Brief Report

Cristian E. Schleich

Departamento de Biología
Facultad de Ciencias Exactas y Naturales
Dto. Biologia, CC 1245, Universidad
Nacional de Mar del Plata-CONICET
Funes 3250, Mar del Plata 7600, Argentina
E-mail: cschleic@mdp.edu.ar

Ontogeny of Spatial Working Memory in the Subterranean Rodent *Ctenomys talarum*

ABSTRACT: *While several works analyzed the spatial learning and memory capacities in adults of subterranean rodents, no study was done examining the development of these cognitive processes in pups of any of those species. Therefore, the development of spatial working memory in the South American subterranean rodent Ctenomys talarum was investigated by analyzing the pups' spatial performance in a delayed alternation task. When a short delay of 1 min was interposed between runs in the Y-maze, 20-day-old pups made more errors than 40- and 60-day-old pups. When longer intervals (10 min) were elapsed between runs, younger pups made approximately twice as many errors as the ones committed by 60-day-old pups, showing the age-dependent development of spatial working memory in this species of subterranean rodent. Increased space use by C. talarum pups, caused first by the appearance of independent exploratory behavior and later by the need of leaving maternal territory and construct a new burrow system, showed some correspondence with the improvements in the pups' spatial working memory performance, suggesting for the importance of this cognitive capacity in developing pups for which spatial learning and memory constitute essential abilities for survival and fitness. © 2010 Wiley Periodicals, Inc. Dev Psychobiol*

Keywords: ontogeny; spatial working memory; subterranean rodents; Ctenomys talarum

INTRODUCTION

Spatial memory, defined as the capacity of recognizing, codifying, storing, and recovering spatial information about the arrangement of items or specific routes in space (Carrillo-Mora, Giordano, & Santamaría, 2009; Nelson, 2005), constitutes a key cognitive function for animals, since most of their vital activities, like foraging, finding mates or avoiding neighbors, require precise spatial orientation (Antinuchi & Schleich, 2003; Costanzo,

Bennett, & Lutermann, 2008; Kimchi & Terkel, 2001). Two different types of spatial memory are identified: working memory, a short-term memory for a location that is used within a testing session, and reference memory, which imply a long-term storage of spatial locations (Carrillo-Mora et al., 2009; Dudchenko, 2004). Contrary to spatial working memory, spatial reference memory usually exhibits more duration and resistance to interference, and could persist from days to months (Carrillo-Mora et al., 2009; Dudchenko, 2004). Besides the functional differences among them, both types of memory are vital to the survival of the animals, particularly for those who inhabit structurally complex habitats (Mastrangelo, Schleich, & Zenuto, 2009).

Subterranean rodents live in extensive and elaborate closed underground galleries (Antinuchi & Schleich, 2003; Bennett & Faulkes, 2000; Burda, 2003; Nevo, 1999). The construction, use, and maintenance of these burrow systems requires well-developed spatial abilities in order to efficiently orientate inside the dark tunnels and

to decrease the high energetic costs associated with digging while extending the burrows for foraging (Luna & Antinuchi, 2006; Luna, Antinuchi, & Busch, 2002; Vleck, 1979). Furthermore, in those species that exhibit aboveground activity, developed orientation abilities are also important to reduce the time exposed to predators on surface by relocating burrow openings rapidly (Mastrangelo et al., 2009). Recent works have shown that subterranean rodents indeed exhibit developed orientation abilities (Antinuchi & Schleich, 2003; Costanzo et al., 2008; Kimchi & Terkel, 2001; Mastrangelo et al., 2009). These works studied diverse aspects of the orientation abilities of adult subterranean rodents, like the spatial learning and memory capacity in *Spalax ehrenbergi* compared to aboveground species (Kimchi & Terkel, 2001), the role of sociality and sex on the spatial abilities in the eusocial Damaraland (*Fukomys damarensis*) and the solitary Cape mole-rats (*Georychus capensis*) (Costanzo et al., 2008), and the effects of an exposure to predatory cues on spatial working and reference memory performance in *Ctenomys talarum* (Mastrangelo et al., 2009). However, in comparison, less attention has been paid to the ontogeny of these cognitive processes in these or other species of subterranean rodents.

C. talarum is a solitary subterranean rodent, member of the family Ctenomyidae, which is distributed in the southern region of Buenos Aires Province, Argentina (Busch et al., 2000). The burrow system of this species exhibits a complex design, consisting of a main axial tunnel and several lateral branches and feeding tunnels, all of them plugged with soil (Antinuchi & Busch, 1992). Although tuco-tucos consume the collected food within their tunnels, most gathering of vegetation occurs aboveground, where tuco-tucos become vulnerable to aerial and terrestrial predators (Busch et al., 2000). During these aboveground excursions, the rapid formation of an accurate representation of the surface environment is crucial to escape from predators by relocating burrow entries rapidly. Concomitant with the need of proper orientation inside the intricate burrows and during the brief foraging bouts on the surface, *C. talarum* displays developed spatial abilities for learning and memorizing spatial structures (Antinuchi & Schleich, 2003; Mastrangelo, Schleich, & Zenuto, 2009, 2010). Although these studies analyzed the spatial skills in adults of this species, no works have been done examining spatial memory in developing pups of this or other species of subterranean rodents.

Pups of *C. talarum* are characterized for displaying an altricial development and three major ontogenetic stages can be differentiated according to the degree of development of their exploratory behavior. First, around Days 15–20 after birth, pups start to display exploratory behavior, leaving the nest site for short periods of time and obtaining information about the novel subterranean environment. Before this period, pups are not able to regulate their body temperature and depend exclusively on their mother to obtain food and maintain their body temperature, spending therefore most of the time in close contact with their mother (Schleich & Busch, 2002; Zenuto, Antinuchi, & Busch, 2002). At about 35–45 days after birth, pups reached adult temperature and become behavioral and physiologically independent (Cutrera, Antinuchi, & Busch, 2003; Zenuto et al., 2002). At this age the weaning period starts, and pups show active exploratory behavior, walking independently around the whole burrow system. Finally, from 60 days old, pups leave the maternal territory and begin to disperse (Malizia, Zenuto, & Busch, 1995). In order to successfully disperse, each individual must be able to construct a burrow and to localize and select food from aboveground, two activities that require highly developed spatial orientation abilities.

According to these key ontogenetic stages, it is expected that pups' orientation capacities would improve concomitantly with the development of the exploratory behavior, reaching the highest performance around the age of dispersal, when learning and memorizing precisely the spatial information available below and aboveground become crucial for survival.

Therefore, the objective of this work was to study the ontogeny of spatial memory in *C. talarum* using a short-term memory task. In particular, I studied the spatial performance of 20-, 40-, and 60-day-old pups in a delayed spatial alternation task.

MATERIALS AND METHODS

Animal Maintenance

Fifteen pregnant females were collected using plastic live traps at the sandy dune belt in Mar de Cobo (37° 45′S, 57° 56′ W, Buenos Aires Province, Argentina) during the 2006–2008 period. Captured individuals were carried to the laboratory and housed individually in plastic cages until pups were born. Both mothers and pups were maintained on a natural photoperiod (10:14 hr L/D). Ambient temperature was kept at $24 \pm 2°C$, whereas relative ambient humidity ranged from 50% to 70%. The animals were daily fed with sweet potatoes, carrots, lettuce, corn, grass, and sunflowers seeds.

Apparatus and Procedure

Spatial memory was assessed using an artificial Y-maze, one of the most common versions of the spatial alternation tasks (Deacon & Rawlins, 2006; Dudchenko, 2004). This task capitalizes the rodents' tendency to choose alternative maze arms or locations when individuals are relocated in the apparatus. In order to alternate or avoid a revisit, each animal must memorize the goal arm chosen in the previous trial. Since

the individuals must remember the location of the arm within a testing session, but not between sessions, it is consider as a spatial working memory task (Dudchenko, 2004).

Spatial alternation was tested in pups of three different ages: 20, 40, and 60 days old. These age categories were chosen because they coincide with key stages in the development of pups' exploratory behavior; 20 days: pups start to left the nest and wander around the burrow, 40 days: mother starts weaning and pups display active exploratory behavior, and 60 days: initiation of pups' dispersal time.

Similar numbers of *C. talarum* pups were randomly assigned to each experimental age (see legend of Fig. 1) and each individual was only tested at one of the time intervals and at one of the three age categories used. To study alternation perform-ance, individuals were introduced into a 10-cm diameter transparent tube Y-maze consisting of an entrance tube (25-cm long) connected to two opposed arms (30-cm long) equipped
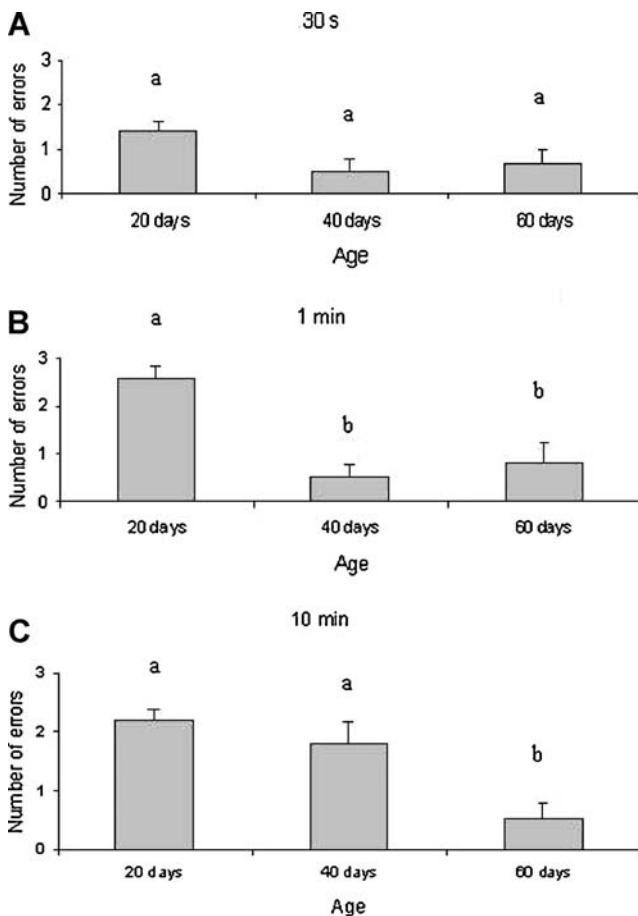

FIGURE 1 Spatial working memory performance of *Ctenomys talarum* pups indicated by the number of errors (mean ± SD) made by 20- (n = 15), 40- (n = 13), and 60- (n = 13) day-old pups in a spatial delayed alternation task (Y-maze). Three different delays of 30 s (A), 1 min (B), and 10 min (C) were interposed between the first and second run in the spatial task. Different letters represent significant differences (p < .05).

with guillotine doors at their entrances. Previous to the start of the experiments, each pup was allowed to enter the Y-maze for 5 min to habituate to the artificial labyrinth.

At the beginning of the spatial alternation trials, the individuals were placed in the entrance tube for 2 min. After this, each pup was allowed to enter one of the arms of the Y-maze, preventing it to enter into the alternate arm (forced-choice alternation). The position of the open or closed arm (right or left) was balanced, but the order was randomly determined. During a 60 s period, the individual explored the open arm which contained different objects (squares, triangles, and balls of different colors) in order to increase its motivation to explore. Then, the pup was picked up and replaced at the entrance of the Y-maze. Two short and one long delay of 30 s, 1 min, or 10 min were interposed between the first and second run (spatial delayed alternation task). After these time periods, the animals were allowed to reenter the maze, which presented both arms opened. The individuals' choices were recorded as correct or wrong if they decided to enter into the alternate arm or into the previously explored arm respectively. After the end of each trial, the Y-maze was washed with tap water and odorless detergent, wiped with alcohol and then allowed to air dry to ensure that no odors remained. Latex gloves were used when handling pups and artificial maze in order to avoid human scent transfer.

At every age (20, 40, and 60 days) and delay time analyzed (30 s, 1 min, and 10 min), each pup was subject to a block of five choice-trials. This number was selected in order to avoid habituation to the task which could be caused by a higher number of trials and to avoid the constant handling of the animals which could induce stressful responses in them, particularly at very young ages (Carrillo-Mora et al., 2009).

Data Analysis

A one-way ANOVA (Sigmastat 3.0) was used to test the hypothesis of no differences in the number of wrong choices (alternation performance) among pups of different ages and at different delay times. When error values did not fit a normal distribution, a logarithmic transformation was applied to the data. Since values were zero in some trials, a constant to this variable was added before transformation. When transformed data did not fit a normal distribution, a nonparametric test (Kruskal–Wallis) was used. A Student–Newman–Keuls Test a posteriori was used to identify differences when results showed statistical differences.

RESULTS

The number of errors made by pups at different ages in the delayed alternation task is shown in Figure 1. Although the mean number of errors tended to be higher in younger pups (20 days old), no statistical differences in the number of errors were found among pups of different ages when a delay of 30 s was interposed between the first and second run in the Y-maze (Kruskal–Wallis test, n20d = 5, n40d = 4, n60d = 3, df = 2, H = 4.55, p = .117). However, the situation was different when longer delays were

introduced. When a 1 min delay was interposed, 20-day-old pups made significantly more errors than older pups (Kruskal–Wallis test, n20d $=$ 5, n40d $=$ 4, n60d $=$ 6, df $=$ 2, $H=10.93$, $p=.004$; post hoc Student–Newman–Keuls Test $p<.05$ for 20 vs. 40 and 20 vs. 60 days old, respectively). No statistical differences in the number of errors were found among pups of 40 and 60 days old. Finally, when a 10 min delay was introduced, 60-day-old pups performed better than 20- and 40-day-old pups (ANOVA, n20d $=$ 5, n40d $=$ 5, n60d $=$ 4, df $=$ 2, $F=9.63$, $p \leq .004$, post hoc Student–Newman–Keuls Test $p<.05$ for 20 vs. 60 and 40 vs. 60 days old, respectively).

DISCUSSION

In rodents, the hippocampus, in conjunction with other neural structures, are generally considered to be involved in the generation of spatial working memory (Broadbent, Squire, & Clark, 2006; Brown, Pagani, & Stanton, 2005; Murray & Ridley, 1999; Wang & Cai, 2006). Therefore, the appearances of some memory functions depend mainly on the maturation of the hippocampus, a brain structure that undergoes significant postnatal growth and development (Altman, Brunner, & Bayer, 1973). The late development of the hippocampus is reflected in the age-dependent emergence of spatial memory observed in various rodent species. First works showed that while 15-day-old pup's rats made random choices in a T-maze, adult levels of correct choices of 70% were observed among 30-day-old pups (Bronstein, Dworkin, & Bilder, 1974). Similarly, Castro, Paylor, and Rudy (1987) and Green and Stanton (1989) found ontogenetic differences in the development of short-memory in rats. Recently, Brown et al. (2005) found that conditional discrimination learning in rats appears between 23 and 30 days after birth, depending on the type of conditional cue utilized.

Young pups of the subterranean rodent *C. talarum* were also impaired in their working memory performance when compared to older individuals. When short delays were interposed between the forced and choice runs in the spatial alternation task, 20-day-old pups made more errors than 40- and 60-day-old pups. This initial improvement in the spatial performance, comprised between 20 and 40 days after birth, coincides with evidence from other behavioral studies which also shown the appearance of spatial working memory in rats between 20 and 30 days after birth (Bronstein et al., 1974; Brown et al., 2005; Castro et al., 1987; Green & Stanton, 1989). Further changes in pups' spatial performance were also observed when longer intervals (10 min) were inserted between runs. In that condition, younger pups made approximately twice errors than the ones committed by 60-day-old pups,

showing the age-dependent development of spatial working memory in this species of subterranean rodent. Comparable results were obtained by Stanton (1982), who found that 14-day-old rats responded to a single-alternation of rewarded task when the interval between trials was 30 s, but 11-day-old pups responded to the pattern only when the interval was 8 s. In line with these results, Castro et al. (1987) showed that while oldest pups performed well in a conditional-spatial discrimination task at all delay intervals, the performance of 25- and 31-day-old pups fell to chance when tested at 60 and 180 s intervals, respectively.

As explained before, spatial memory is a key cognitive function for those animals living in complex habitats, as it is the case of subterranean rodents (Antinuchi & Schleich, 2003; Costanzo et al., 2008; Kimchi & Terkel, 2001). Living within long and structurally complex tunnel systems require highly developed spatial orientation capacities in order to achieve successful foraging, reproduction and territory defense (Mastrangelo et al., 2009). For *Ctenomys* pups, three growing stages are crucial related to the development of spatial abilities. First, from about 15–20 days after birth, pups start to leave their nest for shorts periods of time and move independently (Schleich & Busch, 2002). The appearance of this exploratory behavior, that requires the rapid formation of mental representations of the location of the burrow's nest and the tunnels and branches in order to avoid long isolation episodes, coincides with the emergence of spatial working memory in growing pups observed in the superior performance of 40-day-old pups compared to 20-day-old pups in the alternation task. At this age, the mother starts weaning and refuses to nurse pups, which display active exploratory behavior and move around the whole burrow system searching for food.

Dispersal age is a critical period for young individuals. Several functional changes are implied before and during dispersal (Clobert, Fraipont, & Danchin, 2008). Reaching adult body temperature, thermoregulatory capacity, and independent foraging are important physiological and behavioral factors required by *C. talarum* pups before departing, and all of them are fully achieved previous to dispersal age (Schleich & Busch, 2002; Zenuto et al., 2002). In comparison, less attention has been paid to the development of orientation abilities as another factor influencing dispersal time and success. Leaving the maternal territory implies that each juvenile must be able to construct a new burrow and to localize and select food patches on the surface, two activities that require highly developed spatial orientation abilities (Mastrangelo et al., 2009). In *C. talarum*, the dispersal age was also concomitant with a further enhancement in the spatial ability of postweaned individuals when compared to preweaned ones, suggesting that the development of this

cognitive capacity constitutes an essential attribute to achieve a successful dispersal.

In summary, this study provides the first example of a correlation between changes in exploratory behavior and the development of orientation capacities in a species of subterranean rodent. Increased space use by *C. talarum* pups, caused first by the appearance of independent exploratory behavior and later by the need of leaving maternal territory and construct a new burrow system, corresponded with improvements in the pups' spatial working memory performance, suggesting for the importance of this cognitive capacity in developing pups for which spatial learning and memory constitute essential abilities for survival and fitness. Since no information about the structural development of the hippocampus in *Ctenomys* is available, futures studies should be focus on examining if the behavioral differences in spatial working memory found in *C. talarum* pups may be associated in part to the pattern of neural development in this species of subterranean rodent.

NOTES

REFERENCES

Altman, J., Brunner, R. L., & Bayer, S. A. (1973). The hippocampus and behavioral maturation. Behavioral Biology, 8, 557–596.

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

Antinuchi, D., & Schleich, C. (2003). Spatial memory in a solitary subterranean rodent *Ctenomys talarum* (Rodentia: Ctenomyidae). Belgian Journal of Zoology, 133, 89–91.

Bennett, N. C., & Faulkes, C. G. (2000). African mole-rats: Ecology and eusociality. Cambridge: Cambridge University Press.

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2006). Reversible hippocampal lesions disrupt water maze performance during both recent and remote memory tests. Learning & Memory, 13, 187–191.

Bronstein, P. M., Dworkin, T., & Bilder, B. H. (1974). Age-related differences in rats' spontaneous alternation. Animal Learning & Behavior, 4, 285–288.

Brown, K. L., Pagani, J. H., & Stanton, M. E. (2005). Spatial conditional discrimination learning in developing rats. Developmental Psychobiology, 46, 97–110.

Burda, H. (2003). Adaptations for subterranean life. In: D. G. Kleiman, V. Geist, M. Hutchins, & M. C. McDade (Eds.), Mammals I. Vol. 12 of Grzimek's Animal Life Encyclopedia (pp. 69–78). Farmigton Hills, MI: Gale Group.

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

Carrillo-Mora, P., Giordano, M., & Santamaría, A. (2009). Spatial memory: Theoretical basis and comparative review on experimental methods in rodents. Behavioral Brain Research, 203, 151–164.

Castro, C. A., Paylor, R., & Rudy, J. W. (1987). A developmental analysis of the learning and short-term-memory processes mediating performance in conditioned-spatial discrimination problems. Psychobiology, 15, 308–316.

Clobert, J., de Fraipont, M., & Danchin, E. (2008). Evolution of dispersal. In: E. Danchin, L. Giraldeau, & F. Cézilly (Eds.), Behavioral ecology (pp. 323–259). Oxford University Press. New York.

Costanzo, M., Bennett, N., & Lutermann, H. (2008). Spatial learning and memory in African mole-rats: The role of sociality and sex. Physiology and Behavior, 96, 128–134.

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

Deacon, R. M. J., & Rawlins, J. N. P. (2006). T-maze alternation in the rodent. Nature Protocols, 1, 7–12.

Dudchenko, P. (2004). An overview of the tasks used to test working memory in rodents. Neuroscience and Biobehavioral Reviews, 28, 699–709.

Green, R. J., & Stanton, M. E. (1989). Differential ontogeny of working memory and reference memory in the rat. Behavioral Neuroscience, 103, 98–105.

Kimchi, T., & Terkel, J. (2001). Spatial learning and memory in the blind mole rat (*Spalax ehrenbergi*) in comparison with the laboratory rat and Levant vole. Animal Behaviour, 61, 171–180.

Luna, F., Antinuchi, C. D., & Busch, C. (2002). Digging energetics in the South American rodent *Ctenomys talarum* (Rodentia, Ctenomyidae). Canadian Journal of Zoology, 80, 2144–2149.

Luna, F., & Antinuchi, C. D. (2006). Cost of foraging in the subterranean rodent *Ctenomys talarum*: Effect of soil hardness. Canadian Journal of Zoology, 84, 661–667.

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.

Mastrangelo, M., 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 Behaviour, 77, 685–692.

Mastrangelo, M., Schleich, C. E., & Zenuto, R. R. (2010). Spatial learning abilities in males and females of the subterranean rodent Ctenomys talarum. Ethology, Ecology & Evolution, 22, 101–108.

Murray, T. K., & Ridley, R. M. (1999). The effect of excitotoxic hippocampal lesions on simple and conditional discrimination learning in the rat. Behavioural Brain Research, 99, 103–113.

Nelson, R. J. (2005). Learning and memory. In: R. J. Nelson (Ed.), An introduction to behavioral endocrinology (pp. 721–770). Sunderland, MA: Sinauer Associates.

Nevo, E. (1999). Mosaic evolution of subterranean mammals. Regression, progression, and global convergence. New York: Oxford University.

Schleich, C. E., & Busch, C. (2002). Juvenile vocalizations of *Ctenomys talarum* (Rodentia: Octodontidae). Acta Theriologica, 47, 25–33.

Stanton, M. E. (1982). Performance of 11 and 14-day-old rats on a working memory problem. Behavioral and Neural Biology, 36, 304–310.

Vleck, D. (1979). The energy cost of burrowing by the pocket gopher *Thomomys bottae*. Physiological Zoology, 52, 122–136.

Wang, G., & Cai, J. (2006). Disconnection of the hippocampal–prefrontal cortical circuits impairs spatial working memory performance in rats. Behavioral Brain Research, 175, 329–336.

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