A larger hippocampus is associated with longer-lasting spatial memory

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Volumetric studies in a range of animals (London taxi-drivers, polygynous male voles, nest-parasitic female cowbirds, and a number of food-storing birds) have shown that the size of the hippocampus, a brain region essential to learning and memory, is correlated with tasks involving an extra demand for spatial learning and memory. In this paper, we report the quantitative advantage that food storers gain from such an enlargement. Coal tits (*Parus ater*) a food-storing species, performed better than great tits (*Parus major*), a nonstoring species, on a task that assessed memory persistence but not on a task that assessed memory resolution or on one that tested memory capacity. These results show that the advantage to the food-storing species associated with an enlarged hippocampus is one of memory persistence.

There are at least three constituent aspects to spatial learning and memory: memory capacity (the number of locations remembered), memory persistence (the duration over which a location is remembered), and spatial resolution (the least distance at which remembered locations can be discriminated). Although volumetric studies have shown that several behavioral adaptations are associated with an enlarged hippocampus (1–6), no volumetric study, nor any of a number of experiments examining spatial memory performance (7–16), has yet been able to determine whether the observed enlargement of the hippocampus in food-storing and other species is associated with one, two, or all three of these aspects of memory. If food-storers have a greater memory capacity than the nonstorers, we predict that performance would be similar when there is only one item to remember, but would diverge with increasing number of items, with the nonstorers having a poorer performance when required to remember more items (see Fig. 1*A*). If the species differed only in the spatial resolution of memory, they should achieve similar performance levels when items are far apart, but the food storers should perform better when the items are close together (Fig. 1*B*). If the food storers only have a longer-lasting memory, then the species difference in performance should be similar regardless of number of items or their proximity (Fig. 1*C*; see ref. 17 for a similar argument).

In this experiment, wild-caught food storers [coal tits (*Parus ater*), a species with an enlarged hippocampus] and a related nonstoring species [great tits (*Parus major*)] were tested on all of these three aspects of spatial memory within the same experiment. All birds were presented with one to four white squares on a computer-controlled touch screen. Squares disappeared after a peck was directed at them. Once all squares had been pecked and after a retention interval, the birds were presented with a square in one of these earlier locations and a second square in a new location. They were rewarded for pecking the square in the new location (the "target"), following a spatial nonmatching rule. Memory capacity was tested by varying the number of sample images to be remembered from one to four. The spatial resolution at which the birds had to remember image locations to solve the task was manipulated by presenting the target immediately adjacent to the location of the sample in half of the trials ("near" condition), but farther away on the others ("far"

condition; see Fig. 2). Memory persistence was assessed by manipulating the retention interval between sample and choice by using a titration procedure: After a correct choice the retention interval in the following trial increased, whereas after an error the retention interval was decreased. Retention intervals were increased until the proportion of each bird's correct responses stabilized at 70%. The performance of each bird was assessed by the final retention interval it reached in each experimental condition.

Experiment 1

Materials and Methods. *Animals.* The subjects were eight coal tits and eight great tits (all wild-caught in deciduous woodland in Northumberland, England). All birds were housed individually in wire-mesh cages (77 cm long × 44 cm wide × 44 cm high) and were fed daily with ad libitum water and an insectivorous bird food mixture (Orlux, Sunring Cooke, Greasbrough, Rotherham, U.K.), supplemented by peanuts, sunflower seeds, pine nuts, and wax moth larvae. They were maintained on a 13.5:10.5 h light:dark cycle and under a temperature range of 14–16°C. For both training and experiments, birds were deprived of food at 9:00 a.m. each morning and provided with fresh food when their session had finished. Training and testing began at 10:00 a.m. Birds tested later in the day were provided with nuts at intervals through the day.

Training. All of the birds were trained to peck at one to four white squares (2 cm × 2 cm; the "sample") on a computer-controlled touch screen. Images disappeared after a peck was directed at them. Once all images had been pecked and after a retention interval of 1 sec, birds were presented with a square in one of these earlier locations and a second square in a new location. Correct choices were followed by delivery of a small piece of peanut as a reward; errors were followed by the onset of the intertrial interval. The intertrial interval was always 90 sec.

To give a bird access to the touch screen, a sliding door was pulled off the front of the cage. Before titration of retention intervals, all birds were trained, separately for each sample number, to a criterion of at least 70% correct choices averaged over 3 days. There were 20 trials in each daily training session.

Testing. The birds were tested in 16 blocks of 4 days each. Within each block of days, a day of testing with one sample was followed by a day with two samples, then three and four samples. There were 20 trials in each daily testing session. In total the birds received 160 trials with each of the eight combinations of proximity and sample number. The retention interval (RI) was titrated independently for each condition. The titration proce-
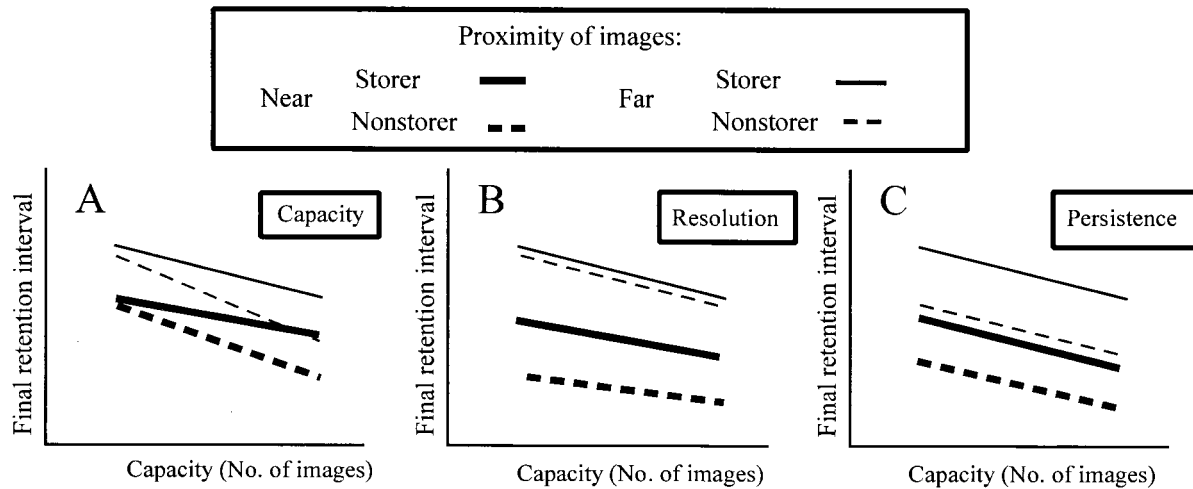
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Fig. 1. Predicted outcomes if an enlarged hippocampus is associated with different memory capacity, resolution, or persistence. The storers are represented by the unbroken lines, the nonstorers by the dashed lines. Heavy lines represent images that are near to each other and thin lines represent images that are far apart. (*A*) If the species differ in memory capacity, performance should be similar when there is little to remember, but diverge as memory load increases. The pair of lines for each group are the predictions for the way in which performance levels should change with increasing numbers of sample images when the choice images differ in their proximity (see also *C*). (*B*) If the species differ in spatial resolution, performance should be similar when the images are far apart, but differ when they are close together. (*C*) If the species differ in memory persistence, the differences should be apparent from the smallest memory load and remain the same as memory load increases, regardless of proximity.

dure was as follows: After a correct choice, the retention interval (beginning at 1 sec) in the following trial increased by 0.3 sec, after an error it decreased by 0.7 sec. That means the retention interval stabilizes when a bird gets no more than 70% of its choices correct. These procedures are summarized in Fig. 2.

We tested for differences in retention intervals by using an

Near condition with one sample image

Far condition with four sample images
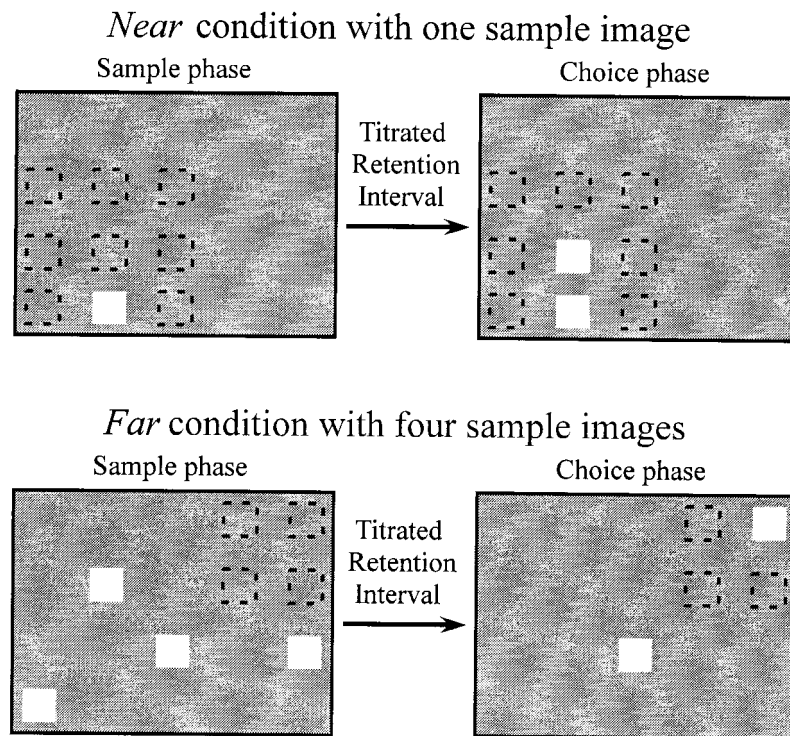
Fig. 2. A schematic of the experimental design showing examples of the variation in the number of sample images and in the distance at which choices were presented. From one to four white squares were displayed in the sample phase. In the Choice phase of the Near condition, one image (and only one, regardless of the number of sample images) was placed in one of the positions immediately adjacent to the target. These positions are marked here by stippled outlines, which were *not* shown to the birds. The birds were presented only with the white squares. In the Choice phase of the Far condition, there was no sample in the positions immediately adjacent to the target. The combination of one to four samples with Near and Far conditions resulted in a total of eight different conditions.
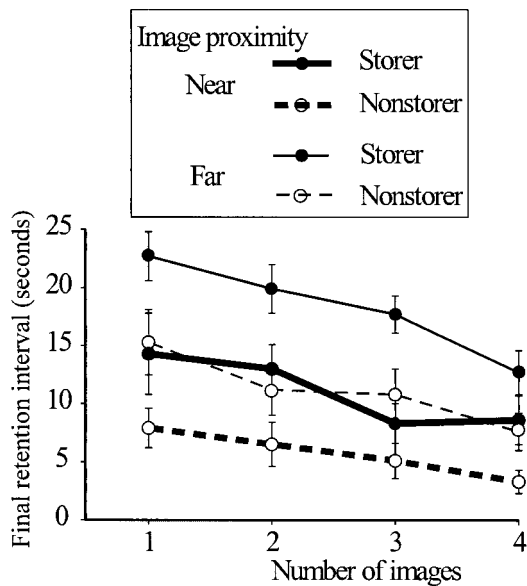
Fig. 3. Final retention intervals (seconds) reached by the birds at the end of the experiment. Data are means and SE. The storers are represented by the filled circles, the nonstorers by the open circles. Heavy lines represent images that are near to each other and thin lines represent images that are far apart.

analysis of variance with species as between-subjects factor and number of samples and proximity as within-subjects factors.

Results. Memory persistence is different between the species in both proximity conditions and from the smallest memory load. The final retention intervals reached in each condition were significantly longer for the coal tits than for the great tits [$F(1,12) = 8.98$, $P < 0.02$; see Fig. 3]. The task became more difficult for both species as the number of sample images, and therefore the memory load, increased [$F(3,36) = 32.68$, $P < 0.0001$]. It was also more difficult for both species when the two images in the choice phase were near rather than far apart [$F(1,12) = 28.21$, $P < 0.0002$]. None of the interactions were significant [Species × Memory load: $F(3,36) < 1$, $P > 0.5$; Species × Proximity: $F(1,12) < 1$, $P > 0.5$; Memory load × Proximity: $F(3,36) = 2.20$, $P > 0.1$].

Discussion. The results from this experiment are consistent with the outcome predicted if the difference between the groups is one of memory persistence (see Fig. 1C). The predicted outcomes shown in Fig. 1 A and B can be excluded. The coal tits reached longer retention intervals than did the great tits. The task became more difficult for both species both as the number of sample images, and therefore the memory load, increased and when the two images in the choice phase were near rather than far apart, but this was equally so for both species. If there is a difference in memory capacity between the two species, we predicted that the great tits would perform less well than the coal tits as the number of images to be remembered increased. This was not the case. If there are differences in the spatial resolution of memory, we predicted similar performance by both species when the images were far apart but that the coal tits would outperform the great tits when the images were close together. This was also not the case. There is only the overall species difference that we expect if the coal tits have a longer lasting memory, and no sign of any other difference.

The distinction between spatial resolution and persistence of memory, however, depends on the simplifying assumption that the spatial resolution of the memory for a location does not

change over time, i.e., that forgetting is an all-or-nothing effect. A bird would either remember a location perfectly, with the same precision as at the time when the memory was laid down, or the bird would not remember that location at all. Only the probability of perfect recall would change over time.

We know that this simplifying assumption is unlikely to be valid, because there is evidence in other species that precision does decrease over time (18–21), i.e., memory degradation is a gradual process. The results of experiment 1 then may be explained by saying that the food storers encoded image locations with better precision than the nonstorers did, but that the rate at which that spatial memory became more indistinct over time was the same in both species. The rate of forgetting (of memories becoming more vague) would be the same, and food storers would only take longer to reach the same point of vagueness as nonstorers because they started out with a more precise representation of location.

To resolve this issue, we ran a further experiment in which we directly tracked the spatial resolution of memory over time. At each of four fixed retention intervals we titrated the distance between a single sample and a goal to the minimum the birds could discriminate. Comparing this minimum distance reached by birds across retention intervals directly should reveal how the spatial resolution of memory changes over time.

Coal tits and great tits that had not been used in experiment 1 were presented with a sample image, which was a white circular spot that disappeared when a bird pecked at it. After a retention interval of 2.5 sec, 5 sec, 10 sec, or 20 sec, the image was displayed again in the same location, together with a target image. The images were initially at a maximum distance of 104 mm apart, center to center (increased to a maximum of 125 mm to allow for worse performance than expected), and presented at a randomly chosen angle. The distance between stimuli was titrated separately for each retention interval such that after a correct choice the distance between stimuli in the next trial was decreased, after an error it was increased. At the end of the experiment we looked for the minimum distance at which each bird's performance level was a stable 70% correct.

Experiment 2

Animals and Testing. Six coal tits and six great tits that had not been used in experiment 1 were tested. As in experiment 1, a delayed non-matching-to-sample rule was used such that the birds were rewarded for pecking at the image in the novel location in the choice phase. During training and testing, birds were presented with a sample image (a white circular spot of 16-mm diameter) that disappeared when a bird pecked at it. After a retention interval of 2.5 sec, 5 sec, 10 sec, or 20 sec, the image was displayed again in the same location, with a target image presented at another location on the screen. The images were initially at a maximum distance of 104 mm apart, center to center (increased to a maximum of 125 mm for birds that performed badly when the images were 104 mm apart), and presented at a randomly chosen angle. The distance between stimuli was titrated separately for each retention interval such that after a correct choice the distance between stimuli in the next trial was decreased by 1.2 mm, after an error it was increased by 2.8 mm. The minimum possible distance, center to center, was 20.8 mm. At the end of the experiment, we looked for the minimum distance at which each bird's performance level was a stable 70% correct. There were 20 trials in each daily session. Birds were tested in blocks of 4 days, 1 day with each retention interval.

We tested for differences in distances by using an analysis of variance with species as between-subjects factor and retention interval as within-subjects factors.
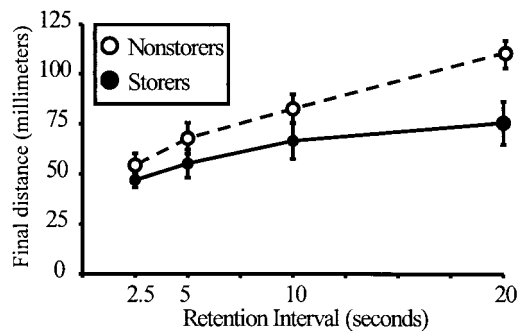
Fig. 4. Final distances between choice images achieved at each of four retention intervals in experiment 2. The storers are represented by the filled circles, the nonstorers by the open circles.

Results. There was no difference between the species in minimum distance reached [$F(1,11) = 4.15$, $P = 0.067$]. However, the coal tits achieved a smaller distance between stimuli than the great tits did at 20 sec, the longest retention interval [interaction between retention interval and distance: $F(3,33) = 2.93$, $P < 0.05$; analysis of simple effects showed that coal tits differed from great tits only at the 20-sec retention interval: $F(1,11) = 7.95$, $P < 0.02$; for all other retention intervals $F < 2$, $P > 0.1$].

Discussion. The results provide evidence for a longer lasting memory in coal tits than in great tits, but the coal tits do not initially store spatial information at greater spatial resolution (Fig. 4). If coal tits differed from great tits only in the spatial resolution of the information initially stored in memory, but not in the rate of decay of that memory, we would have expected to find the same difference in the titrated distance at all retention intervals. This was not the case. If the species differed only in the rate of forgetting, we would expect no difference at short retention intervals, but the coal tits should perform better than the great tits at longer intervals. The data fit this prediction. If the species differed both in accuracy and rate of forgetting, we would expect a significant difference at short retention intervals that increases at longer intervals, but this was not the case.

Conclusions

The results of experiment 2 thus confirmed the conclusions of experiment 1: Over the range of retention intervals tested, the food-storing coal tits differ from the nonstoring great tits only in the persistence of memory. Therefore, we conclude that the coal tits' memory for locations decays at a slower rate. This finding is in contrast to data from previous studies comparing different aspects of spatial memory between storing species that vary in their dependence on food storing. Those data could not be used to distinguish between the possibility of encoding differences or of memory persistence as the explanation for the observed species differences (11–13).

There are limits to the generality of our findings. First, we studied only short-term memory. We do not know whether food storers also differ from nonstorers in long-term memory, and if so, how they might differ. We do not know how short-term and long-term memory interact. It is possible that keeping information for longer in short-term memory helps transfer to long-term memory, analogous to rehearsal effects in human memory (22). Second, we tested memory within the small-scale environment of a touch screen. Hummingbirds' reaction to landmark array displacements depends on the scale of the array and the displacement (23). On the other hand, pigeons reacted to landmark displacement on a touch screen in the same way as to landmark displacements in a larger open field (24).

Despite their limitations, our results provide evidence that the enlargement of the hippocampus in food-storing birds may enable these birds to increase the duration of time over which they can remember spatial information. These data demonstrate which specific aspect of spatial memory is associated with hippocampal enlargement.

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