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Effects of Captivity and Memory-Based Experiences on the Hippocampus in Mountain Chickadees

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The complexity of an animal's physical environment is known to affect the hippocampus. Captivity may affect hippocampal anatomy and this may be attributable to the limited opportunities for memory-based experiences. This has tangential support, in that differential demands on memory can mediate changes in the hippocampus. What remains unclear is whether captivity directly affects hippocampal architecture and whether providing memory-based experiences in captivity can maintain hippocampal attributes comparable to wild-caught conspecifics. Using food-caching mountain chickadees (*Poecile gambeli*), we found that wild-caught individuals had larger hippocampal volumes relative to the rest of the telencephalon than captive birds with or without memory-based food-caching experiences, whereas there were no differences in neuron numbers or telencephalon volume. Also, there were no significant differences in relative hippocampal volume or neuron numbers between the captive birds with or without memory-based experiences. Our results demonstrate that captivity reduces hippocampal volume relative to the remainder of the telencephalon, but not at the expense of neuron numbers. Further, memory-based experiences in captivity may not be sufficient to maintain hippocampal volume comparable to wild-caught counterparts.

Keywords: captivity, hippocampus, mountain chickadee, *Poecile gambeli,* spatial memory

Memory, or the process of using past representations to guide current behavior, has ramifications on territoriality, mate choice, navigation, acquisition of food resources, and many other ecologically relevant behaviors (e.g., Brennan, Kaba, H., & Keverne, 1990; Godard, 1991; Menzel, Brandt, Gumbert, Komischke, & Kunze, 2000; Shettleworth, 1990). Further, differential demands on memory have been shown to affect brain structures differently, specifically reflected in the hippocampus, the region of the brain responsible for spatial memory processing. Animals that have a greater reliance on spatial memory tend to have larger hippocampi and more hippocampal neurons (Healy & Krebs, 1996; Krebs, Sherry, Healy, Perry, & Vaccarino, 1989; Lucas, Brodin, de Kort, & Clayton, 2004; Sherry, Vaccarino, Buckenham, & Herz, 1989). For example, food-storing birds that rely heavily on spatial memory for food retrieval have larger hippocampi than closely related species that do not rely on spatial memory to retrieve food. Similarly, but within a species, male meadow voles (*Microtus pennsylvanicus*) have higher demands on spatial memory as a result of patrolling large territories, which is reflected by larger hippocampi compared with females that reside in significantly smaller home ranges (Jacobs, Gaulin, Sherry, & Hoffman, 1990). Clearly, a relationship exists between demands on spatial memory and the hippocampal architecture, where higher demands on spatial memory appear to contribute to increased hippocampal volume and neuron number.

Similarly, it has been hypothesized that an animal's physical environment can also alter hippocampal volume and neuron numbers. Captivity has been suggested to reduce hippocampal attributes when captive animals are compared with wild-caught conspecifics (Barnea & Nottebohm, 1994; Day, Guerra, Schlinger, & Rothstein, 2008; Smulders, Casto, Nolan Jr., Ketterson, & DeVoogd, 2000). Captivity may represent a less complex environment than the wild, thereby restricting both physical movement and memory-based experiences and demands (van Praag, Kempermann, & Gage, 2000). Thus, within the captive environment, providing a more complex physical environment may allow for greater physical stimulation, encourage increased activity levels, and increase demands on memory, all of which may increase hippocampal volume, neuron number, and neurogenesis rates (Kempermann, Kuhn, & Gage, 1997).

This effect of a more complex captive environment on the hippocampus has been presumed to be mediated through both an arousal response and through learning and memory. The arousal response occurs when increased motor stimulation from navigating a more complex environment increases hippocampal attributes. Studies have shown that increased activity levels in more complex environments are sufficient to increase cell proliferation in the

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brain (e.g., van Praag, Christie, Sejnowski, & Gage, 1999a; van Praag, Kempermann, & Gage, 1999b). In addition, increased learning and memory may be needed in a more complex environment and may directly affect hippocampal attributes (e.g., Kempermann et al., 1997). Therefore, both increased physical activity and memory use can potentially affect the hippocampus in terms of hippocampal size, neuron number, and neurogenesis. However, distinguishing between the effects of memory and motor stimulation has proven difficult. Studies testing the effects of learning and memory while controlling for the arousal response have been equivocal (e.g., Greenough, Cohen, & Juraska, 1999). These conflicting results are generated by some studies showing that hippocampal neurogenesis rates can be increased by spatial learning tasks (Ambrogini et al., 2000; Gould, Beylin, Tanapat, Reeves, & Shors, 1999; Lemaire, Koehl, Le Moal & Abrous, 2000) while others failing to find a link between spatial learning tasks and hippocampal neurogenesis (van Praag et al., 1999a,b).

Moreover, the development and long-term maintenance of hippocampal attributes such as volume and neuron number may require spatial, memory-based experiences ("use it or lose it" hypothesis, Clayton 1995a), which may be sustained in complex environments. In some studies, the use of memory in just a few tasks such as food-caching and food-retrieving lead to the enlargement of the hippocampal structure (Clayton, 1998; Clayton & Krebs, 1995). Specifically, several studies on developing birds found that memory-based experiences in food caching and retrieving led to an increase in hippocampal volume and/or neuron number, while restricting such experiences led to a decrease in hippocampal volume and/or neuron number (Clayton, 1994, 1996, 2001; Clayton & Krebs 1994a; Patel, Clayton, & Krebs, 1997). In these studies, birds were hand-raised, still early in development and had no prior food-caching experience. This may explain why Cristol (1996) found no difference in hippocampal attributes between adult birds that were deprived of food-caching experiences for 26 days versus those who had the opportunity to acquire memory-based experiences; his birds had been in captivity for several years and had a great deal of previous caching experience. Collectively, it is difficult to ascertain if hippocampal plasticity is restricted to ontogeny or if differential environmental conditions, such as captive versus wild animals, and differential memory-based experiences can lead to hippocampal plasticity in adult animals. Furthermore, it is unclear what exactly is affected by the environmental conditions typical of captivity: hippocampal volume, neuron numbers, or both.

Our goal in this study was twofold. First, we tested whether an animal's physical environment would affect hippocampal attributes. Specifically, we tested whether food-caching mountain chickadees (*Poecile gambeli*) housed in captivity differed in hippocampal volume, hippocampal neuron number and neuronal density as compared with fully developed wild-caught conspecifics. We predicted that captivity, with reduced environmental complexity and restricted memory-based experiences (compared with memory-based experiences afforded in the natural environment), would reduce hippocampal volume, neuron number and, potentially, neuron density. Second, we attempted to determine, within the context of captivity, whether differences in hippocampal structure, neuron number, and/or neuron density are based on differential memory-based experiences, while controlling for physical activity. We expected that deprivation of memory-based experiences, mediated through caching and retrieving food items, would decrease hippocampal volume, neuron numbers, and neuronal density, as compared with captive counterparts with memory-based experiences.

Method

Treatment Groups

Twenty-four juvenile male mountain chickadees (3–4 months old) were caught at our 40 feeder grid spread over 10 km along two forest roads near Sagehen Creek in Tahoe National Forest, CA in September of 2007. We matched subjects in pairs based on body weight and randomly assigned each of the birds to one of two captive treatment groups. The two captive treatment groups were composed of birds with either the opportunity to cache and retrieve food items, thus engaging in memory-based experiences (experienced group), or birds deprived of caching and retrieval with no opportunity to engage in memory-based experiences (deprived group). The wild-caught group was comprised of an additional 12 juvenile males that were captured in January 2008 in the same location at the time all birds were sacrificed for the brain analyses. Birds in all three groups in this experiment were the same age (7–8 months old) at the time of sacrifice. The wild-caught birds were measured for body weight and sacrificed immediately.

Husbandry for Captive Birds

Captive birds were individually housed in wire mesh cages ($60 \times 40 \times 60$ cm), with only auditory contact with other subjects. Cages contained two perches, a bathing dish, and two food dishes. Birds were fed once a day with pine nuts, shelled and unshelled sunflower seeds, crushed peanuts, mealworms, and Roudybush (Roudybush Inc., Woodland, CA). Water was provided ad lib for drinking and bathing in a large water dish on the bottom of the cage. Cages and dishes were cleaned weekly. Subjects were maintained at 20°C on a light cycle that mimicked the natural ambient light schedule.

Testing Room

The testing room had two "trees" constructed from wood (8.26 cm $\times$ 8.26 cm $\times$ 238.76 cm), each tree with 20 caching holes and wooden perches. A string with a knot tied in the end was suspended above each hole so the subjects in the experienced group had to remove the knot from the hole to acquire food items. We hung 16 blocks (8.89 cm $\times$ 14.61 cm $\times$ 3.81 cm), divided among three rows, on one wall of the testing room and 16 blocks on the opposite wall. Blocks were staggered between rows. Each block had a hole, a wooden perch, and a string with a knot tied in the end. Again, the knot was suspended above the hole so the subjects in the experienced group had to remove the knot from the hole to acquire food items.

All experimental procedures occurred in the testing room, which was adjacent to the rooms where the birds were housed. Access from the bird's home cage to the testing room was through an opening in the wall connecting each individual bird's cage with the testing room to minimize stress from handling. When testing a subject, the lights in the housing room were extinguished while lights in the testing room remained on. By doing so, the bird was

stimulated to fly toward the light and into the testing room without handling by the experimenter. Once the subject had flown into the testing room, the opening between the rooms was closed and the lights were turned back on in the housing room. Similarly, this process was reversed to motivate the bird to fly back into its cage at the end of each trial (Clayton, 1992, 2001).

Familiarization Period

Each subject was allowed to habituate to the testing room and caching apparatus for 2 hours a day, every other day, for a total of 6 hours. During this period, each bird from the experienced group was familiarized with the testing room and with finding food in the blocks. We randomly baited six sites per 2-hour session so the subjects learned to look for food within the caching array (Pravosudov & Clayton, 2002). We also provided pine nuts and sunflower seeds in the testing room so birds in the experienced group could make caches. By the final familiarization period, all birds in the experienced group had cached and retrieved food from the caching array. The deprived group was allowed the same access to the testing room and the caching apparatus but absolutely no food was available in the testing room at any time during the familiarization period.

Memory-Based Experiences for the Experienced Group

After the familiarization period, birds from the experienced group were allowed in the testing room every other day for 3 months to cache and retrieve food items and to participate in associative learning tasks, thus acquiring memory-based experiences in the lab. The experienced group was allowed to cache and later retrieve their caches over a 2-month period and allowed to perform an associative learning task for 1 month. For all memory-based tasks, food was removed from the cages of the experienced group 30 min before lights off the evening before testing and replaced after the birds had performed in the memory tasks the following day. By doing so, birds were motivated to cache and retrieve during the memory tasks.

During the caching and retrieving task, a dish with pine nuts and sunflower seeds was provided in the testing room. When a bird was allowed in the testing room, we recorded the type and amount of food consumed, as well as where the bird stored food items within the caching array. All observations of testing occurred from behind a one-way mirror. After 10 min, the bird was returned to its home cage and all caches were removed from the caching array. After a 4-hour retention interval, we replaced the bird's caches in the appropriate caching holes, covered the holes with the string and allowed the bird back in the testing room for 20 min, but now the only food available was located in the bird's previous cache locations. We recorded the number of caches recovered and the number and order of caching holes investigated. We considered the bird to have investigated a caching hole if the bird removed the knot from in front of the hole in the caching apparatus (Shettleworth, Krebs, Healy, & Thomas, 1990; Clayton & Krebs, 1994bc; Pravosudov, 2003; Pravosudov & Clayton, 2001, 2002; Pravosudov, Mendoza, & Clayton, 2003).

We also allowed the birds to participate in an associative learning task for one month. In the associative learning task, we stored one seed and allowed the bird to recover the seed using memory (Brodbeck, 1994; Clayton 1995b; Clayton & Krebs, 1994bc). During the learning phase, a bird was allowed into the testing room in which all caching locations were open and only one of them had a clearly visible pine nut. When the bird discovered the nut it was allowed to eat for 2 s, after which lights were turned off and the bird flew back into its home cage. After a 5-min retention interval during which the bird had no food, it was allowed back into the room but now all caching sites were covered by a knot at the end of the string so the bird could use memory to locate previously found food. This is a very common task used in memory experiments and memory performance on food-finding associative learning tests closely resembles cache retrieval performance (Brodbeck, 1994; Shettleworth et al., 1990) and thus should apply to memory-based cache retrieval behavior and associated changes in the hippocampus (Brodbeck, 1994; Shettleworth, 1990; Shettleworth et al., 1990).

Deprived Group

Because hippocampal attributes can be affected by increased activity levels in more complex environments (e.g., van Praag et al., 1999ab), we attempted to equalize this effect between the experienced and deprived groups. To control for potential effects on hippocampal architecture due to the experienced group navigating a more complex environment (i.e., the testing room), we also allowed the deprived group access to the testing room in the same time intervals as the experienced group.

The deprived group was treated in the same manner as the experienced group, with some exceptions. First, right before access to the testing room in the morning, the deprived group was provided with food in their cages for 10 min. By doing so, the birds in the deprived group had the same experience handling food as the experienced group did while in the testing room, but without the opportunity to cache in the testing room. Thus, when each bird from the deprived group was in the testing room, no food was available for caching but the bird could explore the caching apparatus. All caching sites were always covered with a knot at the end of the string. We also made sure birds did not cache food in their home cages. Water was provided in large dishes on the floor and no water bottles were used to prevent birds from caching in water bottles. Cages had no places available to cache food out of bird sight (all parts of the cage had tight fit and we verified daily that birds never cached food between cage parts). We also verified that birds did not cache seeds under tray paper or at any sites on a daily basis. Birds could only drop seeds on the cage floor or leave them on the sides of the feeder where they remained highly visible to the birds. In addition, we removed all food that was outside of the feeder on the daily basis. We think it is unlikely that seeds dropped on the floor constituted the same experience as food caching, as these seeds were clearly visible and were not retrieved. We also think that if birds pushed the seeds outside the cage that would also not constitute memory-based experience. Clayton (2001), using young mountain chickadees, showed that retrieval is more critical for the hippocampal enlargement than just caching, and mountain chickadees that were allowed to only cache but not to retrieve had significantly smaller hippocampal volumes as compared with chickadees that were allowed to both cache and retrieve. In our experiment, deprived birds had absolutely no retrieval experience. Therefore, the main difference between the experienced and deprived groups was in the opportunity to obtain memory-based

caching and cache retrieval experiences, as well as memory-based experience in associative memory tests in the testing room.

Histology

At the end of 3 months, birds were anesthetized with a lethal overdose of Nembutal (0.07 ml of 50 mg/ml Nembutal). The birds were transcardially perfused with 0.1 M phosphate buffered saline for 10 min followed by 15–20 min perfusion of 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were extracted and postfixed in 4% paraformaldehyde for 24 hours before cryoprotection. Brains were cryoprotected in 15% sucrose, then 30% sucrose, and finally flash-frozen on dry ice. Brains were stored at −80°C until sliced.

Brains were sliced on a cryostat (Leica CM 3050S: −20°C) in the coronal plane every 40 μm. Every 4th section was mounted and Nissl-stained with thionin. Slides were coded, thus tissue slices were measured blind to treatment. We measured hippocampal volume, telencephalon volume (telencephalon volume minus hippocampal volume) as a control area, and hippocampal neuron numbers, all estimated with standard stereological methods (Microbrightfield, Inc. StereoInvestigator; Leica M4000B microscope).

Hippocampal and telencephalon volumes were measured in their entirety and estimated with the Cavalieri procedure (Gundersen & Jensen, 1987). Hippocampal volume was measured with a 200-μm grid; telencephalon volume was measured with a 1200-μm grid following our previous work (Pravosudov & Omanska 2005a,b; Pravosudov et al., 2002). Neuron counts were performed with an optical fractionator procedure at 1000 × (West, Slomianka, & Gundersen, 1991). The optimal grid size (250 μm), counting frame (30 × 30 μm), dissector height (5 μm) and frequency of sections sampling (12) has been determined previously in chickadees (Pravosudov & Omanska 2005a,b; Pravosudov et al., 2002). The left and right hemispheres were both measured for volume and neuron counts and then added to produce the given values. There were no significant differences between left and right hippocampal volumes and between the total number of neurons in the left and right hippocampus. After estimating hippocampal volume and neuron number, we also calculated neuron density (neuron number/hippocampal volume).

Statistics

The data met all assumptions for parametric analyses. Differences in telencephalon volume among wild-caught birds, captive birds deprived of memory-based experiences, and captive birds with memory-based experiences were determined by ANCOVA, both with body weight as the covariate and without a covariate. Differences in relative hippocampal volume, hippocampal neuron number, and hippocampal neuron density were determined by ANCOVA, with body weight and telencephalon volume as covariates. The goal of our study was to see whether changes in the environment affect the hippocampus but not the rest of the brain; therefore, we used the remainder of the telencephalon (telencephalon minus hippocampus) as a control area following all similar studies (e.g., Krebs et al., 1989; Clayton, 2001). Using telencephalon as a covariate allows testing whether treatment effects were specific to the hippocampus. We also compared absolute telencephalon volume between the groups to verify that our treatment had no effect on telencephalon volume. Pairwise comparisons were Bonferroni-corrected and we considered all results to be statistically significant if $p \leq .05$.

Results

Telencephalon volume, when adjusted for body weight, was not significantly different among wild-caught birds, captive birds deprived of memory-based experiences, and captive birds with opportunities for memory-based experiences ($F_{2,33} = 0.141$, $p = .869$; Figures 1, 2a). When telencephalon volume was not adjusted for body weight, we obtained comparable results ($F_{2,33} = 0.081$, $p = .922$). When controlling for body weight and telencephalon volume, we also found that hippocampal neuron counts were not significantly different among the three groups ($F_{2,31} = 1.283$, $p = .291$; Figure 3a; with no adjustment for body weight and telencephalon volume, we obtained similar results: $F_{2,33} = 1.414$, $p = .257$). However, treatment did have a significant effect on hippocampal volume and neuron density when accounting for body weight and telencephalon volume (hippocampal volume: $F_{2,31} = 27.85$, $p < .001$; neuron density: $F_{2,30} = 11.73$, $p < .001$; Figures 2b, 3b; nonadjusted volume and neuron density followed a similar pattern: hippocampal volume: $F_{2,33} = 18.21$, $p < .001$; neuron density: $F_{2,32} = 9.52$, $p < .001$). Subsequent pairwise comparisons indicated that wild-caught birds had 26% larger hippocampal volumes that were less dense in neurons than both captive treatments ($p < .001$ for all comparisons; Figures 2b, 3b). However, there were no differences in hippocampal volume and neuron density between the two captive treatments (hippocampal volume, $p = .882$; neuron density, $p \approx 1.0$; Figures 2b, 3b). Unadjusted telencephalon and hippocampal volumes, neuron counts and neuron density yielded similar results.

Discussion

We found that wild food-caching chickadees had larger hippocampi with lower densities of neurons relative to the remainder

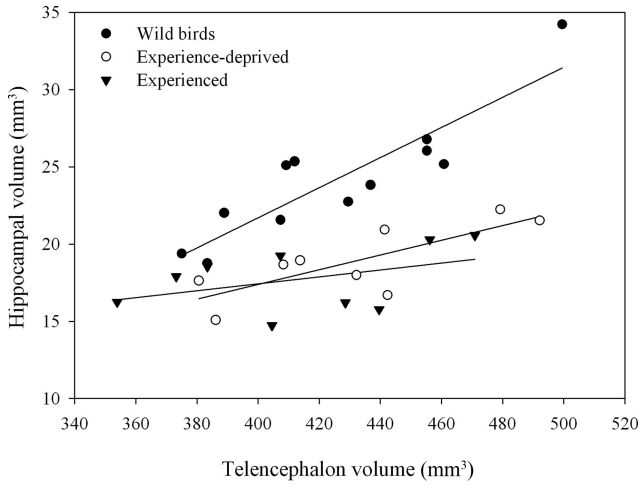

Figure 1. Relationship between telencephalon and hippocampal volumes in three experimental groups of mountain chickadees.

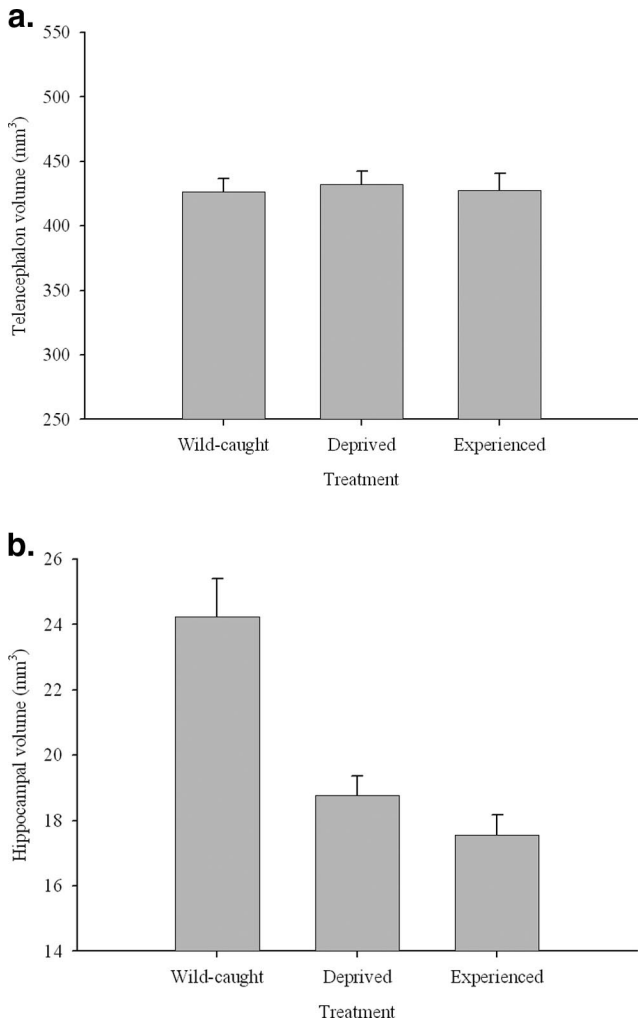

Figure 2. (a) Relative telencephalon volume + *SE* (least squares means from the model with body weight as the covariate) across three treatment groups. Treatment groups were wild-caught birds ($n = 12$), captive birds deprived of memory-based cache retrieval experiences ($n = 12$) and captive birds with the opportunity for memory-based cache retrieval experiences ($n = 12$). No differences across treatments were detected ($p = .869$). (b) Relative hippocampal volume + *SE* (least squares means from the model with body weight and telencephalon volume as the covariates) across three treatment groups. Treatment groups were wild-caught birds ($n = 12$), captive birds deprived of memory-based cache retrieval experiences ($n = 12$), and captive birds with the opportunity for memory-based cache retrieval experiences ($n = 12$). Wild-caught birds had larger hippocampal volumes than either of the captive groups ($p < .001$).

of the telencephalon than both the captive birds with memory-based experiences and captive birds deprived of memory-based experiences. Telencephalon volume did not differ between the three groups and there were also no significant differences in the total number of hippocampal neurons between the three groups. These results suggest that captivity resulted in decreased hippocampal volume, but without affecting the total number of neurons or the remainder of the telencephalon volume, and thus support the findings of other studies in which hippocampal attributes were affected by captivity (Barnea & Nottebohm, 1994; Day et al., 2008; Smulders et al., 2000). For example, Smulders et al. (2000) found that captive dark-eyed juncos (*Junco hyemalis*) had smaller hippocampal formations, relative to the telencephalon, than did their wild counterparts, although they did not estimate the number of neurons. Similarly, Day et al. (2008) found that wild-caught female brown-headed cowbirds (*Molothrus ater obscurus*) had larger hippocampal volumes relative to the telencephalon than did captive females housed socially. Of interest, they found no difference in relative hippocampal volume between wild-caught

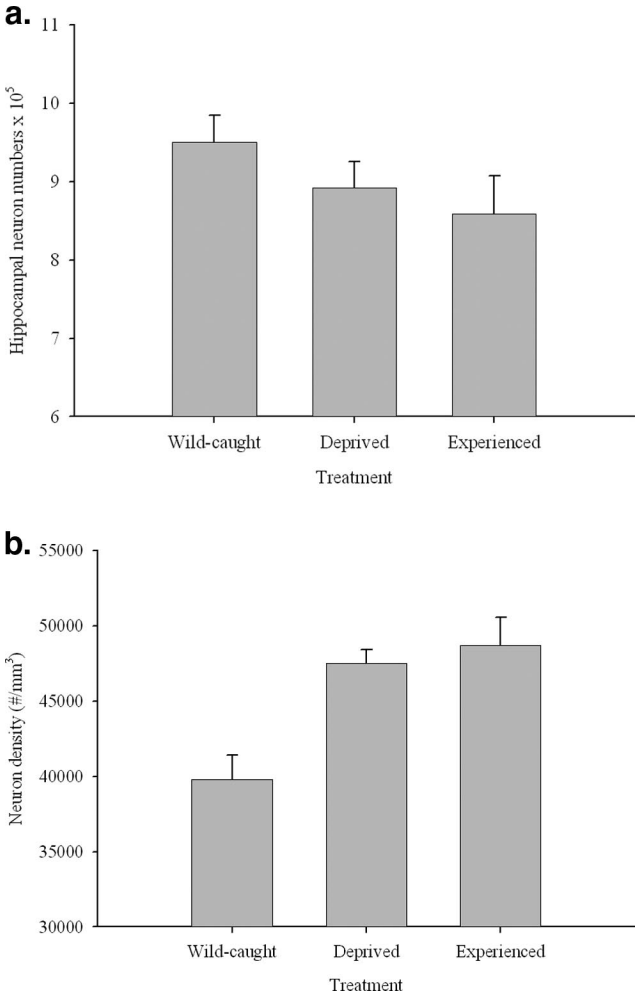

Figure 3. (a) Relative hippocampal neuron counts ($\times 10^6$) + *SE* (least squares means from the model with body weight and telencephalon volume as the covariates) across three treatment groups. Treatment groups were wild-caught birds ($n = 12$), captive birds deprived of memory-based cache retrieval experiences ($n = 12$), and captive birds with the opportunity for memory-based cache retrieval experiences ($n = 12$). No differences across treatments were detected ($p = .291$). (b) Relative neuron density + *SE* (least squares means from the model with body weight and telencephalon volume as the covariates) across three treatment groups. Treatment groups were wild-caught birds ($n = 12$), captive birds deprived of memory-based cache retrieval experiences ($n = 12$), and captive birds with the opportunity for memory-based cache retrieval experiences ($n = 12$). Wild-caught birds had a lower density of neurons than either of the laboratory groups ($p < .001$).

females and captive females housed in isolation, which seems to contradict their main conclusion that restricting nest searching behavior in captive females should cause a decrease in hippocampal volume when compared with wild-caught females. However, they also did not measure the number of neurons. Taken together, our results support the hypothesis that captivity does result in reduced hippocampal volume, but it remains unclear what may be the mechanisms of such reduction as our study showed that neuron numbers were not affected.

Although we found differences in relative hippocampal volume between wild-caught birds and their captive counterparts, we did not find a difference in neuron numbers between wild-caught and captive birds. Thus, captivity did not have a significant effect on total number of neurons present in the hippocampus for the three month duration of our study. We speculate that the larger hippocampal volume in our wild-caught birds may be related to larger neuronal or glial anatomy which was not measured in this study (van Praag et al., 2000). For example, rats placed in complex environments have more dendritic branching and synaptic contacts as compared with conspecifics housed in impoverished conditions, potentially leading to an increase in hippocampal volume (Altschuler, 1979; Fiala, Joyce, & Greenough, 1978; Leggio et al., 2005). In captivity, the demands on memory are likely less than those in the wild; therefore hippocampal anatomy may be adaptively down-regulated through particular neuronal attributes (e.g., dendritic branching), rather than through a reduction in neuron numbers.

We also determined, within our captive environment, that provisioning of memory-based experiences via food caching and cache retrieval, as well as associative learning tasks, was not sufficient to increase hippocampal volume, neuron numbers, or neuron density, as compared with birds deprived of such memory-based experiences. These results agree with those from Cristol (1996), in that the opportunity for memory-based experiences did not alter hippocampal attributes in adult birds. However, our results, together with those of Cristol (1996), contrast with previous studies that found that hand-raised, developing birds in captivity, when provided with memory-based food caching and cache retrieval experiences similar to ours, exhibited significant increases in hippocampal volume and neuron numbers (Clayton, 1994, 1996, 2001; Clayton & Krebs 1994a; Patel et al., 1997).

We have several potential explanations for our results. First, the birds that had the opportunity for memory-based experiences in our study may not have had enough experiences to maintain a larger hippocampus, as compared with birds deprived of memory-based experiences. Although our design allowed for memory-based experiences every other day for 3 months, these opportunities are likely much fewer than those experienced in the wild, and may not have been sufficient to induce differences in hippocampal architecture. It is possible that our experimental manipulation in captive birds may not have produced a sufficiently great difference in cognitive or physical activity between the two experimental groups to establish whether deprivation of cognitive activity and exercise is responsible for the "captivity effect." However, other studies using similar designs with developing birds found increased hippocampal volume and neuron numbers when birds were provided with memory-based cache and retrieval experiences and these differences were apparent in as few as 3 weeks (Clayton, 1994, 1996, 2001; Clayton & Krebs 1994a; Patel et al., 1997). In fact, the work by Clayton (2001) suggests that as few as three food caching and cache retrieval experiences was enough to trigger significant changes in the hippocampal volume in naïve juvenile food-caching mountain chickadees. In our experiment using the same species, we provided significantly more food caching and retrieval experiences to fully grown experienced birds and yet detected no such effects. This suggests that our design and timeframe should have been sufficient to induce changes in hippocampal structure, if such changes were to occur at the same rate in our older birds. Thus, one explanation for this discrepancy is that birds only exhibit plasticity in hippocampal architecture during ontogeny or that hippocampal attributes may be "set" during the first opportunities to engage in memory-based activities. Since we used older birds, as did Cristol (1996), we may have missed the window in which memory-based experiences within captivity could affect hippocampal structure. Our results therefore might suggest that once the hippocampus is fully developed, further memory-based experiences may not be necessary to maintain its volume or neuron numbers. Alternatively, once the hippocampus is fully developed, only very large-scale differences in memory-based experiences may trigger changes in the hippocampal volume, which may explain our results with wild-caught versus captive birds.

It may also be possible that birds in our deprived group had some caching experience in their home cages, which may potentially narrow the difference between the deprived and experienced groups. It does not seem likely, however, because we know from daily censuses that deprived birds did not hide any seeds and so the only experience they might have had was related to dropping seeds on the floor or pushing them through the wire. Clayton (2001) showed that hippocampal growth in mountain chickadees requires cache retrieval rather than just caching and our deprived birds had no retrieval experience.

An alternative explanation is that our results may indicate that motor stimulation is more important in determining hippocampal volume than memory-based experiences in the laboratory. Both captive groups had the opportunity for motor stimulation within the testing room, which may have maintained similar hippocampal architecture, even in the presence of opportunities for memory-based experiences in the experienced group. Several studies found that physical movement, outside of memory-based experiences, is sufficient to increase aspects of the hippocampal anatomy. For example, running has been found to increase neurogenesis and cell proliferation in the hippocampus of mice (van Praag et al., 1999a,b). Similarly, physiological changes in the hippocampus, such as an increase in neurotransmitters and an increase in field potentials, have been shown in enriched environments, possibly due to motor stimulation (Por, Bennett, & Bondy, 1982; Sharp, McNaughton, & Barnes, 1985; van Praag et al., 2000). However, developing birds in studies by Clayton and colleagues (Clayton, 1994, 1996, 2001; Clayton & Krebs 1994a; Patel et al., 1997) had similar opportunities for motor experiences and yet the experience of caching and retrieving provided a strong effect on the hippocampal volume. More testing is needed to understand the effects of memory-based experiences on the hippocampus, perhaps by either providing more opportunities for memory-based experiences in the laboratory or restricting motor activity.

It is also possible that the effects observed were triggered by stress in captivity. Our birds were wild-caught while birds in experiments by Clayton and colleagues were hand-raised. How-

ever, from our previous studies with mountain chickadees in our laboratory conditions, we know that levels of plasma corticosterone return to normal levels within 3 weeks in captivity (Pravosudov et al., 2003), suggesting quick habituation. It might be possible, however, that even a short elevation in corticosterone levels trigger long-term changes in the hippocampus. On the other hand, we did not detect any differences in neuronal numbers within the hippocampus, which suggests that stress levels in captive birds might not have been high.

There have been reports that hippocampal volume might vary seasonally, with the largest hippocampi in August (Smulders, Sasson, & DeVoogd, 1995), although other studies could not replicate such seasonal variation in hippocampal volume (Hoshooley & Sherry, 2004). Regardless, it appears unlikely that seasonal variation in the hippocampal volume, if any such changes occur, had an effect on our experiment because all birds were maintained on a light cycle, reflecting the natural ambient light schedule and captive and wild birds were sacrificed at exactly the same time.

Although we did not find anatomical differences in terms of hippocampal volume, neuron number, and neuron density between our two captive groups of birds, it would be interesting to test whether other changes within the hippocampus may occur as a result of differential memory-based experiences within a captive setting. Although we did not find differences in neuron numbers, there may be differences in the birth and death rates of neurons within the hippocampus, contingent upon memory-based experiences (Gould & Gross, 2002; Gould, Tanapat, Rydel, & Hastings, 2000). Further, differential anatomical changes, such as dendritic branching and synaptic morphology, may have ramifications on processing power, subsequent spatial memory performance, and associated fitness outcomes. While the number of neurons may have been stable regardless of memory use or environmental complexity during our study, dendritic branching and synaptic morphology may have been reduced in our captive birds to adaptively track memory use in the relatively less complex environment. Thus, relatively fast changes in hippocampal volume may be attributable to these changes in dendritic branches. Future work should investigate which specific properties of the hippocampus contribute to volumetric changes not involving neuronal numbers.

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