

Changes in Spatial Memory Mediated by Experimental Variation in Food Supply Do Not Affect Hippocampal Anatomy in Mountain Chickadees (*Poecile gambeli*)

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ABSTRACT: Earlier reports suggested that seasonal variation in food-caching behavior (caching intensity and cache retrieval accuracy) might correlate with morphological changes in the hippocampal formation, a brain structure thought to play a role in remembering cache locations. We demonstrated that changes in cache retrieval accuracy can also be triggered by experimental variation in food supply: captive mountain chickadees (*Poecile gambeli*) maintained on limited and unpredictable food supply were more accurate at recovering their caches and performed better on spatial memory tests than birds maintained on *ad libitum* food. In this study, we investigated whether these two treatment groups also differed in the volume and neuron number of the hippocampal formation. If variation in memory for food caches correlates with hippocampal size, then our birds with enhanced cache recovery and spatial memory per-

formance should have larger hippocampal volumes and total neuron numbers. Contrary to this prediction we found no significant differences in volume or total neuron number of the hippocampal formation between the two treatment groups. Our results therefore indicate that changes in food-caching behavior and spatial memory performance, as mediated by experimental variations in food supply, are not necessarily accompanied by morphological changes in volume or neuron number of the hippocampal formation in fully developed, experienced food-caching birds. © 2002 Wiley Periodicals, Inc. *J Neurobiol* 51: 142–148, 2002;

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INTRODUCTION

Many species of birds and mammals are known to cache food for later use (Vander Wall, 1990) and rely

on memory to recover their caches at a later date (e.g., Shettleworth, 1995; Jacobs, 1995). Laboratory studies have demonstrated that spatial memory performance is impaired by lesions of the hippocampus in food-caching birds (Sherry and Vaccarino, 1989; Hampton and Shettleworth, 1996). In addition, comparative studies have shown that food-caching birds and mammals have a larger hippocampal volume relative to telencephalon than noncaching species (Krebs et al., 1989; Sherry et al., 1989; Jacobs et al., 1990). Similarly, species that cache more intensively have a

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larger hippocampal formation than species that cache less intensively (Healy and Krebs, 1992, 1996; Hampton et al., 1995; Basil et al., 1996). All these findings have led to the idea that there may be an evolutionary selection pressure for increased hippocampal size associated with the enhanced visuo-spatial cognitive demands for more efficient cache retrieval (Krebs, 1990; Krebs et al., 1996).

Comparative studies have shown that the differences in hippocampal size between storers and non-storers arise after the young have fledged from the nest (Healy and Krebs, 1993; Healy et al., 1994) and that the additional hippocampal growth during post-natal development in young food storers depends on food-caching and retrieval experience (Clayton and Krebs, 1994; Clayton, 1995, 1996, 2001). What is less clear is whether experience-dependent changes in hippocampal size also occur in adulthood in association with changes in food-caching behavior and/or in spatial memory.

One way to test this hypothesis is to compare the hippocampal volume and total neuron number of adult food storers in different seasons because food-caching behavior changes seasonally (e.g., Haftorn, 1956; Ludescher, 1980; Pravosudov, 1985). One study reported a seasonal change in hippocampal morphology in food-caching birds: in the black-capped chickadee (*Poecile atricapillus*), both the volume and total number of neurons in the hippocampus were greatest in October when food-caching is at its peak (Smulders et al., 1995, 2000b). In contrast, Barnea and Nottebohm (1994) found no seasonal variation in hippocampal neuron number in black-capped chickadees, although they did find differences in neurogenesis. Similarly, Lavenex and colleagues (2000a, b) found no seasonal variation in volume, neuron number, or cell proliferation rates in the hippocampus of gray squirrels (*Sciurus carolinensis*), a food-caching mammal, which, like chickadees, has a well defined seasonal variation in caching activity (Thompson and Thompson, 1980). All these studies of seasonal variation were based on the assumption that changes in caching behavior are accompanied by variation in spatial memory processing. Based on published data, however, it is not clear that changes in caching behavior and/or spatial memory processing necessarily correlate with changes in volume or neuron number in the hippocampal formation of fully developed, experienced food-caching vertebrates.

In food-caching birds, manipulations of photoperiod result in increased caching activity (Shettleworth et al., 1995) and improved performance on spatial memory tasks (Clayton and Cristol, 1996). The volume of the hippocampus, however, does not seem to

be affected by such manipulations (Krebs et al., 1995). Other environmental factors, such as temperature, hormone levels, and food supply, however, also change seasonally and may be driving the seasonal changes in caching behavior. It is therefore important to understand which, if any, of these factors might affect memory performance, and whether these factors might also influence hippocampal morphology. The fact that food supply changes seasonally has important implications, because birds' survival probability may depend on successful cache retrieval (Pravosudov and Grubb, 1997; Pravosudov and Clayton, 2001). If a bird does not remember a cache during late summer when food is plentiful and it is easy to maintain energy balance, such a lapse should not have a strong effect on the bird's survival. We therefore hypothesized that efficiency of food caching and retrieval may vary in response to energetic demands (Pravosudov and Clayton, 2001). If variation in spatial memory performance is subserved by variation in volume and neuron number of the hippocampal formation, then hippocampal morphology should also respond to these changes in energetic demands.

We previously demonstrated that mountain chickadees (*Poecile gambeli*) maintained on limited and unpredictable food supply were more efficient at retrieving their own caches and performed better on spatial memory tests than chickadees maintained on *ad libitum* food (Pravosudov and Clayton, 2001). These results therefore provided us with the opportunity to test the hypothesis concerning the relationship between changes in hippocampal size and cognition independent of any confounding seasonal effects.

MATERIALS AND METHODS

Twenty-four mountain chickadees were caught using mist-nets during late November 1999 near the Sage Hen Field Reserve of Tahoe National Forest. Birds were transported to the laboratory, placed individually in wire-mesh cages (60 × 42 × 60 cm), and maintained on a 8:16 light/dark cycle at 20°C. Birds were fed a mixture of shelled sunflower seeds, crushed peanuts, and mealworms, and given *ad libitum* water. After 1 week in captivity, birds were randomly assigned to one of two groups. One group was maintained on *ad libitum* food and the other was given a limited and unpredictable food supply. For the limited and unpredictable food supply, birds were given access to food for three or four 20-min intervals per day, resulting in either 60 or 80 min of access to food per day. Each day, we randomly determined whether birds received three or four 20-min feeding intervals. We also randomly spread these intervals throughout the day, without having any two feeding intervals contiguous.

Table 1 Volume of the Hippocampal Formation and the Remainder of the Telencephalon, Total Number of Neurons, and Neuron Density of the Hippocampus in Mountain Chickadees under Limited and Unpredictable Food Supply and *Ad Libitum* Food Supply

Parameter	Unpredictable Food (<i>n</i> = 10)		<i>Ad Libitum</i> Food (<i>n</i> = 11)		<i>t</i> Test Results	Power
	Mean ± SE	CE	Mean ± SE	CE		
Wing length, mm	72.40 ± 0.87		72.36 ± 0.67		<i>t</i> = 0.03, <i>p</i> = .97	
Body mass, g	11.84 ± 0.68		11.62 ± 0.21		<i>t</i> = 0.74, <i>p</i> = .47	
Hippocampus volume, mm ³	12.06 ± 0.43	0.02	12.05 ± 0.61	0.02	<i>t</i> < 0.01, <i>p</i> = .99	0.48
Telencephalon volume, mm ³	306.03 ± 10.95	0.02	309.67 ± 9.03	0.02	<i>t</i> = -0.26, <i>p</i> = .80	0.59
Total number of neurons	936,691 ± 39,837	0.06	907,841 ± 40,661	0.06	<i>t</i> = 0.50, <i>p</i> = .62	0.48
Neuron density, per mm ³	77,789 ± 2096		76,097 ± 9529		<i>t</i> = 0.47, <i>p</i> = .65	0.69

CE represents the average (per group) of the relative variance of individual estimates of volumetric brain measures and the total number of neurons. CEs are not presented for neuron density because it was derived from the other measurements.

After 60 days on different feeding schedules, we tested the birds' spatial memory performance in laboratory tests that lasted 34 more days during which birds were maintained on the same feeding schedules. We used several procedures to test spatial memory performance (Pravosudov and Clayton, 2001). First, birds were tested for their accuracy to retrieve their own caches. Second, birds were tested on one-trial associative memory tasks in which they had to find previously encountered, hidden food that they did not cache themselves. These behavioral experiments demonstrated that birds maintained on limited and unpredictable food supply were more accurate in retrieving their own caches than birds maintained on *ad libitum* food supply. Importantly, there were no significant differences in the amount of cached food between the two groups. Birds maintained on limited and unpredictable food supply also performed better on one-trial associative memory tasks (Pravosudov and Clayton, 2001).

After completion of the behavioral tests (94 days on experimental treatments), we collected blood samples for analyses of plasma corticosterone (Pravosudov et al., 2001). Birds were then anesthetized (0.03 mL of 50 mg/mL Nembutal per bird) and perfused transcardially with 100 mL of phosphate buffer (0.1 M PO₄) followed by 100 mL of 4% paraformaldehyde in phosphate buffer. Birds were decapitated and their brain within the skull was placed in the same fixative for 1 week. The brains were then removed from the skull and postfixed for another week. The brains were cryoprotected in a 30% sucrose solution, frozen, and kept at -20°C until processing. We cut coronal sections at 40 μm on a sliding, freezing microtome. Every fourth section was mounted onto gelatin-coated slides, stained for Nissl with thionin, and coverslipped with Permount. Because of technical problems, only 21 birds were used for measurements of volume and neuron number: 10 birds in the unpredictable food group (eight males and two females) and 11 birds in the *ad libitum* food group (eight males and three females).

We used StereoInvestigator 3.15a (Microbrightfield Inc., Colchester, VT) for all stereological measurements. We used the Cavalieri principle to measure the volume of the

hippocampal formation and the volume of the telencephalon (for details of the method see Gundersen and Jensen, 1987; West and Gundersen, 1990). We determined the boundaries of the hippocampal formation as described in Krebs et al. (1989). To evaluate the precision of our sampling methods, we calculated coefficients of error for volume and neuron count measurements. The relative variance of individual estimates (CE) was calculated using the quadratic approximation (Gundersen and Jensen, 1987) and taking into account the Nugget effect (West et al., 1996). We used a total of 10 to 15 sections per bird to measure the hippocampal volume (1 in 12 sections, 480 μm apart). In accordance with the Cavalieri principle (Gundersen and Jensen, 1987; West and Gundersen, 1990), we chose the first section randomly from the first four sections containing the hippocampus. Pilot studies showed that this sampling scheme was most efficient and provided very low variance of individual estimates (CE, Table 1) and that increasing the number of sections measured did not increase estimation accuracy. To measure the volume of the telencephalon, we used a total of 11 to 15 sections (1 in 16 sections, 640 μm apart), with the first section chosen randomly from the first six sections. Sections were coded prior to analyses and the code was broken after all measurements were completed.

We calculated the total number of neurons in the hippocampal formation using the optical fractionator method (West et al., 1991), which allows the estimation of the absolute number of neurons independently from any measurement of the volume of the structure (West et al., 1991). To count neurons, we used a 100X Neofluar® oil objective (N.A. 1.30) on a Nikon Optiphot® microscope linked to PC-based Stereo Investigator®, and sampled the same sections used for the volumetric measurements. We estimated the number of neurons on the right side and doubled it to obtain the total number of neurons in the hippocampal formation as there were no significant differences between sizes of right and left sides (Sign test, *n* = 21, *z* = 0.87, *p* = .38).

We used two-sample *t* tests to compare the volume of the hippocampus and the remainder of the telencephalon be-

tween groups, because all data did not show a significant departure from the normal distribution. To compare relative hippocampal volume, we performed an ANCOVA on log-transformed (natural logarithm) volume measurements with nonhippocampal telencephalon volume as covariate. We used a power analysis to calculate the power of the tests to detect a 10% difference between groups, taking a 10% change in estimates as the smallest biologically relevant change expected based on published reports. Data presented in Smulders et al. (1995), for example, suggest that the volume of the hippocampal formation in black-capped chickadees might change by as much as 30% in 3 months. Smulders et al. (2000a) also reported that an average of 78 days in captivity resulted in a 13% reduction in hippocampal volume in dark-eyed juncos (*Junco hyemalis*). Data on neuronal incorporation rates in the hippocampus of adult black-capped chickadees also suggest a 40% increase between August and October (Barnea and Nottebohm, 1994).

RESULTS

We found that the volume of the telencephalon without the hippocampal formation was not significantly different between the two treatment groups (Table 1). More importantly, long-term limited and unpredictable food supply had no effect on the volume of the hippocampal formation (Table 1). Hippocampal volume correlated with the volume of the remainder of the telencephalon ($r = 0.51, p = .01$), but the volume of the hippocampal formation relative to the volume of the remainder of the telencephalon did not differ between the two treatment groups [ANCOVA, $F(1, 18) = 0.11; p = .74$, power to detect a 10% difference = 0.90]. Similarly, long-term limited and unpredictable food supply had no effect on total neuron number or neuron density in the hippocampal formation (Table 1). There were no sex differences in any of the estimated parameters (t test, both treatment groups combined, 16 males and 5 females, all p values $> .3$). There were no significant differences between treatments in body size or body mass prior to the experiment (Table 1), but chickadees maintained on limited and unpredictable food had gained significantly more mass than chickadees on *ad libitum* food by the end of the experiment (Pravosudov and Clayton, 2001).

The variance of our volume and neuron number estimates (CE) was low for all measurements (Table 1), which supports the reliability of our methods. Power analysis showed that we had sufficient power to detect biologically relevant differences between treatments (Table 1). The power to detect a 10% difference between groups was more than 48%, and

the power to detect a 20% difference was more than 99% in all tests.

DISCUSSION

Although long-term differences in food supply resulted in changes in cache-retrieval accuracy and performance on spatial memory tasks, the present results show that these changes were not accompanied by changes in volume or total neuron number of the hippocampal formation in experienced food-caching mountain chickadees. Our results therefore provide further experimental evidence in support of the view that changes in food-caching behavior and/or spatial memory performance do not necessarily correlate with changes in hippocampal structure in fully developed experienced food-caching animals (Lavenex et al., 2000a, b; Banta Lavenex et al., 2001). In a recent critique, Bolhuis and Macphail (2001) also argued on theoretical grounds that naturalistic changes in the use of spatial memory in animals do not provide a solid foundation to expect differences in brain size.

One might argue that the length of food treatment was insufficient to produce detectable changes in the hippocampal morphology. This seems unlikely for a number of reasons. In our experiment, we maintained the chickadees on different feeding supplies for about 3 months, a length of time sufficient to produce a difference in cache-retrieval accuracy (Pravosudov and Clayton, 2001). According to Barnea and Nottebohm (1994), this length of time is also more than sufficient to detect significant differences in neuronal incorporation rates in the hippocampal formation of free-ranging or captive black-capped chickadees. Similarly, changes in hippocampal volume in young chickadees of the same species can be detected after just 3 weeks of experience of food caching and cache retrieval (Clayton, 2001). It is therefore unlikely that the length of our experiment was insufficient to detect significant changes in volume or neuron number in the hippocampal formation of chickadees maintained in different food supply conditions.

Another issue relates to the age and previous experience of our birds. In young birds with limited or no caching experience, hippocampal volume has been shown to depend on food caching and retrieval experience (Clayton, 1995, 1996, 2001). On the other hand, Cristol (1996) reported that a 1 month absence of caching experience had no effect on the hippocampal volume in fully developed adult willow tits (*Parus montanus*) that were experienced in food caching. We probably used a mixture of juveniles (more than 4

months old, but less than 1 year old) and adults (more than 1 year old). However, birds were selected randomly for the two experimental groups, and there is no reason to believe that the ratio of juveniles to adults differed between the two treatment groups. The fact that the animals from the two groups did not differ in size or body mass prior to the experiment supports this argument. Furthermore, we trapped our birds at the end of November, after juvenile birds had gone through 3–4 months of extensive food caching (from August to November; Pravosudov, personal observation). We can therefore be confident that all birds used in this study were already fully experienced in food caching before being brought into the laboratory.

Our data provide no support for the hypothesis that hippocampal volume and neuron number correlate with cache retrieval accuracy and spatial memory performance in experienced food-caching birds. Assuming that seasonal changes in caching activity are accompanied by changes in spatial memory performance, our findings parallel previous results showing no correlation between hippocampal size and food-caching intensity in adult birds and mammals (Barnea and Nottebohm, 1994; Krebs et al., 1995; Lavenex et al., 2000a, b; but see Smulders et al., 1995, 2000b). We suggest that changes in volume and neuron number in the hippocampus reported within the same species represent developmental changes in young inexperienced individuals, and that these morphological characteristics do not vary consistently in fully grown, experienced animals (Lavenex et al., 2000a, b; Banta Lavenex et al., 2001).

This hypothesis is in direct contrast with reports by Smulders and colleagues (1995, 2000b) that both volume and neuron number of the hippocampal formation undergo seasonal variations that correlate with changes in caching activity in black-capped chickadees. There are, however, a number of differences between the data presented in Smulders et al. (1995, 2000b) and that of the other studies of food-caching birds (Barnea and Nottebohm, 1994) and some mammals which, like food-caching birds, have well defined seasonal patterns in food caching behavior (Lavenex et al., 2000a, b). One problem is that the birds identified as adults in the studies by Smulders and colleagues were also likely to be a mixture of adults and juveniles, and it is therefore not easy to compare their mixed data set with that of Barnea and Nottebohm (1994), in which juveniles and adults were analyzed separately. The second difficulty is that Smulders et al. (2000b) used an estimate of hippocampal volume to calculate neuron numbers instead of relying on methods that enable estimation of total

neuron number independently from volume estimation (West et al., 1991; Lavenex et al., 2000a, b), a problem that is confounded by the fact that their measurements of hippocampal volume were not only adjusted for variation in telencephalon volume, but also for differential weight changes during fixation between samples. Examining the data presented in Smulders et al. (1995) Lavenex et al. (2000a) suggested that the entire telencephalon volume varied seasonally rather than being specific to the hippocampal formation. As many of these studies have used different species, it is possible that there are some species-specific differences in the relationships between cognition and the hippocampus. However, to support a general claim that changes in food-caching behavior and spatial memory correlate with changes in the hippocampus we should be able to demonstrate this relationship in different species exhibiting similar behavior.

We conclude that there is no good evidence to support the claim that changes in memory correlate with changes in hippocampal morphology, either seasonally or as a result of experimental variation in food supply. Other seasonal factors, such as photoperiod, might still have some effect on the hippocampus in food-caching animals. Whereas one study (Krebs et al., 1995) reported that changes in photoperiod did not result in volumetric changes of the hippocampal formation in food-caching birds, more studies might be warranted to investigate the relationship between photoperiod and the hippocampus. Our results indicate that changes in memory performance, such as those triggered by variations in food supply, are not necessarily subserved by changes in volume or neuron number in the hippocampal formation of experienced food-caching animals. Other potentially less expensive mechanisms (e.g., changes in synaptic plasticity) could provide the neurobiological basis for such changes in memory performance.

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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.
- Basil JA, Kamil AC, Balda RP, Fite KV. 1996. Differences in hippocampal volume among food storing corvids. *Brain Behav Evol* 47:156–164.
- Bolhuis JJ, Macphail EM. 2001. A critique of the neuroecology of learning and memory. *Trends Cogn Sci* 5:426–433.
- 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. 1996. Development of food-storing and the hippocampus in juvenile marsh tits (*Parus palustris*). *Behav Brain Res* 74:153–159.
- 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, Cristol DA. 1996. Effects of photoperiod on memory in food-caching marsh tits. *Anim Behav* 52:715–726.
- 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.
- Gundersen HJG, Jensen EB. 1987. The efficiency of systematic sampling in stereology and its predictions. *J Microsc* 147:229–263.
- Haftorn S. 1956. Contribution to the food biology of tits, especially about storing of surplus 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.
- Hampton RR, Sherry DF, Shettleworth SJ, Khurgle M, Ivy G. 1995. Hippocampal volume and food-storing behavior are related in parids. *Brain Behav Ecol* 45:54–61.
- Hampton RR, Shettleworth SJ. 1996. Hippocampal lesions impair memory for location but not color in passerine birds. *Behav Neurosci* 110:831–835.
- Healy SD, Clayton NS, Krebs JR. 1994. Development of hippocampal specialization in two species of tit (*Parus* spp.). *Behav Brain Res* 613:23–28.
- Healy SD, Krebs JR. 1992. Food-storing and hippocampus in corvids: amount and volume are correlated. *Proc Roy Soc Lond B* 248:241–245.
- Healy SD, Krebs JR. 1993. Development of hippocampal specialization in a food-storing bird. *Behav Brain Res* 53:127–131.
- Healy SD, Krebs JR. 1996. Food-storing and the hippocampus in *Paridae*. *Brain Behav Evol* 47:195–199.
- 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.
- Jacobs LF, Gaulin SJC, Sherry DF, Hoffman GE. 1990. Evolution of spatial cognition: Sex-specific patterns of spatial behavior predict hippocampal size. *Proc Natl Acad Sci USA* 87:6349–6352.
- Krebs JR. 1990. Food-storing birds—adaptive specialization in brain and behavior. *Phil Trans R Soc Lond B* 329:153–160.
- 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 MA, Jacobs LF. 2000a. Sex differences, but no seasonal variations in the hippocampus of food-caching squirrels: a stereological study. *J Comp Neurol* 425:152–166.
- Lavenex P, Steele MA, Jacobs LF. 2000b. 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.
- Ludescher FB. 1980. Fressen und Verstecken von Sämereien bei der Weidenmeise *Parus montanus* im Jahresverlauf unter konstanten Ernährungsbedingungen. *Ökol. Voegl* 2:135–144.
- Pravosudov VV. 1985. Search for and storage of food by *Parus cinctus lapponicus* and *P. montanus borealis* (Paridae). *Zool Zhurnal* 64:1036–1043 (in Russian).
- Pravosudov VV, Clayton NS. 2001. Effects of demanding foraging conditions on cache retrieval accuracy in food-caching mountain chickadees (*Poecile gambeli*). *Proc Royal Soc Lond B* 268:363–368.
- 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, Kitaysky AS, Wingfield JC, Clayton NS. 2001. Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). *Gen Comp Endocrinol* 123:324–331.
- Sherry DF, Vaccarino AL. 1989. Hippocampus and memory for food caches in black-capped chickadees. *Behav Neurosci* 103:308–318.
- Sherry DF, Vaccarino AL, Buckenham K, Hertz RS. 1989. The hippocampal complex of food-storing birds. *Brain Behav Ecol* 34:308–317.
- Shettleworth SJ. 1995. Memory in food-storing birds: From the field to the Skinner box. In: Alleva E, Fasolo A, Lipp H-P, Nadel L, editors. *Behavioral Brain Research in Naturalistic and Semi-naturalistic Settings*. Proceedings of NATO Advanced Study Institute Series: Maratea, It-

- aly. The Hague: Kluwer Academic Publishers, p. 158–179.
- Shettleworth SJ, Hampton RR, Westwood RP. 1995. Effects of season and photoperiod on food storing by black-capped chickadees, *Parus atricapillus*. *Anim Behav* 49: 989–998.
- Smulders TV, Casto JM, Nolan V Jr, Ketterson ED, DeVoogd TJ. 2000a. Effects of captivity and testosterone on the volumes of four brain regions in the dark-eyed junco (*Junco hyemalis*). *J Neurobiol* 43:244–253.
- 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. 2000b. Seasonal changes in neuron numbers in the hippocampal formation of a food-hoarding bird: the black-capped chickadee. *J Neurobiol* 44:414–422.
- Thompson DC, Thompson PS. 1980. Food habits and caching behavior of urban grey squirrels. *Can J Zool* 58:701–710.
- Vander Wall SB. 1990. Food hoarding in animals. Chicago: University of Chicago Press. 445 p.
- 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 subdivisions of the rat hippocampus using the optical fractionator. *Anat Rec* 231:482–497.