



Thermoregulatory development and behavior of *Ctenomys talarum* pups during brief repeated postnatal isolation



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ABSTRACT

In altricial mammals, the role of the mother and siblings throughout pup's early ontogeny is critical to determine "normal" development in neonates. It has been reported that variations in parental investment during pups' development affect thermoregulatory capacity, growth patterns, brain development and behavior during lifetime, such as spatial learning and memory in adults. *Ctenomys talarum* (tuco-tuco) is a solitary subterranean rodent, who inhabits complex burrows and exhibits developed spatial orientation abilities. Tuco-tuco's pups display an altricial development, spending more than 80% of the time in contact with the mother. Throughout weaning period, pups display active exploratory behavior and improvements in their spatial capabilities. Then, we determined the effect of repeated brief postnatal isolations on the acquisition of physiological thermoregulation and the development of spatial learning capabilities in tuco-tuco's pups. As it occurs in wild animals, daily brief isolations (30 min) did not affect the acquisition of adult's body temperature nor resting metabolic rate's development pattern. Moreover, behavioral response and adult spatial abilities of isolated pups were similar to that observed in non-isolated ones. Then, during periods of mother's absence, minor physiological and behavioral adjustments, such as shivering and postural changes, are required to keep *C. talarum* pups within allostasis.

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1. Introduction

In altricial mammals, the normal development of neonates depends on the interactions between the mother and their pups. After birth, pups are completely dependent on its mother for grooming, food and thermoregulation. Therefore, natural or artificial modifications of mother–infants relationships have been demonstrated to produce changes in pups' physiology, neurobiology and behavior in many species (Lehmann and Feldon, 2000; Lévy et al., 2003; Zimmerberg et al., 2003).

A key physiological parameter that may be affected either by maternal separation, time invested by the mother on pups' care and/or litter size, is the development of thermoregulatory capacity (Kuhn and Schanberg, 1998; Antinuchi and Luna, 2002). Obligatory minimum heat production in small altricial mammals develops gradually over the first weeks of life (Piccione et al., 2002; Zenuto et al., 2002). Throughout this period, pups depend on physical contact with their mother and siblings to maintain body temperature, because heat loss exceeds individual heat production (Blumberg and Sokoloff, 1998). Therefore, the role of the mother and siblings through the early stages of pup's ontogeny is critical to determine thermoregulatory capacity in

adults. It has been reported that variations in the extent of parental investment during pups' development affect not only maintenance of body temperature (T_b), but also growth patterns (Newkirk et al., 1998). For example, in the grass mouse (*Akodon azarae*), pups from small litters, with supposedly higher allocation of energy from parents per pup, increased T_b and body weight at higher rates than those from large litters (Antinuchi and Luna, 2002). Besides resource provision, thermal environment under which pups are reared has also strong effects on homeothermy acquisition. In muskrat pups (*Ondatra zibethicus*, MacArthur and Humphries, 1999) and in mink pups (*Mustela vison*, Tauson et al., 2006), low environmental temperatures as a result of prolonged maternal absence, affect thermoregulatory development even if pups are allowed to reduce heat loss by huddling.

Nevertheless, in rats it has been observed that subtle variations in housing, as the number of individuals sharing the same nest during early development, can affect animal's physiology and then, its responses to stressors. Moreover, it has been demonstrated that siblings' physical contact and interaction can mask or even eliminate certain physiological and behavioral plasticity obtained in response to repeated maternal separation in early life (Fournier et al., 2011, 2012).

The postnatal period is also a decisive phase for rodent's brain development (Sampath et al., 2010) and is characterized by a profound hippocampal neurogenesis (at 5–7 post natal days), synaptogenesis (at days 4–11), and dendritic and hypothalamic-pituitary axis (HPA) development (Gutman and Nemeroff, 2002). Consequently, early life

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stressors like maternal separation have lasting changes on brain functions and therefore on behavior during lifetime, particularly affecting social and maternal behavior, and spatial learning and memory in adults (Lévy et al., 2003; Zimmerberg et al., 2003; Sampath et al., 2010), although the extent of the effects of separation depends on the frequency, duration and age at isolation (see Lehmann and Feldon, 2000).

Regarding maternal behavior after separation, it has been shown that, when a mother was reunited with its breeding, there was an immediate approach response, followed by sustained elevated levels of maternal care, expressed in increased licking, grooming and nursing. This improvement in care-taking behaviors towards isolated pups has strong effects on their adult life, since these individuals exhibit less anxiety behaviors and better performance in spatial tasks (Liu et al., 1997; Caldji et al., 1998; Liu et al., 2000).

Ctenomys talarum (tuco-tucos) is a solitary subterranean rodent, member of the family Ctenomyidae, which is distributed in Buenos Aires Province, Argentina (Woods, 1984). They inhabit systems of closed galleries parallel to the surface, which consist of a main axial tunnel and a variable number of lateral branches and feeding tunnels (Antinuchi and Busch, 1992). Concomitant with the complexity in their burrows' design, tuco-tucos display developed spatial orientation abilities (Antenucci and Schleich, 2003; Mastrangelo et al., 2010). Morphological and physiological characteristics of this species are similar to those observed in other subterranean rodents, such as compact bodies, short tails, and strong limbs, while the most conspicuous physiological trait is their low basal metabolic rate (BMR), compared to the surface rodents of similar body mass (Luna et al., 2009).

C. talarum pups are characterized for displaying an altricial development and three major ontogenetic stages can be differentiated according to physiological and behavioral variables. Initially, and until 15–20 days after birth, pups are fed almost exclusively with milk (Schleich and Busch, 2002; Zenuto et al., 2002) and are not capable of maintaining body temperature by their own, depending therefore on their mother to obtain food and thermoregulate (Zenuto et al., 2002; Antinuchi et al., 2007). During this period pups spend more than the 80% of the time in contact with their mother, who leaves nest only for short periods of time (Schleich and Busch, 2002; Zenuto et al., 2002). At about 35–45 days after birth, pups reach adult temperature and become behaviorally and physiologically independent from their mother (Zenuto et al., 2002; Cutrera et al., 2003). At this age, weaning period starts and pups show active exploratory behavior, displaying improvements in their spatial working memory performance. At dispersal age (~70 days old), individuals of *C. talarum* display further enhancements in their spatial abilities (Schleich, 2010).

Although several works studied physiological and behavioral ontogenetic characteristics of *C. talarum* pups with permanent access to the mother and siblings (Zenuto et al., 2002; Cutrera et al., 2003; Schleich and Busch, 2004; Schleich, 2010), no studies were conducted to assess the physiological and behavioral mechanisms that pups employ to face the effect of short maternal absence. In particular, the aim of this study was to evaluate if pups of *C. talarum* compensate for maternal and sibling absence bouts, and if it happens, which are the mechanism involved on the acquisition of physiological thermoregulation and development of spatial learning capabilities. Given that short maternal separation is found in this species and that males do not exert parental care (Zenuto et al., 2001), we hypothesized that 1) pups show behavioral mechanisms to compensate for short mother and siblings' separation to maintain a stable T_b , 2) after weaning, metabolic rate of separated pups is similar than that of not-separated ones, because the former do not require a significant increased heat production to keep T_b stable, 3) after experimental disruption of mother-pup interaction and once together, the separated pup does not display relevant signals and behaviors to ensure physical contact with the mother and siblings to restore T_b , and 4) adult tuco-tucos who went through

short maternal isolation during early life do not exhibit poorer spatial learning capabilities than non-isolated ones.

2. Material and methods

2.1. Animals and maintenance

C. talarum pregnant females were captured using live traps in Mar de Cobo (37° 45' S, 57° 56' W, Buenos Aires Province, Argentina) during the reproductive period. Captured rodents were carried to the laboratory and maintained in plastic cages (0.30 × 0.40 × 0.25 m) with a refuge and wood shavings as nesting material, in a room with photoperiod and ambient temperature (T_a) automatically controlled (12L:12D; 25 ± 1 °C). Individuals were fed with lettuce, carrots, sweet potatoes, and corn and sunflower seeds ad libitum. As these animals do not drink free water, it was not provided. Not all captured pregnant females gave birth in the laboratory, and those who arrived to a successful parturition were only used in case litters were composed by at least 3 pups, otherwise were discarded from the experiment. Treatments started at day 2 after birth.

Animals were cared for in accordance with the guidelines for the capture, handling and maintenance in captivity of mammals of the American Society of Mammalogists (Sikes and Gannon, 2011) and current laws of Argentina.

2.2. Maternal and sibling separation

Due to the difficulty of raising and maintaining several litters in captivity for long periods of time, and to avoid the effects of maternal care variations between litters, we decided to use two siblings of each litter. One of the pups, randomly assigned, was used as "control" and the other as "isolated". To evaluate maternal separation effect, the "isolated" pup was daily separated from the mother and siblings since day 2, whereas the "control" pup remained in the cage in contact with their mother and littermates. Each pup was identified by a small mark, as described by Zenuto (2010).

Generally, two different models of maternal separation can be applied. In the more naturalistic one, pups are separated from their mother for short periods of time, less than one hour, simulating those moments when mother leaves nest to forage. On the other hand, prolonged separations, of more than two hours, are used to provoke extreme stressful conditions (Ladd et al., 2000; Boccia and Pedersen, 2001; Pryce et al., 2005). In order to be consistent with our objective of studying both, the response and characteristics of brief maternal separation comparable to mother's behavior, and to avoid the deleterious effects of prolonged isolation periods that do not occur in wildlife, separation time lasted for 30 min per day (Zenuto et al., 2002; Luna and Antinuchi, 2003). During daily separation, isolated pup was maintained in a single cage, with wood shavings taken from the mother's cage. Throughout experimentation, ambient temperature was 25 ± 1 °C, which is within thermoneutral zone (i.e. 25 °C–30 °C) for pups (Cutrera et al., 2003) and adults (Busch, 1989) of this species and the temperature commonly observed inside burrows (Cutrera and Antenucci, 2004).

2.3. Oxygen consumption ($\dot{V}O_2$) and resting metabolic rate (RMR)

During experiments, body mass (W) and $\dot{V}O_2$ consumption were obtained for isolated and control pups, at days 2, 6, 10, 15, 30 and 60 after birth. Pups' resting metabolic rate was estimated at 25 ± 1 °C by $\dot{V}O_2$ consumption on isolated and control pups from each litter. We used a computerized positive-pressure open-flow respirometric system (Sable System, Las Vegas). Pups were placed individually in a metabolic chamber, which consisted of a transparent acrylic cylinder (approx. 460 mL). Depending on pup's age, air flows entering the metabolic chamber varied from 300 mL/min up to 600 mL/min, controlled

by a mass flow meter (Side-Trak 830, Sierra Instruments, Monterey). Air passed through a CO₂ absorbent (IQB®) and water scrubber (Silica Gel) before and after passing through the chamber. Excurrent air was subsampled at 100 ± 10 mL/min and oxygen concentration was obtained with an FC-1B oxygen analyzer (STPD corrected, Sable Systems, Las Vegas), every 1sec with Datacan V-PC program (Sable System, Las Vegas). $\dot{V}O_2$ values were calculated using the equation 4a of Withers (1977:122). Resting metabolic rate was estimated as the 5 min lowest steady-stable of $\dot{V}O_2$ consumption within the 30 min of measurement.

2.4. Body temperature (T_b), body temperature variation (ΔT_b) and thermal conductance (C)

Depending on pups' age, body temperature was measured as pharyngeal or rectal temperature, with a thermistor (Cole-Parmer) before and after each $\dot{V}O_2$ measurement. ΔT_b was obtained from the difference of these values. Thermal conductance was estimated at thermoneutrality (25 °C, Busch, 1989), as $C = RMR / (T_b - T_a)$, where T_b is body temperature after RMR estimation (Seymour et al., 1998).

2.5. Behavior during and after isolation period

A day after each $\dot{V}O_2$ measurement (at 3, 7, 11, 16, 31 and 61 days after birth), isolated pups were video recorded during the 30 min-separation period from their mother and siblings (Sony digital HD 1080 60i camera recorder, HDR-XR100). During this period, distance traveled inside the cage, movement/rest frequency, shivering, vocalization, coprophagy, grooming, and anogenital area sniffing was obtained.

After the separation period, isolated pups were moved to a transparent cage with the mother and the rest of the litter. As isolated pups tended to look for their mother's belly and stayed below her, observations were made from the bottom of this cage. To maintain odor familiarity, the cage contained little wood shavings and faces from the nest cage. Pups and mother's behavior was recorded for 15 min and later classified into different categories (i.e. physical contact with the mother, huddling among pups, lactation, and cage exploration) and quantified as frequencies.

2.6. Spatial learning

Once pups reached 70 days old (beginning of dispersal period), they were placed into individual cages with the same characteristics as described before. When isolated and control individuals reached 120 days old, we evaluated their spatial learning abilities by measuring the capacity of individuals to solve a longitudinal labyrinth. The maze, made of white PVC tubes with transparent acrylic sheets on the top, consisted of a series of dead end paths (Tauson et al., 2006) and one correct path leading to the goal point at the opposite end of the starting point, resembling the structure of a natural burrow. A food reward (5 g of sweet potato) was placed at the goal point (Fig. 1S; Supplementary material).

We trained animals in two daily trials (one in the morning and one in the afternoon) until ten trials were completed. Before starting each trial, the animal was carried out from its home cage to the starting point of the labyrinths. After two minutes, the tuco-tucos was allowed to enter to the maze and the trial finished when the animal either reached the goal point or after 10 min without obtaining the food reward. At the end of each trial, the animal was returned to its home cage in the transfer tube. The time spent to complete the task and the number of errors (when the animal entered a dead-end path during the test trial) committed by the individuals (spatial learning performance parameters) were recorded during each trial. After this study concluded, animals were released at the site of capture in good physical condition. Even that all pups were raised in captivity, they intuitively constructed their own burrows when were released – no need for

vertical social transmission for development of subterranean habits – (see Vassallo, 2006).

2.7. Statistics

Linear regressions were performed to establish the relationship between W and T_b with age. In both cases, a Student- t test was performed to determine differences among regression slopes for isolated and control pups. Since the gender of the pups cannot be determined at day 2, moment at which pups were randomly assigned to control or isolated group, we excluded sex as a factor in all statistical analyses. A two-way repeated measures ANCOVA was used to test the null hypothesis of no difference in RMR among days between isolated and control pups, without considering their sibling relationship. Body mass was used as a covariate. In addition, repeated measures ANOVA was performed to test for no difference among days and treatments for mass specific RMR, T_b , ΔT_b , and C . Data frequencies of recorded behaviors were transformed to arcsine. One-way repeated measures ANOVA was used to test the null hypothesis of no differences in the recorded behaviors among ages during the isolation period. Friedman test was used only when assumptions of parametric tests were not met. The frequency of behaviors after pups' reincorporation to the litter among days between isolated and control pups was analyzed using repeated measures two-way ANOVA. Spatial learning performance parameters between control and isolated individuals were compared using repeated measures ANOVA (trial as repeated factor).

3. Results

3.1. Body mass

Seven pregnant females gave birth to litters, composed by 3 to 7 pups (mean = 4.29, SD = 1.48, median = 4, $n = 7$, total pups = 30). The mean body mass of pups at day 2 was 10.30 ± 2.18 g. In order to avoid potential unintended effects from handling pups during the first and most critical day after birth, body mass at birth was estimated by the intercept of the regression of W and age (6.45 g). Relationship between W and age fitted to a linear function. No differences in W was observed between control and daily separated pups (Student's- t , $t_{crit} = 1.99$, $T_{obs} < 0.01$) until day 60. For comparative purposes, W data were pooled and regression equation obtained ($W = 1.24 \text{ days} + 6.45$, $r^2 = 0.95$).

3.2. Resting metabolic rate of pups

No differences between isolated and control pups were detected when RMR neither was analyzed with W as a covariate (RMANCOVA, $F_{1, 6} = 2.34$, $P = 0.17$), nor in the interaction between days and condition (i.e. control or isolated pup; $F_{5, 30} = 1.09$, $P = 0.39$). There were differences in the RMR between days ($F_{5, 30} = 6.15$, $P < 0.001$; data of RMR and body mass for each group are presented in Supplementary material [Table 1S]). For comparative purposes mass-specific metabolic rate was analyzed and presented in Table 1. Similarly, no differences in mass-specific RMR between isolated and control pups were observed (RMANOVA, $F_{1, 12} = 1.83$, $P = 0.20$). No differences were detected in the interaction between days and experimental condition ($F_{5, 60} = 0.17$, $P = 0.97$). However, there were differences in mass specific RMR between days ($F_{5, 50} = 23.33$, $P < 0.001$). In both cases, a bimodal pattern in RMR was found. An increase until day 10 was observed, following by a progressive decrease until day 60.

3.3. Body temperature and thermal conductance

There were no differences in regression between T_b and days between isolated and control pups (Student's- t , $t_{crit} = 1.99$, $T_{obs} = 0.14$). Also, no differences were observed in T_b between treatments

Table 1Pooled data of resting metabolic rate (RMR), body temperature (T_b), and delta of body temperature (ΔT_b) of control and isolated pups of *C. talarum* at different ages.

	Day					
	2	6	10	15	30	60
RMR (mLO ₂ /gh)	2.66 ± 0.50 ^a	2.89 ± 0.42 ^a	3.37 ± 0.41 ^b	3.03 ± 0.59 ^{ab}	2.23 ± 0.46 ^c	1.74 ± 0.27 ^d
T_b (°C)	31.6 ± 0.9 ^a	32.3 ± 1.5 ^{ab}	32.3 ± 1.3 ^{ab}	33.0 ± 0.9 ^b	34.6 ± 1.2 ^c	36.2 ± 0.9 ^d
ΔT_b (°C)	-2.4 ± 2.0 ^a	-1.4 ± 1.9 ^{ab}	-0.2 ± 1.0 ^b	0.2 ± 0.8 ^b	0.5 ± 0.6 ^b	0.5 ± 0.6 ^b

Since no differences were observed between isolated and control pups for physiological variables (all, $P > 0.05$), data of frequency was pooled. Different letters indicate statistical differences among days ($P < 0.05$).

(RMANOVA, $F_{1, 12} = 0.22$, $P = 0.65$) but there were variations among days ($F_{5, 60} = 35.89$, $P < 0.001$, Table 1). Interaction between days and experimental condition showed no differences ($F_{5, 60} = 0.71$, $P = 0.62$). Pups' T_b in the first days of life was relatively lower than that observed at day 30 and 60 (Tukey, $P < 0.05$, Table 1). On the other hand, ΔT_b did not differ between neither treatments (RMANOVA, $F_{1, 12} = 0.25$, $P = 0.63$) nor interaction between days and treatment ($F_{5, 60} = 0.72$, $P = 0.61$), but between days ($F_{1, 10} = 15.50$, $P < 0.001$). Particularly, ΔT_b was higher in the first 10 days compared to that observed at days 30 and 60 (all, Tukey, $P < 0.05$). Furthermore, pups under 10 days of age were not able to thermoregulate efficiently (Table 1). Differences in C were observed between conditions (RMANOVA, $F_{1, 10} = 7.40$, $P = 0.02$), being lower in isolated pups (Fig. 1). Furthermore, C decreased from day 2 to day 60, both in control and isolated pups ($F_{5, 50} = 20.18$, $P < 0.001$, Fig. 1). However, no differences were observed in the interaction between day and condition ($F_{5, 50} = 1.99$, $P = 0.10$).

3.4. Behavior during isolation period

No differences were observed neither in the percentage of time that pups spend moving in the cage (mean = $47.2 \pm 0.7\%$, RMANOVA, $F_{5, 25} = 1.80$, $P = 0.15$), nor in the total distance moved within the cage in isolated pups between days (mean = 0.7 ± 0.2 m, $F_{5, 25} = 1.79$, $P = 0.15$). Although, isolated pups shivered more frequently at days 3 and 7 (Friedman, $df = 5$, $P = 0.01$, Table 2). The frequency of pups' vocalization was higher within the first 15 days, compared with days 30 and 60 (Table 2). Moreover, the frequency of coprophagy/anogenital sniffing was not different between days (mean = $1.1 \pm 0.7\%$, Friedman, $df = 5$, $P = 0.53$), while grooming was different ($F_{5, 25} = 2.59$, $P = 0.05$), particularly for the first 15 days compared to day 60 (all, Tukey, $P < 0.04$, Table 2).

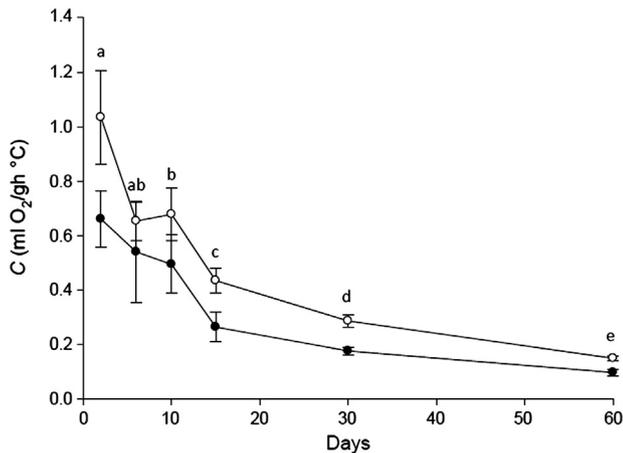


Fig. 1. Relationship between thermal conductance (C) and age of *C. talarum* control (white) and isolated (black) pups. Since differences were observed in C between conditions ($P = 0.02$), and the same statistical pattern among days for each condition ($P < 0.01$), several letters indicate statistical differences among days for each condition, either control or isolated.

3.5. Behavior after isolation period

After separations, pups were returned with the mother and siblings. No differences in the frequency of physical contact with the mother (CM), huddling (H), lactation (LAC) and exploration (EXP) between isolated and control pups were observed (2-way RMANOVA; CM, $F_{1, 6} = 1.34$, $P = 0.29$; H, $F_{1, 6} = 1.75$, $P = 0.24$; LAC, $F_{1, 6} = 2.42$, $P = 0.17$; EXP, $F_{1, 6} = 1.22$, $P = 0.31$). On the other hand, the frequency of CM ($F_{5, 30} = 4.30$, $P < 0.01$), LAC ($F_{5, 30} = 4.72$, $P < 0.01$), or EXP ($F_{5, 30} = 10.63$, $P < 0.01$) were different between days (see Table 3). In general, CM and LAC decreased, but EXP increased, as pups get older. No differences in the frequency of huddling between days were observed ($F_{5, 30} = 0.44$, $P = 0.81$, Table 3). There were no differences in the interaction between experimental condition and day for all variables (CM, $F_{5, 30} = 0.32$, $P = 0.89$; H, $F_{5, 30} = 0.32$, $P = 0.90$; LAC, $F_{5, 30} = 0.31$, $P = 0.90$; EXP, $F_{5, 30} = 0.66$, $P = 0.65$).

3.6. Spatial learning

Both isolated and control individuals showed an improvement in their spatial learning performance after the first trials, and both errors and time to reach the goal maze declined as the number of trials increased (Fig. 2). No differences in the number of neither errors nor latency between isolated and control tuco-tucos were found (RMANOVA, errors, $F_{1, 12} = 0.005$, $P = 0.94$; latency, $F_{1, 12} = 0.74$, $P = 0.40$).

4. Discussion

During the last decades several works showed that exposing mammals to early-life adverse events, including maternal separation or social isolation, provoked profound alterations in the physiology and behavior of stressed individuals. However, the majority of this knowledge comes from the studies performed in laboratory rats subjected to severe and sustained maternal separation, and/or in surface dwelling rodents (Lehmann and Feldon, 2000). In contrast, few works have been conducted in wild species subjected to natural stressful events that happen in their environments, and none of them involved subterranean species. Therefore, and trying to fill this gap in our knowledge, we studied the physiological and behavioral effects of brief maternal separations, similar to the ones suffered by neonates during growing, in the wild subterranean rodent *C. talarum*.

An essential mechanism for maintaining homeostasis in mammals is the control of body temperature, which covers a wide variety of strategies depending on the species, ontogenetic stage and environmental circumstances to which organisms are exposed. In this study, we assessed the development of thermoregulatory capacity in relation to maternal role in the altricial *C. talarum* pups. We found that the daily separation of pups has no effect on thermoregulatory development, compared to those pups with continuous access to the mother and siblings. Moreover, thermoregulatory development pattern for offspring is similar to the one observed by Zenuto et al. (2002). Until day 10, pups are unable to maintain constant T_b during isolation. Thermoregulatory independence in these individuals was reached after 15 days old. Subsequently, T_b increases, reaching adults values up to day 60 (see Table 1).

Table 2

Frequency of shivering, vocalization, and grooming during the isolation period of pups of *C. talarum* at different ages. Different letters indicate statistical differences among days ($P < 0.05$).

% Time	Day					
	3	7	11	16	31	61
Shivering	14.4 ± 4.9 ^a	13.9 ± 5.7 ^a	0.7 ± 0.6 ^b	0.6 ± 0.5 ^b	0 ± 0 ^b	0 ± 0 ^b
Vocalization	30.2 ± 5.7 ^a	28.5 ± 7.3 ^a	9.2 ± 2.0 ^a	10.9 ± 4.0 ^a	2.4 ± 1.3 ^b	2.0 ± 1.0 ^b
Grooming	3.0 ± 1.6 ^a	5.4 ± 3.6 ^a	4.4 ± 1.7 ^{ab}	6.6 ± 2.6 ^{ab}	9.6 ± 3.2 ^b	13.1 ± 2.9 ^b

The ability to maintain the T_b within certain ranges of T_a is determined by the relationship between metabolic heat production and thermal conductance (McNab, 2002). In this case, there was no difference in RMR between isolated and control pups, and both presented two clear phases in their energetic patterns (Table 1): there is a trend in increasing metabolic rate up to day 10, followed by a decrease until day 60. The rapid rise of RMR in early days has been observed in several species (Spiers and Adair, 1986; Berthon et al., 1994). This increase could contribute to T_b gradual raise, but most of the energy expended would be related to the production of new tissue and its maintenance. Along the second phase of the metabolic pattern, i.e. after day 10, RMR starts to decrease. *C. talarum* offspring reach weaning at day 45 (Zenuto et al., 2002), achieving age of dispersal after day 60 (Schleich, 2010). During this period, pups reach adult T_b (Zenuto et al., 2002; Antinuchi et al., 2007; Table 1), however RMR is still relatively high (0.92 mL O₂/gh for adults; Luna and Antinuchi, 2007), probably due to an additional energy requirement related to their growth.

However, isolated pups had a lower C at 25 °C than pups with continuous access to the mother and the rest of the litter (Fig. 1). While there are no differences in RMR and T_b , isolated pups could reduce their C by reducing the exposed surface through a differential posture. During isolation period, pups reduce the exposure of its ventral area, bringing the chest area to the belly, as this area is the most important way of heat loss by conduction (Cutrera and Antenucci, 2004; Luna and Antinuchi, 2007). Moreover, as observed in *C. talarum*, when solitary pups are exposed to temperatures lower than observed in burrows (19 °C), they show a high ΔT_b than pups in contact with their mothers and siblings (see Cutrera et al., 2003). Associated with individual or offspring's clustering strategy (Antinuchi et al., 2007), decrease of surface – volume ratio during periods where pups are isolated is a common behavior within mammals (Seymour et al., 1998).

Although no differences in adult T_b development were observed among isolated and non-isolated pups, several behaviors were detected during isolation period. Shivering in the isolated pups was characteristically observed over the first days during isolation (Table 2), without being detected during mother–offspring contact (data not shown). In particular, the fact that shivering causes heat production by muscle work (Jansky, 1973), and that frequency with which pups do it during isolation has been higher in the first 7 days, indicates that this could be a strategy used for heat production in the absence of physical contact with the mother and siblings. Although shivering was not energetically estimated in this study, this behavior could be important for pups' development as a way for gaining heat, and therefore successfully reach adulthood since no differences in T_b development among pups were

observed. Then, from day 16, shivering was no longer visible and heat loss by conductance was diminished.

Not only physiological parameters of the pups are affected by maternal isolation, but also maternal behavior towards the isolated and non-isolated pups change after returning isolated infants to the nest. Liu et al. (1997) showed that in rats mothers spent more time licking and grooming pups that were removed from their nest for 15 min, than did mothers whose pups were not handled. Similarly, Zimmerberg et al. (2003) found that mothers spent more time with and licked and groomed more frequently isolated pups, than pups that stayed with their mother all time at the nest. Contrary to this, we did not find differences neither in the time spent in physical contact with the mother nor in the duration of the lactation periods between isolated and control pups. This result suggests either that the mother was not capable of recognizing isolated pups or that the time required for an increment in the frequency of care-taking behaviors towards the separated pups may be greater than the time of the experiment. Nevertheless, as separation period was repeated but short, and during the rest of the day pups were in contact with not only the mother but also at least the two siblings, responses to isolation could had been reduced, even eliminated (Fournier et al., 2011, 2012).

Maternal separation has also been shown to produce long-term consequences in adult offspring, including alterations in anxiety behaviors, immune function, HPA responsiveness, and learning and memory in general, and on spatial learning capabilities in particular (Lehmann et al., 2000; Oitzl et al., 2000; Workel et al., 2001; Zimmerberg et al., 2003; Daniels et al., 2004). When reaching adult age, individuals of *C. talarum* that suffered brief separations from their mother and littermates during their early life do not show differences in their spatial learning capabilities when compared to non-isolated ones. This result coincides with the previous works which showed that when individuals were isolated for single or repeated short periods of time (~30 min), they displayed similar spatial performances than control individuals, and did not show the age-related decline in spatial learning and memory performance (Meaney et al., 1991; Lehmann and Feldon, 2000). Moreover, some works also reported that adult rats which experienced brief isolation periods during postnatal days exhibited better spatial abilities than rats that did not suffer that treatment (Lehmann et al., 2002). As a general rule, while brief repeated separations (3–15 min) seem not to affect or even improve spatial abilities in adult organisms, prolonged and repeated isolation periods yield negative impacts on spatial learning and memory (Sandstrom and Hart, 2005; Aisa et al., 2007), although positive effects were also observed (Frisone et al., 2002), showing the complexity of the effects of stress factors on cognitive parameters.

Table 3

Pooled data of frequency of physical contact with the mother (CM), huddling (H), lactation (LAC), and exploration of the nest cage (EXP) of control and isolated pups of *C. talarum* at different ages.

% Time	Day					
	3	7	11	16	31	61
CM	77.5 ± 6.0 ^a	80.0 ± 8.7 ^a	69.2 ± 5.5 ^{ab}	55.8 ± 12.8 ^{bc}	46.7 ± 8.6 ^{bc}	24.8 ± 7.8 ^{bc}
H	30.8 ± 8.6 ^a	22.5 ± 9.3 ^a	35.8 ± 9.3 ^a	29.2 ± 14.1 ^a	12.8 ± 2.9 ^a	18.6 ± 7.9 ^a
LAC	52.5 ± 9.5 ^a	60.0 ± 13.5 ^a	47.5 ± 7.6 ^a	45.0 ± 12.3 ^a	40.0 ± 8.7 ^a	3.3 ± 1.8 ^b
EXP	2.5 ± 1.8 ^a	4.2 ± 4.2 ^{ab}	13.3 ± 5.5 ^{bc}	24.2 ± 6.7 ^{cd}	33.3 ± 5.8 ^d	57.6 ± 8.2 ^e

Since no differences were observed between isolated and control pups for the frequency of different behaviors (all, $P > 0.05$), data was pooled. Different letters indicate statistical differences among days ($P < 0.05$).

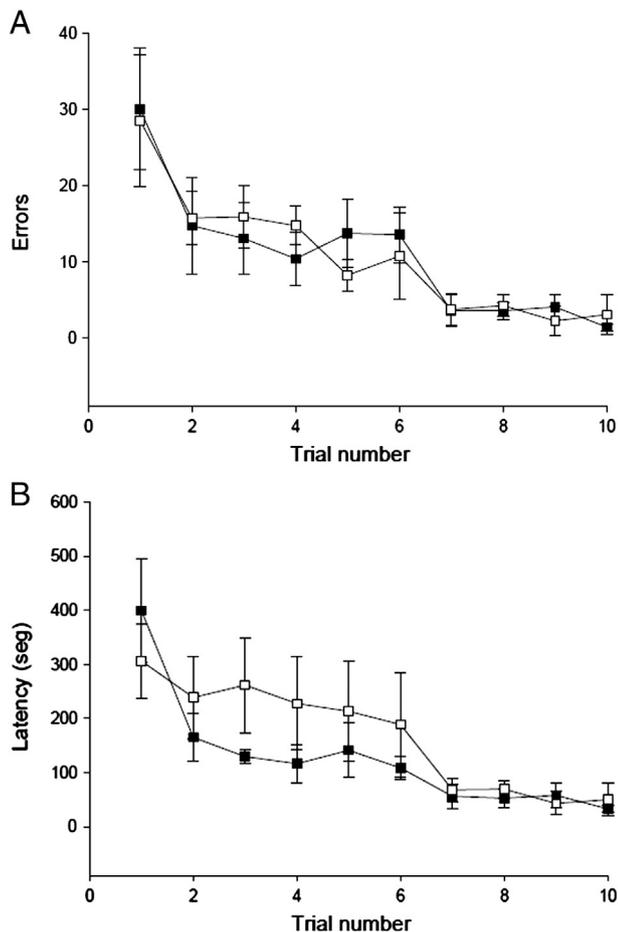


Fig. 2. Mean number (\pm SE) of errors (A) or latency (B) to reach the goal during each trial of spatial learning of control (white) and isolated (black) individuals of *C. talarum* in the longitudinal labyrinth.

5. Conclusion

In conclusion, pups of *C. talarum* daily isolated for 30 min showed no differences neither in the acquisition of adult's T_b nor in the RMR's development pattern, behavioral response and adult spatial abilities compared with pups who had permanent access to their mother and littermates. Even that behavioral parameters show a high interindividual variation (Table 2, Fig. 2), and must be taken with caution, one point that might be further explored is the presence of visible shivering during isolation at early days. Although shivering might not be completely functional at this age, it is the main thermogenic mechanism during adulthood (Luna et al., 2012), and may be responsible for the similar thermoregulatory development among isolated and control individuals. Thus, when *C. talarum* offspring face periods in which mother is absent, minor physiological and behavioral adjustments, such as shivering and postural changes, comprise part of pup-mother behavioral repertoire employed to keep individuals within the limits of allostasis.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.cbpa.2014.03.008>.

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