Neuroscience 255 (2013) 76–85

SCATTER HOARDING AND HIPPOCAMPAL CELL PROLIFERATION IN SIBERIAN CHIPMUNKS

Y. PAN,[a] M. LI,[a] X. YI,[c] Q. ZHAO,[a] C. LIEBERWIRTH,[b] Z. WANG[b] AND Z. ZHANG[a*]

[a] *State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, Datun Road, Chaoyang District, Beijing 100101, PR China*

[b] *Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL 32306, USA*

[c] *College of Agriculture, Henan University of Science and Technology, Xining Road 35, Luoyang 471003, PR China*

Abstract—Food hoarding, especially scatter hoarding and retrieving food caches, requires spatial learning and memory and is an adaptive behavior important for an animal's survival and reproductive success. In the present study, we examined the effects of hoarding behavior on cell proliferation and survival in the hippocampus of male and female Siberian chipmunks (*Tamias sibiricus*). We found that chipmunks in a semi-natural enclosure displayed hoarding behavior with large individual variations. Males ate more scatter-hoarded seeds than females. In addition, the display of hoarding behavior was associated with increased cell proliferation in the hippocampus and this increase occurred in a brain region-specific manner. These data provide further evidence to support the notion that new cells in the adult hippocampus are affected by learning and memory tasks and may play an important role in adaptive behavior.

Key words: hoarding, hippocampus, BrdU, Ki67, cell proliferation.

INTRODUCTION

Food hoarding represents an important behavioral adaptation to seasonal and unpredictable changes in food resources (Pravosudov and Clayton, 2002; Pravosudov and Smulders, 2010). Food-hoarding behavior can generally be divided into two categories: larder hoarding (storing food caches in one larder) and scatter hoarding (storing food in multiple locations scattered throughout the animal's home range) (Morris, 1962; Vander Wall, 1990). Scatter and larder hoarding reflect two different strategies of defending cache losses against intra- and inter-specific cache pilferage (Vander Wall and Jenkins, 2003). By scattering food from a concentrated site into separate caches, a scatter hoarder can increase the dispersion of a resource occurring in a rich patch to the point where it is less economic for a competitor to steal from a cache than to forage for food (Smith and Reichman, 1984). Scatter hoarding is more beneficial especially when an animal cannot defend its caches from inter- and intra-specific competitors. However, scatter hoarding is more energy-costly than larder hoarding and requires good spatial memory in managing and relocating seeds, whereas larder hoarding usually depends on the ability to defend intruders to avoid the loss of cached food. It is worth mentioning that hoarding behaviors also affect seed fates and seedling establishment (Vander Wall, 1990). Scatter hoarding is essential to seed regeneration of many tree species and results in mutual co-evolution between plants and animals. In contrast, larder hoarding often has negative effects on seedling establishment.

Scatter hoarders are known to create large numbers of caches. For example, a single Clark's nutcracker (*Nucifraga columbiana*) may store up to 100,000 seeds during one autumn season (Vander Wall, 1990). European tits, such as the Crested tit (*Parus critatus)* and Willow tit (*Parus montanus*), may create anywhere from 100,000 to 500,000 individual caches per year (Haftorn, 1956; Brodin, 1994). In mammals, the gray squirrel (*Sciurus carolinensis*) creates thousands of scattered caches in the autumn to use during the winter months (Thompson and Thompson, 1980). The Merriam's kangaroo rat (*Dipodomys merriami*) scatter-hoards extensively and depends on food caches during long periods of drought, which may last up to several years (Leaver, 2000). High memory capacity and unique mechanisms of spatial memory are essential for scatter hoarders to manage and relocate such large numbers of caches.

Indeed, the relationship between spatial memory and scatter hoarding has been demonstrated in a variety of animal species including birds (Shettleworth and Krebs, 1982; Vander Wall, 1982; Kamil and Balda, 1985; Hitchcock and Sherry, 1990) and rodents (Smith and Reichman, 1984; Jacobs and Liman, 1991; Vander Wall, 1991; Jacobs, 1992; Macdonald, 1997). One of the brain areas known to play an important role in spatial memory is the hippocampus (O'keefe and Nadel, 1978; Shettleworth, 2003; Sherry, 2005; Smulders,

*Corresponding author. Tel: +86-10-62554027; fax: +86-10-62565689.
E-mail address: zhangzb@ioz.ac.cn (Z. Zhang).
Abbreviations: ANOVA, analysis of variance; BrdU, 5-bromo-2′-deoxyuridine; DAB, 3′-diaminobenzidine; DG, dentate gyrus; PBS, phosphate buffered solution; PBT, Triton X-100 in 0.1 M phosphate buffered solution; SNK, Student–Neuman–Keul's; SVZ, subventricular zone.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.09.065

2006). For example, chickadees (*Poecile atricapillus*) with hippocampal lesions can still store seeds but cannot remember where they put them (Sherry and Vaccarino, 1989). Hippocampal lesions decrease the performance of chickadees and juncos (*Junco hyemalis*) on a spatial delayed matching-to-food samples task but do not interfere with the performance on a color matching task (Hampton and Shettleworth, 1996). Although not being examined in food hoarding, hippocampal lesions have been shown to impair the performance in traditional spatial memory tests in laboratory rodents (McNaughton et al., 1989; Ferbinteanu et al., 1999; Xavier et al., 1999; Gilbert et al., 2001). While many studies have demonstrated that behavioral performance requiring spatial memory (such as food hoarding) is associated with hippocampal volume (Krebs et al., 1989, 1990; Sherry et al., 1989; Healy and Krebs, 1992, 1996; Brodin and Lundborg, 2003), other studies have shown inconsistent results (Volman et al., 1997; Brodin and Lundborg, 2003; Brodin and Grubb, 2005). It has been suggested that volume measurements are highly sensitive to various factors and rely on many subtle changes in the structure of the brain (Roth et al., 2010). Therefore, it may be important to focus on more specific and detailed analyses of the hippocampus, such as assessing neurogenesis (Pravosudov and Smulders, 2010; Roth et al., 2010). Interestingly, recent studies have shown that in rodents under laboratory conditions, newly proliferated cells in the adult hippocampus are associated with enhanced performance in spatial learning and memory tasks (Gould et al., 1999; Lemaire et al., 2000; Snyder et al., 2005; Leuner et al., 2006). In birds, hippocampal neurogenesis is associated with seasonal changes in food hoarding (Barnea and Nottebohm, 1994) and species differences in food-hoarding behavior (Hoshooley and Sherry, 2007). However, detailed evidence is still lacking for the interactions between food hoarding and adult hippocampal cell proliferation and survival in mammalian species.

The Siberian chipmunk (*Tamias sibiricus*) is a diurnal, solitary rodent species that occurs in the Xiaoxing'anling Mountain area (Jin et al., 2004). Studies conducted in the field as well as in semi-natural enclosures have shown that both male and female chipmunks can scatter hoard and retrieve seeds (Jiao et al., 2011; Yi et al., 2011). In a recent study, Siberian chipmunks were found to prune the radicle of germinating white oak (*Quercus mongolica*) acorns before caching and to re-prune them after scatter hoarding to postpone acorn germination and radicle growth, indicating that they can actively manage their hoarded seeds (Yang et al., 2012). It has been suggested that this species is the main agent of seed predation, dispersal, and hoarding of several local tree species (Yi et al., 2008; Yi and Zhang, 2008). In the present study, we took advantage of this animal model to compare the seed-hoarding behavior of males and females in a semi-natural enclosure and to see whether this behavior correlated to changes in hippocampal cell proliferation and survival, which in turn, may play a role in hoarding behavior.

EXPERIMENTAL PROCEDURES

Subjects and housing conditions

Forty male and 19 female Siberian chipmunks (*T. sibiricus*) were captured using live-traps during the autumn of 2010 (experiment 1) and 2012 (experiment 2) in the forest of the Xiaoxing'anling mountain area (elevation at about 750 m, 45° 58′N, 129° 08′E) in China. The subjects were housed individually in cages made with a steel frame and wire mesh (90 (H) × 40 (W) × 50 (L) cm). They were housed at ambient room temperature (15–20 °C during the day and 10–15 °C at night) under natural photoperiod (14L:10D h). They were provided with carrots, peanuts, tree seeds (Korean pines, *Pinus koraiensis* and white oak, *Q. mongolica*) collected from the local forests, and water *ad libitum*. All procedures complied with the guidelines for the animal use and care as stipulated by the Institute of Zoology, Chinese Academy of Sciences.

Enclosure

The semi-natural enclosures (10 × 10 m) were established in an open and flat area. Vegetation coverage within each enclosure was more than 95% with an average height of 20 cm. The enclosure walls were built using bricks with a smooth surface and the walls extended approximately 2.5 m above and 0.5 m below the ground to prevent subjects from escaping. Similar enclosures have been successfully used in previous studies investigating hoarding behavior of chipmunks (Vander Wall et al., 2008; Yi et al., 2011; Yang et al., 2012) and other rodent species (Chang et al., 2009, 2010). An artificial nest constructed using bricks (20 × 15 × 30 cm) with a plastic water bowl were placed in a corner of the enclosure to allow animals to rest and drink freely. An area of 0.5 × 0.5 m at the center of each enclosure was designated as the seed station. Seeds of Korean pines were used in the present study. The seeds were tagged using an established method (Yi and Zhang, 2008). Briefly, a hole with a 0.3-mm diameter was drilled through the husk far from the embryo of each seed to avoid damaging the cotyledon or the embryo. A flexible plastic tag (2.5 × 3.5 cm, < 0.3 g) was tied through the hole of each seed using a thin 10-cm long steel thread. All tags were numbered to allow seeds to be easily relocated and identified. This method has been used successfully in previous studies without having noticeable effects on seed removal and hoarding behaviors displayed by rodents (Xiao et al., 2006; Gómez et al., 2008).

Experimental procedure

Two experiments were conducted using the semi-natural enclosures. Experiment 1 was designed to test individual and sex differences in the expression of hoarding behavior and its effects on cell proliferation in the hippocampus. Thirty-nine field-captured adult Siberian chipmunks (20 males and 19 females) were weighed and then randomly assigned into one of two treatment groups. In the control group (eight males and eight