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 & Macpail 2001; Macpail & 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 & Macpail 2001; Macpail & Bolhuis 2001; Brodin & Lundborg 2003;
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 definitely 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°51ʹ N, 147°49ʹ W); Prince George, BC (53°54ʹ N, 122°47ʹ W); Missoula, MT (46°51ʹ N, 114°07ʹ W); Fort Collins, CO (40°39ʹ N, 105°11ʹ W); and Manhattan, KS (39°08ʹ N, 96°37ʹ W). These sites were chosen specifically 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.

(b) Tissue preparation
Brains were extracted immediately after capture. Birds were anaesthetized (0.07 ml of 50 mg ml⁻¹ Nembutal) and perfused transectionally 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 μm 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 our 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 μm grid; telencephalon volume was measured with a 1200 μm 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 !. A 250 μm grid with a 30×30 μm counting frame, 5 μm dissector height and 2 μm 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, F1,56 = 1.143, p = 0.290; neuron numbers: F1,62 = 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 (°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>
<thead>
<tr>
<th>Location</th>
<th>Body mass (g)</th>
<th>Brain mass (g)</th>
<th>Absolute hippocampal volume (mm³)</th>
<th>Absolute number of hippocampal neurons (10⁶)</th>
<th>Relative hippocampal volume (mm³)a</th>
<th>Relative number of hippocampal neurons (10⁶)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fairbanks, AK</td>
<td>11.37 (0.17)</td>
<td>0.77 (0.01)</td>
<td>28.15 (0.87)</td>
<td>1.952 (0.161)</td>
<td>494.26 (15.55)</td>
<td>28.38 (0.85)</td>
</tr>
<tr>
<td>Prince George, BC</td>
<td>10.87 (0.17)</td>
<td>0.80 (0.02)</td>
<td>26.60 (0.82)</td>
<td>1.877 (0.120)</td>
<td>469.26 (15.55)</td>
<td>28.45 (0.85)</td>
</tr>
<tr>
<td>Missoula, MT</td>
<td>11.42 (0.30)</td>
<td>0.73 (0.01)</td>
<td>25.07 (0.77)</td>
<td>1.735 (0.104)</td>
<td>461.90 (21.90)</td>
<td>27.04 (0.98)</td>
</tr>
<tr>
<td>Fort Collins, CO</td>
<td>11.65 (0.19)</td>
<td>0.76 (0.02)</td>
<td>25.03 (0.79)</td>
<td>1.727 (0.105)</td>
<td>485.82 (25.03)</td>
<td>27.04 (0.98)</td>
</tr>
<tr>
<td>Manhattan, KS</td>
<td>12.84 (0.24)</td>
<td>0.74 (0.02)</td>
<td>23.15 (0.63)</td>
<td>1.393 (0.153)</td>
<td>459.10 (14.41)</td>
<td>22.50 (1.01)</td>
</tr>
</tbody>
</table>

a Least-squares means calculated from a GLM analysis using telencephalon and body mass as covariates.
The observed relationship between latitude, climate and brain morphology of chickadees along our environmental gradient followed exactly the predicted pattern. 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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