

Hippocampal volumes and neuron numbers increase along a gradient of environmental harshness: a large-scale comparison

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Environmental conditions may provide specific demands for memory, which in turn may affect specific brain regions responsible for memory function. For food-caching animals, in particular, spatial memory appears to be important because it may have a direct effect on fitness via the accuracy of cache retrieval. Animals living in more harsh environments should rely more on cached food, and thus theoretically should have better memory to support cache retrieval, which may be crucial for survival. Consequently, animals in harsh environments may benefit from more neurons within a larger hippocampus (Hp), a part of the brain involved in spatial memory. Here, we present the first large-scale test of the hypothesis that Hp structure is related to the severity of the environment within a single food-caching species (the black-capped chickadee, *Poecile atricapillus*) with a large range encompassing a great diversity of climatic conditions. Hp size in birds collected at five locations along a gradient of environmental harshness from Alaska to Kansas ranked perfectly with climatic severity. Birds from more harsh northern climates (defined by lower ambient temperature, shorter day length and more snow cover) had significantly larger Hp volumes and more Hp neurons (both relative to telencephalon volume) than those from more mild southern latitudes. Environmental pressures therefore seem capable of influencing specific brain regions independently, which may result in enhanced memory, and hence survival, in harsh climates.

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

1. INTRODUCTION

Memory is an important mechanism used to gather, retain and recall information about the world. As such, it is probably important for survival. We know that many factors can produce increased demands on memory (Shettleworth 1998), which in turn may potentially affect memory function. However, it is not clear how selection influences memory, its underlying neural mechanisms and which specific pressures might be most relevant to produce changes in function and the mechanism(s).

Climatic severity has been suggested in food-storing animals as one possible selective factor affecting memory (Krebs *et al.* 1989; Sherry *et al.* 1989, 1992). Since many food-storing species are also non-migratory, food caching (storing food in numerous locations for retrieval at a later time) is thought to be an adaptation to survive harsh winter conditions (Krebs *et al.* 1989; Sherry *et al.* 1989; Pravosudov & Grubb 1997; Pravosudov & Lucas 2001). Cache retrieval is facilitated frequently by memory, of which spatial memory appears to be particularly important (Krebs *et al.* 1989; Sherry *et al.* 1989; Vander Wall 1990). Theory (Pravosudov & Lucas 2001) predicts that selection on cache retrieval ability, and hence spatial memory, is a function of environmental severity characterized by low ambient temperature, high snow cover and reduced day length, all of which demand more efficient foraging. There is probably then strong selection pressure for accurate memory, especially in harsh climates (e.g. high latitudes) where caching and retrieving food become

more important for survival. Still, how memory is linked to fitness and how selection works on the mechanisms of enhancing memory remain unclear.

One possibility is that selection on spatial memory may involve the hippocampus (Hp), the region of the brain that, in part, supports memory function. The importance of the Hp for spatial memory has been well demonstrated in both mammals and birds (Sherry & Vaccarino 1989; Sherry *et al.* 1989; Hampton & Shettleworth 1996). A leading thought is that the volume of the Hp is directly related to memory capability (Krebs *et al.* 1989). As suggested by the adaptive specialization hypothesis, selection may produce a specialized phenotype (a larger Hp region) for a specific important task (memory-based cache retrieval; Krebs *et al.* 1989; Sherry *et al.* 1989; Garamszegi & Eens 2004; Lucas *et al.* 2004). However, support for this hypothesis is not without controversy (Shettleworth 1995; Bolhuis & Macphail 2001; Macphail & Bolhuis 2001; Brodin & Lundborg 2003; Brodin & Bolhuis 2008). In fact, many interspecific comparative studies fail to show consistent patterns, leaving the overall connections between memory, caching intensity and the brain ambiguous. These discrepancies in the observed relationship between Hp volume and memory may be due in part to inconsistencies in the data collection and methodology of numerous independent studies, as well as ecological confounds and invalid assumptions related to phylogeny and the phenotypes of ancestral species (Bolhuis & Macphail 2001; Macphail & Bolhuis 2001; Brodin & Lundborg 2003;

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de Kort & Clayton 2006; Brodin & Bolhuis 2008). In addition, many of these studies considered only volumetric measurements of the Hp while ignoring the number of neurons, which might be a better indicator of hippocampal function (Roth & Dicke 2005).

Brodin & Bolhuis (2008) suggested that the best support to date for the adaptive specialization hypothesis is a study by Pravosudov & Clayton (2002), which suggested a direct link between environmental conditions, spatial memory and the Hp. Upon comparing the behaviour and brain morphology of a single species of food-caching birds from a single harsh (high latitude) location and a single mild (lower latitude) location under identical laboratory conditions, Pravosudov & Clayton (2002) found that those from the harsh environment had significantly more accurate memory, as well as larger Hp volume and more Hp neurons, and cached more food. However, the support for the direct link between the environment and the brain is limited to this comparison of only two populations. It therefore remains unclear whether the relationship between the environment and the brain is robust, and whether it will hold as an ecologically relevant pattern among many different climates and locations.

To definitively test the relationship between the environment and the Hp as it might relate to the adaptive specialization hypothesis, we compared the Hp volume and the total number of Hp neurons in a single species, the black-capped chickadees (*Poecile atricapillus*), on a large geographical scale at five locations along a latitudinal gradient of environmental harshness across its range in North America (figure 1). According to our hypothesis, survival benefits provided by better spatial memory should be more pronounced in populations living in more harsh, energetically demanding environments, in which food caches are likely to be especially important. Thus, we predict that birds living at northern latitudes, where the climate is harsh (figure 1) and survival may be highly dependent upon cached food, will have larger Hp formations containing more neurons than those living at more southern latitudes.

2. MATERIAL AND METHODS

(a) Collection sites

We compared the hippocampal volume and total number of Hp neurons of black-capped chickadees (*P. atricapillus*) at five locations across North America: Fairbanks, AK (64°51' N, 147°49' W); Prince George, BC (53°54' N, 122°47' W); Missoula, MT (46°51' N, 114°07' W); Fort Collins, CO (40°39' N, 105°11' W); and Manhattan, KS (39°08' N, 96°37' W). These sites were chosen specifically along a latitudinal gradient of climate severity (figure 1). At each site, 12 birds were captured at feeders. While it would be best to collect the birds at the peak of caching at each location in order to maintain consistency and to maximize the potential effects 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 two-month period of intensive autumn caching, from mid-September (AK) to late October (KS) 2007, based on the literature (Brodin 2005; Pravosudov 2006).

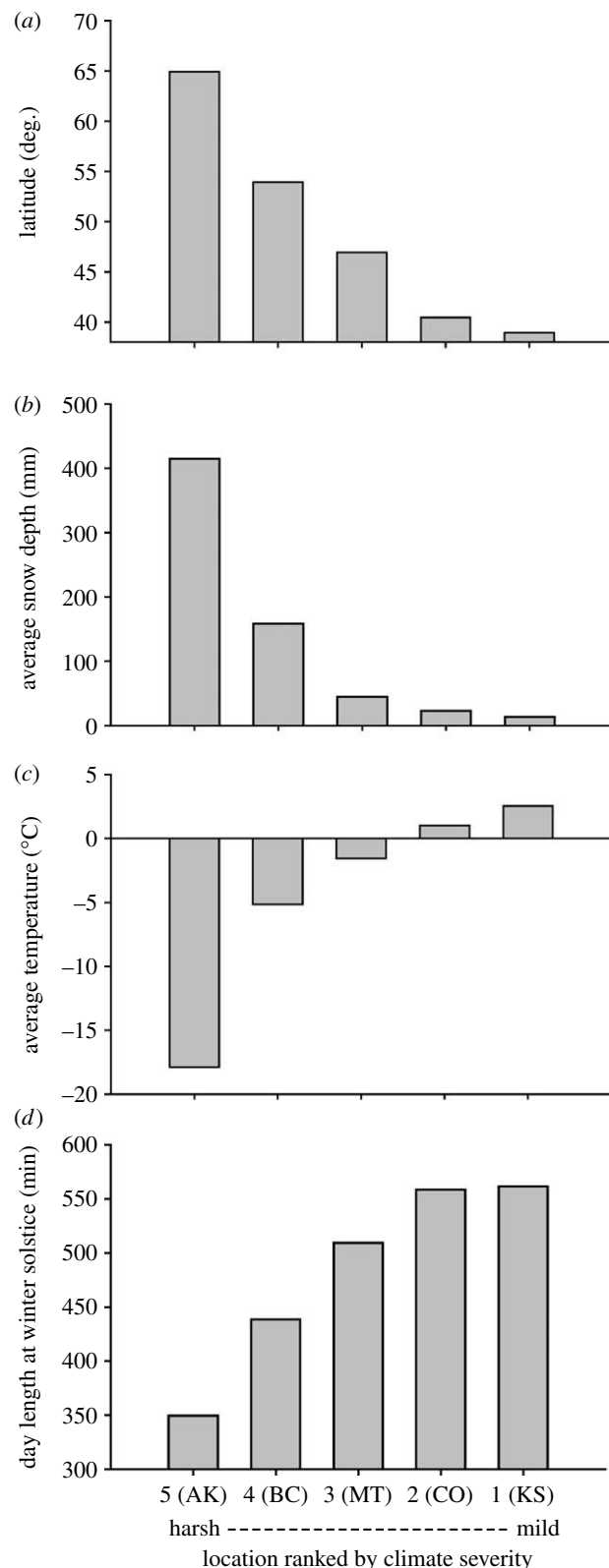


Figure 1. Collection locations ranked by (a) latitude and (b–d) climatic severity. All measures of climate severity decrease as latitude decreases. All ranked correlations of latitude and climate variable are perfect (Spearman $\rho = 1$, $p < 0.001$).

(b) Tissue preparation

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 4 per cent paraformaldehyde in 0.1 M phosphate buffer. The brains were post-fixed

in 4 per cent paraformaldehyde for 7 days before cryoprotection in 15 per cent and then 30 per cent sucrose, and then frozen at -80°C for storage.

(c) Tissue analysis

Tissue was cut into $40\ \mu\text{m}$ coronal sections on a Leica CM 3050S cryostat at -20°C . Every fourth section was mounted and stained with thionin Nissl stain. Hp volumes and neuron numbers were estimated with modern stereological methods using STEREOINVESTIGATOR software (Microbrightfield, Inc., Colchester, VT) and Leica microscope (M4000B). Both the Hp and telencephalon were measured in their entirety. We measured the Hp (the hippocampal formation or hippocampal complex) as per Krebs *et al.* (1989). Brain volumes were estimated with the Cavalieri procedure (Gundersen & Jensen 1987). Hp volume was measured with a $200\ \mu\text{m}$ grid; telencephalon volume was measured with a $1200\ \mu\text{m}$ grid. The optimal grid size and frequency of sections sampling has been determined previously (Pravosudov & Omanska 2005). Neuron counts were performed with an optical fractionator procedure (West *et al.* 1991) at $1000\times$. A $250\ \mu\text{m}$ grid with a $30\times 30\ \mu\text{m}$ counting frame, $5\ \mu\text{m}$ dissector height and $2\ \mu\text{m}$ guards was used as in the previous studies of chickadees (Pravosudov & Omanska 2005). We calculated a coefficient of error (CE) to estimate precision with the nugget effect for both neuron counts (CE mean (s.e.) = 0.097 (0.003)) and volume (CE mean (s.e.) = 0.016 (0.000)). The left and right hemispheres were measured independently and summed to produce the reported total values. There were no significant differences between left and right hippocampal volume and between the total number of neurons in right and left Hp (repeated-measures general linear model (GLM). Hp, $F_{1,56} = 1.143$, $p = 0.290$; neuron numbers: $F_{1,62} = 0.036$, $p = 0.850$). All brains were measured blind to location.

(d) Climate data

Climatological data are represented as the average over the winter months (November to March) of yearly averages from 1971 to 2000. Data from AK, MT, CO and KS were obtained from the National Oceanic and Atmospheric Administration (2008) climate database. Data for BC were obtained from the Environment Canada climate database (Environment Canada 2008). We used average winter temperature ($^{\circ}\text{C}$), average snow depth (mm) and day length (min) during winter solstice as our climate variables of interest.

(e) Statistical analysis

We tested the specific prediction that Hp volume and neuron count would decline with latitude, i.e. a decline in climate severity. Thus, we used an ordered heterogeneity test to assess the predicted relationship among sites ranked by environmental harshness (Gaines & Rice 1990). We analysed Hp volume and neuron count after controlling for telencephalon volume and body mass within a GLM; we report least-squares means in the analyses (table 1). The ordered heterogeneity test followed Rice & Gaines (1994).

3. RESULTS

Hp volume and the total number of Hp neurons relative to the remainder of telencephalon and body mass increased continuously with an increase in latitude and climate severity (perfect rank correlation of latitude and

Table 1. Average (s.e.m.) morphological data for black-capped chickadees collected at five locations along a gradient of environmental harshness. (Locations ranked by climate severity from harsh to mild.)

Location	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) ^a	relative number of hippocampal neurons ($\times 10^6$) ^a	dates collected (2007)
Fairbanks, AK	11.37 (0.17)	65.88 (0.70)	0.77 (0.01)	28.15 (0.87)	1.952 (0.161)	494.26 (15.55)	28.38 (0.85)	2.030 (0.128)	18–20 Sept
Prince George, BC	10.87 (0.17)	67.05 (0.64)	0.80 (0.02)	26.60 (0.82)	1.877 (0.126)	502.61 (21.94)	27.04 (0.98)	2.013 (0.148)	29 Sept to 1 Oct
Missoula, MT	11.42 (0.30)	65.83 (0.58)	0.73 (0.02)	25.07 (0.77)	1.735 (0.104)	446.90 (21.90)	25.19 (0.85)	1.745 (0.128)	10 Oct
Fort Collins, CO	11.65 (0.19)	67.19 (0.42)	0.76 (0.01)	25.03 (0.87)	1.727 (0.115)	485.82 (11.84)	25.04 (0.79)	1.736 (0.120)	15–17 Oct
Manhattan, KS	12.84 (0.24)	69.15 (0.73)	0.74 (0.02)	23.15 (0.63)	1.578 (0.098)	459.10 (14.91)	22.50 (1.01)	1.393 (0.153)	21–23 Oct

^a Least-squares means values calculated from a GLM analysis using telencephalon and body mass as covariates.

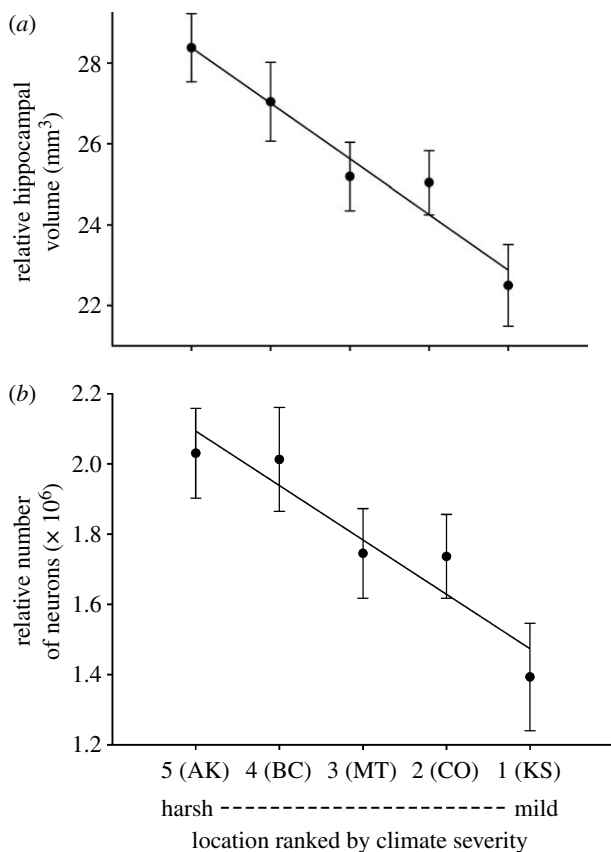


Figure 2. Relative (a) hippocampal volume and (b) neuron numbers (least-squares means) across locations ranked by climate severity. Chickadee Hp volumes (ordered heterogeneity test, $r_s P_c = 0.997$, $k=5$, $p<0.001$) and neuron numbers ($r_s P_c = 0.944$, $k=5$, $p<0.001$) significantly decreased with latitude and climate severity. Both rank Hp volumes and neuron numbers perfectly correlated with rank latitude and climate (all Spearman $\rho = 1$, $p<0.001$). Analysis of absolute Hp volumes ($r_s P_c = 0.999$, $k=5$, $p<0.001$) and neuron counts ($r_s P_c = 0.760$, $k=5$, $p<0.01$) did not change the observed patterns.

climate severity; figure 1*a–d*; all $p<0.001$, Spearman rank correlation). Black-capped chickadees in harsh climates had larger relative Hp ($p<0.001$, ordered heterogeneity test; figure 2*a*) and more Hp neurons ($p<0.001$, ordered heterogeneity test; figure 2*b*) than their conspecifics in more mild climates. We observed a perfect rank correlation of Hp measures, latitude and all climate variables (all $p<0.001$, Spearman rank correlation; compare figures 1 and 2). Absolute Hp volume and the total number of neurons also followed exactly the same pattern (all $p<0.01$; table 1).

4. DISCUSSION

The observed relationship between latitude, climate and brain morphology of chickadees along our environmental gradient followed exactly the predicted pattern. Our results demonstrate that as latitude increases and climates become more harsh, chickadees increase their Hp volumes and Hp neuron numbers (both in absolute terms and relative to telencephalon volume). Furthermore, our results suggest that this relationship is quite robust, as it follows the same pattern when observed across the wide geographical range of North America.

This variation in the Hp volume may be explained by the adaptive specialization hypothesis (Krebs *et al.* 1989; Sherry *et al.* 1989; Garamszegi & Eens 2004; Lucas *et al.* 2004). In this study, we have removed the ecological confounds and phylogenetic assumptions of previous work by examining the relationship between the brain and the environment in a single species. In addition, we have demonstrated the relationship between brain morphology and the environment not only with Hp volume but also with Hp neuron counts, which may be more meaningful for variation in memory capabilities. Furthermore, while caching intensity may potentially correlate with environmental conditions, the actual severity of the environment may be a better predictor of the degree of potential dependency on cached food for survival. Ultimately, it is not just the number of caches, but the ability to accurately retrieve those caches that is probably most crucial for fitness. It is this ability to retrieve caches during demanding times that may connect the environment with memory and the brain (Pravosudov & Clayton 2002).

Thus, our results provide strong support for the idea of the adaptive specialization hypothesis. As climate becomes more harsh, chickadees may have increasing demands for more caches and for better memory, and respond by increasing their Hp volumes and the associated neuron numbers. In addition, Pravosudov & Clayton (2002) showed that birds from a harsh climate had higher caching rates and better spatial memory than those from a mild climate. Together, these data demonstrate that the relationship between Hp structure and climate is robust on a large scale, which supports the hypothesis that environmental conditions demanding better memory for survival result in an enlarged Hp with more neurons. Particularly striking is that both Hp and neuron number correlated perfectly with every variable measured to assess climate harshness as theoretically predicted (Pravosudov & Lucas 2001). Thus, in more harsh environments, better memory may be facilitated through a larger Hp and more neurons leading to increased fitness. Our data suggest that this effect can be limited to a specific region of the brain, in our case the Hp, and thus specific environmental pressures may affect Hp volumes and neuron numbers independent of other brain regions.

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