

# Dominance-Related Changes in Spatial Memory Are Associated with Changes in Hippocampal Cell Proliferation Rates in Mountain Chickadees

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**ABSTRACT:** It is well established that spatial memory is dependent on the hippocampus in both mammals and birds. As memory capacity can fluctuate on a temporal basis, it is important to understand the mechanisms mediating such changes. It is known that early memory-dependent experiences in young animals result in hippocampal enlargement and in increased neurogenesis, including cell proliferation and neuron survival. It is less clear, however, whether temporal changes in spatial memory are also associated with changes in hippocampal anatomy and cell proliferation in fully grown and experienced adult animals. In a previous study, we experimentally demonstrated that socially subordinate mountain chickadees (*Poecile gambeli*) showed inferior spatial memory performance compared to their dominant group mates, in the absence of significant differences in baseline corticosterone levels. Here we investigated whether these differences in memory between dominant and subordinate birds were associated with

changes in the hippocampus. Following memory tests, chickadees were injected with 5-bromo-2'-deoxyuridine to label dividing cells and sacrificed 2 days after the injections. We found no significant differences in volume or the total number of neurons in the hippocampal formation between dominant and subordinate chickadees, but subordinate birds had significantly lower cell proliferation rates in the ventricular zone adjacent to both the hippocampus and mesopallium compared to the dominants. Individuals, which performed better on spatial memory tests tended to have higher levels of cell proliferation. These results suggest that social status can affect cell proliferation rates in the ventricular zone and support the hypothesis that neurogenesis might be involved in memory function in adult animals. © 2004

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**Keywords:** spatial memory; hippocampus; cell proliferation; neurogenesis; mountain chickadee

## INTRODUCTION

It is widely recognized that the hippocampus plays an important role in spatial memory in both mammals and birds, and the relationship between spatial memory and the hippocampus has been a topic of many

studies (Jacobs, 1995; Krebs et al., 1996). In young and inexperienced animals, it has been demonstrated that early memory-based experiences result in increased hippocampal volume with more neurons and increased cell proliferation rates (Clayton and Krebs, 1994; Clayton, 1995, 2001; Patel et al., 1997). These studies showed that a few experiences suffice to trigger hippocampal growth in young birds, but further experience has no additional effect (Clayton, 1995). Thus, an important remaining question is which processes in the hippocampus mediate temporary changes in spatial memory in adult, fully experienced animals.

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Hippocampal neurogenesis, a process of neuron proliferation and survival in adult brains of both mammals and birds, has been implicated in mediating memory and learning in adult animals (Barnea and Nottebohm, 1994; Gould et al., 1999; Kempermann, 2002; Nottebohm, 2002; Prickaerts et al., 2004). Despite the widespread belief that the link between hippocampal neurogenesis and spatial memory has been well established in birds (Drapeau et al., 2003), there is, in fact, only a single study in which such a link has been proposed (Barnea and Nottebohm, 1994). In that pioneering study, Barnea and Nottebohm (1994) demonstrated that neuron recruitment rates varied seasonally in a food-caching bird, the black-capped chickadee (*Poecile atricapillus*), with the highest rates in October when food caching was supposedly at its peak. The positive association between food caching intensity and neuron recruitment rates suggested that neurogenesis might be involved in spatial memory function, because these birds rely on spatial memory, at least in part, to retrieve their cached food. It is important to note that Barnea and Nottebohm (1994) measured the neuron recruitment rates and did not distinguish which component of this process, cell proliferation or neuron survival, was responsible for the seasonal variation. A critical assumption of this study is that food-caching birds also have the best spatial memory during the most intensive food-caching period (Barnea and Nottebohm, 1994). Whereas this finding is ground breaking, it does not establish a solid link between memory and neurogenesis (cell proliferation and/or neuron survival) for several reasons: (a) individual birds vary in their caching behavior, yet nothing was known about the individuals used in the analyses; (b) there are no good data on seasonality of natural food caching activity of wild black-capped chickadees, or whether an additional caching peak occurs during early spring, as in European parids (Haftorn, 1956; Pravosudov, 1985); (c) it is not known if birds have better memory during the peak of long-term caching, or during cache retrieval that might occur much later (Pravosudov and Grubb, 1997). Barnea and Nottebohm (1994) assumed that caches are retrieved shortly after storing, but there are no data available on timing of cache-retrieval in black-capped chickadees. Some data showing that caches are recovered shortly after storing come from studies of marsh tits (*Parus palustris*) with artificial feeders in fairly southern latitudes (Sherry, 1989). But there is evidence that cache retrieval in parids might occur several months after caching (Sherry, 1989; Pravosudov and Grubb, 1997). Parids also often re-cache many previously made caches in new locations for future use (Sherry, 1989; Pravosudov and Grubb,

1997). Because the studies reporting short-term retrieval cannot account for recaching, we simply do not have much data on when naturally made caches are retrieved for the final use. Therefore, the relationship between memory performance and neurogenesis (including both cell proliferation and neuron survival) in individual birds remains unclear, and we cannot be sure that neurogenesis and spatial memory performance are indeed linked based on Barnea and Nottebohm's (1994) study.

Smulders et al., (1995, 2000) also reported that the volume of the hippocampus and the number of hippocampal neurons varied seasonally in black-capped chickadees, with the largest hippocampus occurring in October, supposedly the most intensive food caching period. They argued that food-caching birds increase their hippocampal volume by adding more neurons when demands for spatial memory are highest and then reduce it when memory demands decrease. Lacking specific information regarding memory and food caching activity, however, it remains impossible to draw any conclusions about the relationship between hippocampal volume and memory. To determine whether changes in hippocampal cell proliferation rates, hippocampal volume, and/or hippocampal neuron numbers are associated with changes in spatial memory we need to compare the brains of animals in which memory performance has been established previously.

In a previous behavioral experiment, we tested randomly matched dominant-subordinate pairs of mountain chickadees and found that in comparison with dominant birds, subordinates (1) showed significantly less efficient memory-based cache retrieval, (2) performed significantly worse on a spatial memory task, but not on a nonspatial memory task, and (3) had baseline plasma corticosterone levels indistinguishable from dominant chickadees (Pravosudov et al., 2003). Here we investigated the brains of these birds and specifically tested whether differences in spatial memory found between dominants and subordinates were associated with differences in (a) relative hippocampal volume, (b) the total number of hippocampal neurons, and (c) hippocampal cell proliferation rates. We also tested whether individual performance on spatial memory tests correlated with individual levels of cell proliferation in the hippocampus.

## METHODS

Twenty-four mountain chickadees were trapped near Sage Hen, Tahoe National Forest, CA, 14–18 November, 2001, using mist nets near feeders baited with sunflower seeds. All

birds were at least 4–5 months old, and all most likely had extensive experience in food caching in the wild, as mountain chickadees cache quite intensively in September and October (pers. obs.). All birds were transported into the laboratory at the University of California Davis, placed individually in cages (60 × 42 × 60 cm) and maintained on a 8:16-h light:dark cycle at approximately 20°C throughout the entire experiment. They were given water with vitamins *ad libitum* and fed a mixture of pine nuts, shelled sunflower seeds, crushed peanuts, and mealworms. We used Zoogen, Inc. (Davis, CA) for DNA sexing using red blood cells and then we formed male–male and female–female pairs matched by wing length and placed each pair into adjacent cages (Pravosudov et al., 2003). Pairs of adjacent cages were separated by a metal partition that could be removed, making one 120 × 42 × 60 cm cage. All cages were individually connected to an experimental room (325 × 218 × 312 cm), which was used for memory experiments. We determined social status of birds by allowing each pair into the experimental room and observing their behavior through one-way glass. We assigned dominance rank by recording either aggressive interactions or passive displacements (Pravosudov et al., 2003). After dominance ranks we determined, we allowed birds to interact within their adjacent cages by removing the partition. To avoid excessive aggression, we started by allowing the birds to interact for only 2 h per day, gradually increasing this time to 6.5 h per day, from January 4 to January 21, 2002. We tested spatial memory performance of individual chickadees on a cache retrieval task and on spatial and nonspatial versions of a one-trial associative learning task between January 21 and March 2, 2002 (Pravosudov et al., 2003). On March 2, 2002, we injected all birds once with a 50 mg/kg 5-bromo-2'-deoxyuridine (BrdU) solution and then sacrificed them for the brain analyses 2 days later. All birds had 57 days of dominance interactions before the BrdU injection.

Birds were anesthetized (0.03 mL per bird of 50 mg/mL Nembutal–sodium solution) and perfused transcardially with 100 mL of phosphate buffer (0.1 M PO<sub>4</sub>) followed by 100 mL of 4% paraformaldehyde in phosphate buffer. After perfusion, birds were decapitated and their brain (within the skull) was placed in 4% paraformaldehyde for 1 week. We then removed the brains from the skull and postfixed them in 4% paraformaldehyde for an additional week, after which all brains were cryoprotected in a 30% sucrose solution, frozen, and kept at –20° until processing. We cut coronal sections at 40 μm on a sliding, freezing microtome, and collected every section in phosphate-buffered saline (PBS, 0.1 M, pH 7.4). Every fourth section was mounted onto gelatin-coated slides, Nissl-stained with thionin, and coverslipped with Permount for measurements of the hippocampal formation volume and the total number of hippocampal neurons. Sections for BrdU immunohistochemistry (also every fourth section) were stored at –20° in a cryoprotectant solution until processing.

## Volumetric Measurements and Neuron Counts

We used StereoInvestigator software (version 3.15a, Microbrightfield, Colchester, VT) for all stereological measurements. To measure the volume of the hippocampal formation and the telencephalon on Nissl-stained slides, we used the Cavalieri principle (Gundersen and Jensen, 1987; West and Gundersen, 1990), employed successfully before with chickadees (Pravosudov and Clayton, 2002; Pravosudov et al., 2002). We determined the boundaries of the hippocampal formation as described in Krebs et al. (1989). We used a total of 14 to 18 sections per bird for hippocampal measurements (480 μm apart, 1 in 12 sections). The first section was chosen randomly from the first four sections containing the hippocampus, according to the Cavalieri principle (Gundersen and Jensen, 1987; West and Gundersen, 1990). This sampling method has proven to be most efficient in chickadees by providing low variance of individual estimates (Pravosudov and Clayton, 2002; Pravosudov et al., 2002). We used a total of 14 to 18 sections (640 μm apart, 1 in 16 sections), with the first section chosen randomly from the first four sections to measure the volume of the telencephalon. All sections were coded prior to the analyses, so all measurements were performed blind with respect to bird identity and experimental design.

To calculate the total number of hippocampal neurons we used the optical fractionator method (West et al., 1991) on the same Nissl-stained sections that were used for the volumetric measurements. This method allows estimation of the absolute number of neurons independently from the estimates for the hippocampal volume (West et al., 1991). We used a ×100 Neofluar oil objective on a Nikon Optiphot microscope linked to the PC-based StereoInvestigator system. We used the right half of the hippocampal formation to estimate the number of neurons and then doubled that number to get the total number of hippocampal neurons because there were no significant size differences between the right and left halves of the hippocampal formation (Sign test,  $z = 0.20$ ,  $p = 0.84$ ).

To evaluate the precision of our sampling scheme, we calculated coefficients of error for both volumetric and neuron count measurements. We calculated the relative variance of individual estimates (CE) by using the quadratic approximation (Gundersen and Jensen, 1987) and taking into account the Nugget effect (West et al., 1996). The variance of estimates was low for telencephalon volume (mean CE = 0.01, range 0.01–0.02), hippocampal volume (mean CE = 0.02, range 0.01–0.03), and neuron counts (mean CE = 0.06, range 0.05–0.09), which supports the reliability of our measurements and our sampling scheme.

## BrdU Labeling

We followed the procedure described in Lavenex et al. (2000a). We used free-floating sections at room temperature. We first rinsed the sections in 0.1 M PBS (4 × 10 min), then incubated in 2 N HCl for 1 h, rinsed in 0.1 M borate

buffer (pH 8.5) for 15 min, then in PBS ( $2 \times 10$  min), and finally incubated in  $1 \mu\text{g/mL}$  proteinase K in PBS for 30 min. All sections were then washed in PBS ( $3 \times 5$  min), placed in 0.5% hydrogen peroxide in PBS for 30 min, washed again in PBS ( $3 \times 5$  min), and incubated in a blocking solution of 0.1 M PBS with 1 mg/mL bovine serum albumin (BSA), IgG (secondary antibody, Vectastain ABC Kit, Vector Laboratories) and 0.3% Triton X-100 for 1 h. We then incubated all sections overnight in primary anti-BrdU antibody (Beckton Dickinson; 1:500 in PBS/BSA/Triton), rinsed them in PBS ( $3 \times 5$  min), and incubated in antimouse biotinylated secondary antibody (Vectastain ABC kit) in 0.1 M PBS/BSA/Triton. All sections were then washed in PBS ( $3 \times 5$  min), incubated in ABC reagent (Vectastain ABC kit) in PBS/BSA/Triton, rinsed in PBS ( $3 \times 5$  min), and treated for 10 min in 0.5 mg/mL 3,3'-diaminobenzidine mixed in 0.05 M Tris buffer, pH 7.4 with 0.01%  $\text{H}_2\text{O}_2$ . Finally, all sections were washed in PBS ( $3 \times 5$  min), Nissl stained in thionin for 1.5 min, mounted on gelatin-coated slides and coverslipped with Permount.

We counted BrdU-labeled cells in the ventricular zone (VZ) adjacent to the hippocampus (HP) and to the mesopallium (M, formerly hyperstriatum ventrale) following Patel et al. (1997). No labeled cells were detected anywhere else in the hippocampus. All labeled cells were counted in the ventricular zone of both right and left hippocampal halves throughout the entire thickness of the section on every 12th section ( $480 \mu\text{m}$  apart) using a  $\times 100$  Neofluar oil objective on a Nikon Optiphot microscope linked to the PC-based StereoInvestigator system.

## Statistical Analyses

We used analyses of variance and covariance for all brain data. All data (raw or log-transformed) met the assumptions for parametric statistical procedures. For analyses of relative hippocampal volume, total number of hippocampal neurons and number of BrdU-labeled cells, we used the volume of the telencephalon without the hippocampal formation as a covariate. We used a standard power analysis to calculate the power of nonsignificant tests to detect a 30% difference between the compared groups as a biologically relevant difference based on published reports (Barnea and Nottebohm, 1994; Smulders et al., 1995; MacDougall-Shackleton et al., 2003).

## RESULTS

There were no significant differences between dominants and subordinates in either wing length,  $F(1, 20) = 0.18$ ,  $p = 0.67$ , or body mass,  $F(1, 20) = 0.98$ ,  $p = 0.33$ , at the time the birds were sacrificed.

The volume of telencephalon minus the hippocampal formation did not differ significantly between dominant and subordinate chickadees,  $F(1, 21) = 0.01$ ,  $p = 0.98$ . There was no significant difference

between dominants and subordinates in the absolute volume of the hippocampal formation (Fig. 2);  $F(1, 22) = 0.92$ ,  $p = 0.35$ . Relative volume of the hippocampal formation (controlled for the volume of the rest of the telencephalon) was also not significantly different between dominants and subordinates,  $F(1, 20) = 1.21$ ,  $p = 0.29$ , while it positively and significantly correlated with the telencephalon volume,  $F(1, 20) = 7.34$ ,  $p = 0.01$ . Statistical power to detect a 30% difference between the groups in the hippocampal volume was 0.99.

The total number of the hippocampal neurons did not differ significantly between dominant and subordinate chickadees (Fig. 3);  $F(1, 21) = 0.13$ ,  $p = 0.72$ ; statistical power to detect a 30% difference between the groups was 0.99.

All BrdU-labeled cells were located near the ventricular zone (Fig. 1). Compared to dominants, subordinate chickadees had significantly fewer BrdU-labeled cells in the ventricular zone adjacent to the hippocampus (Fig. 4);  $F(1, 15) = 7.12$ ,  $p = 0.02$ , and adjacent to the mesopallium,  $F(1, 15) = 7.14$ ,  $p = 0.02$ . The number of all counted BrdU-labeled cells in the ventricular zone was also significantly lower in subordinate birds,  $F(1, 15) = 7.33$ ,  $p = 0.02$ . We also analyzed cell proliferation rates in relation to individual birds memory performance presented in Pravosudov et al. (2003) across both groups. Compared to individuals with lower cell counts, birds with more BrdU-labeled cells in the ventricular zone adjacent to the hippocampus demonstrated significantly better memory performance measured as fewer number of sites inspected in order to find the first two caches on a cache-retrieval task [Pravosudov et al., 2003; linear regression,  $t(12) = 2.91$ ,  $p = 0.01$ ; Fig. 5]. The same significant correlation was also present between memory performance on a cache-retrieval task and cell proliferation rates in the ventricular zone adjacent to the mesopallium [linear regression,  $t(12) = 2.78$ ,  $p = 0.02$ ; Fig. 6]. In contrast, there was no significant relationship between spatial memory performance on a cache-retrieval task and either hippocampal volume [linear regression,  $t(17) = 0.31$ ,  $p = 0.76$ ; Fig. 7] or the total number of hippocampal neurons [linear regression,  $t(17) = 0.66$ ,  $p = 0.52$ ; Fig. 8].

There were no significant differences between males and females in any of the measured brain parameters ( $p > 0.12$ ).

## DISCUSSION

Our study produced the following results: (a) individual differences in spatial memory that were associated

with dominance status, were not related to differences in either hippocampal volume or the total number of hippocampal neurons; (b) subordinate chickadees had significantly lower cell proliferation rates in the ventricular zone (both adjacent to the hippocampus and to the mesopallium) and inferior spatial memory performance compared to their dominant group mates; (c) cell proliferation rates in the ventricular zone of individual birds correlated with individual levels of spatial memory performance.

Experimental birds consisted of individuals that were at least 4–5 months old but we did not differentiate between younger and older birds. Whereas in natural parid flocks older birds seem to have a higher social status than younger ones, this relationship seems to derive from the fact that older birds almost always have a prior residency in such groups, while Koivula et al. (1993) experimentally demonstrated that age per se has no effect on dominance status. Additional evidence that age has no direct effect on dominance status comes from a study showing that plumage coloration can be used to predict social status but not age in black-capped chickadees (Mennill et al., 2003). We formed our pairs randomly from unfamiliar birds, so relative age of these birds should have no bearing on the determination of their social status. Developmental studies also suggest that young food caching birds need little memory-based experience to develop the hippocampus to its adult size (Clayton, 1995, 2001), and all our birds most likely had extensive food-caching experience during August–October prior to their capture (Pravosudov, pers. obser.).

Our study demonstrated that dominance-induced changes in spatial memory were clearly associated with changes in cell proliferation rates in the ventricular zone adjacent both to the hippocampus and to the mesopallium. At the same time, performance on a nonspatial memory task did not differ significantly between dominant and subordinate chickadees (Pravosudov et al., 2003). These results suggest that (1) social interactions may have a direct effect on cell proliferation rates in the ventricular zone, and (2) neurogenesis, as a process may be involved in hippocampus-dependent spatial memory mediation in fully developed birds. Similar findings have been reported for mammals (Prickaerts et al., 2004) and Barnea and Nottebohm (1994) also proposed that hippocampal neurogenesis might be linked to spatial memory function in birds using individuals with unknown history of memory performance. Our study investigated cell proliferation rates in individuals for which memory performance was established, and it appears to be the first true demonstration that specific changes in spatial memory can be associated with

changes in hippocampal cell proliferation rates in birds. Alternatively, because we found that dominance-related differences in cell proliferation rates were not specific to the ventricular zone adjacent only to the hippocampus and subordinates had lower cell proliferation rates in the ventricular zone adjacent both to the hippocampus and to the mesopallium, it remains possible that these differences were unrelated to changes in spatial memory. Also, in our study we could not determine whether the labeled cells were neurons because of a short time span between BrdU injection and the time the birds were sacrificed. Thus, we cannot predict how many of the new cells would become functional hippocampal neurons. However, we assume that the number of new neurons is likely to be proportional to the number of proliferating cells.

We used only a single low-dose (50 mg/kg) BrdU injection in our study. Gould and Cross (2002) suggested that such a low dose might label only a fraction of the dividing cells, and higher doses are necessary to label all dividing cells. Rakic (2002), on the other hand, suggested that high and multiple doses of BrdU might produce numerous artifacts and result in overestimation of proliferating cell numbers. There are also some indications that high BrdU doses could be detrimental to animals' health (Drapeau et al., 2003). Even if our study underestimated the total number of proliferating cells, we used the same low BrdU dose for both dominants and subordinates, and thus our results provide a relative comparison between them. It remains possible that dominance status influenced the rate of BrdU uptake (Gould and Gross, 2002), which could bias our results. Gould and Gross (2002) argued that many factors, such as stress and hormone manipulations, which could change the blood flow or blood–brain barrier permeability could affect BrdU uptake. In our study, dominants and subordinates did not significantly differ in baseline corticosterone levels (Pravosudov et al., 2003), and there was a significant correlation between individual memory performance and the number of BrdU-labeled cells, so we think it is unlikely that BrdU uptake was affected by dominance interactions, although we cannot completely rule it out.

Mammalian studies have demonstrated repeatedly that stress has a negative effect on hippocampal neurogenesis (Gould and Tanapat, 1999; Fuch et al., 2001; Tanapat et al., 2001; Czeh et al., 2002). Tanapat et al. (2001) showed that a simple exposure to a predator's odor caused a reduction in neurogenesis rates in rats. Whereas some of the mammalian studies induced stress through treatments not naturally experienced by wild animals, our experiment explored the effects of dominance relationships commonly experi-

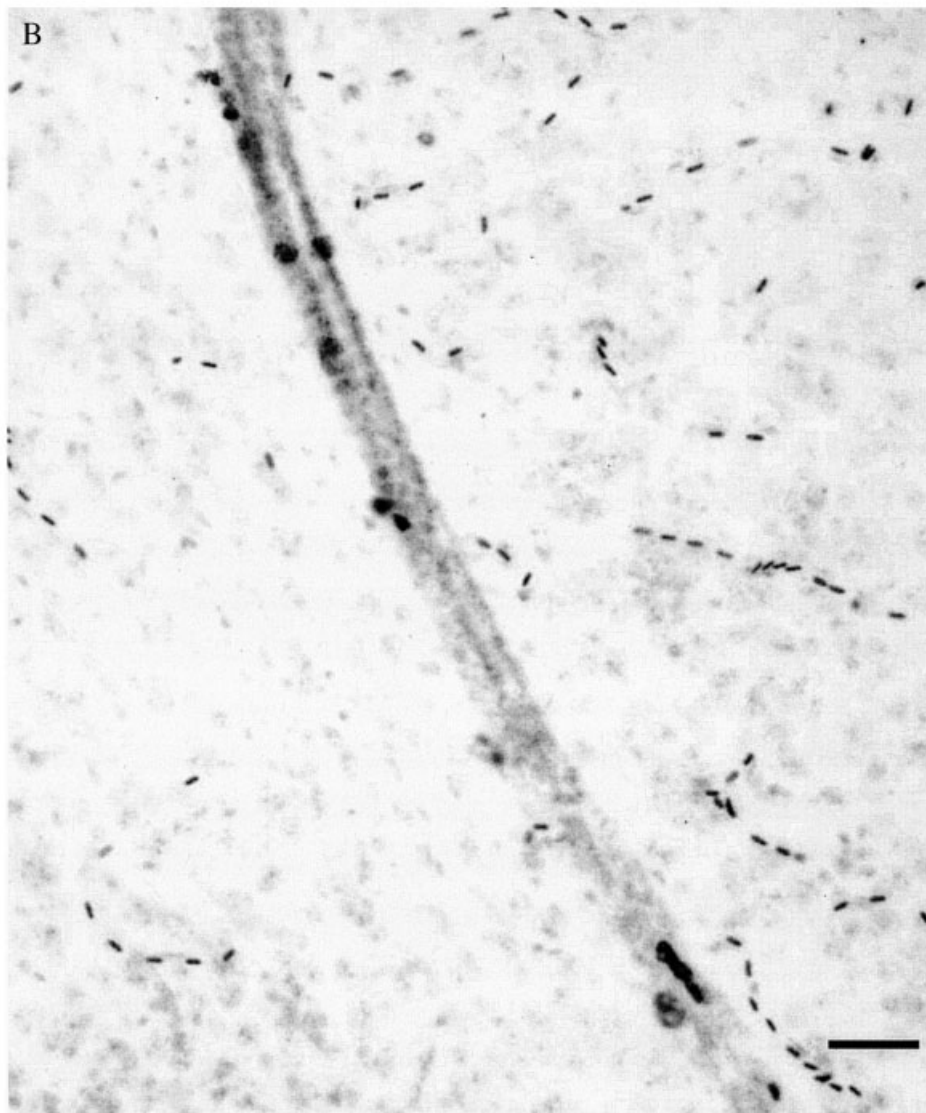
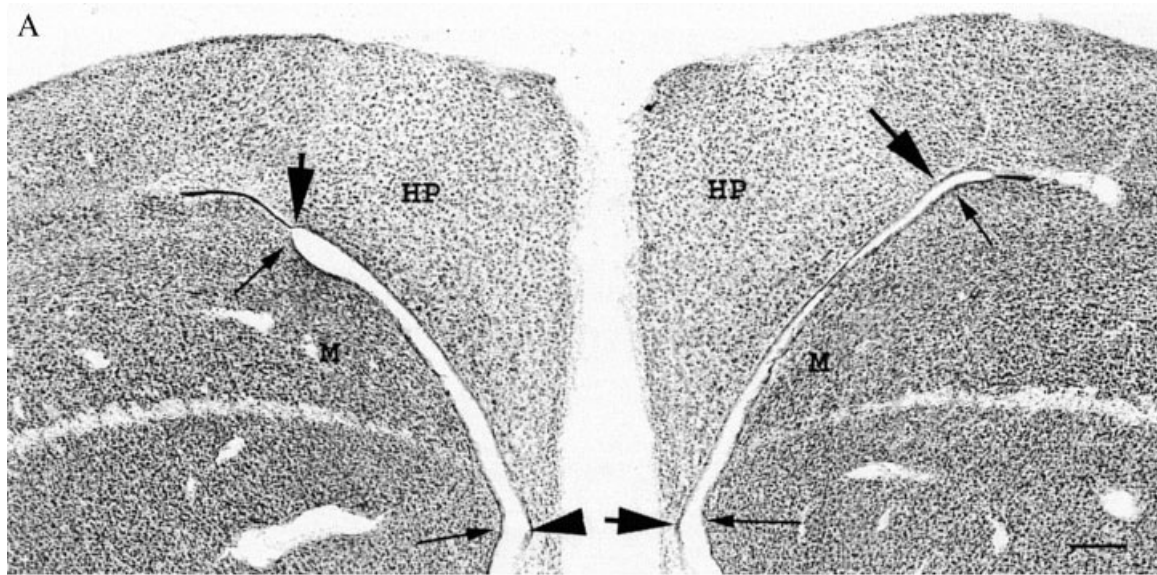
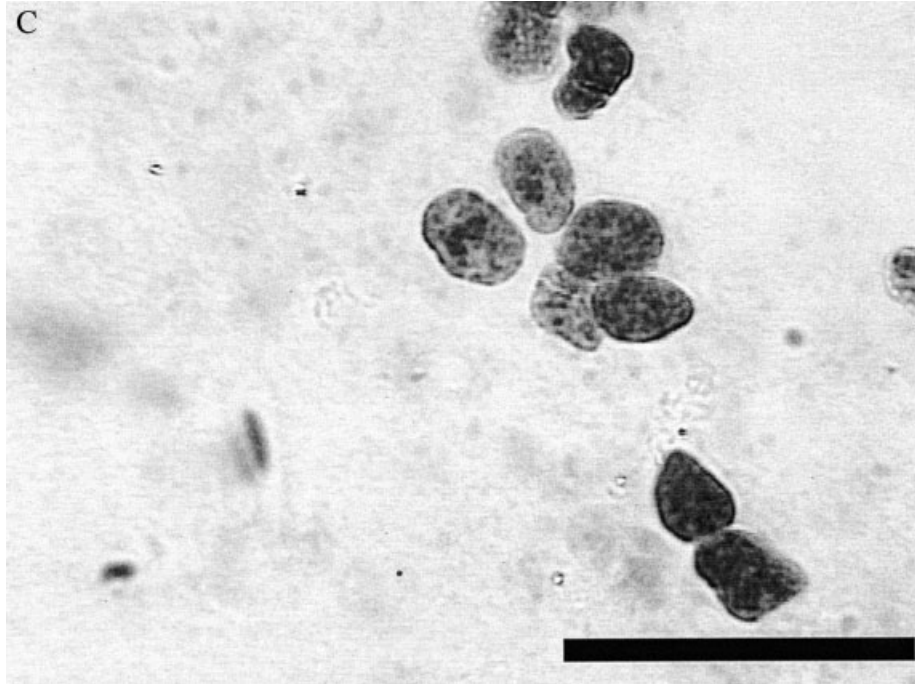


Figure 1



**Figure 1 (Continued)**

enced by food-caching parids during the winter. Like the mammalian studies, our findings suggest that stress of social subordination negatively affects cell proliferation rates. Stress in animals is usually associated with elevated levels of glucocorticoid hormones, and it is assumed that elevated glucocorticoids directly impact neurogenesis rates (Gould and Tanapat, 1999; Tanapat et al., 2001). The fact that social subordination was not associated with elevation in baseline corticosterone levels in our study (Pravosudov et al., 2003) suggests that elevation of glucocorticoid hormones may not be necessary for social experiences to affect the hippocampal cell proliferation process.

It has been suggested that neurogenesis might be involved in learning and memory (Barnea and Nottebohm, 1994, Gould et al., 1999; Shors et al., 2001; Kempermann, 2002; Nottebohm, 2002). Our study also supports this hypothesis and shows that the relationship between neurogenesis and memory is similar

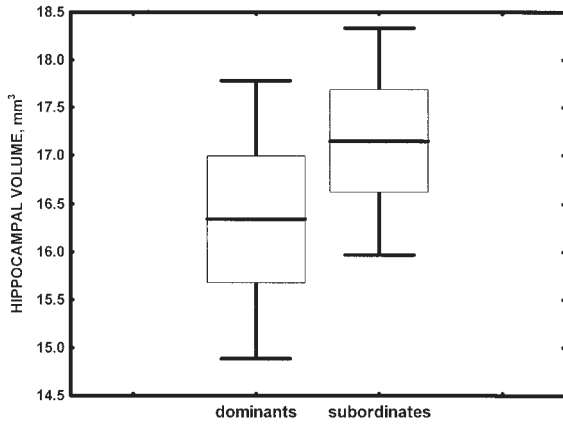
in both mammals and birds. Although we only measured cell proliferation rates and not the survival or fate of these new cells, our conclusions still concern neurogenesis as a process, which consists of both cell proliferation and neuron survival (Prickaerts et al., 2004). Most of the evidence for the relationship between neurogenesis and learning comes from correlational studies, which cannot establish a causal link between the two (Banta Lavenex et al., 2001). Although our results also do not demonstrate a causal relationship between hippocampal cell proliferation and memory, they do provide support to the idea that neurogenesis and learning might be linked in both mammals and birds.

In our study, dominance-induced changes in spatial memory were not associated with changes in either hippocampal volume or the total number of hippocampal neurons. These results contradict the hypothesis of Smulders et al. (1995, 2000) that food-caching birds increase their hippocampal volume by adding more neurons when memory demands are the highest.

Numerous other studies also do not support the findings of Smulders et al. (1995, 2000). Barnea and Nottebohm (1994) did not find seasonal variation in the total number of hippocampal neurons in the same species while reporting seasonal variation in neuronal recruitment rates. Hoshooley and Sherry (2004) found

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**Figure 1** (A) Photograph of a coronal section showing the ventricular zone area adjacent to the hippocampus (between the large arrowheads) and the mesopallium (between the small arrowheads). Scale bar = 250  $\mu\text{m}$ . (B) Area of the ventricular zone with BrdU-labeled cells. Scale bar = 25  $\mu\text{m}$ . (C) BrdU-labeled cells. Scale bar = 25  $\mu\text{m}$ .

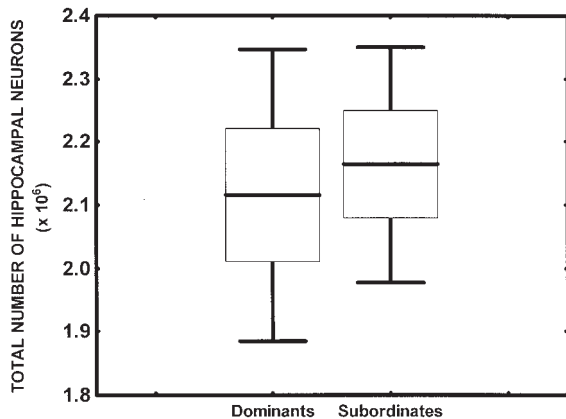


**Figure 2** Absolute hippocampal volume of dominant and subordinate mountain chickadees. Bars represent S.E. and whiskers represent 95% confidence interval.

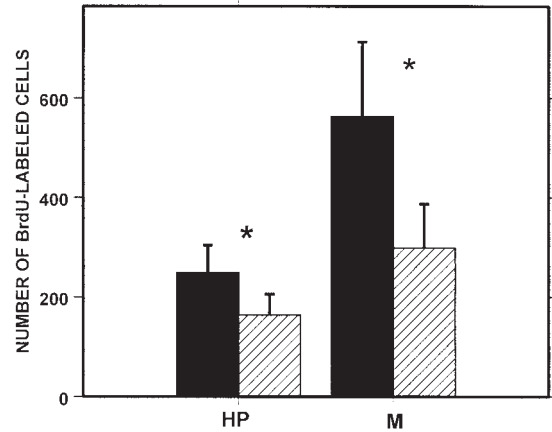
no significant seasonal variation in the hippocampal volume or neuron numbers in black-capped chickadees, again the same species as in the Smulders et al. (1995, 2000) studies. Food-caching gray squirrels (*Sciurus carolinensis*), while exhibiting clear seasonal variation in caching patterns, also do not appear to have seasonal variation in the hippocampal volume or neuron number (Lavenex et al., 2000a,b).

As seasonal changes in the brain could be associated with a variation in the photoperiod, it is reasonable to predict that such changes, if any, could be triggered by the photoperiod, yet several studies showed no effect on the hippocampal volume (Krebs et al., 1995; MacDougall-Shackleton et al., 2003).

Results reported here and our previous study, which showed that spatial memory performance was affected by unpredictable food supply (Pravosudov

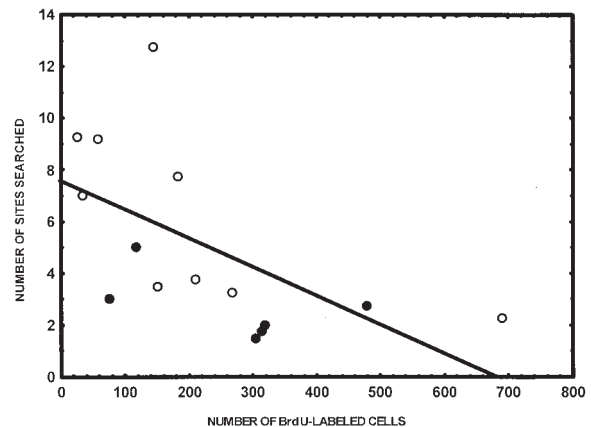


**Figure 3** Total number of hippocampal neurons in dominant and subordinate mountain chickadees. Bars represent S.E. and whiskers represent 95% confidence interval.

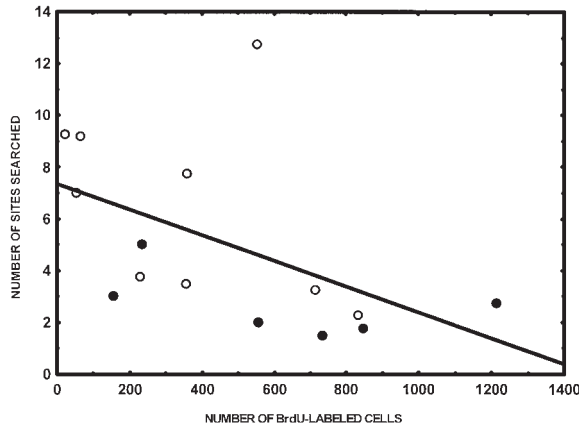


**Figure 4** The number of counted BrdU-labeled cells in the ventricular zone of the hippocampus (HP) and the mesopallium (M) in dominant (solid bars) and subordinate (hatched bars) chickadees. \* $p < 0.05$ .

and Clayton, 2001; Pravosudov et al., 2002), demonstrate that changes in spatial memory were not associated with variation in hippocampal volume or the total number of hippocampal neurons. Finally, Cristol (1996) showed that hippocampal volume in adult willow tits (*Parus montanus*) was also not affected by a 1-month absence of caching experience. Altogether, it appears that hippocampal volume or the total number of neurons do not change in response to caching experience or variation in spatial memory in fully grown animals. Instead, alternative mechanisms, such as the rate of cell turnover, appear to be involved in mediation of temporary changes in spatial memory.

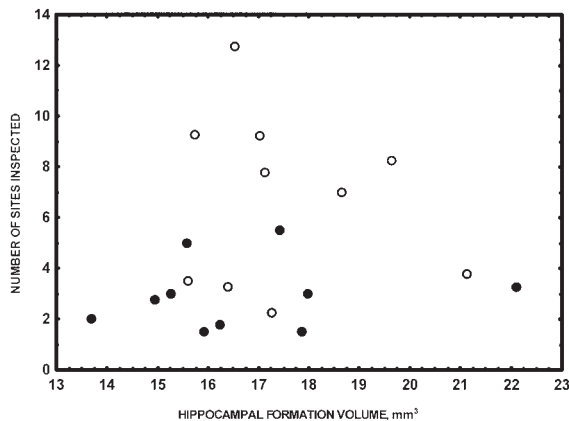


**Figure 5** The relationship between spatial memory performance on a cache-recovery task (measured as the number of sites inspected in order to recover first two caches; Pravosudov et al., 2003) and cell proliferation rates in the ventricular zone adjacent to the hippocampus. Dominants = filled circles; subordinates = open circles.

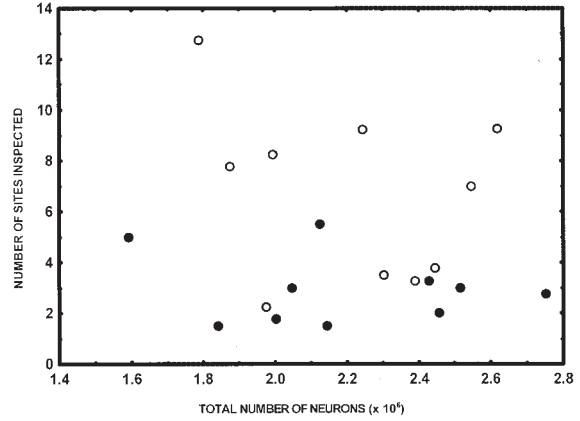


**Figure 6** The relationship between spatial memory performance on a cache-recovery task (measured as the number of sites inspected in order to recover first two caches; Pravosudov et al., 2003) and cell proliferation rates in the ventricular zone adjacent to the mesopallium. Dominants = filled circles; subordinates = open circles.

Interestingly, two other studies failed to demonstrate significant seasonal variation in cell proliferation rates (Lavenex et al., 2000a; Hoshoooley and Sherry, 2004) or in apoptosis (Hoshoooley and Sherry, 2004) in food-caching gray squirrels and black-capped chickadees. Furthermore, Hoshoooley and Sherry's (2004) results suggest that seasonal variation in neuron recruitment rates reported in Barnea and Nottebohm (1994) either stemmed specifically from seasonal variation in neuron survival rates or that the seasonal trend in neurogenesis suggested by Barnea and Nottebohm (1994) is not typical for food-caching animals. In any case, the



**Figure 7** The relationship between spatial memory performance on a cache-recovery task (measured as the number of sites inspected in order to recover first two caches; Pravosudov et al., 2003) and hippocampal volume. Dominants = filled circles; subordinates = open circles.



**Figure 8** The relationship between spatial memory performance on a cache-recovery task (measured as the number of sites inspected in order to recover first two caches; Pravosudov et al., 2003) and the total number of hippocampal neurons. Dominants = filled circles; subordinates = open circles.

significance of seasonal variation in neurogenesis in food-caching birds remains unclear unless we can confirm that memory capacity changes along with seasonal caching rates.

Our study supports the hypothesis that hippocampal neurogenesis might be involved in mediating spatial, hippocampal-dependent memory in birds. We showed that social dominance relationships could affect, specifically, cell proliferation rates in the ventricular zone without influencing the volume or the total number of neurons, and that levels of individual performances on spatial memory tests were related to individual levels of cell proliferation in the ventricular zone. It remains to be established whether dominance status can affect hippocampal neuron survival rates and apoptosis to better understand the regulation and function of neuronal replacement in the adult brain.

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## REFERENCES

- Banta Lavenex P, Lavenex P, Clayton NS. 2001. Comparative studies of postnatal neurogenesis and learning: a critical review. *Avian Poultry Biol Rev* 12:103–125.
- Barnea A, Nottebohm F. 1994. Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proc Natl Acad Sci USA* 91:11217–11221.
- Clayton NS. 1995. The neuroethological development of food-storing memory: a case of use it or lose it! *Behav Brain Res* 70:95–101.
- Clayton NS. 2001. Hippocampal growth and maintenance depends on food-caching experience in juvenile mountain chickadees (*Poecile gambeli*). *Behav Neurosci* 115:614–625.
- Clayton NS, Krebs JR. 1994. Hippocampal growth and attrition in birds affected by experience. *Proc Natl Acad Sci USA* 91:7410–7414.
- Cristol DA. 1996. Food storing does not affect hippocampal volume in experienced adult willow tits. *Behav Brain Res* 81:233–236.
- Czeh B, Welt T, Fischer AK, Erhardt A, Scmitt W, Muller MB, Toschi N, Fuchs E, Keck ME. 2002. Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: effects on stress hormone levels and adult hippocampal neurogenesis. *Biol Psychiatry* 52:1057–1065.
- Drapeau E, Mayo W, Aurousseau C, Le Moal M, Piazza P-V, Abrous DN. 2003. Spatial memory performance of aged rats in the water maze predicts levels of hippocampal neurogenesis. *Proc Natl Acad Sci USA* 100:14385–14390.
- Fuch E, Flugge G, Ohl F, Lucassen P, Vollmann-Honsdorf GK, Michaelis T. 2001. Psychosocial stress, glucocorticoids, and structural alterations in the tree shrew hippocampus. *Physiol Behav* 73:285–291.
- Gould E, Gross CG. 2002. Neurogenesis in adult mammals: some progress and problems. *J Neurosci* 22:619–623.
- Gould E, Tanapat P. 1999. Stress and hippocampal neurogenesis. *Biol Psychiatry* 46:1472–1479.
- Gould E, Tanapat P, Hastings NB, Shors TJ. 1999. Neurogenesis is adulthood: a possible role in learning. *Trends Cogn Sci* 3:186–192.
- Gundersen HJG, Jensen EB. 1987. The efficiency of systematic sampling in stereology and its predictions. *J Microsc* 147: 229–263.
- Jacobs LF. 1995. Adaptive patterns of hippocampal size and space use in wild rodents. In: Alleva E, Fasolo A, Lipp H-P, Nadel L, editors. *Studies of the brain in naturalistic settings (NATO Advanced Studies Institute Series)*. Dordrecht: Kluwer Academic Press, p 311–322.
- Haftorn S. 1956. Contribution to the food biology of tits, especially about storing of surplus of food. Part IV. A comparative analysis of *Parus atricapillus* L., *P. cristatus* L., and *P. ater* L. *Kgl Norske Vidensk Selsk Skr* 4:1–54.
- Hoshooley JS, Sherry DF. 2004. Neuron production, neuron number and structure size are seasonably stable in the hippocampus of the food-storing black-capped chickadee (*Poecile atricapillus*). *Behav Neurosci* 118:345–355.
- Kempermann G. 2002. Why new neurons? Possible functions for adult hippocampal neurogenesis. *J Neurosci* 22:632–638.
- Koivula K, Lahti K, Orell M, Rytkonen S. 1993. Prior residency as a key determinant of social dominance in the willow tit (*Parus montanus*). *Behav Ecol Sociobiol* 33: 283–287.
- Krebs JR, Clayton NS, Hampton RR, Shettleworth SJ. 1995. Effects of season and photoperiod on food storing and the hippocampus in a food-storing and a non-storing passerine bird. *NeuroReport* 6:1701–1704.
- Krebs JR, Clayton NS, Healy SD, Cristol DA, Patel SN, Jolliffe AR. 1996. The ecology of the avian brain: food-storing memory and the hippocampus. *Ibis* 138:34–46.
- Krebs JR, Sherry DF, Healy SD, Perry VH, Vaccarino AL. 1989. Hippocampal specialization of food-storing birds. *Proc Natl Acad Sci USA* 86:1388–1392.
- Lavenex P, Steele M, Jacobs LF. 2000a. The seasonal pattern of cell proliferation and neuron number in the dentate gyrus of wild adult eastern grey squirrels. *Eur J Neurosci* 12:643–648.
- Lavenex P, Steele MA, Jacobs LF. 2000b. Sex differences, but no seasonal variations in the hippocampus of food-caching squirrels: a stereological study. *J Comp Neurol* 425:152–166.
- MacDougall-Shackleton SA, Sherry DF, Clark AP, Pinkus R, Hernandez AM. 2003. Photoperiodic regulation of food storing and the hippocampus volume in black-capped chickadees, *Poecile atricapillus*. *Anim Behav* 65:805–812.
- Mennill DJ, Doucet SM, Montgomerie R, Ratcliffe LM. 2003. Achromatic color variation in black-capped chickadees, *Poecile atricapilla*: black and white signals of sex and rank. *Behav Ecol Sociobiol* 53:350–357.
- Nottebohm F. 2002. Neuronal replacement in adult brain. *Brain Res Bull* 57:737–749.
- Patel SN, Clayton NS, Krebs JR. 1997. Spatial learning induces neurogenesis in the avian brain. *Behav Brain Res* 89:115–128.
- Pravosudov VV. 1985. Search for and storage of food by *Parus cinctus lapponicus* and *P. montanus borealis* (Paridae). *Zool Zhurnal* 64:1036–1043.
- Pravosudov VV, Clayton NS. 2001. Effects of demanding foraging conditions on cache retrieval efficiency in food-caching mountain chickadees (*Poecile gambeli*). *Proc R Soc Lond B* 268:363–368.
- Pravosudov VV, Clayton NS. 2002. A test of the adaptive specialization hypothesis: population differences in caching, memory, and the hippocampus in black-capped chickadees (*Poecile atricapilla*). *Behav Neurosci* 116: 515–522.
- Pravosudov VV, Grubb TC Jr. 1997. Energy management in passerine birds during the non-breeding season: a review. *Curr Ornithol* 14:189–234.
- Pravosudov VV, Lavenex P, Clayton NS. 2002. Changes in spatial memory mediated by experimental variation in

- food supply do not affect hippocampal anatomy in mountain chickadees (*Poecile gambeli*). *J Neurobiol* 51:142–148.
- Pravosudov VV, Mendoza SP, Clayton NS. 2003. The relationship between dominance, corticosterone, memory, and food caching in mountain chickadees (*Poecile gambeli*). *Horm Behav* 44:93–102.
- Prickaerts J, Koopmans G, Blokland A, Scheepens A. 2004. Learning and adult neurogenesis: survival with or without proliferation? *Neurobiol Learn Mem* 81:1–11.
- Rakic P. 2002. Adult neurogenesis in mammals: an identity crisis. *J Neurosci* 22:614–618.
- Sherry DF. 1989. Food storing in the paridae. *Wilson Bull* 1989:289–304.
- Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould, E. 2001. Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 410:372–376.
- Smulders TV, Sasson AD, DeVoogd TJ. 1995. Seasonal variation in hippocampal volume in a food-storing bird, the black-capped chickadee. *J Neurobiol* 27:15–25.
- Smulders TV, Shiflett MW, Sperling AJ, DeVoogd TJ. 2000. Seasonal changes in neuron numbers in the hippocampal formation of a food-hoarding bird: the black-capped chickadee. *J Neurobiol* 44:414–422.
- Tanapat P, Hastings NB, Rydel TA, Galea LAM, Gould, E. 2001. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J Comp Neurol* 437:496–504.
- West MJ, Gundersen HJG. 1990. Unbiased stereological estimation of the number of neurons in the human hippocampus. *J Comp Neurol* 296:1–22.
- West MJ, Ostergaard K, Andreassen OA, Finsen B. 1996. Estimation of the number of somatostatin neurons in the striatum: an in situ hybridization study using the optical fractionator method. *J Comp Neurol* 370:11–22.
- West MJ, Slomianka L, Gundersen HJG. 1991. Unbiased stereological estimation of the total number of neurons in the subdivision of the rat hippocampus using the optical fractionator. *Anat Rec* 231:482–497.