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Gould, Kristy L.; Gilbertson, Karl E.; Hrvol, Andrew J.; Nelson, Joseph C.; Seyfer, Abigail L.; Brantner, Rose M.; and Kamil, Alan C., "Differences in Relative Hippocampus Volume and Number of Hippocampus Neurons among Five Corvid Species" (2013). *Avian Cognition Papers*. 6.
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Differences in Relative Hippocampus Volume and Number of Hippocampus Neurons among Five Corvid Species

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Abstract

The relative size of the avian hippocampus (Hp) has been shown to be related to spatial memory and food storing in two avian families, the parids and corvids. Basil et al. [*Brain Behav Evol* 1996;47: 156-164] examined North American food-storing birds in the corvid family and found that Clark's nutcrackers had a larger relative Hp than pinyon jays and Western scrub jays. These results correlated with the nutcracker's better performance on most spatial memory tasks and their strong reliance on stored food in the wild. However, Pravosudov and de Kort [*Brain Behav Evol* 67 (2006), 1-9] raised questions about the methodology used in the 1996 study, specifically the use of paraffin as an embedding material and recalculation for shrinkage. Therefore, we measured relative Hp volume using gelatin as the embedding material in four North American species of food-storing corvids (Clark's nutcrackers, pinyon jays, Western scrub jays and blue jays) and one Eurasian corvid that stores little to no food (azure-winged magpies). Although there was a significant overall effect of species on relative Hp volume among the five species, subsequent tests found only one pairwise difference, blue jays having a larger Hp than the azure-winged magpies. We also examined the relative size of the septum in the

five species. Although Shiflett et al. [*J Neurobiol* 51 (2002), 215-222] found a difference in relative septum volume amongst three species of parids that correlated with storing food, we did not find significant differences amongst the five species in relative septum. Finally, we calculated the number of neurons in the Hp relative to body mass in the five species and found statistically significant differences, some of which are in accord with the adaptive specialization hypothesis and some are not.

Keywords: Corvids, Food storing, Hippocampus, Septum, Telencephalon

Introduction

Scatter hoarding is a foraging strategy that has been studied extensively in two avian families: the Corvidae, which includes crows, jays, magpies, and nutcrackers, and the Paridae, which includes chickadees and tits. This strategy allows birds to utilize times of food abundance to their advantage by caching extra food for future use. Some birds return later in the same day or within a few days to retrieve their caches, while others use longer-term storage

to provide a large percentage of their diet throughout the winter, relying very heavily on the caches they make in the fall. Birds that scatter hoard use spatial memory to relocate the caches they made [Balda, 1980; Sherry et al., 1981; Vander Wall, 1982; Sherry, 1984; Kamil and Balda, 1985]. Comparative work within the corvid family has shown that performance on spatial memory tasks in the laboratory is related to scatter-hoarding behavior. Corvids that store more food and rely on it more heavily usually outperform birds that store less food and rely on it less [Balda and Kamil, 1989; Kamil et al., 1994; Olson et al., 1995; Gould-Beierle, 2000]. This same pattern of performance is seen when comparing corvids that do not scatter-hoard to those that do [Clayton and Krebs, 1994]. Comparative work within the Parid family shows a similar trend in some studies [Krebs, 1990; Clayton and Krebs, 1994; McGregor and Healy, 1999] but not others [Healy and Krebs, 1992; Healy, 1995; Healy and Suhonen, 1996].

In terms of functionality, the hippocampus (Hp) is important in the formation of memory in general, and spatial memory and navigation more specifically. The avian Hp has been shown to be directly analogous to the mammalian Hp in terms of neuroanatomy and physiology [Krayniak and Siegel, 1978; Casini et al., 1986; Erichsen et al., 1991; Krebs et al., 1991; Shapiro and Wieraszko, 1996; Szkeley and Krebs, 1996; Siegel et al., 2000; Smulders and DeVogd, 2000; Gould et al., 2001; Siegel et al., 2002; Shiflett et al., 2004; Shimizu et al., 2004]. When the avian Hp is lesioned or temporarily deactivated, birds have a difficult time with spatial navigation [Bingman et al., 2005], spatial tasks [Hampton and Shettleworth, 1996; Shiflett et al., 2003], and finding hidden food [Sherry and Vaccarino, 1989]. The avian Hp is also evolutionarily homologous to that in mammals [Colombo and Broadbent, 2000; Jarvis et al., 2005].

Neuroanatomically, the relative size of the Hp has been shown to be related to spatial memory and scatter hoarding when comparing species within the two avian families in question [corvids: Healy and Krebs, 1992; parids: Hampton et al., 1995; Basil et al., 1996; Healy and Krebs, 1996]. Healy and Krebs [1992] investigated Hp volume relative to body weight in European scatter-hoarding corvids and ranked birds in three categories of food storing: little to no food storing (jackdaw *Corvus monedula* and alpine chough *Pyrrhocorax graculus*), moderate food storing (European crow *Corvus corone*, European magpie *Pica*, rook *Corvus frugilegus*, Asian red-billed blue magpie *Cissa erythrorhynch*), and heavy food storing (European jay *Garrulus glandarius*). They found that Hp volume relative to body mass (BM) was associated

with the degree of food-storing behavior, with a positive correlation between the estimated amount of food-hoarding behavior and the relative volume of the Hp. They also showed that European magpies (scatter hoarders) have a significantly larger relative Hp than jackdaws (non-scatter hoarders).

Basil et al. [1996] examined four North American scatter-hoarding species in the corvid family: Clark's nutcrackers, Western scrub jays, pinyon jays, and grey-breasted jays (now called Mexican jays). The residuals from linear regressions of Hp on telencephalon showed substantial deviations, with the nutcracker and scrub jay having positive residuals, while the pinyon jay and grey-breasted jay had negative residuals. The nutcracker residuals were the largest, which correlate with the nutcracker's better performance on most spatial memory tasks [Balda and Kamil, 1989; Kamil et al., 1994; Olson et al., 1995] and their strong reliance on stored food in the wild [Vander Wall and Balda, 1981]. However, sample sizes were small and it is unknown whether the residuals were significantly different among the four species.

Brodin and Lundborg [2003] did a meta-analysis on all of the corvid and parid Hp volume data sets available at the time and did not find a significant relationship between food-caching and Hp volume. They also collected data in additional individuals of four species and found no significant differences when compared to their previous measurements, so the original differences that were found among species were not due to discrepancies in measuring. Brodin and Lundborg used the hoarding categories of Healy and Krebs [1992, 1996], which included non-hoarders, non-specialized hoarders, and specialized hoarders. They concluded that the reason for the discrepancy between previous studies and their own was how food-storing behavior was defined and categorized, as well as the small numbers of individuals used in previous studies.

In their meta-analysis, Brodin and Lundborg [2003] pooled all data for each family, instead of looking at North American and Eurasian birds separately. Lucas et al. [2004] took the continent where the bird lives into account because Eurasian birds tend to be larger in weight and have larger brains in general than North American birds. They used the same data sets as Brodin and Lundborg [2003], but controlling for continent. With continent thus included in the analysis, there was a significant relationship between food-storing and Hp volume in both parids and corvids. However, they found no differences in Hp volume among the corvids they used without factoring in continent and only found significant differences between corvids that store no food and those

that store food even when accounting for continent. There were no differences in Hp volume between corvids that they categorized as non-specialized hoarders (e.g. Western scrub jay) and specialized hoarders (e.g. Clark's nutcracker). Garamszegi and Eens [2004] continued by adding a larger data set of non-caching birds and controlling for phylogenetic associations and found a significant relationship between food storing and Hp volume, even without factoring in continent. However, Garamszegi and Lucas [2005] concluded that the continent difference in relative Hp size is robust and found in many species of birds, but is most likely independent of food hoarding. The reason for the differences found between birds on the two continents is still not clear. What we might conclude from these series of studies is that direct neuroanatomical comparisons between North American and European corvids and parids may not be valid. However, Pravosudov and de Kort [2006] argued that this may be a premature conclusion based on results showing that their sample of Western scrub jays had an overall relative brain size that was larger than any of the European corvids that have been measured.

While it appears that there is some debate as to a correlation between relative Hp and food storing within the corvid family, there also appear to be problems with the only multispecies North American data set of corvid Hp measurements [Basil et al., 1996]. Pravosudov and de Kort [2006] raised questions about the methodology used in the Basil et al. study, specifically the use of embedding materials and recalculation for tissue shrinkage. Basil et al. embedded their tissue in paraffin before slicing. However, Pravosudov and de Kort, as well as more recent studies done in parids and corvids, froze the tissue before slicing it. While Basil et al. corrected for tissue shrinkage due to the use of paraffin embedding, Pravosudov and de Kort argue that the correction used may not have been accurate and that the measurements in the Basil et al. study may be incompatible with other measurements done with birds using frozen tissue. Therefore, they feel that including the Basil et al. data within a meta-analysis or comparing it to frozen tissue should be avoided. Pravosudov and de Kort measured the Hp in 21 Western scrub jays in their study and found that those birds had a larger absolute and relative Hp volume than those of Basil et al., when corrected for shrinkage [1996]. This indicates that shrinkage may indeed be a problem with this data set when trying to compare it to other data sets that were prepared differently.

Another aspect of Basil et al. [1996] was that differences among species in relative Hp volume were not

statistically analyzed in a similar way to other studies. Residuals of the regressions were plotted and qualitatively compared, but were not statistically analyzed further. Therefore, even though the Clark's nutcrackers had a larger positive residual than Western scrub jays, and the other two species, pinyon jays and Mexican jays, had negative residuals, there were no significant effects reported.

Basil et al. [1996] is the only study measuring Hp volume within North American corvids to date. Because this study used a different method to prepare the tissue than most others, it is probably not appropriate to include it within a larger meta-analysis due to problems with shrinkage of tissue. Our purpose here was to directly analyze tissue that has been processed in a similar way to other studies. We also wanted to determine if there were differences in septum volume. We therefore calculated relative Hp and septum volume based on the standard methodology used by others (slicing frozen tissue) in three of the North American scatter-hoarding corvids investigated in Basil et al., Clark's nutcrackers (*Nucifraga columbiana*), pinyon jays (*Gymnorhinus cyanocephalus*), and Western scrub jays (*Aphelocoma californica*). We also included another North American scatter-hoarding corvid, the blue jay (*Cyanocitta cristata*). We were interested in the blue jay because there is only one data point for relative Hp volume in this species [Sherry et al., 1989]. All four species scatter hoard to varying degrees for both short- and long-term recovery [Balda, 1980; Darley-Hill and Johnson, 1981; Johnson and Adkisson, 1985; Balda, 1987]. We also included an additional Eurasian corvid, the azure-winged magpie (*Cyanopica cyana*) because this species does not scatter hoard or cache food routinely and if they do cache, it is only highly valued food items for short periods of time [Turcek and Kelso, 1968; Canario et al., 2002]. The azure-winged magpie has not been included in any Hp analyses in the past.

We also examined the relative size of the septum in these five species of birds. The septum shares reciprocal connections with the Hp, and in mammals, these connections are important in the acquisition and consolidation of spatial memory [Chrobak et al., 1989; Poucet et al., 1991; Chrobak and Napier, 1992; Poucet and Buhot, 1994; Walsh et al., 1998; Smith and Pang, 2005]. Similar reciprocal connections between septum and Hp are found in the avian brain [Krayniak and Siegel, 1978; Szekely and Krebs, 1996; Szekely, 1999]. Shiflett et al. [2002] showed a difference in septum volume relative to telencephalon amongst three species of parids, with the scatter-hoarding species having a larger relative septum than the species that do not store food. However, Pravosudov [2009]

found no differences in septum volume due to nutritional deprivation in Western scrub jays, while nutritional deprivation did cause differences in the volume of the Hp. He concludes that the role of the septum may be less crucial to spatial memory than the Hp because nutritionally deprived birds perform worse on spatial memory tasks and have a smaller Hp, but do not differ in septum volume.

Finally, we also measured the number of neurons in the Hp of each individual within the five species of corvids to look for species differences. While volumetric analysis may be an easy method to use in the search for neurobiological differences among species, volume may not be the dimension by which species differences in the Hp express themselves. Roth et al. [2010] suggested that future research should focus on, among other things, the number of neurons. Previous studies have shown correlations between Hp volume and the number of neurons in the Hp in birds of the Paridae; the larger the Hp, the more neurons within the Hp. Healy et al. [1994] found that food-storing marsh tits had both a larger Hp and more neurons within the Hp than non-food-storing blue tits. Smulders et al. [2000] found a larger number of neurons in the Hp of food-storing black-capped chickadees in the fall than at other times of the year, which coincides with a larger autumnal Hp volume as well (but see Hoshoooley and Sherry [2004]). Finally Pravosudov and Clayton [2002] and Roth and Pravosudov [2009] found within-species differences in different populations of the blackcapped chickadee. Birds from harsher climates had both a larger Hp and more neurons within the Hp than birds from milder climates.

Materials and Methods

The original research reported herein was performed under guidelines established by the Institutional Animal Care and Use Committee at the University of Nebraska.

Four Clark's nutcrackers, 4 pinyon jays, 5 Western scrub jays, 5 blue jays, and 4 azure-winged magpies were used in this analysis. Clark's nutcrackers were captured from a wild population in North Central Colorado and had been in captivity for 7-14 years. Pinyon jays were captured from a wild population near Flagstaff, Ariz., USA, and had been in captivity for 11-13 years. Scrub jays were captured from a wild population in Flagstaff and had been in captivity for approximately 5 years. Blue jays were taken from nests in Lincoln, Nebr., USA, and hand raised in the laboratory. They had been in captivity for 4-11 years. Azure-winged magpies were captured from a banded wild population near Badajoz, Spain, that has been studied for a number of years by Carlos de la Cruz. They had been in captivity for approximately 5 years.

Tissue Preparation

All birds were anesthetized with Nembutal and perfused transcardially with 0.9% saline and 0.1% sodium nitrite in a 0.1 M sodium phosphate buffer solution. Brains were removed immediately and placed in 30% sucrose-4% paraformaldehyde-phosphate buffer until they sank and then embedded in 10% gelatin-30% sucrose. The gelatin blocks were placed in 4% paraformaldehyde-phosphate buffer, frozen, and sliced coronally at 40 μm . Sections were collected and every 6th section was mounted and stained with cresyl violet.

Tissue Analysis

The overall volume of the Hp, septum, and entire telencephalon were measured using boundaries determined in accordance with published cytoarchitecture criteria [telencephalon: Karten and Hodos, 1967; Hp: Krebs et al., 1989; septum: Shiflett et al., 2002] (Figure 1). Brain sections were visualized on a Zeiss Axioskop 40 microscope and captured on an AxioCam MRc5 digital camera. Axio Vision LE software was used to outline the surface area of each section. Volumes were calculated by multiplying the surface area by the distance between the center planes of the measured sections.

Brain sections were later viewed on an Olympus BH-2 microscope under a Nikon Plan Apo $\times 60/1.4$ oil immersion lens for cell counting in the Hp and an Olympus D Plan $\times 4/0.10$ dry objective lens for Hp boundary tracing. An Optronics MicroFire digital camera mounted to the microscope was used to project the image from the microscope. MicroBrightField's software, Stereo Investigator (SI) β version 10, June 2010, was used to draw boundaries and estimate cell populations (MBF Bioscience, Williston, Vt., USA). Stereo Investigator's optical fractionator workflow was used to generate neuron population estimates.

Slides were viewed first under a $\times 4$ D Plan objective. The boundaries where neuron counting was to take place were traced around the Hp as defined in accordance with published cytoarchitecture criteria (same boundaries were used as in volumetric analysis). Neurons were identified using the standard criteria of the presence of Nissl-stained cytoplasm, identifiable nucleoli, and shape. Counting grid size was between 400×400 and $800 \times 800 \mu\text{m}$. Counting frame size was $37 \times 37 - 40 \times 40 \mu\text{m}$ throughout the study and a consistent grid size was used within each individual bird. The frame size and grid size were adjusted for each species based on the size of the Hp. Dissector height remained at 6 μm , with 2- μm guard zones above and below the dissector. For all measures, a Gunderson coefficient of error was calculated to estimate precision for the neuron counts. Mean coefficient of error and standard error for each species was: nutcrackers (0.04, 0.003), pinyon jays (0.05, 0.003), scrub jays (0.04, 0.005), blue jays (0.04, 0.003), and magpies (0.04, 0.003).

Results

North American Scatter-Hoarding Species: Volume Analyses

First, we analyzed the results of the four species that reside in North America (Clark's nutcracker, pinyon jay, Western scrub jay, and blue jay) because of previous

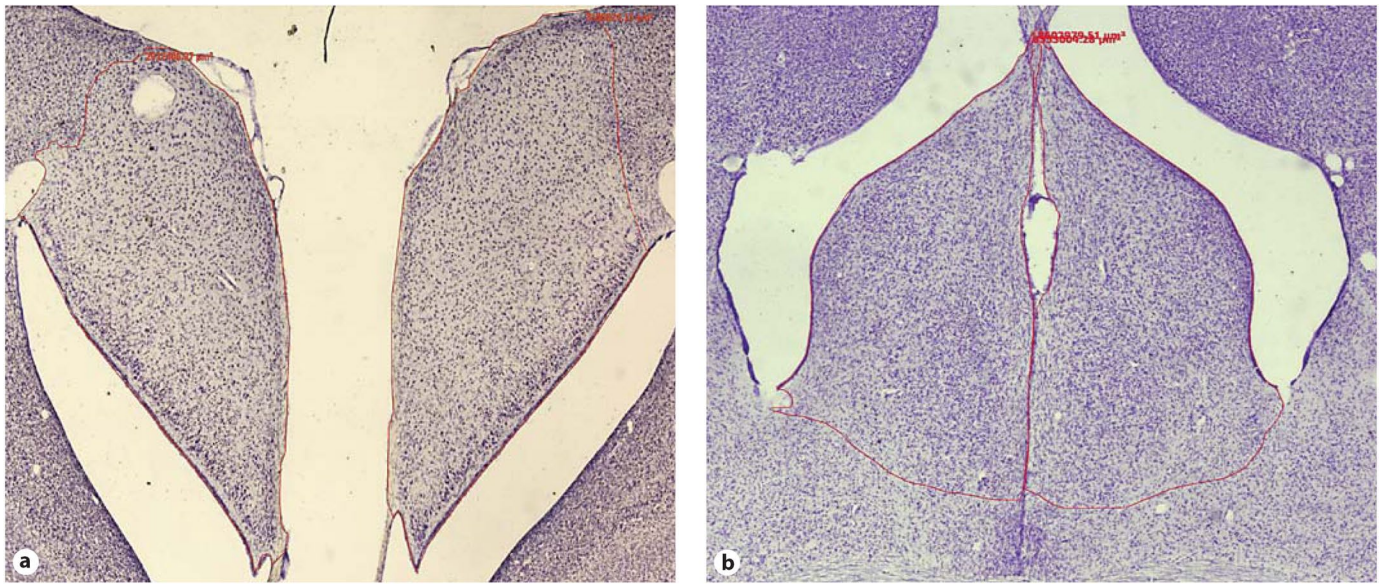


Figure 1. Photomicrographs of a 40-µm section of Western scrub jay Hp (a) and septum (b) with the boundaries outlined in each.

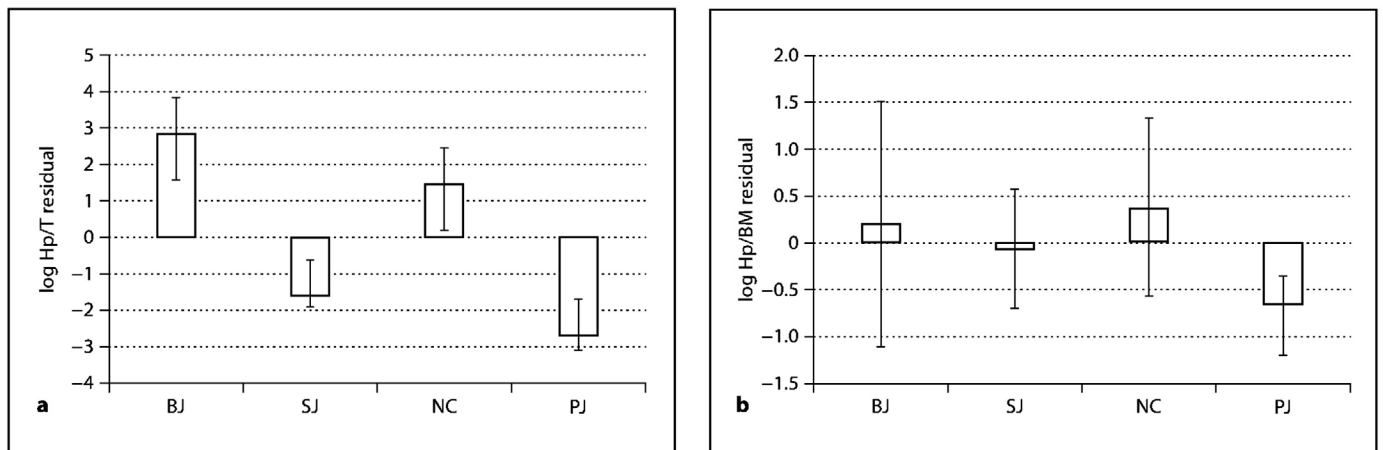


Figure 2. The residuals and SD for Hp volume relative to the remainder of the telencephalon (T) volume (a) and Hp volume relative to BM (b) in the four North American species of corvids. BJ = Blue jay; SJ = Western scrub jay; NC = Clark's nutcracker; PJ = pinyon jay.

research showing that North American food-hoarding species have smaller brains than their Eurasian counterparts [Lucas et al., 2004], making direct comparisons more difficult. We found a significant increase in log Hp volume as log BM increased ($R = 0.489$, $F_{1,16} = 5.025$, $p = 0.040$). We also found a significant increase in log Hp volume as log telencephalon increased ($R = 0.608$, $F_{1,16} = 9.392$, $p = 0.007$).

We tested for differences among the four species with a one-way ANOVA of the residuals of log Hp on log telencephalon (Figure 2a) and the residuals of log Hp on log

BM (Figure 2b). We found no significant differences for log Hp on log telencephalon ($F_{3,14} = 1.83$, $p = 0.190$) or on log BM ($F_{3,14} = 1.68$, $p = 0.22$).

We found no significant effect of log septum regressed on log BM ($R = 0.187$, $F_{1,16} = 0.557$, $p = 0.459$). However, we found a linear trend of log septum regressed on log telencephalon ($R = 0.458$, $F_{1,16} = 4.254$, $p = 0.056$). Therefore, septum volume does not increase as BM increases, but there is a positive trend in septum volume as telencephalon volume increases.

We tested for species differences with a one-way

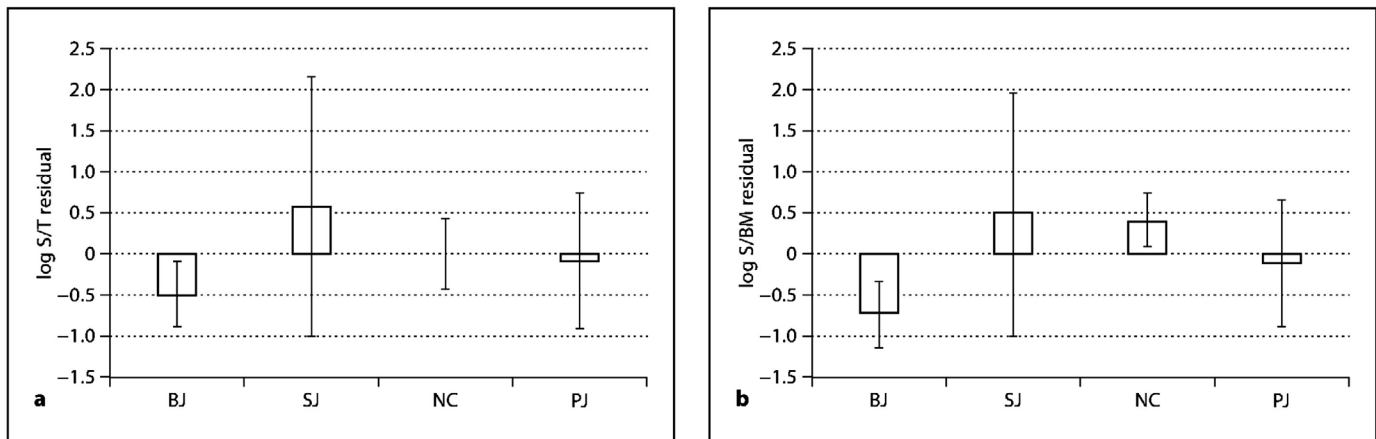


Figure 3. The residuals and SD for septum (S) volume relative to the remainder of the telencephalon (T) volume **(a)** and septum volume relative to BM **(b)** in the four North American species of corvids. BJ = Blue jay; SJ = Western scrub jay; NC = Clark's nutcracker; PJ = pinyon jay.

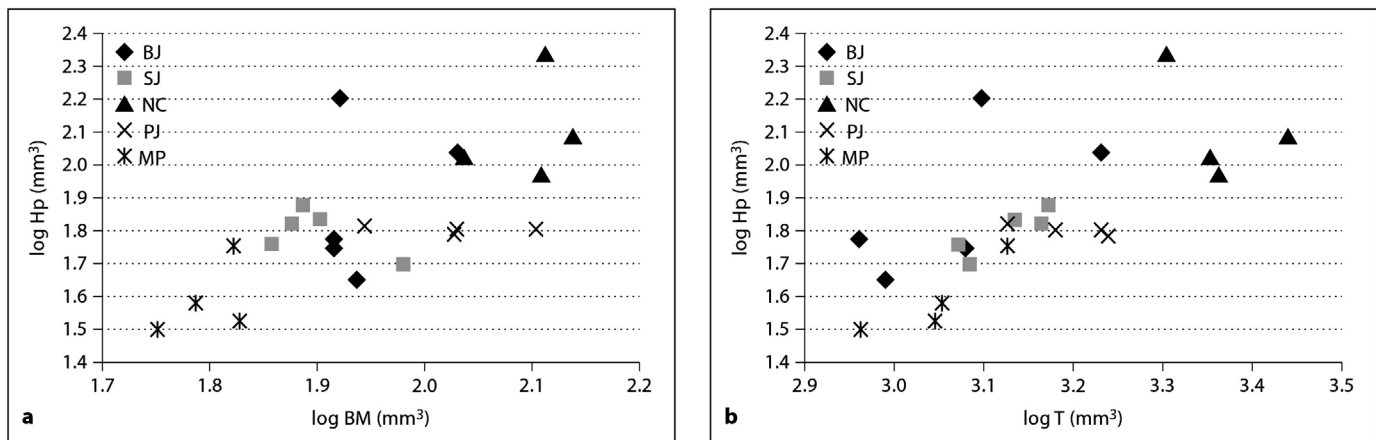


Figure 4. log Hp volume plotted against log BM **(a)** and log telencephalon (T) volume **(b)**. BJ = Blue jay; SJ = Western scrub jay; NC = Clark's nutcracker; PJ = pinyon jay; MP = azure-winged magpie.

ANOVA of the residuals of log septum on log telencephalon (Figure 3a) and the residuals of log septum on log BM (Figure 3b). We found no significant differences for log septum on log telencephalon ($F_{3,14} = 1.01$, $p = 0.420$) or on log BM ($F_{3,14} = 1.93$, $p = 0.170$).

We calculated the power of these ANOVAs as a function of the difference between the means assuming that the means were evenly spaced in terms of the dependent variable. For simplicity, we also scaled the differences between the means in terms of the total variance in the data (using standard deviations, SD). Thus we determined the SD of the data set and calculated the probability of

detecting effects of ± 1.0 , ± 1.5 , and ± 2.0 SD, which were 0.448, 0.757, and 0.927, respectively. Lucas et al. [2004] reported a power of 0.54 with an effect size of $r^2 = 0.390$, which corresponds to an effect size of ± 1.6 SD [Cohen, 1988]. Comparatively then, our power of 0.757 (± 1.5 SD) is significantly greater than that of Lucas et al. [2004].

All Species: Volume Analyses

We then analyzed the data including the North American species and the azure-winged magpie. We found a significant linear effect of log Hp regressed on log BM ($R = 0.681$, $F_{1,20} = 17.309$, $p < 0.0001$). As BM increases, Hp

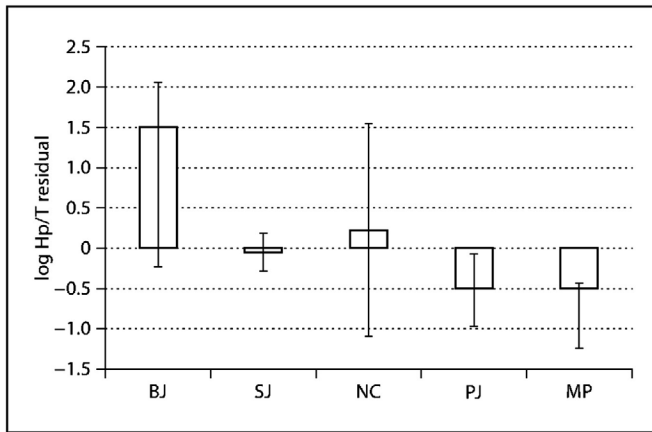


Figure 5. The residuals and SD for the Hp volume relative to the remainder of the telencephalon (T) volume in all five species of corvids. BJ = Blue jay; SJ = Western scrub jay; NC = Clark's nutcracker; PJ = pinyon jay; MP = azure-winged magpie.

volume increases (Figure 4a). We also found a significant linear effect of log Hp regressed on log telencephalon ($R = 0.608$, $F_{1,20} = 18.948$, $p < 0.0001$). As telencephalon volume increases, Hp volume increases (Figure 4b).

We tested for species differences with a one-way ANOVA of the residuals of log Hp on log telencephalon, and found a significant effect ($F_{4,17} = 3.007$, $p = 0.048$; Figure 5). A post hoc Tukey honestly significant difference analysis indicated that only the difference between blue jays (mean residual = 0.9217) and azure-winged magpies (mean residual = -0.8370) was significant. A one-way ANOVA of the residuals of log Hp on log BM, however, found no significant differences among species ($F_{4,17} = 1.432$, $p = 0.266$).

We found significant linear effects of log septum regressed on log BM ($R = 0.44$, $F_{1,20} = 4.814$, $p = 0.040$) and on log telencephalon ($R = 0.551$, $F_{1,20} = 8.726$, $p = 0.008$). When all five species are included in the analysis, septum volume increases as BM (Figure 6a) and telencephalon volume (Figure 6b) increase.

We tested for differences amongst species with a one-way ANOVA. We found no significant effects of the residuals of log septum on log telencephalon ($F_{4,17} = 1.875$, $p = 0.161$) or of the residuals of log septum on log BM ($F_{4,17} = 1.377$, $p = 0.284$).

In order to determine the power of these ANOVAs, we calculated the SD of the data set and calculated the probability of detecting effects of ± 1.0 , ± 1.5 , and ± 2.0 SD, which were 0.671, 0.947, and 0.999, respectively. Again, our power of 0.947 with an effect size of ± 1.5 SD is much

larger than the power of Lucas et al. [2004] of 0.54, with an effect size of ± 1.6 SD.

New World Jays: Volume Analyses

We also performed separate analyses including just the three North American jay species (Western scrub jay, pinyon jay, and blue jay). Lucas et al. [2004] placed Clark's nutcrackers with the North American jays when doing their continent analysis of relative Hp volume, but did not take continent of origin into account (although they did acknowledge that Clark's nutcrackers are more closely related to the Eurasian corvids and have a similar relative Hp volume). Clark's nutcrackers, while found in North America, are old world in origin [Hope, 1989] and most closely related to the Eurasian nutcracker (*Nucifraga caryocatactes*) [Ericson et al., 2005]. Therefore, their evolutionary relationships are very different from that of the three new world jay species and this may also be reflected in the evolution of the Hp, spatial memory, and food-storing behavior as well.

We did not find a significant increase in log Hp volume as log BM increased ($R = 0.041$, $F_{1,12} = 0.020$, $p = 0.890$) or as log telencephalon increased ($R = 0.334$, $F_{1,12} = 1.508$, $p = 0.243$). When we tested for differences with a one-way ANOVA of the residuals of log Hp on log BM ($F_{2,11} = 0.566$, $p = 0.583$) or of log Hp on log telencephalon ($F_{2,11} = 1.854$, $p = 0.202$), no significant effects were found.

We also did not find a significant increase in log septum volume as log BM increased ($R = 0.151$, $F_{1,12} = 0.279$, $p = 0.607$) or as log telencephalon increased ($R = 0.304$, $F_{1,12} = 1.223$, $p = 0.290$). When we tested for species differences with a one-way ANOVA of the residuals of log Hp on log BM ($F_{2,11} = 1.773$, $p = 0.215$) or of log Hp on log telencephalon ($F_{2,11} = 1.218$, $p = 0.333$), there were no significant effects.

Comparisons with Previous Volumetric Research

When the absolute Hp volumes of our scrub jays ($n = 5$), pinyon jays ($n = 4$), and nutcrackers ($n = 4$) are compared species by species with those in Basil et al. [1996] (scrub jays $n = 2$, pinyon jays $n = 2$, and nutcrackers $n = 4$) adjusted for 23% shrinkage, there are significant differences between the two studies for all three species (one-way ANOVA for scrub jays: $F_{1,5} = 17.25$, $p = 0.038$; pinyon jays: $F_{1,4} = 483.36$, $p < 0.001$; nutcrackers: $F_{1,6} = 7.31$, $p = 0.035$; Table 1), with the larger Hp volumes in the current study. A similar analysis of Hp volume relative to telencephalon volume found that our scrub jays ($F_{1,5} = 28.93$,

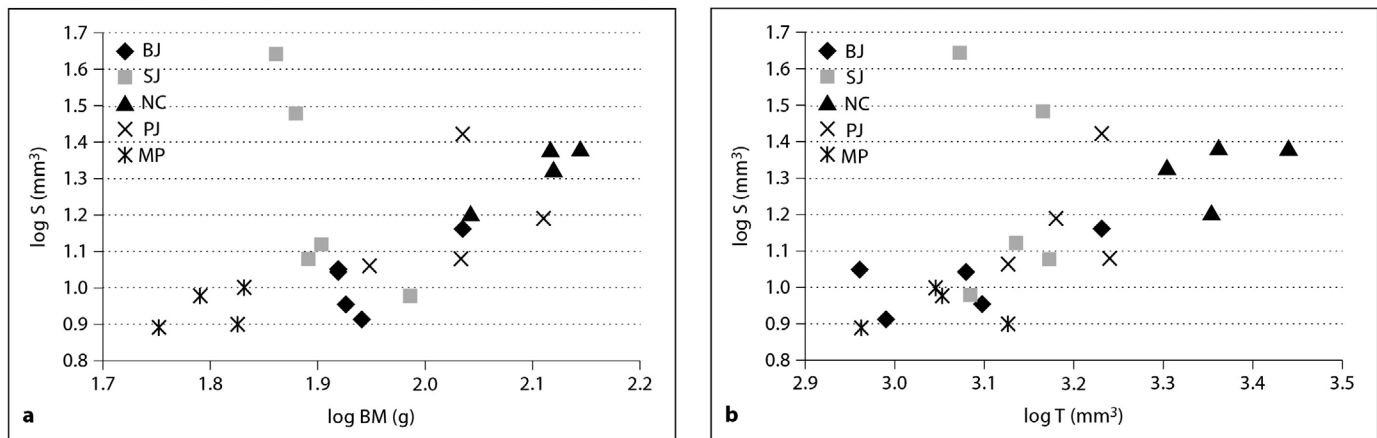


Figure 6. log septum (S) volume plotted against log BM (**a**) and log telencephalon (T) volume (**b**). BJ = Blue jay; SJ = Western scrub jay; NC = Clark's nutcracker; PJ = pinyon jay; MP = azure-winged magpie.

$p = 0.0003$) and pinyon jays ($F_{1,4} = 11.11$, $p = 0.029$) had a larger relative Hp volume than the birds of Basil et al., but the nutcrackers did not ($F_{1,6} = 2.27$, $p = 0.182$; Table 2). Absolute telencephalon volume of our birds and those of Basil et al. adjusted for shrinkage show significant differences between the studies for scrub jays ($F_{1,5} = 7.82$, $p = 0.038$) and nutcrackers ($F_{1,6} = 11.75$, $p = 0.014$), but not pinyon jays ($F_{1,4} = 2.32$, $p = 0.202$; Table 3).

We also compared our scrub jay results with those of Pravosudov and de Kort [2006] and Basil et al. [1996]. Our scrub jays were intermediate in terms of absolute size, both in telencephalon and Hp (Table 4).

Sherry et al. [1989] analyzed the Hp and telencephalon volume in 1 blue jay using standard methodology. Hp volume was 45.68 mm^3 and telencephalon volume was 996.91 mm^3 . Our blue jays ($n = 5$) had both a larger mean Hp volume (85.39 mm^3) and telencephalon volume ($1,126.6 \text{ mm}^3$). We are not certain why there is such a large discrepancy, but small sample size and natural variation in size might account for it. One of our blue jays had Hp and telencephalon volumes similar to the blue jay in Sherry et al. (Hp = 44.51 mm^3 and telencephalon = 915.19 mm^3). At any rate, the mean relative Hp of blue jays in our study was the largest in our data set, but statistically different only from the azure-winged magpies.

All Species: Neuron-Counting Analyses

Number of neurons relative to BM was skewed (skewness = 1.747), meaning the data were not normally distributed. Therefore, we used a non-parametric Kruskal-Wallis test, which does not assume a normal distribution. The test revealed significant differences among species in respect to

Table 1. Clark's nutcrackers, pinyon jays, and Western scrub jays mean absolute Hp volume in mm^3 (SD) and sample size in two independent studies.

Species	Our study	n	Basil et al. [1996]	n
Clark's nutcrackers	134.78 (184.43)	4	57.99 (14.16) ¹	4
Pinyon jays	63.25 (1.53)	4	40.13 (0) ¹	2
Western scrub jays	62.91 (9.9)	5	32.06 (3.6) ¹	2

1. Adjusted for shrinkage.

Table 2. Clark's nutcrackers, pinyon jays, and Western scrub jays mean Hp relative to the remainder of the telencephalon in mm^3 (SD) and sample size in two independent studies.

Species	Our study	n	Basil et al. [1996]	n
Clark's nutcrackers	0.0601 (0.0321)	4	0.0337 (0.0080) ¹	4
Pinyon jays	0.0407 (0.0059)	4	0.0281 (0.0021) ¹	2
Western scrub jays	0.0468 (0.0040)	5	0.0307 (0.0004) ¹	2

1. Adjusted for shrinkage.

number of neurons in the Hp relative to BM [χ^2 (4, $n = 22$) = 15.4, $p = 0.004$]. In pairwise Mann-Whitney U tests between species, significant differences were found between nutcrackers and the other four species: blue jays ($z = -2.449$, $p = 0.014$), pinyon jays ($z = -2.449$, $p = 0.014$), scrub jays ($z = -2.021$, $p = 0.043$), and azure-winged magpies ($z = -2.309$, $p = 0.021$; Figure 7a). Significant

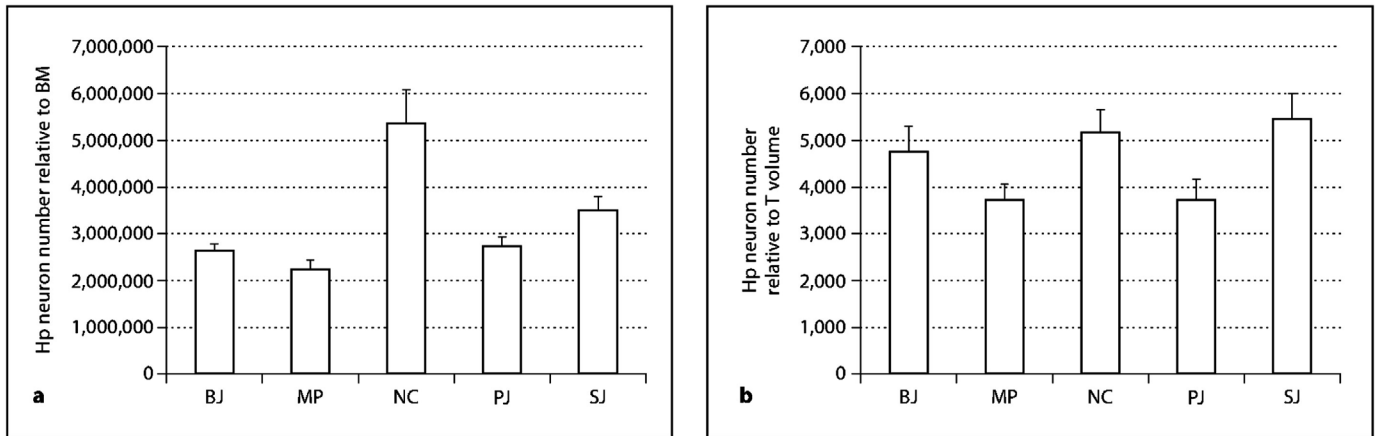


Figure 7. The number of neurons in the Hp relative to BM **(a)** and telencephalon (T) volume **(b)** for each of the five species of birds. Error bars are SE. BJ = Blue jay; SJ = Western scrub jay; NC = Clark's nutcracker; PJ = pinyon jay; MP = azure-winged magpie.

Table 3. Clark's nutcrackers, pinyon jays, and Western scrub jays mean absolute telencephalon volume in mm³ (SD) and sample size in two independent studies.

Species	Our study	n	Basil et al. [1996]	n
Clark's nutcrackers	2,191.29 (340.14)	4	1,717.21 (219.04) ¹	4
Pinyon jays	1,510.15 (184.43)	4	1,429.87 (80.61) ¹	2
Western scrub jays	1,278.54 (131.19)	5	1,094.6 (55.6) ¹	2

1. Adjusted for shrinkage.

Table 4. Mean absolute Hp volume (in mm³), SD, and sample size of Western scrub jays in three independent studies.

Study	Hp volume	SD	n
Current study	62.91	9.9	5
Pravosudov and de Kort [2006]	73.19	6.9	21
Basil et al. [1996]	32.061	3.6	2

1. Adjusted for shrinkage.

differences were also found between the scrub jays and the other three species: blue jays ($z = -2.205$, $p = 0.027$), pinyon jays, ($z = -.96$, $p = 0.05$), and azure-winged magpies ($z = -2.309$, $p = 0.021$; fig. 7a). There were no other species differences.

Number of neurons relative to telencephalon volume was not skewed (skewness = 0.237), meaning the data

were normally distributed. Therefore we analyzed the data using a parametric one-way ANOVA of the residuals of log neuron count on log telencephalon volume and did not find significant differences among species ($F_{4,16} = 2.79$, $p = 0.062$; Figure 7b). We also analyzed the data using a nonparametric Kruskal-Wallis test, so that the results could be compared to those of the previous Kruskal-Wallis test and found no significant differences among species [$\chi^2(4, n = 22) = 7.921$, $p = 0.095$]. In both cases, the results were not statistically significant, but showed a trend toward species differences.

Discussion

Hp: North American Species

We found no significant differences in relative Hp volume amongst the four species of North American scatterhoarding corvids: Clark's nutcrackers, pinyon jays, Western scrub jays, and blue jays. Previous work suggested that relative Hp size might be correlated with food storing and spatial memory ability in four species of North American corvids [Basil et al., 1996], and differences associated with dependence on cached food have been reported in many studies, as reviewed in the Introduction. However, due to differences in methodology and statistical analyses (discussed in Pravosudov and de Kort [2006]), we think it more likely that this association is either much smaller than thought or nonexistent among the four North American species we studied. Our birds were similar to those of Basil et al. in that they had been in captivity for

substantial amounts of time and were older. Therefore, the major difference is the way in which the tissue was processed. However, our scrub jays and pinyon jays had a larger Hp relative to the remainder of the telencephalon than the same species in Basil et al., but the nutcrackers were not significantly larger. This suggests that Basil et al. may have found that their nutcrackers had a larger relative Hp because their two jay species had relative Hp volumes that were smaller than normal.

Hp: All Species

When the data from all five species were combined in a single analysis, there was a significant effect of species, and the only pairwise contrast that was significant was the blue jay–azure-winged magpie comparison. The blue jays did have the larger mean relative Hp of the four North American species, which was not predicted *a priori*. With only one data point representing blue jay–relative Hp volume [Sherry et al., 1989], we believe this is an interesting finding. Blue jays have not been extensively used to study either food-storing behavior or spatial memory in the laboratory, but they extensively use long-term storage of acorns and beech nuts in the wild [Darley-Hill and Johnson, 1981; Johnson and Adkisson, 1985]. Therefore, their spatial memory ability might be interesting to investigate further. However, since there were no statistically significant differences among our four North American corvids, it appears that relative Hp size may not be a good indicator of spatial memory performance. Therefore, it is not certain how blue jays would compare to the other species on such tasks. However, our results are similar to those of Healy and Krebs [1992], who found significant Hp volume differences between food-storing corvids (Eurasian jays) with non-food-storing corvids (jackdaws). If azure-winged magpies store food, it is very little [Turcek and Kelso, 1968; Canario et al., 2002]. The significant difference between blue jays and magpies in Hp volume then reflects a similar trend.

Our blue jays were the only hand-reared birds in our study. While we have no way of knowing whether hand-rearing may lead to differences in brain development, this might explain the significant difference between blue jays and azure-winged magpies in relative Hp volume, although this would require that hand-rearing has differential effects on the growth of the Hp versus the growth of the remainder of the telencephalon. Roth et al. [2012] have shown that hand-raised black-capped chickadees have significantly smaller relative Hp volumes compared to their wild-caught counterparts. However, there were no significant differences in total neuron number and

neurogenesis in the Hp compared to wild-caught chickadees. In a previous study, LaDage et al. [2009] demonstrated that wild-caught mountain chickadees have a significantly larger Hp volume immediately after capture than after approximately 6 months in captivity, but there was no difference in neuron number. Although our blue jays were hand raised, all of our species had been in captivity for at least 6 months, an environment in which Hp volume decreases. This suggests that Hp volume was equally influenced by captivity across species, which was therefore not an important variable contributing to volume differences.

The inclusion of the European azure-winged magpies provided data for a species of corvid with little to no scatter hoarding, a useful addition for meta-analyses of corvids in the future. Of the five species, the magpie had the largest mean negative residual. However, direct comparisons between Eurasian and North American corvids might be biased because of differences in brain size of corvids from the different continents [Lucas et al., 2004; Garamszegi and Lucas, 2005], although the azure-winged magpies had the smallest brain size. Nevertheless, based on natural history, one might predict that the magpies would have relatively small Hp volumes, and this was the case.

Hp: Western Scrub Jays

Pravosudov and de Kort [2006] claimed that Western scrub jays may have the largest Hp volumes relative to BM among all investigated corvids based on their results from 21 birds. We did not find Western scrub jays to have the largest relative Hp volume by any of our measures, although we did find our scrub jays had a larger relative Hp than those of Basil et al. [1996]. Our sample size is smaller than that of Pravosudov and de Kort, and our birds were older and had been in captivity longer and these differences may have played a role in our results. The scrub jays from Pravosudov and de Kort were hand raised, while those in our study and the study of Basil et al. were not. Interestingly, the birds with the largest relative Hp in our study were also hand raised, the blue jays. While there is no known reason why hand raising may cause a larger relative Hp, it would be interesting to investigate further.

It may also prove important that the scrub jays in our study and that of Basil et al. [1996] were from Northern Arizona, while the scrub jays in Pravosudov and de Kort [2006] were from Northern California. Recent classification has established distinct subspecies of the Western scrub jay, with birds in Arizona classified as Woodhouse's scrub jays and birds in California classified as California

scrub jays [Rice et al., 2003]. The two subspecies are differentiated in terms of ecology; California scrub jays eat and cache acorns, while Woodhouse's scrub jays eat and cache pinyon pine seeds [Curry et al., 2002]. This could also lead to differences in caching behavior, spatial memory, and Hp, and could be another potential explanation for the larger Hp of Pravosudov and de Kort's birds.

Septum

Unlike the results from parids reported by Shiflett et al. [2002], we did not find significant differences in relative septum volume in any of our comparative analyses. This could be because the North American corvids we studied all scatter hoard food, while Shiflett et al. compared food-hoarding and non-food-hoarding species. However, even when we included the azure-winged magpie, in which scatter hoarding is quite limited, we did not find significant septum differences. It would be interesting to look for neurobiological differences in the septum among corvids who scatter hoard and others that do not cache, such as the Eurasian jackdaw.

General Discussion - Volume Measurements

All of the North American birds included in our study engage in scatter hoarding, albeit in differing amounts, and this could be important. Even though there are differences in the natural history of scatter hoarding among these species, all birds that engage in this behavior use spatial memory to create and relocate their caches. Therefore, they are all relying on an Hp-based spatial memory system when recovering caches. A gross analysis such as analyzing Hp volume, therefore, might not tap into the subtle differences within the Hp that may be the basis for differences in spatial memory ability. The research that showed significant differences in Hp volume among Eurasian corvids (Healy and Krebs [1992]) may have found these differences because they were comparing food-storing corvids (Eurasian jays) with non-storing corvids (jackdaws). The difference between scatter hoarding and no scatter hoarding may be big enough to be reflected in Hp volume differences. And, there may be evolutionary differences in how increased spatial memory demands are reflected in changes in brain tissue in North American birds. This may also be why the only significant difference we found was between a food-hoarding species (the blue jay) and one that stores little to no food (the azure-winged magpie).

The absence of species differences in measures of either Hp or septum amongst the North American corvid species is contrary to the adaptive specialization hypothesis [Krebs et al., 1989; Sherry et al., 1989; Krebs et al., 1996]. There appears to be no straightforward correspondence between differences in natural history (scatter hoarding) and the underlying brain areas associated with spatial memory. But we know that there are differences in spatial memory ability among at least three of our North American species on many laboratory tasks (Clark's nutcracker, pinyon jay, and scrub jay) that do correlate with differences in natural history (as well as similar differences among parids [e.g. Krebs, 1990; Healy, 1995]). This behavioral evidence offers compelling reason to think that there are differences in the neural substrate underlying spatial memory abilities in these birds. But this does not specify Hp volume as a critical variable, and research has begun to focus on other aspects of the underlying neurobiology [e.g. Szekely, 1999; Hoshooley and Sherry, 2004].

Neuron Counts

We found species differences in the number of neurons in the Hp relative to body size, with Clark's nutcrackers having the largest relative number of neurons, followed by Western scrub jays. There were no significant differences amongst the remaining species.

The significant results for the nutcrackers coincide with the large amount of food they hoard, their reliance on that stored food and their spatial memory ability in the laboratory [Vander Wall and Balda, 1981; Balda and Kamil, 1989; Kamil et al., 1994; Olson et al., 1995]. It is also consistent with the adaptive specialization hypothesis [Krebs et al., 1989; Sherry et al., 1989; Krebs et al., 1996], as nutcrackers are very highly specialized for food hoarding with excellent spatial memory abilities. However, the number of neurons in the Hp of pinyon jays seems counterintuitive based on the amount of food they store, their reliance on that stored food [Balda, 1980, 1987], and their spatial memory ability in the laboratory [Balda and Kamil, 1989; Kamil et al., 1994]. Although Pravosudov and de Kort [2006] suggest that Western scrub jays cache more heavily than previously reported, there are no field data in support of this idea, to the best of our knowledge (although it is clear that they cache readily and show episodic-like memory in the laboratory [Clayton and Dickinson, 1998]). The available field data on pinyon jays and Western scrub jays [Turcek and Kelso, 1968; Ligon, 1978] suggest that pinyon jays store more food than Western

scrub jays from Arizona and New Mexico. This suggests that pinyon jays would have more Hp neurons than scrub jays, as does the relatively strong performance of pinyon jays on several spatial memory tasks compared to scrub jays (from Arizona and New Mexico).

The Western scrub jay, while it stores less food and relies on that food less than the nutcracker or pinyon jay, has more Hp neurons than the pinyon jay. The scrub jay routinely does more poorly on spatial memory tasks in the laboratory when compared to Clark's nutcracker, but sometimes performs in a similar manner to the pinyon jay. Pravosudov and de Kort [2006] argued that the Western scrub jay may have the largest Hp volumes relative to BM among all investigated corvids. While we did not find volume effects, our scrub jays did have more Hp neurons than all other corvids in our study except the Clark's nutcracker. The reason for the large number of neurons in the scrub jay Hp is unclear.

When it comes to blue jays, we know that they do store a large amount of food and most likely rely fairly heavily on those hoards throughout the fall and winter [Darley-Hill and Johnson, 1981; Johnson and Adkisson, 1985]. However, we know very little about their spatial memory ability. While the blue jays were the only species to have a significantly larger Hp volume than the azure-winged magpies, they did not have significantly larger numbers of neurons relative to BM. This is another example of how different species may have evolved different neuroanatomical means for spatial memory.

The number of neurons in the Hp relative to the telencephalon was not significantly different among species. However, it approached significance ($p = 0.062$). Nutcrackers, scrub jays, and blue jays had higher mean neuron numbers than the other two species. The reason for the blue jay's large difference in relative neuron number when looking at the BM versus the telencephalon is not known. Blue jays also had a larger Hp relative to the telencephalon than the azure-winged magpies, while having a larger (but nonsignificant) number of neurons relative to the telencephalon than the magpies as well.

Overall Conclusions

The results of this study suggest that relative Hp volume and neuron number are correlated in some way with hoarding behavior and spatial memory performance, but not as precisely as suggested by the adaptive specialization hypothesis. Significant differences in relative Hp volume were only found between blue jays and azure-winged magpies. This failure to find significant differences in Hp

volume among the four North American corvid species is consistent with the mixed results of previous studies. Basil et al. [1996] found no statistically significant differences among four North American corvids in relative Hp volume. Healy and Krebs [1992] did a linear regression of relative Hp volume against degree of food storing in European corvids and found a significant relationship. They did not, however, look for overall species differences in Hp volume among all of their corvid species. They did however directly compare relative Hp volume in the food-storing magpie and the non-food-storing jackdaw and found the magpie had a significantly larger relative Hp. This is similar to our finding of a significantly larger relative Hp in the food-storing blue jay compared to the non-food-storing azure-winged magpie.

It is also necessary to remember that spatial memory is not a singular, homogenous cognitive trait. Smulders et al. [2010] point out that if a species has adaptations in spatial memory having to do with food-hoarding behavior, those adaptations could be in spatial resolution as well as in the capacity and duration of the memory, or any combination of the three. Hp volume may be correlated with all three aspects of adaptive spatial memory, and therefore this could explain the lack of significant differences amongst the four North American scatter-hoarding species of birds that we found. Imagine, for example, that one species has a larger memory capacity, but another has better spatial resolution. Since both abilities may utilize the Hp, the species may not differ in Hp volume, but show differences in spatial memory tasks of capacity or resolution. An understanding of how spatial memory has been selected for in each species with regard to scatter hoarding would help us make better predictions about both neurological and behavioral differences.

There may be other factors, including other brain differences and other types of memory, that might explain the natural history and behavioral differences among our species of corvids more effectively. For example, the relatively large number of neurons in the scrub jay Hp might relate to their excellent performance in experiments on episodic-like memory [Clayton and Dickinson, 1998]. More generally, it has been proposed that the Hp plays an extensive role in memory and cognition, well beyond the requirements of memory for cache locations. For example, Eichenbaum [2006] and Jacobs [2006] have argued for a significant role for the Hp in a common, flexible, and relational engine underlying configural learning.

Despite these likely complications, our study demonstrates significant differences in Hp neuron number between Clark's nutcrackers and the four other corvids

investigated, as well as the Western scrub jay and the remaining three corvids. While the results for the nutcrackers fits *a priori* hypotheses based on the adaptive specialization hypotheses, other results do not. For example, pinyon jays often show better spatial memory than scrub jays, depending on the type of task involved. This pattern is consistent with the ideas of Smulders et al. [2010]. Pinyon jays, while not having a larger Hp or more Hp neurons compared to other corvid species, may be using one specific aspect of spatial memory that allows them to perform well on certain spatial memory tasks. This aspect may not be reflected in volume or neuron number differences in the Hp.

These overall results also contribute towards reconciling the findings of Basil et al. [1996] with more recent research, and provide new data points for future analyses of Hp volume and function among food-hoarding birds. These results also suggest that the adaptive specialization hypothesis is too narrow in scope, especially in view of the emerging picture of multiple Hp function. Future research should focus on further analysis of other potential differences within the Hp among these species, including factors such as the size of dendritic trees, number of synapses, and axonal size, as Roth et al. [2010] suggest.

Acknowledgments — We would like to thank Rick Bevins and Ming Li for the use of their laboratory space at the University of Nebraska for perfusions and C. de la Cruz of la Universidad de Extremadura, Badajoz, Spain, for providing the azure-winged magpies.

We would also like to thank Bob Jacobs and Lori Driscoll (Colorado College) for the use of their laboratory space for the cell-counting portion of the study and Susan Hendricks at MBF Bioscience for critical technological support and methodological insight throughout cell counting. Feedback and discussion with Jennifer Basil was helpful in preparing this article. K.L. Gould was supported by a George and Jutta Anderson Faculty Sabbatical Award from the Luther College. K.E. Gilbertson, A.J. Hrvol, J.C. Nelson, A.L. Seyfer, and R.M. Brantner were supported by student research funds from the Luther College. K.L. Gould and K.E. Gilbertson were also supported by a Short-Term Consultation Grant from the Midstates Consortium for Math and Science. This research was also supported in part by NIMH Grant ROI-MH069893.

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