral alterations and cognitive deficits in adulthood (Janeway 2001; Champagne et al. 2003; Pryce and Feldon, 2003; Kaffman and Meaney, 2007; Kosten et al. 2012).

Spatial navigation is recognized as one of the cognitive functions that may be altered by maternal stress. Glucocorticoids and other hormones released by the mother in response to stress during pregnancy and lactation, as well as variations in maternal care, can negatively influence brain development in areas involved in spatial learning and memory (Lemaire et al. 2000, Yang et al. 2006, Lupien et al. 2009). These deficiencies in spatial skills can have a profound impact on the adult life of the offspring. For example, avoiding a food source that has been depleted, remembering the location of a safe site to avoid being predated, or returning to the nest to feed young are activities that require a developed ability to orient and navigate, particularly crucial in habitats with high structural complexity (Winter and Stich 2005).

Contrary to aboveground, spatial navigation in subterranean habitats must be based on restricted sensory information, because of the limited use of auditory or visual cues (Reichman and Smith 1990; Kimchi and Terkel 2004; Brachetta et al. 2014). In the particular case of subterranean rodents, which construct complex and extensive burrows with multiple chambers and openings, it is expected that their spatial cognitive abilities are under strong selection pressure (Nevo 1999; Bennett and Faulkes 2000). For these species, the ability to learn and memorize tunnel systems is decisive for feeding, breeding, and territory defense in these complex subterranean systems (Antinuchi and Schleich 2003), especially
considering the high energetic costs associated with the construction and maintenance of their tunnels (Luna and Antenucci, 2006; Mastrangelo et al. 2009).

Ctenomys talarum (Talas tuco-tuco; Thomas 1898) is a subterranean herbivorous rodent distributed in sandy soils of the coast of the province of Buenos Aires, Argentina. This species inhabits gallery systems parallel to the soil surface, which generally consist of a tunnel that functions as the main axis and several lateral branches (Antinuchi and Busch, 1992). Coincident with the structural complexity of their caves, adult tuco-tucos exhibited a high capacity to learn and memorize longitudinal mazes (Antinuchi and Schleich, 2003, Mastrangelo et al. 2009). Individuals of *C. talarum* often emerge for short periods of time to collect vegetation that they then consume in their burrows (Busch et al. 2000; del Valle et al. 2001; Schleich and Zenuto, 2007). During these aboveground excursions, and also when dispersing (from 60 days of age), individuals are exposed to terrestrial and aerial predators (Vassallo et al. 1994, Busch et al. 2000, Canepuccia 2005). In addition, predatory events by dogs and domestic cats have also been detected in peri-urban areas (C. E. Schleich, pers. comm.).

Previous studies on adult tuco-tucos showed that direct and indirect exposure to predatory cues produces a stress response that negatively affects their spatial cognitive abilities (Mastrangelo et al. 2009; Brachetta et al. 2014). Likewise, exposure to predator odor generates a state of anxiety and induces behavioral changes associated with reduced activity and the appearance of defensive behaviors (Brachetta et al. 2015). In a recent study, the relationship between behavioral and physiological responses to stress was observed, finding that predator cues act as stressors that trigger a differential increase in plasma cortisol and anxious behaviors (Brachetta et al. 2018). Recently, these non-lethal predatory effects were found to extend to the next generation, with variations in the behavior of juveniles from mothers exposed to predator cues during pregnancy (Brachetta et al. 2018). Therefore, and considering the vital importance of the spatial abilities for the particular way of life of subterranean rodent species, and to the variety of effects that the exposure to predatory cues generate in this species, we will study how predatory stress during pregnancy and/or lactation may affect the development of spatial cognitive abilities of *C. talarum* progeny.

We expect that maternal stress, generated by the presence of natural predator cues, will affect the spatial abilities of the progeny. Particularly, a compromise in spatial performance is predicted; this alteration will be reflected as an increase in the time required to learn a longitudinal maze (and the number of errors committed), as well as a decrease in the ability to evoke the maze after the learning stage has finished. These effects on the cognitive abilities of the progeny, will be more noticeable when mothers are exposed to the same stress factor on two instances (pregnancy and lactation), than when it occurs on a single instance (pregnancy or lactation). On the other hand, both blood cortisol levels and the neutrophil/lymphocyte (N/L) ratio are expected to be higher in the pups of those females that were exposed to the stress factor during pregnancy and lactation than the pups of those females that were exposed to the stressor only once.

**Materials And Methods**
Animals

Pregnant females of *Ctenomys talarum* (n = 20) were captured using live traps in dunes close to Mar Azul locality (37°15'19" S, 56°58'04" W, Buenos Aires Province, Argentina) during the breeding season of this species (August-October). Females were housed in individual plastic boxes provided with half terracotta pot as a refuge and wood shavings as nesting material. The room was kept with controlled temperature and photoperiod (25±2°C, L:W 12:12 photoperiod). Pregnant individuals were fed daily *ad libitum* with sweet potato, corn, lettuce, sunflower seeds and fresh grass, and after 7 days of acclimatization to captivity, they were randomly assigned to the different experimental groups. At the end of the experiments, all animals (mothers and offspring) were returned to the capture site in good health conditions.

Capture, handling and captive maintenance of all animals were performed according to the guidelines approved by the American Society of Mammalogy (Sikes et al. 2016) and current laws in Argentina. All procedures were reviewed and approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the FCEyN of the UNMdP (RD – 2022–86).

Maternal Stress Procedure

Females were assigned to the following treatments: exposure to stress during pregnancy (P+), during lactation (L+), in both periods (P+/L+) or control group (P-/L-). In this way, the following groups were obtained:

- **P+/L+:** stress during fetal development and lactation period (n females = 5, n offspring = 12).
- **P+/L-:** stress during fetal development (n females = 6, n offspring = 14).
- **P-/L+:** stress during lactation period (maternal care) (n females = 5, n offspring = 14).
- **P-/L-:** control without exposure to stress (n females = 5, n = 12).

Exposure of females to stress was based on cues indicative of predator presence (urine odor + cat fur odor + immobilization). In groups exposed during pregnancy, the procedure was performed at the end of acclimatization to laboratory conditions and repeated after 15 days. Since the females were captured with signs of advanced pregnancy, it was estimated that they were close to completing the second month of pregnancy (of a total of approximately 95 days of gestation), so the stress factor was applied twice to each female prior to the birth of the offspring. In the groups exposed during lactation, the dams were exposed to the stress factor on days 2 and 8 post-partum.

The pups were housed in the same box with their mother until 60 days of age (weaning period), at which time they were separated and housed in individual boxes until they reached adulthood (6 months) and began the evaluation of their spatial abilities, particularly learning and spatial memory.

Maternal Exposure to predator stress
Exposure of dams to predator cues was simulated by simultaneous exposure to shavings impregnated with cat urine odor (5 min) and immobilization of individuals using a cloth impregnated with domestic cat fur odor, restraining and releasing them intermittently for a period of 2 min (see Mastrangelo et al. 2009). In this way, a sequence of predator attack and prey escape was simulated. To obtain the fur odor, a cat was allowed to rest on a piece of cloth for a period of 7 days. Then, it was stored in a zip lock bag at -20°C until used in the treatments. Shavings impregnated with cat urine were obtained 24 h before treatments and also stored in zip bags.

Progeny Spatial abilities

To evaluate spatial capabilities, the performance of each individual was tested in a longitudinal labyrinth designed to simulate the structure of a *C. talarum* tunnel system. It comprised a series of dead-end paths and one correct path leading to the goal point at the opposite end of the start point (Fig. 1). A food reward (piece of sweet potato and grass) was placed at the goal point. The length of the labyrinth (9-10m) was within the range of length of natural burrows for this species (14 ± 8m). Animals were maintained at 80% of the initial body mass during the experiments to increase their motivation to explore and learn the maze. A total of 12 trials were performed in 6 days (2 trials per day), one in the morning and one in the afternoon. Before starting each trial, the animal was transported in a transfer tube to the start point of the labyrinths, where it remained in the dark for a habituation period of 1 min. Then, it was allowed to enter the longitudinal labyrinth and the trial ended when the animal reached the food reward or when 10 min had elapsed, if the reward was not obtained. Using a zenithal camera, the complete trials were filmed and the time spent to complete the task (latency) and the number of errors (entries to the dead-end paths) made by individuals (spatial performance parameters) were obtained from recordings.

Spatial memory of the individuals was evaluated 30 days after the end of the maze learning period, repeating the same protocol of the learning trial but only once. The variables analyzed were again the errors made and the latency to achieve the reward in the maze.

Blood collection

At the end of the memory test, blood samples were taken to obtain different indicators of the stress and health condition of the individuals. For this purpose, individuals were anesthetized by placing absorbent cotton soaked in 100% SCOTT-CASSARÁ inhalation anesthetic isofluorane in a container and exposing the individuals to it for approximately 20 seconds. A syringe with a butterfly connected to a heparinized microcapillary tube was used to obtain the blood sample from the suborbital sinus. Manipulation of the animals did not last more than 3 minutes so as not to affect cortisol levels as a consequence of the procedure (Vera et al. 2011).

Analysis of physiological parameters

1. Cortisol: plasma cortisol levels were quantified using a DRG EIA 1887 kit by Elisa assay (Validation in Vera et al., 2019).
2. Neutrophil/lymphocyte ratio (N/L): N/L is a stress parameter that is proposed to be particularly associated with chronic stress (Davis and Maney 2008). In *C. talarum*, increases in N/L ratio were verified during specifically stressful situations (such as brief immobilization) and chronic stress (Vera et al. 2008, 2019). Blood smears were fixed in 70% methanol for 10 min and stained with May Grunwald-Giemsa. Then under a microscope (Olympus CX 31, Tokyo) all cell types (neutrophils, eosinophils, basophils, and monocytes) were counted under 45x magnification in a total of 200 leukocytes from which the N/L ratio was calculated.

3. Glucose: Blood glucose levels are used to assess the short-term effects of exposure to a stressor and/or health condition (e.g., captivity and dietary restriction, Vera et al. 2008, Schleich et al. 2015). Glucose was determined using a glucometer immediately after blood collection (Accu-Chek Active Roche Diagnostics, Range: 10–600 mg/dl).

**Statistical analysis**

All statistical comparisons involved a nested design in which the parameters to be measured (latency and number of errors) were analyzed for each offspring during and at the end of the learning period (trial 12) and 30 days after the end of the learning period (memory trial). For this, a mixed model was fitted with "Mother" and "Animal" as random factor, where the animals are nested in the mothers (1 single level of the factor Mother per Animal). The same analysis was used for the measurement of physiological parameters: plasma cortisol, N/L ratio and blood glucose. For the data obtained in the learning period, comparisons also involved the same individuals on different occasions, which generates a repeated measures structure to the analysis. Differences in responses to experimental treatments were evaluated by nested and repeated measures ANOVAs using the "nlme" package (Pinheiro, Bates, DebRoy, Sarkar, and R Core Team, 2014 in R (R Core Team)). The normality of the residuals was evaluated with Shapiro-Wilk tests. In those cases where the assumption was not met, the data were previously transformed (log10.time, power.errors). 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 treatments).

**Results**

**Spatial learning**

There was a significant effect of trial on both the time taken to complete the maze (latency) and the number of errors, with both decreasing as trials elapsed (Latency: p = 0.0021 gl = 533 t= -3.091; Errors: p = 0.0002 gl = 533 t=-3.783 Fig. 2A and B).

No effect of treatments on latency to finish the maze was observed (P+: p = 0.833 gl = 16, t = 0.213; L+: p = 0.225 gl = 16 t=-1.261; P +L+: p = 0.662 gl = 16 t = 0.444), while a decrease in the number of errors made by the offspring of stressed dams during lactation was recorded (L+: p = 0.02 gl = 16 t=-2.562; P+: p = 0.71 gl = 16 t=-0.378; P +L+: p = 0.361 gl = 16 t = 0.94; Fig. 2B).
No significant interactions were found between Assay, Pregnancy and Lactation for either variable (Latency: p = 0.458 gl = 533 t=-0.741; Errors: p = 0.227 gl = 531 t=-1.207).

Spatial memory

No effect of predator stress during pregnancy or lactation on the number of errors and time spent by offspring individuals during the spatial memory test was observed (Latency- P+: p = 0.203 gl = 15 t = 1.329, L+: p = 0.709 gl = 15 t = 0.379; Errors- P+: p = 0.853 gl = 15 t = 0.187; L+: p = 0.584 gl = 15 t=-0.558, Fig. 3A-B). A significant interaction was observed between stress during Pregnancy and Lactation on the latency to complete the maze (P + L+: p = 0.0251 gl = 15 t=-2.488; Fig. 3A), while no interaction was found between both stress factors with respect to errors (P + L+: p = 0.181 gl = 15 t=-1.4; Fig. 3B).

Figure 3A: Boxplot plot representing median and quartiles of the time required (log s) by C. talarum individuals to finish the longitudinal maze during the spatial memory trial. Different capitalized letters indicate significant differences in values.

Physiological parameters

Plasma cortisol levels

No significant effect or interaction of any factor was found on plasma cortisol levels (P+: p = 0.603 gl = 15 t = 0.53, L+: p = 0.643 gl = 15 t = 0.472, P + L+: p = 0.681, gl = 15 t=-0.419; Fig. 4A).

Blood glucose levels

No significant effects of either stress factor were observed (P+: p = 0.161 gl = 15 t= -1.474; L+: p = 0.168 gl = 15 t=- 1.445), although a significant interaction of both factors on blood glucose level was detected (Pregnancy and lactation stress: p = 0.033 gl = 15 t = 2.341) (Fig. 4B).

Neutrophils/lymphocytes ratio

No significant effect or interaction of any factor was found (P+: p = 0.402 gl = 15 t=-0.861, L+: p = 0.993 gl = 15 t = 0.008, P + L+: p = 0.967 gl = 15 t=-0.041 Fig. 4C).

Discussion

Exposure to predators or their cues have sustained effects that extend beyond the survival of individuals (Clinchy et al 2013). Predation risk also affects prey by altering their behavior, modifying daily activity patterns, space use, feeding habits for example (Lima and Dill 1990; Lima 1998; Monclús et al 2005).

Exposure to predatory stress during pregnancy and lactation triggers behavioral and neurological changes in the progeny (Bremner and Vermetten, 2001). When internal homeostasis is altered, the pituitary-adrenal and sympathetic-adrenal axes are activated, generating the appearance of anxiogenic-type behaviors (Davis, 1998). Previous studies in rodents found that, when exposed to a stress factor,
pregnant female rats secrete higher levels of glucocorticoids (corticosterone) that diffuse into the placenta and alter the development of the HPA axis in the offspring, resulting in its hyperactivity in adult life, caused by a deterioration of negative feedback (Barbazanges et al. 1996, Fowden et al. 2022). This diffusion of corticosterone to the offspring may also occur through lactation in cases where exposure to stress occurred during this period (Catalani et al. 2000, Stead et al. 2021). On the other hand, pregnant females under stress show impaired maternal behavior, i.e., stress can directly affect maternal care through the neuroendocrine systems that normally regulate this behavior (Bauer et al. 2016; van Steenwyk et al. 2018). For example, females exposed to stress during pregnancy were observed to exhibit lower levels of oxytocin receptors and, in consequence, lower levels of grooming behavior towards their offspring (Champagne and Meaney, 2006). Thus, the effects of environmental adversity may also be transmitted during the lactation period across generations through a non-genomic mechanism such as maternal care (Champagne and Meaney, 2006).

Stress during the early stages of development can generate a wide variety of effects on offspring. Predatory risk during embryo/fetus formation can affect maternal investment in both offspring number and offspring size and development (Cattelan et al. 2020). At the behavioral level, and as a result of prenatal stress exposure, increases in behaviors denoting anxiety have been observed in the progeny (Akatsu et al, 2015; Weinstock, 2015; Zohar et al, 2015). Similar results are also found regarding depression-related behaviors (assessed in rodents in anhedonia and/or forced swim tests) that are also increased in those animals that were exposed to prenatal stress (Abe et al, 2007; Van Lieshout and Boylan, 2010, Enayati et al 2020). Several of these behavioral effects are linked to the negative effects of maternal stress on the long-term development and functioning of the HPA axis in the offspring (Weinstock 2015).

In terms of spatial abilities, maternal stress can induce alterations in neural networks that can later be expressed in cognitive deficits in spatial learning and memory. For example, studies in Sprague-Dawley rats showed that prenatal stress negatively affected offspring's abilities in spatial learning and memory test, although at older ages this was only observed in females (Wu et al. 2007). Contrariwise, after prenatal stress exposure, there was an impairment of spatial learning in adult male rats, whereas no effect was found in adult females (Weinstock, 2011; Zuena et al. 2008). Pregnant Wistar rats stressed daily during the last week of pregnancy negatively affected spatial learning abilities in males (Szuran et al. 2000), while when stressed with cold water immersion, it altered learning and memory processes in the offspring, regardless of age and sex (Guerrero Aguilera et al. 2017). On the other hand, in adult Wistar males, prenatal sound stress slowed the rate of spatial learning acquisition and decreased memory retention (Barzegar et al. 2015; Modir et al. 2014). Finally, and contrary to most studies, adult SD pups improved their ability to learn in the Morris water maze when exposed to high prenatal stress consisting of restraint three times a day (Zuena et al. 2008), whereas in guinea pigs the effect of prenatal light exposure stress on spatial abilities depended on the timing of stressor application, with both negative or no effects found (Kapoor et al. 2009).
Diverse results were also obtained when pups received relatively poor maternal care (poor licking/cleaning), causing impaired spatial learning (Liu et al. 2000b) but also improved memory for stressful events –known as contextual conditioning- (Champagne et al. 2008; Bagot et al. 2009). Similarly, pups deprived of maternal care showed impaired spatial learning in the water maze (Oomen et al. 2010), but improved fear-conditioned memory (Oomen et al. 2011).

Previous studies in C. talarum showed that prenatal exposure to predator stress modified juvenile responses in two behavioral tests. In the open field, juveniles of stressed mothers decreased the time moving and the distance traveled (Brachetta et al. 2018), whereas in the elevated maze, individuals spent less time in the transparent arms (Brachetta et al. 2018). Both results denote a general anxiety state of individuals whose mothers were stressed during pregnancy, characterized by reduced exploratory activity and avoidance of open spaces.

In the present work, individuals of *Ctenomys talarum* from all experimental groups improved their performance in the longitudinal maze as learning trials progressed. However, and contrary to expectations, no negative effects of maternal stress were observed on the spatial learning ability of offspring upon reaching adulthood. Moreover, offspring of mothers stressed during lactation (L+) showed a slight improvement in their learning ability, exhibiting a lower number of errors. A similar picture was found when evaluating spatial memory, since no deficiencies were observed in individuals from the stressed groups, and even tuco-tucos from mothers stressed during lactation and pregnancy (P + L+) were faster in completing the maze, although they made a similar number of errors as individuals from the other groups.

Regarding physiological parameters, no effect of the treatments was found neither in cortisol levels nor in the neutrophil/lymphocyte ratio, while only a slightly significant effect on blood glucose was observed in stressed individuals during pregnancy and lactation. Both cortisol (or corticosterone) and neutrophil/lymphocyte ratio are two parameters frequently used to assess the impact of environmental stressors or the effect of stressful activities (Davis and Maney 2018). Previous studies showed that prenatal stress affects the functioning and development of the HPA axis in the offspring, producing variations in basal glucocorticoid values as well as in their response to stressors (Weinstock 2015). Previous studies in *C. talarum* showed that prenatal exposure to predator cues did not affect cortisol values in offspring, although behavioral variations were observed in the progeny of stressed mothers (Brachetta et al. 2018). However, the physiological results of the present study seem to be concomitant with the assessment of cognitive parameters, suggesting both for the absence of an effect of maternal stress (during pregnancy and/or lactation) on the physiological condition of individuals.

What could be the determinants of this absence of negative effects of maternal stress on the cognitive abilities of *C. talarum* progeny? A first aspect to evaluate is the particular characteristics of the stressors used. Most of previous studies, where detrimental effects of prenatal and/or lactational stress were found, used induction protocols that included artificial stressors. Immobilization, in conjunction with or without bright light, is one of the most commonly used stressors in pregnant females (Weinstock 2017).
These immobilizations, far from being brief, are performed for periods ranging from 30 min to 6 hs, and can be repeated several times a day or for several days (Ward, 1972; Vallee et al. 1997; Williams et al. 1999; Lesage et al. 2004; Van den Hove et al. 2005). Also, immobilization is used in conjunction with others such as electric shock or exposure to intense noise and/or light (Yaka et al. 2007; Makham et al. 2010; Schulz et al. 2011; Paris and Frye 2011; Bourke et al. 2013; Zohar et al. 2016). Even within immobilization stress there are different protocols, with restraint tubes as the most common one but also extreme cases like prone restraint procedure (fixation of the trunk and all four legs extended flush with a surface with adhesive tape; Crawley et al. 2001). In summary, the stressors to which mothers are normally subjected are artificial and present little or no relation to those they would experience in a natural environment (Koolhaas et al. 1997). In contrast, in the present work, the stress factor used was a transient immobilization with a cat-scent impregnated cloth, thus trying to simulate a natural predatory event. Likewise, the frequency of repetition of the stress factor (two events during pregnancy and/or two during lactation spaced several days apart) could represent a possible natural situation faced by mothers in their environment. These differences between the type of stressor (artificial or natural) as well as the intensity of exposure (acute or chronic) may explain the diversity of effects on the behavior and physiology of the progeny of stressed mothers.

Another condition that can influence the transgenerational effects of stress is the species used in the experiments. Both the direction and magnitude of responses are often particular to each species and its context, leading to a wide and unpredictable variability of results (Sheriff et al. 2017). However, most maternal stress studies have been done using laboratory model species, which are bred according to the researcher's needs and produced through intense selection, resulting in highly inbred individuals (Boonstra 2013). Moreover, they are bred in artificial environments with unlimited food, no predators, no diseases and favorable environmental conditions (Boonstra, 2012). In this study, maternal stress (presence of a natural predator cues) was evaluated in a wild rodent species of subterranean habits and altricial development, whose ecology differs markedly from the ones present in laboratory species, so the effects of stress in these free-living species may be quite different from those obtained in model animals. Incorporating studies in wild animals is necessary to understand and elucidate how animals cope with different challenges in the wild and how the stress axis acts and responds to those conditions (Boonstra 2013).

Finally, living in a changing or challenging environment often favors increased cognitive abilities, allowing individuals to learn and respond to the challenges of their environment more appropriately (Vardi et al 2020). At the neuronal level, the hippocampus plays a preponderant role in the processing of spatial information, its functioning and development being affected by experience (Cnotka et al 2008). Several studies in vertebrates showed that complex (or enriched) environments can stimulate neural development and improve cognitive abilities (Vardi et al 2020). In this study, the litters of stressed females were reared in simple boxes, lacking of the structural complexity of the natural burrows. This situation could affect the spatial abilities of the progeny, and therefore, hide the effects of the maternal stress on these cognitive features. This possibility remains to be investigated.
Conclusion

In conclusion, the results obtained show that the effects of maternal stress can present varied and not strictly negative consequences, although these were the most reported cases. In the present study, the spatial abilities of the offspring, as well as the physiological parameters associated with stress, were not negatively affected by the stress suffered by the mother during pregnancy and lactation.

Although in the last decade studies on the effects of maternal stress have been increasing and took more relevance, it should be noted that much remains to be investigated. It is evident the need of studying this process from a holistic perspective, covering from physiology to ecology and evolution, and taking into account spatial and temporal scales (Sheriff et al. 2017). The use of laboratory models and protocols normally used for stress induction has proven to be useful in understanding how the response to stress exposure is modulated, which has been of great relevance in biomedical studies. However, if we want to understand the effects of stress in nature it is necessary to expand studies to wild species, both in the field and in the laboratory, seeking to evaluate the results under natural conditions (or as close as possibly to the existing challenges in nature) to correctly assess the adaptive potential of the phenotypes that arise as a result to stress (Sheriff et al. 2017).

Declarations

Competing interests: The authors declare no competing interests.

Ethical Approval

All procedures were revised and approved by the local committee for animal use and care in research (Comité Institucional para el Cuidado y Uso deAnimales de Laboratorio, CICUAL, FCEyN-UNMdP, RD 2022-86).

Competing interests

The authors declare that they have no conflict of interest.

Authors' contributions

Iribarne J. and Schleich C. Conceived and designed the analysis; Collected the data; Contributed data or analysis tools, Wrote the paper. Brachetta V. and Zenuto R. Conceived and designed the analysis; Collected the data; Contributed data or analysis tools. Kittlein M. Performed the analysis. All authors reviewed the manuscript.

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Availability of data and materials

Research data are not shared. The data that support the findings of this study are available from the corresponding author upon reasonable request.

References


Figures
Figure 1

Image of the longitudinal maze used to assess learning and spatial memory in Ctenomys talarum individuals.
Figure 2

A: Boxplot plot representing median and quartiles of the time required (log s) by individuals of C. talarum to finish the longitudinal maze over twelve trials. The dashed lines represent the fit to the linear mixed-effects model used.

B: Boxplot plot representing median and quartiles of the number of errors made by individuals of C. talarum to finish the longitudinal maze over twelve trials. The dotted lines represent the fit to the linear mixed-effects model used.

Figure 3

A: Boxplot plot representing median and quartiles of the time required (log s) by C. talarum individuals to finish the longitudinal maze during the spatial memory trial. Different capitalized letters indicate significant differences in values.

B: Boxplot plot representing median and quartiles of the number of errors made by individuals of C. talarum until the end of the longitudinal maze during the spatial memory test.
Figure 4

Boxplot plot representing median and quartiles of physiological parameters obtained at the end of the spatial memory assay in C. talarum individuals whose mothers were exposed to the different treatments during pregnancy and lactation: A. Blood cortisol B. Glucose C. N/L ratio. Different capitalized letters indicate significant differences in values.