

Is hippocampal volume affected by specialization for food hoarding in birds?

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The hypothesis that spatial-memory specialization affects the size of the hippocampus has become widely accepted among scientists. The hypothesis comes from studies on birds primarily in two families, the *Paridae* (tits, titmice and chickadees) and the *Corvidae* (crows, nutcrackers, jays, etc.). Many species in these families store food and rely on spatial memory to relocate the cached items. The hippocampus is a brain structure that is thought to be important for memory. Several studies report that hoarding species in these families possess larger hippocampi than non-hoarding relatives, and that species classified as large-scale hoarders have larger hippocampi than less specialized hoarders. We have investigated the largest dataset on hippocampus size and food-hoarding behaviour in these families so far but did not find a significant correlation between food-hoarding specialization and hippocampal volume. The occurrence of such an effect in earlier studies may depend on differences in the estimation of hippocampal volumes or difficulties in categorizing the degree of specialization for hoarding or both. To control for discrepancies in measurement methods we made our own estimates of hippocampal volumes in 16 individuals of four species that have been included in previous studies. Our estimates agreed closely with previous ones, suggesting that measurement methods are sufficiently consistent. Instead, the main reasons that previous studies have found an effect where we did not are difficulties in assessing the degree of hoarding specialization and the fact that smaller subsets of species were compared than in our study. Our results show that a correlation between food-hoarding specialization and hippocampal volume cannot be claimed on the basis of present data in these families.

Keywords: food hoarding; hippocampus; memory; *Paridae*; *Corvidae*

1. INTRODUCTION

Food-hoarding bird species frequently use spatial memory to relocate caches (Sherry *et al.* 1981; Shettleworth & Krebs 1982, 1986; Vander Wall 1982; Sherry 1984; Kamil & Balda 1985; Sherry & Vaccarino 1989; Shettleworth *et al.* 1990; Balda & Kamil 1992; Clayton 1993; Clayton & Krebs 1994; Brodin & Kunz 1997; Clayton & Dickinson 1999) and many species store thousands of seeds and nuts in scattered locations over large areas (Swanberg 1951; Haftorn 1956, 1959; Pravosudov 1985; Brodin *et al.* 1994). Memory in food-hoarding birds has been studied primarily in two families, the *Corvidae* (crows, jays, nutcrackers, magpies, etc.) and the *Paridae* (tits, titmice and chickadees).

To retain many separate locations in memory over extended periods of time must require a highly specialized memory. This has led to the hypothesis that bird species that store large numbers of food items will experience adaptive specialization of their spatial-memory capacity. The hippocampus, a structure that is located dorsomedially at the back of the telencephalon (the main part of the forebrain), is thought to be important for learning and memory. In birds, the hippocampus proper and the adjacent parahippocampus (figure 1) are frequently treated together as the hippocampal complex (Krebs *et al.* 1989; Sherry *et al.* 1989). We follow this tradition here, but for simplicity we henceforward refer to these structures together as the hippocampus.

Several studies suggest that the hippocampus is enlarged in food-storing birds compared with non-hoarding relatives and that it increases in size with the degree of specialization for food hoarding (Krebs *et al.* 1989; Sherry *et al.* 1989; Healy & Krebs 1992, 1996; Healy *et al.* 1994; Hampton *et al.* 1995; Basil *et al.* 1996). This has inspired studies on humans. Professional taxi drivers who have passed an especially difficult spatial task (such as learning the streets in London by heart) have been reported to possess an enlargement of a part of the hippocampus (Maguire *et al.* 2000). Recently, Bolhuis & Macphail (2001) criticized what they call the neuroecological approach and questioned whether behavioural adaptations can be detected in brain morphology. In particular, they questioned the increase in hippocampal volume with the degree of specialization for spatial memory, which can be seen as a 'core hypothesis' of neuroecology (Healy & Krebs 1992, 1996; Hampton *et al.* 1995; Basil *et al.* 1996). Our aim in this study was to evaluate whether there is a significant correlation between food-hoarding specialization and hippocampal volume. The amalgamation of data from earlier studies with our own new data has given us the largest dataset on hippocampal volumes in corvids and parids analysed so far.

2. MATERIAL AND METHODS

(a) Choice and treatment of birds

We measured the hippocampal volumes of five blue tits (*Parus caeruleus*; a non-hoarding parid), seven willow tits (*P. montanus*; a hoarding parid), one jackdaw (*Corvus monedula*; a non-hoarding corvid) and three Eurasian jays (*Garrulus glandarius*; a

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hoarding corvid). The birds were collected and sacrificed under permits M149-98 and M231-99 from the Local Ethical Review Committee for Animal Experimentation. The rationale for the choice of species was to obtain data for one hoarder and one non-hoarder of similar size in each of the two families that was already represented in the literature. All birds were collected in winter so that yearlings in the storing species would have experienced one autumn of food storing.

We anaesthetized the birds with a lethal intraperitoneal injection of sodium pentobarbital and then perfused them transcardially with phosphate-buffered saline solution, followed by 4% formalin solution. After removal, their brains were left in formalin solution and then prepared with 30% sucrose formalin prior to microtome sectioning. Finally, they were stained using the Thionine–Nissl procedure before mounting on slides. This procedure is the same as that used in earlier studies of this type (Krebs *et al.* 1989; Sherry *et al.* 1989). We cut the telencephalon (including the hippocampus) into 40 µm frozen sections in the coronal plane on a sliding freezing microtome and collected all sections in phosphate-buffered saline. To avoid systematic deviations that may occur owing to periodicity we randomly selected one out of each group of five adjacent sections for staining with cresyl violet.

(b) Image measurements

We measured the hippocampal and telencephalon areas directly on photographs using CANVAS v. 8.0 (Deneba software). We inserted a 1 mm ruler with 0.01 mm markings in each hippocampus photograph in figure 1 and a conventional ruler in telencephalon photographs to calibrate the scale. A convenient scale on a 17 inch computer screen would typically be a magnification of *ca.* 100× for the hippocampus. To calculate the volume we multiplied the areas by the average distance between the cuts of 200 µm. Using this method means that we assumed that the shape and area were essentially the same between two adjacent cuts. This is a reasonable assumption except at the anterior and posterior ends, where the diameters of adjacent cuts will increase and decrease, respectively. These parts account for only a small fraction of the volume, and the increase at the anterior end will essentially be balanced by the decrease at the posterior end.

We defined the boundaries of the telencephalon and the hippocampal areas as in Krebs *et al.* (1989) and Sherry *et al.* (1989). The lateral border between the parahippocampus and the hyperstriatum may be difficult to find even on the best microscopic preparations (figure 1), a fact that has rarely been discussed in previous studies. Illustrations of hippocampus cuts in birds typically show the mid-section of the hippocampus (Krebs *et al.* 1989; Sherry *et al.* 1989; Barnea & Nottebohm 1994; Hampton *et al.* 1995; Basil *et al.* 1996) where this border is easy to identify, at least in parids (figure 1*b*). In the frontal and posterior parts of the hippocampus, however, the position of this border is subject to judgement (figure 1*a,c,d*). To minimize the risk of errors, four people measured every cut independently: the author that did not make the preparations (A.B.) and three students who were hired and trained for this purpose. During this work the slides were marked in code so that the identity of the bird was not known.

(c) The assignment of species to hoarding categories

We used the hoarding categories suggested by Healy & Krebs (1992, 1996) (table 1) and, for species not categorized by these

authors, we have adjusted similar rankings from other studies to their categories (table 1). The categories are:

- (i) non-hoarders;
- (ii) non-specialized hoarders; and
- (iii) specialized hoarders.

Typical category (ii) species store small amounts of food for short time intervals while typical category (iii) species store thousands of items in the autumn to be used as winter food. The logical prediction from a neuroecological perspective is that hippocampal volumes should be relatively small in non-hoarders, larger in non-specialized hoarders and largest in specialized hoarders (Healy & Krebs 1992, 1996; Hampton *et al.* 1995; Basil *et al.* 1996).

In four species there are studies that suggest that the categorization should be different from the one used by Healy & Krebs (1992, 1996). We have, therefore, also included an alternative categorization based on these suggestions (table 2).

(d) Comparisons with literature data

Sources of data on hippocampal volumes are given in table 3. As our own data agreed well with previous measurements (see § 3) we added our own data to these. To calculate mean values for each species we averaged all the individual values of hippocampal volume, telencephalon volume and body mass.

All previous studies on this subject have used similar techniques for the preparations, except for Basil *et al.* (1996) who embedded brains in paraffin instead of freezing them. As paraffin-embedded tissues will shrink relative to frozen tissues we use the correction factor suggested by Basil *et al.* (1996) to make their data comparable with others.

Hippocampal volumes can be compared relative to either body mass or telencephalon volume. We feel that the first comparison is more intuitive but also include the second to control for confounding effects, for example, the daily variation in body mass in individual birds. We test the residuals from the mean family regression lines with one-way ANOVAs.

3. RESULTS

(a) Hippocampal volume and body size

Other things being equal, organ size should correlate with body mass. Hippocampal volume increased with body mass ($r = 0.87$, $n = 12$, $p < 0.001$) and telencephalon volume ($r = 0.94$, $n = 12$, $p < 0.001$) in corvids (figure 2) but neither with telencephalon volume ($r = 0.43$, $n = 9$, $p = 0.24$) or body mass ($r = 0.08$, $n = 9$, $p = 0.84$) in parids (figure 3). This non-significant result may seem surprising but probably depends on the fact that most parids are of similar body size, with only one species, the great tit *P. major* being clearly larger than the others. For the sake of consistency we use the regression line to calculate residuals in both families. If the species from both families are pooled, hippocampal volume increases with both body mass ($r = 0.95$, $n = 21$, $p < 0.001$) and telencephalon volume ($r = 0.97$, $n = 21$, $p < 0.001$) (figure 4).

(b) Species comparisons of hippocampal volumes

In corvids, residual hippocampal volumes did not differ between the hoarding categories, relative to either body mass ($F_{2,9} = 1.09$, $p = 0.38$, one-way analysis of variance

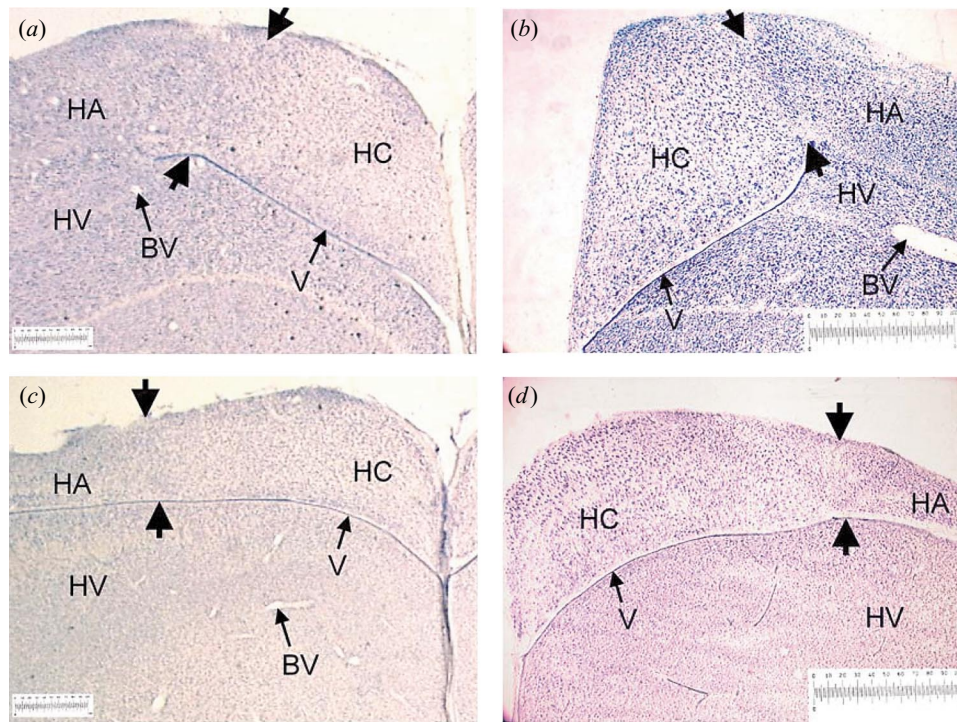


Figure 1. Coronal sections of the hippocampal complex (HC) and adjacent structures in (a,c) a Eurasian jay and (b,d) a willow tit. The sections are at (a) 2.2 mm or 10%, (b) 1.8 mm or 14%, (c) 7.6 mm or 35% and (d) 5.8 mm or 46% of the total distance from the rostral end. The HC is located dorsomedially above the lateral ventricle (V). For jays the right side is shown and for willow tits the left side is shown. The lateral border towards the hyperstriatum accessorium (HA) is indicated by bold arrows. HV is hyperstriatum ventrale, BV is blood vessel. Scale bar, 1 mm.

(ANOVA) or telencephalon volume ($F_{2,9} = 0.58$, $p = 0.58$, one-way ANOVA). In relation to body mass, the Eurasian jay had the relatively largest hippocampus of all corvids in category (iii) (figure 2a). Two other species in this category, of which one was Clark's nutcracker (*Nucifraga columbiana*), lie near the line (figure 2a). The nutcracker is probably the most specialized hoarder of all species in this study (Vander Wall & Balda 1977, 1981; Vander Wall 1990; Basil *et al.* 1996). The fourth species in this category, the pinyon jay (*Gymnorhinus cyanocephalus*), had a smaller hippocampus than expected. Out of the less specialized hoarders in category (ii), the magpie (*Pica pica*) had the relatively largest hippocampus of all corvids. The five others fell on (one species) or below (four species) the line (figure 2a). One of the non-hoarders fell clearly below the line while the other ended up just below it. The comparisons relative to brain size show a similar picture with small changes: the most notable was that the jay, classified in category (iii), now has the relatively largest hippocampus of all corvids (figure 2b).

In corvids the alternative categorization meant moving only the rook from category (ii) to category (iii) (table 2). The differences from the results given in the previous paragraph were small, with no significant differences between the categories relative to body mass ($F_{2,9} = 0.98$, $p = 0.41$, one-way ANOVA) or telencephalon volume ($F_{2,9} = 0.26$, $p = 0.77$, one-way ANOVA).

In parids, residual hippocampal volumes also did not differ significantly between the hoarding categories, relative to either body mass ($F_{2,7} = 0.61$, $p = 0.57$, one-way ANOVA) or telencephalon volume ($F_{2,7} = 0.73$, $p = 0.52$, one-way ANOVA). Additionally, in this family the distributions of residual hippocampal volumes were similar

relative to body mass and telencephalon volume. Out of the species classified in category (iii), the willow tit had a clearly larger hippocampus than predicted while the mountain chickadee (*P. gambeli*) lies below the mean. Just as in corvids, one species in category (ii), the marsh tit (*P. palustris*), lies clearly above the line. The two non-hoarding species, the great tit (*P. major*) and the blue tit, lay on or below the line.

Also, if we instead apply the alternative hoarding categories (table 2), there were no significant differences between the categories relative to body mass ($F_{2,7} = 2.46$, $p = 0.17$, one-way ANOVA) or telencephalon volume ($F_{2,7} = 2.00$, $p = 0.22$, one-way ANOVA).

To pool the two families will increase sample size and hence the power of the tests. However, just as for the families separately, the hippocampal volumes did not differ significantly between the categories relative to body mass ($F_{2,18} = 1.07$, $p = 0.37$, one-way ANOVA) or telencephalon volume ($F_{2,18} = 1.50$, $p = 0.25$, one-way ANOVA).

(c) The reliability of estimates

There was no significant difference between observers in our study ($F_{3,48} = 1.23$, $n = 16$, $p = 0.31$, repeated-measures ANOVA). For three species, the blue tit, the willow tit and the jackdaw, our estimates were very close to earlier ones (table 4). In the Eurasian jay, the average hippocampal volume was 28% smaller than in the only previous study (Healy & Krebs 1992). This was expected, however, since our birds were smaller (159.3 ± 9.0 (standard deviation, s.d.) g, $n = 3$) than in the previous study (192.4 ± 3.4 (s.d.) g, $n = 2$). This means that relative to body size our estimates agree with previous data in the jay.

Table 1. Degree of specialization for food hoarding in different species.

species	category ^a	source
corvids		
scrub jay (<i>Aphelocoma coerulescens</i>)	ii	Vander Wall & Balda (1981); Basil <i>et al.</i> (1996)
pinyon jay (<i>Gymnorhinus cyanocephalus</i>)	iii	Vander Wall & Balda (1981); Marzluff & Balda (1992)
grey-breasted jay (<i>A. ultramarina</i>)	ii	Basil <i>et al.</i> (1996)
blue jay (<i>Cyanocitta cristata</i>)	iii	Darley-Hill & Johnson (1981)
alpine chough (<i>Pyrrhocorax graculus</i>)	i	Healy & Krebs (1992)
Clark's nutcracker (<i>Nucifraga columbiana</i>)	iii	Tomback (1980); Vander Wall & Balda (1981)
jackdaw (<i>Corvus monedula</i>)	i	Healy & Krebs (1992)
red-billed blue magpie (<i>Cissa erythrorhyncha</i>)	ii	Healy & Krebs (1992)
magpie (<i>Pica pica</i>)	ii	Birkhead (1990)
Eurasian jay (<i>Garrulus glandarius</i>)	iii	Chettleburgh (1952); Bossema (1979)
rook (<i>Corvus frugilegus</i>)	ii	Healy & Krebs (1992)
Eurasian crow (<i>Corvus corone</i>)	ii	Healy & Krebs (1992)
parids		
bridled titmouse (<i>Parus wollweberi</i>)	ii	Hampton <i>et al.</i> (1995)
willow tit (<i>Parus montanus</i>)	iii	Haftorn (1956, 1959); Pravosudov (1985); Brodin (1994)
coal tit (<i>Parus ater</i>)	ii	Healy & Krebs (1996)
blue tit (<i>Parus caeruleus</i>)	i	Haftorn (1959); Sherry (1989); Healy & Krebs (1996)
marsh tit (<i>Parus palustris</i>)	ii	Healy & Krebs (1996)
Mexican chickadee (<i>Parus sclateri</i>)	ii	Hampton <i>et al.</i> (1995)
black-capped chickadee (<i>Poecile atricapillus</i>)	ii	Sherry (1989); Healy & Krebs (1996)
mountain chickadee (<i>Parus gambeli</i>)	iii	Haftorn (1974); Pravosudov & Clayton (2001)
great tit (<i>Parus major</i>)	i	Haftorn (1959); Sherry (1989); Healy & Krebs (1996)

^a The species were classified as (i) non-hoarders; (ii) non-specialized hoarders; or (iii) specialized hoarders (see § 2c for details).

Table 2. Alternative categorization of the degree of specialization for food hoarding.

species	new category	source
rook	3	Källander (1978)
coal tit	3	Haftorn (1956); Brotons (1999)
marsh tit	3	Haftorn (1959)
black-capped chickadee	3	Hampton <i>et al.</i> (1995), A. Brodin (unpublished data)

4. DISCUSSION

(a) *Why is there an effect in previous studies?*

The answer to this question is simple: previous studies include fewer species than our study. In a comparison of Eurasian corvids, Healy & Krebs (1992) included only one species classified as a specialized hoarder, the Eurasian jay. This species has an unusually large hippocampus that is not representative of other species in this category, for example, the nutcracker (figure 2). A comparison of American corvids does not claim a general trend, merely that the nutcracker has a larger hippocampus than the other species included in that study (Basil *et al.* 1996). Compared with corvids in general, however, the nutcracker seems to possess only an average-sized hippocampus.

In addition, in their comparison of parids, Healy & Krebs

(1996) classified only one species, the willow tit, as a specialized hoarder. Just as does the jay among the corvids, this species has a very large hippocampus compared with the rest of the family (figure 3). A comparison of American parids was based on only three species. The black-capped chickadee *P. atricapillus* possessed a larger hippocampus and stored more in the laboratory than two other species (Hampton *et al.* 1995). The fact that one species has a larger hippocampus than two others is weak support for a general trend in a whole family. Further, Healy & Krebs (1996) placed this species in category (ii), suggesting that it may not be a highly specialized hoarder.

Two studies compare families (Krebs *et al.* 1989; Sherry *et al.* 1989) or subgroups of families rather than species within families. Comparisons of families are less strong as evidence than comparisons within families since the taxa being compared will be more distantly related. If, for example, sparrows and parids are compared, many factors other than food hoarding could affect hippocampus size, factors that would not be present in a comparison of hoarding and non-hoarding species in the same family. Furthermore, a difference between families does not tell us whether a large hippocampus has evolved as a result of adaptations for food hoarding or whether a large hippocampus is a preadaptation for the evolution of food hoarding.

(b) *Are different studies comparable?*

Considering the large variation in hippocampal volumes between individuals, the agreement between our estimates

Table 3. Sources of hippocampal volumes.
(Number of individuals analysed (*n*) is given in parentheses.)

species	source
corvids	
scrub jay (2), pinyon jay (2), grey-breasted jay (2), Clark's nutcracker (4)	Basil <i>et al.</i> (1996)
blue jay (1)	Sherry <i>et al.</i> (1989)
alpine chough (1), carrion crow (2), jackdaw (13), Eurasian jay (2), magpie (13), red-billed blue magpie (1), rook (1)	Healy & Krebs (1992)
Eurasian jay (3), jackdaw (1)	this study
parids	
black-capped chickadee (1), willow tit (2), marsh tit (11), blue tit (10), coal tit (1), great tit (1)	Healy & Krebs (1996)
black-capped chickadee (5), Mexican chickadee (2), bridled titmouse (3)	Hampton <i>et al.</i> (1995)
black-capped chickadee (3)	Sherry <i>et al.</i> (1989)
black-capped chickadee (25)	Pravosudov & Clayton (2002)
mountain chickadee (21)	Pravosudov <i>et al.</i> (2002)
willow tit (7), blue tit (5)	this study
marsh tit (9), blue tit (6)	Healy <i>et al.</i> (1994)

and earlier data in the four species that we measured was very good. Also, the agreement between the four observers in our study was very good. These observers worked independently and blindly, not expecting the results we account for here beforehand. This suggests that with some training the borders of the hippocampus can be identified with sufficient accuracy. Still, we think that studies of this type benefit from the use of several independent observers in combination with blind measurements owing to the subjectiveness of the method.

(c) Should hippocampal volume be expected to correlate with degree of specialization for food hoarding?

We did not find any correlation between degree of specialization for food hoarding and hippocampal volume, either if we used categories from previous studies or if we used an alternative categorization. There may be many reasons for this. First of all, the hippocampus may not be used for the storage of spatial memories. Hippocampal studies in mammals suggest that the cerebral cortex may be more important than the hippocampus for the storage of memories (Brown 2000). Even if birds do not have a cerebral cortex, it is still possible that memories are transferred to some other brain structure in an analogous way. Morphological effects of memory adaptations would then be expected to occur in this other brain structure rather than in the hippocampus. An argument against this is the fact that no one has yet discovered such a transfer in birds. In addition, lesions of the hippocampus in the black-capped chickadee reduced recovery success to chance levels, suggesting that the lesions interrupted memory storage (Sherry & Vaccarino 1989).

If we assume that the avian hippocampus is important for spatial memory, variations in cognitive abilities may still not be observable in brain morphology. This will be the case either if spatial-memory capacity does not correlate with hippocampal volume or if it does correlate but the morphological effects are too small to be detected. In the first case, an evolutionary approach of the neuroecological type would not be possible (Bolhuis & Macphail

2001). Macphail & Bolhuis (2001) discuss this and instead advocate a general process view. According to this view natural variation in morphology cannot be used to understand the cognitive functioning of the brain. In the second case, effects of memory adaptations would be detectable in hippocampus morphology only if the data were sufficiently accurate. As there are many problems associated with the data we have compiled in this study we believe that it is premature to draw conclusions about whether there are effects (but small) or not. In § 4d we will discuss these problems and suggest improvements that would increase the possibility of detecting such effects.

(d) What could be improved?

One variable in particular, the degree of specialization for food hoarding, is very subjective. To detect small effects we would need to know not only whether birds are specialized hoarders but also how specialized they are. The categorizations (tables 1 and 2) are based not on standardized observations but on studies with a variety of different methods, ranging from episodic observations in the field to controlled experiments in laboratories. Most suggestions are based on observations of hoarding intensities, and, as highly specialized hoarders store more items than less specialized ones, these are probably a good indicator of the degree of specialization.

It is easier to control for confounding factors in a laboratory than in the field. The problem when laboratory experiments are compared is that they have been done in different laboratories, with different regimes for food, temperatures, handling of birds, experimental designs and so on. Hoarders will then differ in motivation, training, etc. The most objective categorization would be achieved if all species under comparison could be tested with the same design in the same set-up. However, given economic factors, the varying geographical ranges of different species in the world, size differences between species and so on, this will probably never be possible.

Systematic observations of hoarding intensities in the field are another way to sample hoarding propensity.

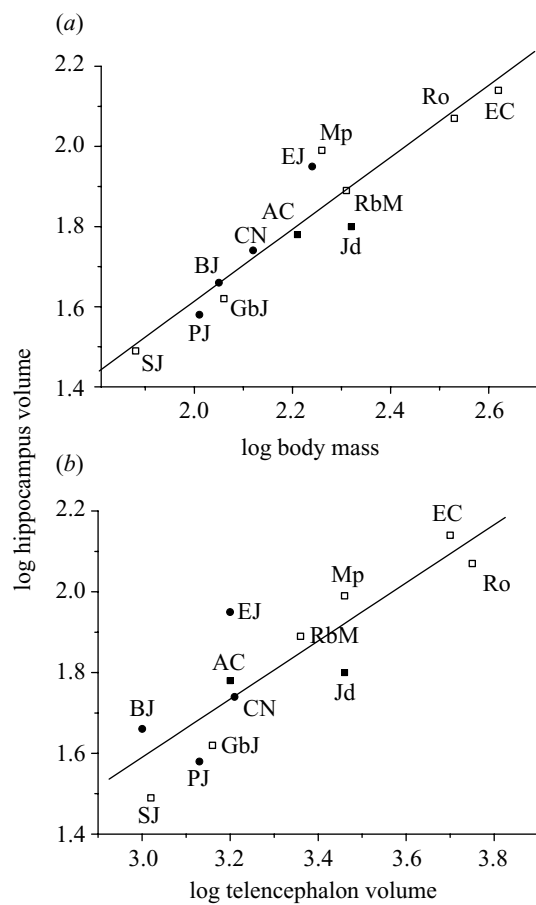


Figure 2. Log hippocampal volume in corvids plotted against (a) log body mass and (b) log telencephalon volume. The increase in volume with both body mass ($y = -0.31 + 0.96x$) and brain volume ($y = -0.18 + 0.9x$) is significant (see § 3a). SJ, scrub jay (*Aphelocoma coerulescens*); PJ, pinyon jay (*Gymnorhinus cyanocephalus*); GbJ, grey-breasted jay (*A. ultramarina*); BJ, blue jay (*Cyanocitta cristata*); AC, alpine chough; CN, Clark's nutcracker; Jd, jackdaw; RbM, red billed blue magpie; Mp, magpie; EJ, Eurasian jay; Ro, rook; EC, Eurasian crow. The black squares show non-hoarders (category (i)), white squares show non-specialized hoarders (category (ii)) and black circles show specialized hoarders (category (iii)).

Many field observations of hoarding have been made at feeders with supplemental food but these will not be reliable since supplemental food will induce increased hoarding rates (Brodin 1992, 1993). Instead, hoarding intensities recorded under unmanipulated conditions should be used. Older field studies, however, suffer from the disadvantage that hoarders cannot be recognized as individuals, which makes calculations of average hoarding rates unreliable. Systematic field studies of natural hoarding rates in individually colour-banded birds have been performed in only two of the species listed in table 1, the willow tit (Pravosudov 1985; Brodin 1994; Brodin *et al.* 1996) and the coal tit (Brotons 1999).

More and better data on the degree of hoarding specialization would also make it possible to improve the crude scale from three to four or five categories, or maybe even to a continuous scale. For example, species that store only rarely, such as the crow (*C. corone*), are now in category (ii) together with species that store more regularly, such

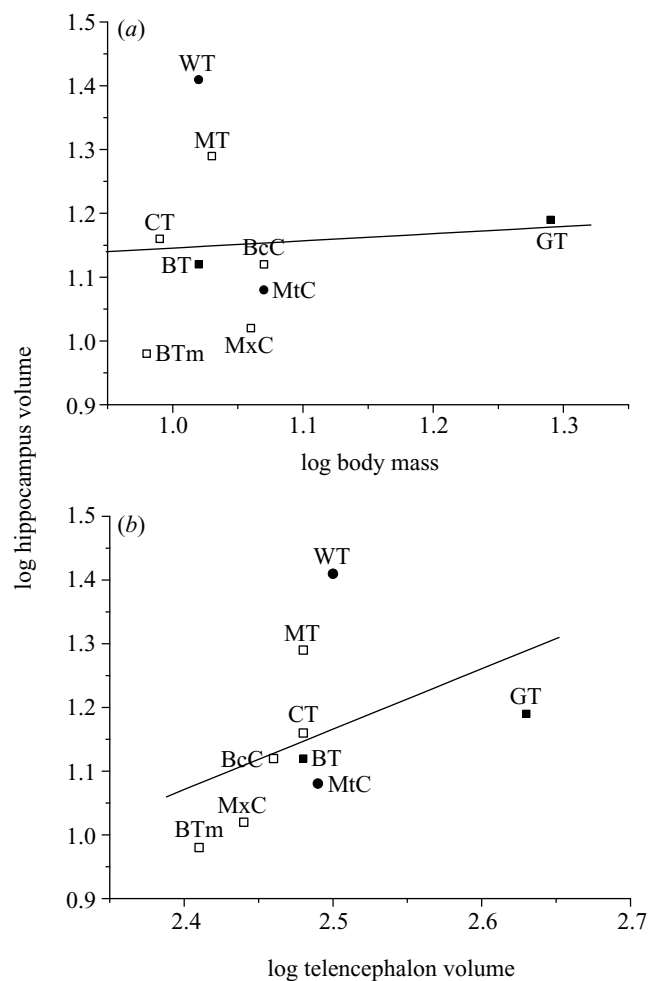


Figure 3. Log hippocampal volume in parids plotted against (a) log body mass ($y = 1.03 + 0.11x$) and (b) log telencephalon volume ($y = -1.20 + 0.95x$). Neither the correlation with body mass or with brain volume was significant (see § 3a). BTm, bridled titmouse; WT, willow tit; CT, coal tit (*P. ater*); BT, blue tit; MT, marsh tit; MxC, Mexican chickadee (*P. sclateri*); BcC, black-capped chickadee; MtC, mountain chickadee (*P. gambeli*); GT, great tit. Symbols as in figure 2.

as the magpie and the rook. Some parid species such as the coal tit (*P. ater*) and the marsh tit store thousands of seeds in an autumn. Still they seem to be less specialized hoarders than, for example, the willow tit. Such species may fit in a category that is intermediate between the present categories (ii) and (iii). 'Average hoarding intensities' sampled in the field during the main hoarding period could possibly be used as a basis for a continuous scale.

If hoarders use other means than memory to relocate caches, there is no reason to expect that they should possess a more developed memory than non-hoarding relatives. Macphail & Bolhuis (2001) recently concluded that food hoarders do not perform better than non-hoarders in spatial-memory tasks. It has been suggested by fieldworkers that parids do not remember caches they retrieve after long retention intervals (Haftorn 1956; Gibb 1960; Grubb & Pravosudov 1994; Brodin & Ekman 1994). There is no reason to expect that long-term hoarders that do not rely on memory for retrieval should require special memory adaptations. The decay of memory for caching locations has been investigated in only three species,

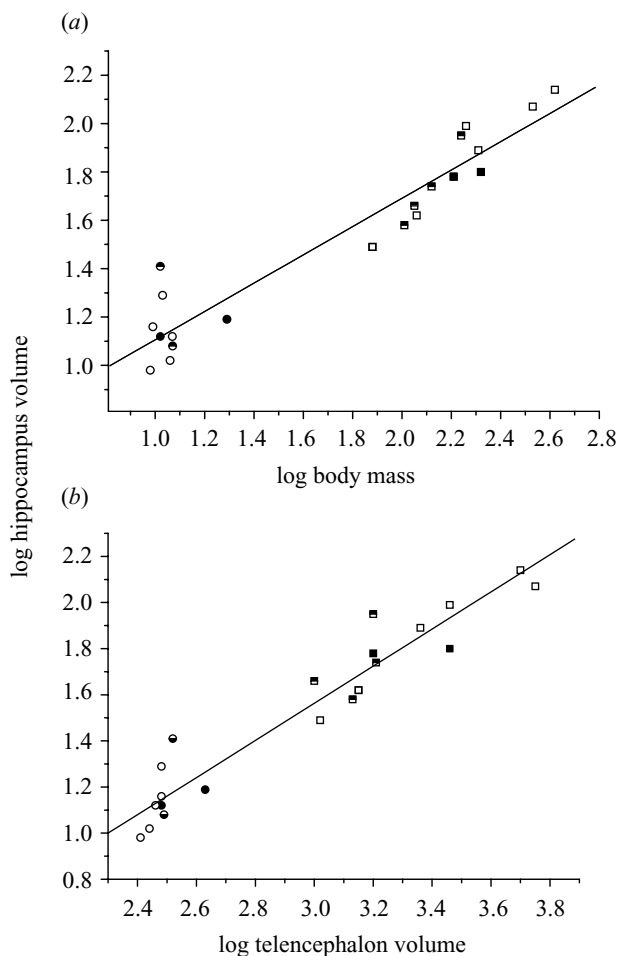


Figure 4. Pooled log hippocampal volume in corvids and parids plotted against (a) log body mass ($y = 0.52 + 0.58x$) and (b) log telencephalon volume ($y = -0.85 + 0.81x$). Corvids are represented by squares and parids by circles. Non-hoarders are represented by black symbols, category (ii) hoarders by white symbols and category (iii) hoarders by half-filled symbols. Species representation in (a) can be understood from figures 2a and 3a, and species in (b) can be understood from figures 2b and 3b.

Clark's nutcracker (Balda & Kamil 1992), the black-capped chickadee (Hitchcock & Sherry 1990) and the willow tit (Brodin & Kunz 1997). Out of these, only the nutcracker seemed to possess a memory that spans several months. Only species that have been shown to use memory to retrieve caches after long retention intervals (such as from autumn to winter) should be included in category (iii).

In addition to problems in assigning species to hoarding categories, there may also be problems in the estimates of hippocampal volumes. Most hippocampus cuts in American corvids originate from one study in which cuts were embedded in paraffin instead of frozen (Basil *et al.* 1996). This technique means that cuts shrink relative to frozen cuts, and, if the degree of shrinkage is larger than suggested by the authors (23%), three long-term hoarders in figure 2 may end up above the regression line, depending on the magnitude of shrinkage. However, this can explain only why we did not find a correlation in the regression against body mass since the telencephalon would be expected to shrink in the same way as the hippocampus.

Table 4. Comparison of hippocampal volumes ($\text{mm}^3 \pm \text{s.d.}$) between this and earlier studies.

species	this study	earlier studies
willow tit	25.7 ± 1.3	27.0 ± 8.3^b
blue tit	13.7 ± 1.3	$13.1 \pm 3.2^{b,c}$
Eurasian jay	77.4 ± 9.0	108.0 ± 11.3^d
jackdaw	66.4^a	62.4 ± 6.5^d

^a $n = 1$.

^b Healy & Krebs (1996).

^c Healy *et al.* (1994).

^d Healy & Krebs (1992).

Many species that are potentially important for a study of this type could not be included as there are no data on their hippocampal volumes. Long-term hoarding is supposedly more important for survival in regions with long and cold winters than in milder regions, and special adaptations for hoarding are most likely to occur in species inhabiting such regions. Nothing is known about the two most northerly distributed parid species, the Siberian tit (*P. cinctus*) and the boreal chickadee (*P. hudsonicus*), or the most northerly corvid species, the Siberian jay (*Perisoreus infaustus*) and the grey jay (*P. canadensis*), all of which are large-scale food hoarders (Haftorn 1974; Pravosudov 1985; Waite & Reeve 1992; Ekman *et al.* 1996). Also missing is the Eurasian nutcracker (*N. caryocatactes*), a species that, together with Clark's nutcracker, is considered to be the most specialized hoarder of all bird species (Swanberg 1951; Vander Wall 1990).

It is possible that comparisons need to be made at an even lower taxonomic level than the species level. If populations within a species are compared, factors might be controlled for that otherwise would make small differences in hippocampal volume difficult to detect. Healy & Krebs (1996) placed the marsh tit in category (ii), non-specialized hoarders, whereas Haftorn (1959) reported that marsh tits store even more than willow tits in the field. It is possible that both classifications are correct. Marsh tits from Oxford may be less specialized hoarders than marsh tits from central Scandinavia. Pravosudov & Clayton (2002) compared hippocampal volumes and hoarding propensities in northern and southern populations of black-capped chickadees, one from Anchorage, AK, USA and the other from Windsor, CO, USA. The Alaska chickadees stored more, retrieved caches more accurately and possessed larger hippocampi.

Smulders *et al.* (1995) and Patel *et al.* (1997) reported that there may be differences in hippocampal volumes within the same individual, for example between seasons. Black-capped chickadees possessed larger hippocampi in autumn, the main hoarding period, than in winter. If such changes occur as a consequence of food hoarding, they may be larger in highly specialized hoarders than in less specialized ones. It may then be easier to detect differences in autumn than in winter. For several reasons this is not an argument against our conclusions.

First, our conclusion of the absence of an effect of hoarding on the hippocampus does not rely on our own birds but on measurements from previous studies that report a significant effect of storing on hippocampal vol-

ume. Second, if the hippocampus is enlarged only in autumn and not when long-term hoarders retrieve caches in winter, the enlargement is not a consequence of the storage of long-term memories. Then there is little reason to predict larger hippocampi in specialized long-term hoarders than in short-term hoarders. Third, it is questioned whether seasonal changes of hippocampal volumes really occur. Barnea & Nottebohm (1994) found a higher rate of neurogenesis in the hippocampus during autumn than during the rest of the year, but detected no ensuing increase in volume. Krebs *et al.* (1995) created seasonal conditions indoors in an effort to replicate the results of Smulders *et al.* (1995) under controlled laboratory conditions. In the 'autumn state' the chickadees stored more seeds but hippocampal volumes did not change. Pravosudov *et al.* (2002) found no effects on hippocampal anatomy of experimentally induced changes in spatial memory.

Finally, there may be more efficient methods than comparing species or populations. An independent contrast analysis (Felsenstein 1985) might show whether food-hoarding specialization and hippocampal volume tended to evolve together. This method, however, cannot be used since there are no modern species-level phylogenies covering Eurasian and American species together, either in corvids or in parids. Also, the problem of assigning species to hoarding categories would still remain. Changing the method will not help as long as the input data are unreliable.

(e) *Conclusions*

In conclusion, we did not find any support for an increase in hippocampal volume with the degree of specialization for food hoarding in the very two families in which the evidence for this is strongest (Krebs *et al.* 1989; Healy & Krebs 1992, 1996; Hampton *et al.* 1995; Basil *et al.* 1996). It is possible that the adaptationistic approach used by neuroecologists may be wrong and that a general-purpose approach (Macphail & Bolhuis 2001) is more correct.

Another possibility is that a correlation exists but the available data are not sufficiently good to detect it. Data for more species of corvids and parids are needed on:

- (i) storing behaviour under natural conditions;
- (ii) hippocampal volumes; and
- (iii) memory longevity.

In addition, for the species included in this study more data are needed, but data collected with standardized methods. When reliable data on both hoarding propensity and hippocampal volumes are available for many species it will be possible to determine whether a neuroecological approach is possible or not.

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REFERENCES

- Balda, R. P. & Kamil, A. C. 1992 Long-term spatial memory in Clark's nutcracker, *Nucifraga columbiana*. *Anim. Behav.* **44**, 761–769.
- Barnea, A. & Nottebohm, F. 1994 Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proc. Natl Acad. Sci. USA* **91**, 11 217–11 221.
- Basil, J. A., Kamil, A. C., Balda, R. B. & Fite, K. V. 1996 Difference in hippocampal volume among food storing corvids. *Brain Behav. Evol.* **47**, 156–164.
- Birkhead, T. 1990 *The magpies*. London: Poyser.
- Bolhuis, J. J. & Macphail, E. M. 2001 A critique of the neuroecology of learning and memory. *Trends Cogn. Sci.* **5**, 426–433.
- Bossema, I. 1979 Jays and oaks: an eco-ethological study of symbiosis. *Behaviour* **70**, 1–117.
- Brodin, A. 1992 Cache dispersion affects retrieval time in hoarding Willow Tits. *Ornis Scand.* **23**, 7–12.
- Brodin, A. 1993 Low rate of loss of willow tit caches may increase the adaptiveness of long-term hoarding. *Auk* **110**, 642–646.
- Brodin, A. 1994 The role of naturally stored food supplies in the winter diet of the boreal Willow Tit *Parus montanus*. *Ornis Svecica* **4**, 31–40.
- Brodin, A. & Ekman, J. 1994 Benefits of food hoarding. *Nature* **372**, 510.
- Brodin, A. & Kunz, C. 1997 An experimental study of cache recovery by hoarding willow tits after different retention intervals. *Behaviour* **134**, 881–890.
- Brodin, A., Lahti, K., Lens, L. & Suhonen, J. 1994 Do crested tits, *Parus cristatus*, store more food at northern latitudes? *Anim. Behav.* **48**, 990–993.
- Brodin, A., Lahti, K., Lens, L. & Suhonen, J. 1996 A northern population of Willow Tits *Parus montanus* did not store more than southern ones. *Ornis Fennica* **73**, 114–118.
- Brotons, L. 1999 Chapter 7: food hoarding in the coal tit. Anonymous winter ecology of tits (Paridae) in boreal and subalpine forests: social organisation and the role of food hoarding. PhD thesis, Universitat de Barcelona, Barcelona, Spain, pp. 68–81.
- Brown, M. V. 2000 Neuronal correlates of recognition memory. In *Brain, perception, memory. Advances in cognitive neuroscience* (ed. J. J. Bolhuis), pp. 185–208. Oxford University Press.
- Chettleburgh, M. R. 1952 Observations on the collection and burial of acorns by Jays in Hainault forest. *Br. Birds* **14**, 359–364.
- Clayton, N. 1993 Lateralization and unilateral transfer of spatial memory in marsh tits. *J. Comp. Physiol. A* **171**, 799–806.
- Clayton, N. S. & Dickinson, A. 1999 Memory for the content of caches by scrub jays (*Aphelocoma coerulescens*). *J. Exp. Psychol.: Anim. Behav. Processes* **25**, 82–91.
- Clayton, N. S. & Krebs, J. 1994 Memory for spatial and object-specific cues in food-storing and non-storing birds. *J. Comp. Physiol. A* **174**, 371–379.
- Darley-Hill, S. & Johnson, W. C. 1981 Acorn dispersal by the blue jay (*Cyanocitta cristata*). *Oecologia* **50**, 231–232.
- Ekman, J., Brodin, A., Bylin, A. & Sklepkovych, B. 1996 Selfish long-term benefits of hoarding in the Siberian jay. *Behav. Ecol.* **7**, 140–144.
- Felsenstein, J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15.
- Gibb, J. A. 1960 Populations of tits and goldcrests and their food supply in pine plantations. *Ibis* **102**, 163–208.
- Grubb, T. C. & Pravosudov, V. V. 1994 Toward a general theory of energy management in wintering birds. *J. Avian Biol.* **25**, 255–260.

- Haftorn, S. 1956 Contribution to the food biology of tits especially about storing of surplus food. Part IV. A comparative analysis of *Parus atricapillus* L., *P. cristatus* L. and *P. ater* L. *Kgl Norske Vidensk Selsk Skr.* **4**, 1–54.
- Haftorn, S. 1959 The proportion of spruce seeds removed by the tits in a Norwegian spruce forest in 1954–55. *Kgl Norske Vidensk Selsk Forh.* **32**, 121–125.
- Haftorn, S. 1974 Storage of surplus food by the Boreal Chickadee (*Parus hudsonicus*) in Alaska, with some records on the Mountain chickadee (*P. gambeli*) in Colorado. *Ornis Scand.* **5**, 145–161.
- Hampton, R. R., Sherry, D. F., Shettleworth, S. J., Khurgel, M. & Ivy, G. 1995 Hippocampal volume and food-storing behavior are related in parids. *Brain Behav. Evol.* **45**, 54–61.
- Healy, S. D. & Krebs, J. R. 1992 Food storing and the hippocampus in corvids: amount and volume are correlated. *Proc. R. Soc. Lond. B* **248**, 241–245.
- Healy, S. D. & Krebs, J. R. 1996 Food storing and the hippocampus in Paridae. *Brain Behav. Evol.* **47**, 195–199.
- Healy, S. D., Clayton, N. S. & Krebs, J. R. 1994 Development of hippocampal specialisation in two species of tit (*Parus* spp.). *Behav. Brain Res.* **61**, 23–28.
- Hitchcock, C. L. & Sherry, D. F. 1990 Long-term memory for cache sites in the black-capped chickadee. *Anim. Behav.* **40**, 701–712.
- Källander, H. 1978 Hoarding in the rook (*Corvus frugilegus*). *Anser* (Suppl. 3), 124–128.
- Kamil, A. C. & Balda, R. C. 1985 Cache recovery and spatial memory in Clark's nutcracker (*Nucifraga columbiana*). *J. Exp. Psychol.: Anim. Behav. Processes* **11**, 95–111.
- Krebs, J. R., Sherry, D. F., Healy, S. D., Perry, H. & Vaccarino, A. L. 1989 Hippocampal specialization of food-storing birds. *Proc. Natl Acad. Sci. USA* **86**, 1388–1392.
- Krebs, J. R., Clayton, N. S., Hampton, R. R. & Shettleworth, S. J. 1995 Effects of photoperiod on food-storing and the hippocampus in birds. *Neuroreport* **6**, 1701–1704.
- Macphail, E. M. & Bolhuis, J. J. 2001 The evolution of intelligence: adaptive specializations versus general process. *Biol. Rev.* **76**, 341–364.
- Maguire, E. A., Gadian, D. G., Johnsrude, I. S., Good, C. D., Ashburner, J., Frackowiak, R. S. J. & Frith, C. D. 2000 Navigation-related structural change in the hippocampi of taxi drivers. *Proc. Natl Acad. Sci. USA* **97**, 4398–4403.
- Marzluff, J. M. & Balda, R. P. 1992 *The pinyon jay—behavioral ecology of a colonial and cooperative corvid*. London: Poyser.
- Patel, S. J., Clayton, N. S. & Krebs, J. R. 1997 Spatial learning induces neurogenesis in the avian brain. *Behav. Brain Res.* **89**, 115–128.
- Pravosudov, V. V. 1985 Search for and storage of food by *Parus cinctus lapponicus* and *P. montanus borealis*. *Zool. Zh.* **64**, 1036–1043.
- Pravosudov, V. V. & Clayton, N. S. 2001 Effects of demanding foraging conditions on cache retrieval accuracy in food-caching mountain chickadees (*Poecile gambeli*). *Proc. R. Soc. Lond. B* **268**, 363–368. (DOI 10.1098/rspb.2000.1401.)
- Pravosudov, V. V. & Clayton, N. C. 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.
- Pravosudov, V. V., Lavenex, P. & Clayton, N. C. 2002 Changes in spatial memory mediated by experimental variation in food supply do not affect hippocampal anatomy in mountain chickadees (*poecile gambeli*). *J. Neurobiol.* **51**, 142–148.
- Sherry, D. F. 1984 Food storage by Black-capped Chickadees: memory for the location and contents of caches. *Anim. Behav.* **32**, 451–464.
- Sherry, D. F. 1989 Food storing in the paridae. *Wilson Bull.* **101**, 289–304.
- Sherry, D. F. & Vaccarino, A. L. 1989 Hippocampus and memory for food caches in black-capped chickadees. *Behav. Neurosci.* **103**, 308–318.
- Sherry, D. F., Krebs, J. R. & Cowie, R. J. 1981 Memory for the location of stored food in marsh tits. *Anim. Behav.* **29**, 1260–1266.
- 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.
- Shettleworth, S. J. & Krebs, J. R. 1982 How do marsh tits find their hoards: the roles of site preference and spatial memory. *J. Exp. Psychol.: Anim. Behav. Processes* **8**, 354–375.
- Shettleworth, S. J. & Krebs, J. R. 1986 Stored and encountered seeds: a comparison of two spatial memory tasks in marsh tits and chickadees. *J. Exp. Psychol.: Anim. Behav. Processes* **12**, 248–257.
- Shettleworth, S. J., Krebs, J. R., Healy, S. D. & Thomas, C. M. 1990 Spatial memory of food-storing tits (*Parus ater* and *P. atricapillus*): comparison of storing and nonstoring tasks. *J. Comp. Psychol.* **104**, 71–81.
- Smulders, T. V., Sasson, A. D. & DeVoogd, T. J. 1995 Seasonal variation in hippocampal volume in a food-storing bird, the Black-Capped Chickadee. *J. Neurobiol.* **27**, 15–25.
- Swanberg, P. O. 1951 Food storage, territory and song in the thick-billed Nutcracker. In *Proc. Xth Int. Ornith. Congr., Uppsala, Sweden, 1950* (ed. S. Hörstadius), pp. 545–554.
- Tomback, D. F. 1980 How nutcrackers find their stores. *Condor* **82**, 10–19.
- Vander Wall, S. B. 1982 An experimental analysis of cache recovery in Clark's nutcracker. *Anim. Behav.* **30**, 84–94.
- Vander Wall, S. B. 1990 *Food hoarding in animals*. University of Chicago Press.
- Vander Wall, S. B. & Balda, R. P. 1977 Coadaptations of the Clark's nutcracker and the pinon pine for efficient seed harvest and dispersal. *Ecol. Monogr.* **47**, 89–111.
- Vander Wall, S. B. & Balda, R. P. 1981 Ecology and evolution of food-storage behavior in conifer-seed-caching corvids. *Z. Tierpsychol.* **56**, 217–242.
- Waite, T. A. & Reeve, J. D. 1992 Gray Jay scatterhoarding behavior, rate maximization, and the effect of local cache density. *Ornis Scand.* **23**, 175–182.

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