

Variation in hippocampal morphology along an environmental gradient: controlling for the effects of day length

Timothy C. Roth II*, Lara D. LaDage and Vladimir V. Pravosudov

Department of Biology, University of Nevada, 1664 North Virginia Street, MS 314, Reno, NV 89557, USA

Environmental conditions may create increased demands for memory, which in turn may affect specific brain regions responsible for memory function. This may occur either via phenotypic plasticity or selection for individuals with enhanced cognitive abilities. For food-caching animals, in particular, spatial memory appears to be important because it may have a direct effect on fitness via their ability to accurately retrieve food caches. Our previous studies have shown that caching animals living in more harsh environments (characterized by low temperatures, high snow cover and short day lengths) possess more neurons within a larger hippocampus (Hp), a part of the brain involved in spatial memory. However, the relative role of each of these environmental features in the relationship is unknown. Here, we dissociate the effects of one theoretically important factor (day length) within the environmental severity/Hp relationship by examining food-caching birds (black-capped chickadee, *Poecile atricapillus*) selected at locations along the same latitude, but with very different climatic regimes. There was a significant difference in Hp attributes among populations along the same latitude with very different climatic features. Birds from the climatically mild location had significantly smaller Hp volumes and fewer Hp neurons than birds from the more harsh populations, even though all populations experienced similar day lengths. These results suggest that variables such as temperature and snow cover seem to be important even without the compounding effect of reduced day length at higher latitudes and suggest that low temperature and snow cover alone may be sufficient to generate high demands for memory and the hippocampus. Our data further confirmed that the association between harsh environment and the hippocampus in food-caching animals is robust across a large geographical area and across years.

Keywords: hippocampus; neuron; environmental gradient; black-capped chickadee; caching; food hoarding

1. INTRODUCTION

Memory is an important trait used to gather, retain and recall information about the environment. As such, it is probably important for survival. We know that many environmental factors can produce increased demands on memory [1], which in turn may potentially affect memory function. However, it is not clear how variation in these traits is produced and which aspects of the environment might be most relevant for changes in the function and the underlying mechanism(s) of memory. It is possible that variation in memory is a function of one or two prevailing factors of the environment. Alternatively, multiple aspects of the environment may affect memory in an additive or multiplicative fashion, thereby making the relationship between the environment, memory and the brain more complex.

Environmental severity has been suggested as one possible factor affecting memory and other cognitive abilities in a variety of different animals [1–3]. For example, in many food-storing animals the degree of environmental severity (traditionally characterized by ambient temperature, snow cover and day length) may be especially important [4–7]. Many temperate zone food-storing species are non-migratory and store food in numerous locations (i.e.

scatter-hoard) when resources are abundant (i.e. autumn) for retrieval at a later time when resources are scarce (i.e. winter). This behaviour is thought to be an adaptation to survive harsh winter conditions when ambient food is unavailable [4,5,8,9]. The retrieval of these scattered food stores (or caches) is facilitated in part by memory, of which spatial memory appears to be particularly important [4,5]. Theory (e.g. [9]) predicts that selection on cache retrieval ability, and hence spatial memory, may be a function of environmental severity, where low ambient temperature, high snow cover and reduced day length demand more efficient foraging in order to maintain positive energy balance. Thus, there are probably strong demands for accurate memory, especially at high latitudes where environmental severity is increased and caching and retrieving food becomes more important for survival. Although it is possible that some caching species do not use memory for the retrieval of long-term caches, it is clear that chickadees use memory for short-term retrieval [10], which should still improve daily survival in harsh environments (e.g. [9]). In addition, the ability to retrieve caches should be more important than caching intensity, unless more caching automatically results in more successful retrieval.

Enhanced memory in harsh environments may potentially be facilitated via a change in the hippocampus (Hp), the region of the brain responsible in part for spatial

* Author for correspondence (tcroth@unr.edu).

memory [5,11,12]. More demanding situations (e.g. more harsh environments) seem to result in specialized phenotypes (e.g. a larger Hp region) that underlie a specific cognitive task (e.g. memory-based cache retrieval). This pattern may be produced by the selection of enhanced traits in specific environments (e.g. adaptive specialization) [4,5,13,14] or may be the consequence of phenotypic plasticity whereby enhancements in the Hp are produced due to increased caching and increased memory use itself. In either case, our previous work [7] provides large-scale support for the association between environmental harshness and the hippocampus. Black-capped chickadees from harsh northern latitudes possessed significantly larger Hp with more neurons than those from more mild southern latitudes, a relationship that was also found in a preliminary study by Pravosudov & Clayton [6]. Thus, the evidence for the relationship between environmental severity and the Hp is robust in this species and holds as an ecologically relevant pattern across a latitudinal gradient composed of locations with differing climates.

However, the relative contribution of the three primary theoretical environmental factors (temperature, snow cover and day length) to this relationship is unknown. As diurnal birds cannot forage in the dark, day length may potentially comprise a large part of environmental severity, as it limits the amount of time available to accumulate necessary food reserves to survive the night. Because of this constraint, there may be increased demands for spatial memory function in birds from higher latitudes (i.e. shorter day lengths) in order to procure enough food for overnight survival. This may explain in part why northern chickadees have larger Hp with more neurons compared with their more southern conspecifics. However, northern environments are also characterized by lower ambient temperatures with more precipitation (snow) during the winter (see figure 1 and [7]), which could also contribute to latitudinal differences in memory and the hippocampus. Low ambient temperatures increase the metabolic needs of animals, while snow cover may reduce food availability and accessibility. Successful cache recovery may be especially crucial in these conditions in order to meet higher energetic demands.

To dissociate the importance of the compounding effects of day length on the relationship between environmental severity and the Hp, we compared the Hp volume and total number of Hp neurons in the same species, the black-capped chickadee (*Poecile atricapillus*), on a large geographical scale at five locations across its range in North America (figure 1). We specifically chose three of these locations (Seattle, Washington (WA); Grant, Minnesota (MN); Presque Isle, Maine (ME)) at approximately the same latitude that experience similar day lengths but very different climates. The other two locations (Anchorage, Alaska (AK); Mt. Vernon, Iowa (IA)) were used as a latitudinal comparison to ensure the robustness of our previous work [7]. According to our hypothesis, better spatial memory and increased Hp attributes should be more pronounced in populations living in more harsh, energetically demanding environments in which dependence on food caches is likely to be especially important. Thus, if day length itself is an important constraint in the relationship between environmental severity and the Hp, then we might expect no

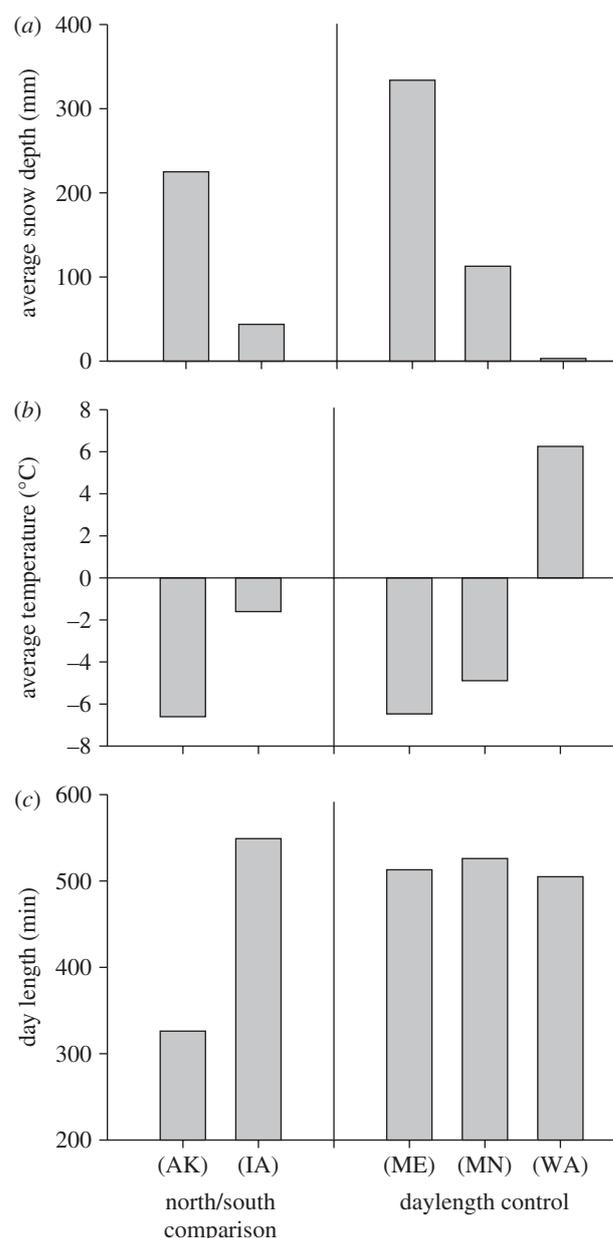


Figure 1. Collection locations sorted by the north/south comparison (left panels) and the latitudinal/day length control (right panels). Within each panel, the locations are ranked by environmental severity, as determined by (a) average snow cover (mm) and (b) average temperature ($^{\circ}\text{C}$) during the winter period (see text for details). Day length (min) at the winter solstice (c) differs for the locations in the left panel, but is similar in those on the right.

significant differences among the populations at the same latitude that experience differences in temperature and snow cover. If, however, other climatic variables (e.g. temperature and snow cover) are important independent of day length, then we should see differences among the populations along the same latitude that follow the same pattern as previously reported [7] of increased Hp volume and neuron number in more severe environments. We do not imply that birds use any of these factors as direct cues for the production of Hp attributes. Instead, we are dissociating the importance of the constraint on the time available to forage (day length) from the constraints of energetic demands (temperature) and access to food (snow cover). Because the MN and ME locations

experience colder temperatures and greater snow cover than the WA location, we expect bigger Hp with more neurons in the MN and ME locations when compared with the WA location. Finally, we expect the relationship between AK and IA to be consistent with our previous examination of populations on a latitudinal gradient [7], that is birds from AK should have much larger Hp volume and more neurons than those from IA.

2. METHODS

(a) Collection sites

We compared the hippocampal (Hp) volume and total number of Hp neurons of black-capped chickadees (*Poecile atricapillus*) at five locations across North America: Anchorage, AK (61°07' N 149°44' W); Presque Isle, ME (46°39' N 68°00' W); Grant, MN (45°05' N 92°57' W); Seattle, WA (47°40' N 122°18' W); and Mount Vernon, IA (41°51' N 91°25' W). These sites were chosen specifically (i) to compare populations with different climates at approximately the same latitude/day lengths (WA, MN, and ME; figure 1) and (ii) to make a comparison with the previous study by Roth & Pravosudov [7] on a latitudinal gradient (AK and IA). At each site, 12 birds were captured at feeders. While it would be best to collect birds at the peak of caching at each location to maintain consistency and to maximize the potential effects of food caching on the brain, due to logistics, we could not determine specifically when that peak occurred at each site. Thus, we chose to collect chickadees during the period of intensive autumn caching from mid September to late October based on the literature [15,16] and our previous work [7].

(b) Tissue preparation

Tissue preparation was performed exclusively by T.C.R. and followed Roth & Pravosudov [7], as necessary for proper comparison (see [17]). Brains were extracted immediately after capture. Birds were anaesthetized (0.07 ml of 50 mg ml⁻¹ Nembutal) and perfused transcardially with 0.1 M phosphate buffered saline for 10 min followed by 15–20 min of 10 per cent methanol-free formalin (from paraformaldehyde) made with 0.1 M phosphate buffer. Brains were post-fixed in the 10 per cent methanol-free formalin solution for 7 days, cryoprotected in 15 per cent and then 30 per cent sucrose, and then frozen at -80°C for storage. Tissue was cut into 40 µm coronal sections on a Leica CM 3050S cryostat at -20°C. Every 4th section was mounted and Nissl stained with thionin.

(c) Tissue analysis

Tissue analysis was performed exclusively by T.C.R. and followed Roth & Pravosudov [7], as necessary for proper comparison (see [17]). Hp volume and neuron numbers were estimated with stereological methods using Stereo-Investigator software (Microbrightfield, Inc.) and Leica microscope (M4000B). Both the Hp and telencephalon were measured in their entirety. We measured the Hp as per Krebs *et al.* [4] and our previous work. We measured the telencephalon as an estimate of the brain region generally associated with cognitive ability to be used as a covariate to control for overall size of the brain [4]. Brain region volumes were estimated with the Cavalieri procedure [18]. Hp volume was measured with a 200 µm grid; telencephalon volume was measured with a 1200 µm grid. The optimal grid size and frequency of sections sampling has been determined previously [6,19]. Neuron counts were performed with an optical fractionator procedure [20] at 1000x. A 250 µm

grid with a 30 × 30 µm counting frame, 5 µm dissector height, and 2 µm guards was used as in previous studies of chickadees [19]. We calculated a coefficient of error to estimate precision with the nugget effect for both neuron counts (CE mean (s.e.) = 0.098 (0.002)) and volume (CE mean (s.e.) = 0.016 (0.0001)). There were no significant differences between left and right hippocampal volume and between the total number of neurons in the right and left Hp (Repeated-measures GLM: Hp, $F_{1,55} = 0.401$, $p = 0.807$; Neuron numbers: $F_{1,55} = 0.842$, $p = 0.505$), thus the hemispheres were summed to produce the reported total values. All brains were measured blind to location.

(d) Climate data

Temperature (°C) and snow depth (mm) are represented as the average over the winter months (November–March) of yearly averages from 1971 to 2000. Day length (min) was measured during the winter solstice. Data were obtained from the National Oceanic and Atmospheric Administration climate database [21].

(e) Statistical analysis

We analysed Hp volume and neuron count after controlling for telencephalon volume within a general linear model; we report least squares means in the analyses and figures (see table 1). The inclusion of body mass as a covariate has been used in the past, but may lead to erroneous interpretations (Roth *et al.* in preparation; see also [17]). We note that the relationships in this analysis are, nevertheless, quite robust, and the inclusion of either telencephalon volume and/or body mass does not change the interpretation of the results (see §3 for statistics). In addition, the use of Hp volume as a covariate in the analysis of neuron number has been used in the past. This analysis produces a least squared mean functionally equivalent to an analysis of density. We include this analysis for completeness in the results, but also note that the interpretation remains the same. Tukey post hoc analyses were performed for a comparison between the three locations at the same latitude. A planned comparison analysis was performed between AK and IA as we expect a directional difference between them. There was no significant sex bias in sampling ($\chi^2 = 2.986$, d.f. = 4, $p = 0.560$) and no significant difference between the sexes in Hp volume ($F_{1,58} = 2.081$, $p = 0.155$) or neuron number ($F_{1,58} = 0.405$, $p = 0.527$); therefore, data for both sexes were pooled. The age of our birds was unknown, but there is no reason to suspect any systematic bias in age across different locations, as we sampled all populations using the same methodology.

3. RESULTS

There was a significant difference in both the Hp volume ($F_{4,55} = 41.106$, $p < 0.0001$) and total neuron count ($F_{4,55} = 28.149$, $p < 0.0001$) in black-capped chickadees from different locations along the latitudinal gradient when using Te volume as a covariate (table 1, figure 2). The inclusion of Hp volume as a covariate in the analysis of total neuron numbers did not change the interpretation ($F_{4,55} = 18.159$, $p < 0.0001$, even though the effect of the Hp in the model was significant; $F_{1,55} = 8.085$, $p < 0.006$). Likewise, the exclusion of covariates (Hp: $F_{4,56} = 6.612$, $p < 0.0001$; Neuron: $F_{4,56} = 29.383$, $p < 0.0001$) and the inclusion of body size (Hp: $F_{4,54} = 8.417$, $p < 0.0001$; Neuron: $F_{4,54} = 27.882$, $p < 0.0001$) did not change the results.

Table 1. Average (s.e.m.) morphological data for black-capped chickadees collected at five locations with varying environmental harshness. Locations ranked by latitude from high to low.

location (latitude)	body mass (g)	wing length (mm)	brain mass (g)	absolute hippocampal volume (mm ³)	absolute number of hippocampal neurons ($\times 10^6$)	telencephalon volume (mm ³)	relative hippocampal volume (mm ³)*	relative number of hippocampal neurons ($\times 10^6$)	dates collected (2008)
Anchorage, AK (61°N)	11.10 (0.16)	66.04 (0.76)	0.80 (0.02)	27.20 (0.64)	2.582 (0.065)	511.41 (18.36)	27.38 (0.73)	2.603 (0.094)	18–20 Sep
Seattle, WA (47°N)	11.19 (0.24)	61.69 (0.63)	0.70 (0.01)	22.65 (0.66)	1.719 (0.105)	430.25 (14.03)	22.33 (0.74)	1.680 (0.095)	22 Oct
Presque Isle, ME (46°N)	11.60 (0.23)	67.50 (0.48)	0.78 (0.02)	26.08 (0.99)	2.537 (0.106)	497.13 (22.85)	26.17 (0.72)	2.548 (0.092)	28 Sep
Grant, MN (45°N)	11.64 (0.25)	65.54 (0.61)	0.78 (0.01)	25.91 (0.72)	2.560 (0.092)	497.20 (13.34)	26.00 (0.72)	2.570 (0.092)	09 Oct
Mt. Vernon, IA (41°N)	12.11 (0.26)	65.08 (0.75)	0.74 (0.02)	24.06 (0.45)	1.609 (0.076)	482.53 (19.88)	24.06 (0.72)	1.609 (0.091)	13–16 Oct

* Least squares means values calculated from a GLM analysis using telencephalon volume as a covariate.

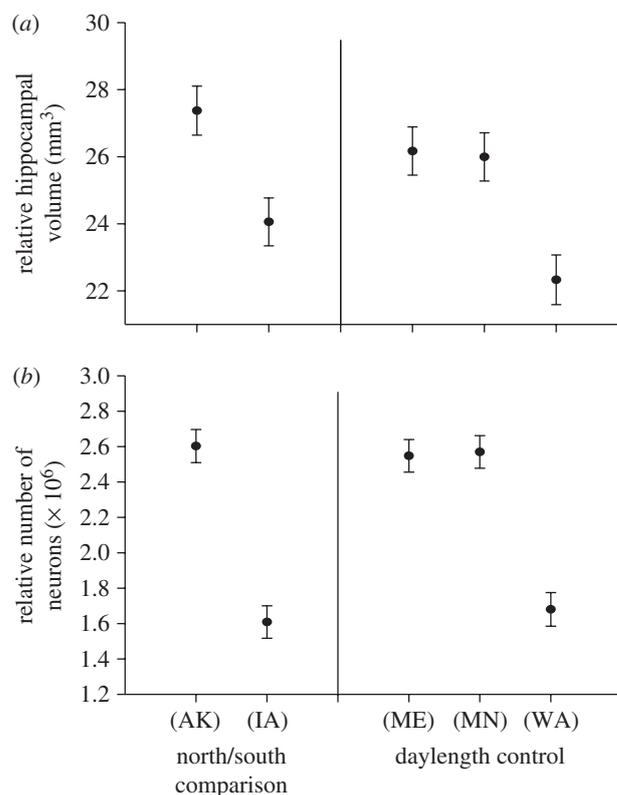


Figure 2. (a) Relative hippocampal volume and (b) relative neuron numbers, (least squares means) across locations controlling for telencephalon volume. Collection locations are sorted as figure 1 (north/south comparison, left panels; latitudinal/day length control, right panels). Within each panel, the locations are ranked by environmental severity.

Based on post hoc comparisons of ME/MN/WA and a planned comparison analysis between AK/IA, there were large and significant differences among the locations (see figure 2). Along the same latitude, there was a stark and significant effect of climate. The Hp volume ($ps < 0.008$) and neuron numbers ($ps < 0.0001$) were significantly smaller in birds from the Washington location, but not different between the Maine and Minnesota locations (all $ps > 0.999$). The birds from the northern most location (Alaska) had significantly larger Hp ($p < 0.0001$) and more Hp neurons ($p < 0.0001$) than those from the southern most location (Iowa) as predicted.

4. DISCUSSION

There was a significant difference in Hp attributes among populations along the same latitude with very different climatic features. Birds from the relatively mild location of Washington had significantly smaller Hp volumes and fewer Hp neurons than the birds from the more climatically harsh populations of Maine and Minnesota, even though all three populations experience similar day lengths. Maine and Minnesota were not different from each other (figure 2). These results suggest that day length may not be the main variable that affects caching intensity and thus may not be solely responsible for the previously reported relationship between latitude and Hp attributes. Rather, the other variables of theoretical interest (temperature and snow cover) seem to be the relevant factors in this relationship independent of the

constraint of day length. We cannot comment on the importance of day length in other contexts such as the regulation of seasonal changes in sexual behaviour. However, in regard to our study examining the explanation for the relationship between latitude and Hp attributes, day length does not seem to be the main driving force. In addition, the observed relationship between latitude, environmental severity, and Hp morphology of chickadees along our north/south environmental gradient followed our prediction. These results are consistent with our previous studies in this same system [6,7] and suggest that the relationship between environmental severity, memory and the Hp is robust.

This study expands our understanding of the variables important in the relationship between environmental severity and the brain in two ways. First, we have removed the potential compounding effects due to latitude/day length and still showed a significant pattern between the severity of the environment and Hp attributes. This suggests that although the theoretical basis for limited foraging time via day length may indeed explain some amount of the variation observed, it is not the primary explanation. This is not to say that day length has no effect on memory of caching birds, but that other variables such as temperature and snow cover appear to be important even at the same day length, at least in the context of our study. Second, we have replicated the results found previously [6,7]. This suggests a strong and robust pattern across the latitudinal gradient that is associated with environmental harshness.

Overall, we have support for the theoretical relationship that more severe climates with higher demands for enhanced memory require and thereby produce larger Hp with more neurons due to the need for advanced memory. We acknowledge that our control for the effects of day length includes a single comparison at the population level. However, these locations were chosen *a priori* specifically to address this issue and the results follow our predictions. Future studies should attempt to include and dissociate additional environmental variables that may impact this relationship such as food availability, resource distributions and structural habitat features. In addition, future work should experimentally investigate the possible causes of these differences in hippocampal morphology by dissociating the effects of genetic adaptation to the local climate from the influence of phenotypic plasticity and individual memory-based experiences.

We offer our gratitude to our many colleagues who facilitated our chickadee collection across North America: C. Handel and Y. Gilles at the United States Geological Survey's Alaska Science Center and P. Lincoln; J. Johnston, J. Monahan, and the Office of the President at the University of Maine, Presque Isle; R. Zink and J. Collins at the University of Minnesota; R. Black at Cornell College; D. Perkel, the Perkel lab, and N. Philips at the University of Washington and E. Sprong Suiter. We would also like to thank A. Roth for assistance in the field and A. Brodin and an anonymous reviewer for critical evaluation of our manuscript. This research was supported by grants from the National Science Foundation (IOB-0615021) and the National Institutes of Health (MH079 892, MH076 797) to

V.V.P. birds were collected under state permits 08-064 (AK), 2008-248 (ME), 14 869 (MN), SC862 (IA), 08-161 (WA), and federal permits MB022 532 in accordance with UNR IACUC approval (A05/06-35).

REFERENCES

- Shettleworth, S. J. 1998 *Cognition, evolution, and behavior*. Oxford, UK: Oxford University Press.
- Dukas, R. 1998 Evolutionary ecology of learning. In *Cognitive ecology* (ed. R. Dukas), pp. 129–174. Chicago, IL: University of Chicago Press.
- Shettleworth, S. J. 2009 The evolution of comparative cognition: is the snark still a boojum? *Behav. Process.* **80**, 210–217. (doi:10.1016/j.beproc.2008.09.001)
- Krebs, J. R., Sherry, D. F., Healy, S. D., Perry, V. H. & Vaccarino, A. L. 1989 Hippocampal specialization of food-storing birds. *Proc. Natl Acad. Sci. USA* **86**, 1388–1392. (doi:10.1073/pnas.86.4.1388)
- Sherry, D. F., Vaccarino, A. L., Buckenham, K. & Herz, R. S. 1989 The hippocampal complex of food-storing birds. *Brain Behav. Evol.* **34**, 308–317. (doi:10.1159/000116516)
- Pravosudov, V. V. & Clayton, N. S. 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. (doi:10.1037/0735-7044.116.4.515)
- Roth, T. C. & Pravosudov, V. V. 2009 Hippocampal volume and neuron numbers increase along a gradient of environmental harshness: a large-scale comparison. *Proc. R. Soc. B* **276**, 401–405. (doi:10.1098/rspb.2008.1184)
- Pravosudov, V. V. & Grubb, T. C. 1997 Management of fat reserves and food caches in tufted titmice (*Parus bicolor*) in relation to unpredictable food supply. *Behav. Ecol.* **8**, 332–339. (doi:10.1093/beheco/8.3.332)
- Pravosudov, V. V. & Lucas, J. R. 2001 A dynamic model of short-term energy management in small food-caching and non-caching birds. *Behav. Ecol.* **12**, 207–218. (doi:10.1093/beheco/12.2.207)
- Brodin, A. 2005 Mechanisms of cache retrieval in long term hoarding birds. *J. Ethol.* **23**, 77–83. (doi:10.1007/s10164-005-0147-5)
- Sherry, D. F. & Vaccarino, A. L. 1989 Hippocampus and memory for food caches in black-capped chickadees. *Behav. Neurosci.* **103**, 308–318. (doi:10.1037/0735-7044.103.2.308)
- Hampton, R. R. & Shettleworth, S. J. 1996 Hippocampal lesions impair memory for location but not color in passerine birds. *Behav. Neurosci.* **110**, 831–835. (doi:10.1037/0735-7044.110.4.831)
- Lucas, J. R., Brodin, A., de Kort, S. R. & Clayton, N. S. 2004 Does hippocampal size correlate with the degree of caching specialization? *Proc. R. Soc. Lond. B* **271**, 2423–2429. (doi:10.1098/rspb.2004.2912)
- Garamszegi, L. Z. & Eens, M. 2004 The evolution of hippocampus volume and brain size in relation to food hoarding in birds. *Ecol. Lett.* **7**, 1216–1224. (doi:10.1111/j.1461-0248.2004.00685.x)
- Brodin, A. 2005 Hippocampal volume does not correlate with food-hoarding rates in the black-capped chickadee and willow tit. *Auk* **122**, 819–828. (doi:10.1642/0004-8038(2005)122[0819:HVDNCW]2.0.CO;2)
- Pravosudov, V. V. 2006 On seasonality in food-storing behaviour in parids: do we know the whole story? *Anim. Behav.* **71**, 1455–1460. (doi:10.1016/j.anbehav.2006.01.006)

- 17 Roth, T. C., Brodin, A., Smulders, T. V., LaDage, L. D. & Pravosudov, V. V. 2010 Is bigger always better? A critical appraisal of the use of volumetric analysis in the study of the hippocampus. *Phil. Trans. R. Soc. B* **365**, 915–931. (doi:10.1098/rstb.2009.0208)
- 18 Gundersen, H. J. G. & Jensen, E. B. 1987 The efficiency of systematic sampling in stereology and its prediction. *J. Microsc.-Oxf.* **147**, 229–263.
- 19 Pravosudov, V. V. & Omanska, A. 2005 Dominance-related changes in spatial memory are associated with changes in hippocampal cell proliferation rates in mountain chickadees. *J. Neurobiol.* **62**, 31–41. (doi:10.1002/neu.20065)
- 20 West, M. J., Slomianka, L. & Gundersen, H. J. G. 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. (doi:10.1002/ar.1092310411)
- 21 National Oceanic and Atmospheric Administration. 2010 *Climate data 1971–2000*. <http://www.weather.gov/climate>.