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Food storing does not affect hippocampal volume in experienced adult willow tits

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Abstract

Food-storing bird species have a larger hippocampal region than closely related non-storing species, and the avian hippocampal region appears to be involved in spatial memory for the locations of stored food. In the present study, willow tits (*Parus montanus*) that were at least 4 years old, and had previously stored food, were trained to store and retrieve seeds in an aviary. After training, control birds were deprived of any opportunities to store seeds, while experimental birds stored 17 seeds and recovered 5 seeds, on average, each day. After 26 days of this treatment there was no detectable difference between the two treatment groups in volume, neuron density, or total neuron number of the hippocampal region. This is in contrast to an earlier study in which a similar degree of food-storing experience caused enlargement of the hippocampal region in young birds with no previous food-storing experience.

Keywords: Hippocampus; Food storing; Spatial memory; Neural plasticity; *Parus montanus*

The hippocampal region (dorsomedial cortex, hereafter 'hippocampus') is involved in the memory used by some species of birds in retrieving stored food [10]. Food-storing species have larger hippocampal regions than non-storing species in the same families [11,12] and among food-storing birds, the size of the hippocampal region is correlated with the degree of dependence on stored food [2,7]. Ablation of the hippocampus in black-capped chickadees (*Parus atricapillus*) reduced retrieval performance for stored seeds to that expected by birds searching at random [13].

In a recent study with young, naive marsh tits (*Parus palustris*) it was found that the experience of retrieving and storing seeds caused enlargement of the hippocampus, while prevention of food-storing caused a decrease in hippocampal volume [6]. In addition to volumetric differences, they found cellular differences between the treatment groups: the experienced birds had more neurons, and a lower proportion of apoptotic cells in the hippocampus. The neural plasticity exhibited by these young marsh tits may reflect a developmental phenomenon found only during the onset of food storing, in which case older birds that have previously stored food should reveal no effect of experience on hippocampal volume. Alternatively, food-storing behavior may be linked to hippocampal volume throughout life as part of a seasonal cycle of storing behavior [1,14].

The goal of the present study was to examine the effect of storing experience on hippocampal volume in adult birds with previous food-storing experience. To do this I used willow tits (*Parus montanus*) that were at least 4 years old and had stored food extensively in captivity and presumably in the wild. Birds in one treatment group (hereafter 'storers') were allowed to store seeds for 15 min each day for 26 days, and to retrieve these seeds after a retention interval of 2–3 h. Control subjects were also allowed into the same test room for the same amount of time each day, but were given only powdered seeds that could not be stored.

Subjects were 8 willow tits (6 male, 2 female) captured in Finland in October 1990. It has been demonstrated that free-living willow tits depend on stored seeds for survival during the winter [3]. Subjects had been in captivity for over 3 years, and had been trained to store seeds a year earlier in the same test room. Birds were housed in $0.77 \times 0.44 \times 0.44$ m ($l \times w \times h$) wire cages

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attached by remotely controlled doors to a test room (3.8 × 3.9 × 2.4 m), and were maintained on 10:14 h light–dark cycle. They were fed a mix of peanut, hulled sunflower seed, hemp seed, and commercial insectivorous bird food, all of which had been ground into a fine powder to prevent subjects from storing it in their cages. Food was removed from cages 30 min before lights went out, and returned to each bird after it had completed its trials the following day. Training and test trials began at approximately 09.00 h GMT, after the birds had experienced 1–2 waking hours of food deprivation.

To ensure that treatment groups contained subjects with equal levels of performance at a food-retrieval task requiring spatial memory, I tested all birds at a one-trial associative memory task before assigning treatment groups. This task, described previously [5], was designed to mimic retrieval of a stored seed, but under conditions where motivation and site preferences could be controlled. (The dependent variable is number of visits required to relocate a hidden food reward). Beginning on 6 December 1993, subjects received 10 days of training at the one-trial associative memory task, and then were tested, in random order, on 5 sequential days. Four birds were assigned to each treatment group by forming several random groupings, and then accepting the first assignment in which the mean performance was exactly equal for the two treatment groups.

To ensure that all subjects began in the same condition and underwent the same training, each was given food-storing experience in the test room from 12–17 January. The test room was equipped with 75 food storage sites made by drilling holes in each of 5 tree branches that were mounted vertically in umbrella holders. Each hole was hidden by a small piece of cloth that could be pushed aside by the birds during storing or retrieval. Each bird was given two 10-min training sessions per day with a bowl containing peanut pieces and hemp seeds available in the test room. The location of each stored seed was recorded, and after each session, all seeds were removed. Before each bird's retrieval session I replaced all seeds stored during their first session and removed the food bowl.

Birds were given food-storing or control experience every day from 19 January through 13 February. The test room was equipped with new tree branches which were replaced and rearranged weekly. Each storer was allowed into the room for 15 min, with a bowl of peanut pieces and hemp seeds available. Control birds were also allowed into the room for 15 min, but had only powdered seeds available. As in the training sessions, after all subjects had been allowed into the room for 15 min, I allowed each bird back into the room for 5 min, and recorded the number of seeds retrieved. The retention interval was 2–3 h. Seeds were stored and retrieved by all storers in each session, and the controls actively searched empty sites during storing and retrieval sessions.

On 14 February birds were perfused transcardially with 0.75% saline followed by 10% formal saline after receiving a lethal overdose of sodium pentabarbitone. Brains were dissected out and postfixed for 7 days in 10% formal saline. They were then mounted in paraffin, sectioned in the coronal plane at 15 μm, and stained with Cresyl violet. Using a 10 × photographic enlarger I traced the boundaries of the hippocampus, ectostriatum (a well-defined cortex region which was to serve as a non-hippocampal control area), and the remainder of the telencephalon on every tenth section. A Wacom digitizer was used to determine the area of each section, and volume was calculated using the formula for volume of a truncated cone. Neurons were counted using the same equipment and protocol described in Healy and Krebs [8], with one exception. Whereas they counted 3 adjacent fields in each of 3 regions of one hemisphere (9 fields per brain), I counted a single field in each region of both hemispheres (6 fields per brain). From neuron density (mean number of neurons/mm^3) and hippocampal volume I estimated the total number of neurons per hippocampus. All of the histological preparations and measurements were done blind.

The results of the one-trial associative memory task were compared to the expectation for random search using a one-tailed z-test. The hippocampal (and ectostriatal) volumes of birds in each treatment group were compared using Mann–Whitney U-tests on the residuals of a regression of hippocampal (or ectostriatal) volume against volume of remainder of telencephalon. Neuron density and estimated total number of neurons in the hippocampus were compared using t-tests.

Subjects performed the one-trial associative memory task at better-than-chance levels ($\bar{x} = 2.9 \pm 0.75$ visits, $z = 1.98$, df$=6$, $P < 0.05$), indicating that they remembered the locations of previously encountered seeds. During the 26-day treatment, storers stored, on average, 17 seeds in 15 locations per session (range 2–34 seeds in 2–30 sites). All storers found and ate previously stored seeds in each retrieval session ($\bar{x} = 5.39$ seeds/session, range 1–14).

The treatment groups did not differ significantly in residual hippocampal volumes (controls $\bar{x} = -0.01 \pm 0.06$; storers $\bar{x} = 0.01 \pm 0.07$, $U = 6$, $U' = 10$, $P = 0.6$; Fig. 1). Another way of comparing relative hippocampus size is to divide each bird's hippocampal volume by the volume of the rest of its telencephalon. The mean for each treatment group was identical (0.06 ± 0.01). Treatment groups also had similar residual ectostriatal volumes (controls $\bar{x} = 0.01 \pm 0.06$; storers $\bar{x} = -0.01 \pm 0.07$, $U = 7$, $U' = 9$, $P = 0.8$). Treatment groups did not differ significantly in neuron density (controls $\bar{x} = 97289 \pm 7961$ neurons/mm^3; storers $\bar{x} = 86335 \pm 4967$ neurons/mm^3, $t = 2.3$, df$=6$, $P = 0.1$), or estimated total

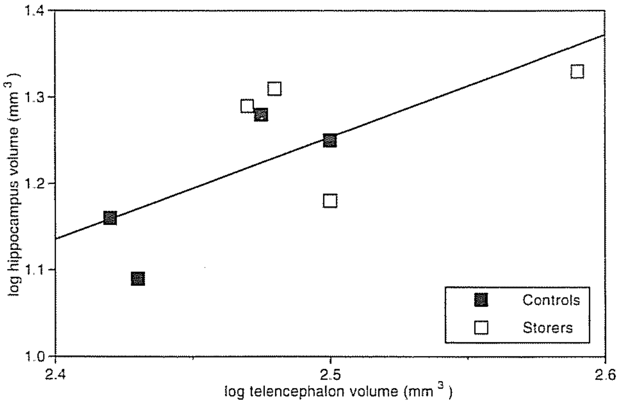

Fig. 1. Regression of log-transformed volumes of hippocampus and remainder of the telencephalon for willow tits receiving daily food-storing experience and control birds given no seeds to store. There was no significant difference between treatment groups in the residuals from the regression line.

neurons per hippocampus (controls $\bar{x}=1{,}536{,}991 \pm 202{,}778$ neurons; storers $\bar{x}=1{,}637{,}210 \pm 177{,}828$ neurons, $t=0.7$, df$=6$, $P=0.5$).

After 26 days of either daily food storing or complete deprivation from food storing, there was no detectable difference in the volume of the hippocampus, or the density and estimated number of hippocampal neurons of the birds in the two treatment groups. Although sample size was relatively low, there is no trend to suggest that a larger sample would reveal an effect of food-storing experience on the hippocampus. This contrasts with results of a previous study on young marsh tits, in which groups of 3–6 birds that stored seeds for 20 min every day or every third day for 23 days exhibited an increase in hippocampal volume when compared with controls deprived of any food-storing experience [6]. There are 3 major differences between that experiment and the present study which could explain the contrasting results. Most importantly, the earlier experiment used young, hand-reared birds that had never been exposed to opportunities for food storing. There was no apparent relationship between age and the effect of food-storing experience on hippocampal volume, suggesting the possibility that experience-induced plasticity might endure throughout life. However, that experiment included only birds ranging in age from 35 to 115 days. The present experiment, using experienced birds that were at least 4 years old, suggests that the effect of food-storing experience on hippocampal volume is either restricted to younger birds or is a one-time phenomenon that occurs at whatever age a bird first stores food.

The recent discovery of seasonal or photoperiod effects on hippocampal volume [14], rate of neurogenesis [1], and food storing and accuracy of spatial memory [4] in food-storing birds suggest another possible explanation for the lack of an effect of food-storing experience on hippocampal volume: subjects in the present experiment were tested in autumn and early winter, whereas the young marsh tits that exhibited hippocampal plasticity in the Clayton and Krebs [6] study were tested in summer and early autumn. However, birds in both studies were maintained on the same constant winter photoperiod (N.S. Clayton, personal communication), thus, seasonal differences could only explain the differing results if hippocampal sensitivity to food-storing experience has endogenous cycles that do not respond to photoperiod.

The third obvious methodological difference between the two studies is that the present study used willow tits, while the previous work was on marsh tits. Although there was no reason to predict that the relationship between food-storing experience and hippocampal size would differ between these morphologically similar congeners, it would also be imprudent to assume that the contrasting results are due only to age differences. Further study is needed to determine whether: (1) as in willow tits, experienced adult marsh tits fail to exhibit the hippocampal plasticity reported in young of that species; (2) naive young birds of other food-storing species exhibit the same relationship between volume of the hippocampus and food-storing experience reported for young marsh tits; and (3) whether the sensitivity of the hippocampus to any effects of experience are limited to certain photoperiods or seasons.

Another recent study suggests that the hippocampus in breeding-aged black-capped chickadees remained unchanged despite photoperiod-induced increases in food storing [9]. On the basis of present evidence I cannot conclude that the hippocampus in adult willow tits exhibits the experience-related plasticity reported in young food-storing birds of the same genus.

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