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Sex Differences in the Effects of Captivity on Hippocampus Size in Brown-Headed Cowbirds (*Molothrus ater obscurus*)

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In brood parasitic cowbirds, hippocampus (Hp) size is correlated with environmental spatial memory demands. Searching for host nests is the presumed causal factor influencing cowbird Hp size, because Hp volumes vary across species, sexes, and seasons according to nest-searching participation. Brown-headed cowbirds have female-only nest searching and, at least in the eastern subspecies, a larger Hp in females than in males, suggesting that nest searching influences cowbird Hp size. We predicted that female brown-headed cowbirds housed in aviaries lacking host nests would have a smaller Hp than wild-caught females whereas males would be unaffected. We found that the Hp was smaller in captive females, but not males, compared to their wild-caught counterparts. This did not appear to be due to general effects of an impoverished environment on all brain regions. Our results imply that interruption of nest searching in cowbirds prevents seasonal increase in Hp size in females. Future studies should isolate which behavioral differences between wild and captive birds contributed to captivity-induced changes in Hp volume in females while not affecting males.

Keywords: cowbird, hippocampus, sex differences, brain plasticity, brood parasitism

Variation in environmental spatial demands is related to variation in hippocampus (Hp) size among closely related species, between sexes, and across seasons in mammalian, avian, and reptilian species. For example, food-storing behaviors in birds (Clayton, 1995a; Hampton, Sherry, Shettleworth, Kurgel, & Ivy, 1995; Hampton & Shettleworth, 1996; Healy & Krebs, 1992; Krebs, Sherry, Healy, Perry, & Vaccarion, 1989; Sherry, Vaccarino, Buckenham, & Herz, 1989; Volman, Grubb, & Schuett, 1997) and rodents (Jacobs, 1992; Jacobs & Spencer, 1994), foraging-related rate of movement in lacertid lizards (Day, Crews, & Wilczynski, 1999), home-range size in voles (Gaulin, 1992; Jacobs, Gaulin, Sherry, & Hoffman, 1990; Sherry, Jacobs, & Gaulin, 1992), and breeding-habitat complexity in petrels (Abbott, Walsh, Storey, Stenhause, & Harley, 1999) are all associated with enlarged Hp size, presumably due to the demands of spatial memory. In addition, seasonal variation in food-storing demands is associated with seasonal changes in Hp volume (Smulders, Sasson, & DeVoogd, 1995) in black-capped chickadees and rodents (Jacobs, 1996). Males have a larger Hp than females in a vole species in which males have larger home ranges but not in vole species that do not have sex differences in home range (Gaulin, 1992). These results provide evidence that environmental or experiential seasonal changes can alter Hp volume and that natural and sexual selection on a variety of spatial-related skills has produced a larger Hp in some species or sexes than in others.

Although correlations of Hp volume with ecological demands for spatial ability are numerous, the specific mechanisms that contribute to such adult variation in the volume of the Hp are not well understood. We do know that species-specific reactions to spatial experience (food storing or other spatial learning tasks) during development contribute to differences between some species of food-storing versus non-food-storing birds. In adults, food-storing experience alone does not appear to alter the volume of the Hp in some species (Cristol, 1996); although combined with other seasonal effects, it may increase Hp volume, cell number, or new cell recruitment in other species such as the black-capped chickadee (Barnea & Nottebohm, 1994; Smulders et al., 1995; Smulders, Shiflett, Sperling, & DeVoogd, 2000). Changes in Hp neuroanatomy across seasons that are correlated with seasonal demand

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for spatial memory appear to be triggered directly by changes in photoperiod in some mammals (Prendergast, Nelson, & Zucker, 2002). In eastern gray squirrels, however, seasonal changes in photoperiod and food storing are not accompanied by changes in Hp volume (Lavenex, Steele, & Jacobs, 2000). In black-capped chickadees, a food-storing bird with seasonal changes in both Hp volume and food-storing behavior, photoperiod does not appear to induce changes in Hp volume or cell proliferation even though photoperiod can induce changes in food-storing behavior (Macdougall-Shackleton, Sherry, Clark, Pinkus, & Hernandez, 2003). Furthermore, causal links between behavioral traits that are related to species and sex differences in Hp size, for example, food storing and mate searching, and actual Hp growth or attrition have not been established in most cases. Thus, more studies are needed that examine mechanisms for changes in Hp volume and that demonstrate that changes in the behavior proposed to alter Hp volume actually do so.

Cowbirds provide an interesting group in which to examine the link between behavior and Hp volume. These brood parasites show species, sex, and seasonal variation in Hp volume in concordance with demands for locating host nests to parasitize when compared to other species in the same family, the Icteridae, that are not parasitic (Clayton & Reboreda, 1997; Reboreda, Clayton, & Kacelnik, 1996; Sherry, Forbes, Khurgel, & Ivy, 1993). In addition, males and females search for host nests together in some cowbird species whereas only the female searches for hosts in others. The Hp is larger in females than in males only in those species studied in which the females search for nests alone (Reboreda et al., 1996; Sherry et al., 1993). This is a particularly interesting result as it counters the more common male bias in Hp volume in rodents (Roof & Havens, 1992). Furthermore, Hp size in two species of parasitic cowbirds appears to be seasonally plastic as Hp volume is larger in the summer when breeding and thus nest searching is taking place than in the winter nonbreeding season. Additionally, sex differences in Hp size seen in the breeding season are not found in the nonbreeding season in a species with female-only nest searching, the shiny cowbird (*Molothrus bonariensis*; Clayton & Reboreda, 1997).

Together, these results suggest that searching for nests is related to Hp volume. Thus, we hypothesized that preventing cowbirds from searching for nests should result in a decrease in Hp volume. To prevent nest searching while reducing the general effects of deprivation (Gage, 2002; McEwen, 1999; Rosenzweig & Bennett, 1996), we held brown-headed cowbirds in large mixed-sex outdoor aviaries where they could have normal social interactions but without the provision of host nests for egg laying. Additionally, we created a specifically impoverished group by transferring some females from the outdoor aviaries to comparatively small isolation chambers. Because we used brown-headed cowbirds for this study, we expected females, but not males, to be sensitive to prevention from nest searching as only females normally search for nests. Thus, we predicted that preventing birds from nest searching by holding them in captivity would selectively decrease the volume of the female Hp but not the male Hp compared to wild-caught counterparts. If general deprivation is the main difference between captive and wild-caught birds, then both sexes should show decreased Hp volume as a result of captive housing and females housed in isolation chambers should show a greater decrease in Hp volume than aviary-housed captive males and females.

Method

Subjects and Housing Conditions

Thirty-two brown-headed cowbirds (*M. ater obscurus*) were captured in walk-in traps in southern California in 1999 and 2000 along the Santa Clara River or in Simi Valley (both in Ventura County) as part of a federal- and state-run cowbird population control program designed to aid the endangered least Bell's vireo (*Vireo bellii pusillus*; Griffith & Griffith, 2000). Because of possible population differences in ecology and neuroanatomy (Uyehara & Nairns, 1992) in cowbirds and because differences in neuroanatomy are known to occur as a result of differential experience and/or age (Clayton, 1995b; Healy, Gwinner, & Krebs, 1996), we attempted to select only adult sexually mature birds that were from the local Ventura population using wing length to identify subspecies and plumage characteristics to identify age classes (Fleischer & Rothstein, 1988; Lowther, 1993). Among adult males, second year (SY) individuals and after second year (ASY) males show different patterns of daily movements (Rothstein, Yokel, & Fleischer, 1986), so we selected only ASY males. All females were adults, minimum SY, as determined by wing cord, but they could not be further aged into SY and ASY. Because all females mate and parasitize nests as yearlings (Darley, 1983; Lowther, 1993; Payne, 1973; Rothstein et al., 1986), all were in breeding condition and were presumed to be engaged in nest searching when caught. It is possible that females were, on average, younger than males. However, all birds were breeding adults and thus brains were unlikely to be more plastic in females because of age. All birds were killed between May and June, a period that coincides with the breeding season in California cowbirds (Payne, 1973).

We had three conditions: wild-caught birds, birds held captive in aviaries, and birds housed in isolation chambers (females only). Captive birds were six males (CM) and five females (CF) captured between late April and early July of 1999 and housed in a large outdoor aviary ($1.2 \times 2.7 \times 6.0$ m) in mixed-sex groups until killed between May 12 and June 30 of 2000, approximately 1 year after their capture. An additional five females (ISO), similarly held in the outdoor aviaries for 1 year, were placed in visual and acoustic isolation chambers (internal diameter $53 \times 28 \times 30$ cm) on natural photoperiods on April 1, 2000, about when breeding normally begins in southern California. These birds were killed 5 weeks later on May 8, 2000. Wild-caught breeding birds, 10 males (WCM) and 6 females (WCF) were captured between May 8 and June 20 of 2000 and were killed within 10 days of capture (a nonsignificant negative correlation between Hp size and capture time for WCM and WCF suggest this brief captivity was insufficient to alter Hp size). As wild-caught birds came in from the field, we killed them in matched pairs with captive birds. All birds in both years were caught at one of the two sites in Ventura County. All birds were given food (Roudybush Maintenance Crumble; Roudybush, Woodland, California) and water ad libitum. All conditions were approved by the University of California Institutional Animal Care and Use Committee (Protocol 185). Captive males and females used in this study were all in apparent breeding condition based on courting and mating behaviors.

Histology and Morphometrics

Birds were injected intramuscularly with a ketamine (50 mg/kg)/xylazine (20 mg/kg) mixture followed by 8 mg/kg sodium pentobarbital and perfused transcardially with 0.8% saline and neutral buffered formalin (NBF). Brains were postfixed in NBF for 11 or 12 days then transferred to 30% sucrose in 0.1 *M* Phosphate Buffer until sinking. Brains were cut in the coronal plane on a cryostat at 40 μm; every fifth section was mounted on a slide and Nissl stained with cresyl violet. Slides were coded so that individuals doing measurements were unaware of the sex and treatment of individuals. We computed the volume of the Hp, telencephalon (Tel), and nucleus rotundus (NRot), a visual, thalamic nucleus. The Tel and NRot were measured as control regions to determine whether captivity affected brain regions outside the Hp and to control for shrinkage of tissue during processing by adjusting Hp volume for Tel volume. Volume was computed by capturing images of slices with MRGrab (Carl Zeiss, Inc., Thornwood, NY) software via a light-microscope equipped with an AxioCam MRc digital camera (Zeiss) and measuring surface areas. Our sampling procedures and volume calculations followed standard stereological methods (Gundersen et al., 1988). Standard neuroanatomical landmarks were used to delineate the boundaries of the brain regions. In birds, the Hp is bounded by the midline, the lateral ventricle, and the brain surface. Laterally it can be distinguished from the apical part of the hyperpallium (HA) by an obvious increase in cell density at the start of the HA (Figure 1A; Day, Westcott, & Olster, 2005; Sherry et al., 1989). Following previous work with brown-headed cowbirds, we measured the Tel from the first slice in which Hp appeared to the last slice in which we measured Hp (Sherry et al., 1993). On sections in which the telencephalic hemispheres were joined with the diencephalon, prominent markers such as the occipito-mesencephalic tract and the anterior commissure were used to determine telencephalic boundaries (Brenowitz, Lent, & Kroodsma, 1995). The NRot boundaries are particularly clear in Nissl-stained sections (Figure 1B). Hp and NRot were measured on every mounted slice (distance between slices 200 μm), and Tel was measured on every other mounted slice (distance between slices 400 μm). We measured both the left and right Hp and Tel on four wild-caught male brains and found no significant laterality (paired *t* test, $p > .05$). In addition, no laterality in Hp volume was found in a previous study that measured six male and five female brown-headed cowbirds (Uyehara & Nairns, 1992). However, the left eye–right hemisphere system in birds has been shown to perform more spatial information processing than the other hemisphere (Clayton & Krebs, 1993), and sex differences in Hp volume favoring males occur only in the right dentate gyrus of rats (Roof, 1995; Roof & Havens, 1992; Tabibnia, Cooke, & Breedlove, 1999). Thus, we continued measurements only on the right, more "spatial," side of all brains, acknowledging the possibility that we could miss laterality differences if they exist in this species. After all brain regions were measured, we randomly selected four slices per brain region from each bird and remeasured these areas. All regions showed a mean variation of less than 3.5% between first and second measurements.

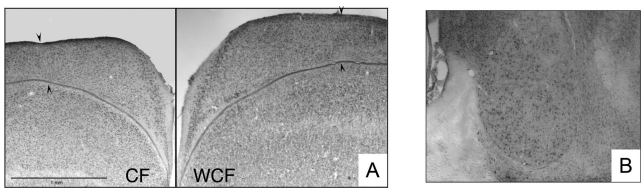

Figure 1. Photomicrographs of hippocampus (A) and nucleus rotundus (B). In A, arrows indicate hippocampus boundaries. The left section is from a captive female (CF) and right section is from a wild-caught female (WCF). We attempted to take photos at the same rostral-caudal position for CF and WCF.

Statistical Analyses

Whether comparisons were significant or not was not affected by log transformation, thus we used raw values. We subtracted the volume of the Hp from Tel volumes (TelH) so that these two measures were independent. To compare volumes of each brain region across groups, we performed one-way analyses of variance (ANOVAs) followed by Bonferroni/Dunn post hoc tests using an alpha level corrected for all possible pairwise comparisons in SuperAnova (Version 1.11; Abacus Concepts, Berkeley, CA). Variation in the size of the Tel can affect Hp volume; therefore, we used simple regressions to examine the relationship between Hp volume and TelH volume using Statview (Version 5.0; SAS Institute, Cary, NC). Note that simple regression analyses showed that weight did not covary with Hp size, and thus weight was not considered further in analyses. To examine changes in brain region volume adjusted for changes in the covariate, we used a general linear model (GLM; SPSS 14; SPSS Inc., Chicago, IL). To determine whether we met the assumptions for performing GLM, we first ran the model with a covariate–treatment group interaction term to verify that slopes were not significantly heterogeneous across groups. We then ran the model without the covariate interaction term and used Levene's test to check for significant difference in error variance across groups before interpreting results. GLM was followed by comparison of covariate-adjusted means using Sidak test adjusting for multiple comparisons. For all comparisons, the alpha or multiple-comparison corrected alpha was set at $p < .05$. Error bars on graphs and error values reported in text are standard errors of the mean.

Results

Hp and Tel

The absolute volume of the Hp was smaller in the two captive female groups (CF = 8.6 ± 0.3, ISO = 9.1 ± 0.4) than in wild-caught females (WCF = 11 ± 0.3) and the two male groups (CM = 10.8 ± 0.3, WCM = 10.4 ± 0.3; see Figure 2): main group effect, $F(4, 27) = 9.93$, $p < .0001$, pairwise values all ps $< .0001$. Hp volume was not significantly different in CM and WCM. Thus, captivity does not appear to alter the size of the male Hp, but both groups of captive females (CF and ISO) had a smaller Hp than wild females. The TelH was larger in the two male groups (CM = 250.3 ± 7.5, WCM = 249.4 ± 6.1) compared to the two captive female groups (CF = 209.2 ± 10.3, ISO = 207.5 ± 4.5; see Figure 3), $F(4, 27) = 8.98$, $p < .0001$, pairwise values all ps $< .0002$. The TelH of WCF (234.8 ± 4.5) was not significantly

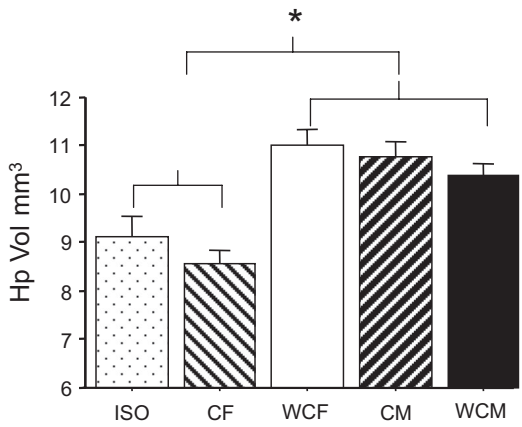

Figure 2. Mean hippocampus (Hp) volume for captive males (CM) and females (CF), wild-caught males (WCM) and females (WCF), and isolated females (ISO). Asterisk above brackets indicates that the hippocampus was significantly smaller in both captive female groups than in WCF as well as CM and WCM. The two male groups did not differ from each other.

different from any other group (post hoc p value was not significant). Thus, TelH size is not altered by captivity in males or females. However, both CF and ISO have a smaller TelH volume compared to both CM and WCM.

We found a significant positive relationship between Hp and TelH (see Figure 4; $R^2 = 0.45$, $p < .0001$). We thus examined changes in Hp volume controlling for changes in TelH, the relative Hp volume (RHp). There was no significant interaction between TelH and treatment, $F(4, 22) = 1.01$, $p = .42$. Thus, the interaction term was removed. Variance in RHp was not significantly heterogeneous among groups, $F(4, 27) = 0.22$, $p = .93$. There was a significant treatment effect, $F(4, 26) = 4.47$, $p = .007$ (see Figure 5). Post hoc test showed that WCF had a larger RHp volume than CF. No other groups differed from each other (WCF vs. WCM, $p = .12$). Thus, aviary housing affected females but not males.

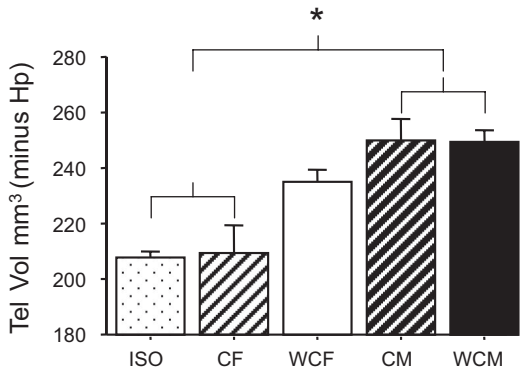

Figure 3. Mean telencephalon volume (Tel Vol) minus hippocampus (Hp) volume (TelH) for captive males (CM) and females (CF), wild-caught males (WCM) and females (WCF), and isolated females (ISO). Asterisk above brackets indicates that TelH size is smaller in both captive female groups compared to both male groups. TelH size did not differ among female groups.

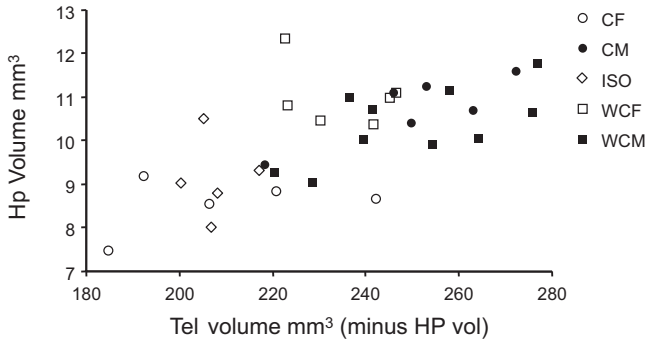

Figure 4. Mean hippocampus (Hp) volume plotted against telencephalon (Tel) volume (minus Hp volume) for groups studied ($R^2 = 0.45$, $p < .0001$). CF = captive females; CM = captive males; ISO = isolated females; WCF = wild-caught females; WCM = wild-caught males.

Nucleus Rotundus

There were no significant differences in the size of the NRot, our control region, across groups (CF = 1.24 ± 0.36, CM = 1.56 ± 0.45, WCF = 1.29 ± 0.32, WCM = 1.80 ± 0.21, ISO = 1.58 ± 0.26), $F(4, 27) = 0.62$, ns. The size of NRot was not correlated with Tel volume ($R^2 = 0.001$, $p = .88$), therefore we did not need to perform analyses adjusting for Tel size. However, we note that correcting NRot volume for Tel volume as we did for Hp did not result in significant differences among groups.

Discussion

Our results demonstrate sex-specific changes in the size of brain regions in birds and agree with the hypothesis that experience with nest searching is necessary for the seasonal increase of Hp volume seen in brood-parasitic female cowbirds (Clayton & Reboreda, 1997). Female brown-headed cowbirds held for 1 year in large outdoor aviaries and killed during the breeding season had a significantly smaller Hp and Hp volume controlling for Tel size than WCF. Hp volume was similar in CM and WCM brown-headed cowbirds. In addition to these sex-specific effects, the effects of captivity also appeared to be specific to the Hp. Al-

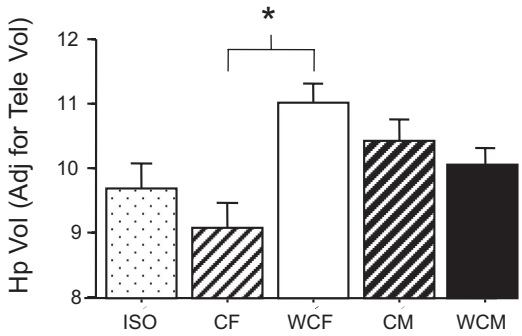

Figure 5. Hippocampal volume (Hp Vol) controlling for the effects of telencephalon volume (Tele Vol; minus Hp Vol). Hp Vol is significantly smaller in captive females than in wild- caught females. ISO = isolated females; CF = captive females; WCF = wild-caught females; CM = captive males; WCM = wild-caught males.

though the Tel (minus Hp) of CF and ISO was smaller than either male group, there was no significant change in this Tel volume in CF or ISO compared to WCF or between CM and WCM. In addition, neither captive conditions nor isolating conditions had any effect on the volume of the NRot.

Changes in Hp volume did not appear to be the result of general effects of an impoverished environment. CM did not have a smaller Hp than WCM. ISO had a Hp volume reduced in size compared to WCF, but this treatment did not cause an additional reduction in Hp volume compared to CF (although it should be noted that longer isolation periods could possibly reduce Hp volume). Furthermore, when adjustments for changes in Tel size were made, Hp volume did not differ between ISO and WCF. Thus, it appears that housing cowbirds in large outdoor aviaries without host nests induces either a female-specific reduction in Hp volume compared to wild-caught cowbirds or a failure of the Hp to grow in captive birds compare to wild-caught birds. Although we cannot differentiate between these two possibilities with these data, we believe it is more likely that differences between WCF and CF are due to a lack of seasonal increase in Hp volume in CF rather than an induced decrease in Hp volume in CF. Although not yet demonstrated for brown-headed cowbirds, the nest searching sex(s) of other species of cowbirds have a larger Hp in the breeding season than in the nonbreeding season (Clayton & Reboreda, 1997). We propose that CF did not undergo the normal expansion of the Hp that occurs during the breeding season in WCF. Further, experiments will be necessary to isolate the particular variable that led to differences between CF and WCF; possibilities include nest searching per se, behaviors otherwise related to female breeding, physiological events altered by captivity in females only, and sex-specific reactions to captive conditions.

Experience and the Hp

Any experience-driven change in Hp volume, whether as a result of nest searching or some related behavior, is an example of adult plasticity of the Hp. Experience-driven changes in neuroanatomical and neurochemical characteristics of the Hp occur as a result of environmental enrichment, learning regimes, and exercise in both young and adult laboratory rodents (Kempermann, Kuhn, & Gage, 1997; Kozorovitskiy et al., 2005; Rosenzweig & Bennett, 1996; van Praag, Kempermann, & Gage, 1999). In birds, experience-driven changes in Hp volume have been demonstrated in juvenile marsh tits, a food-storing bird (Clayton, 1995b, 1996). Although adult Hp plasticity in food-storing birds is supported by studies using black-capped chickadees (Smulders et al., 1995, but see Cristol, 1996), it appears that captive conditions more restrictive than our outdoor aviaries (smaller aviary, all males) can result in a decrease in Hp volume (Barnea & Nottebohm, 1994; Smulders, Casto, Nolan, Ketterson, & DeVoogd, 2000). It is not clear what social or environmental factors excluded by captivity may have played a role in changes in Hp volume, but these studies suggest that experiences in addition to exclusion from nest searching can alter Hp volume. Thus, we must consider the possibility that experience with nest searching is not the driving force behind the changes we see in Hp size. Instead, the behavior thought to drive changes in Hp volume may coincide with other behavioral or physiological variables that are the actual cause of the sex-specific neuroanatomical changes.

Behavioral Variables Altered by Captivity

In addition to prevention of nest searching, there are several behaviors prevented in captivity that might affect females more than males. These include changes in the ability to form dominance hierarchies and to defend breeding territories. In the wild, both male and female cowbirds have dominance hierarchies. In captivity, the dominant male in a cage (Dufty & Wingfield, 1986) shows mate guarding and excludes subordinate males from courting females (Rothstein et al., 1986). Females show less clearly defined dominance in captivity (Rothstein et al., 1986), possibly because the resources that they normally defend, host nests, are absent. The lack of spatial defense could affect both spatial memory requirements and hormone profiles that, in turn, could affect Hp size (Galea, Spritzer, Barker, & Pawluski, 2006; Parducz et al., 2006). In fact, one could suggest that while females were prevented from nest searching and the tracking of other females, males were still involved in keeping track of females and other males—a task that is also likely dependent on the Hp (Day, 2003; Volman et al., 1997). In addition, the establishment of dominance itself can affect Hp cell proliferation or incorporation (Kozorovitskiy & Gould, 2004; Pravosudov & Omanska, 2005). Dominant rats have more new cells in the Hp than subordinates (Kozorovitskiy & Gould, 2004; Pravosudov & Omanska, 2005). Similarly, in mountain chickadees (*Poecile gambeli*), dominant birds show higher levels of cell proliferation in the Hp (Pravosudov & Omanska, 2005). It will be important to determine if the effects seen in mountain chickadees generalize to female cowbirds.

In addition to these social behaviors, captivity has a direct effect on a female cowbird's reproductive condition. Although CM still court and copulate in a manner similar to that seen in free-ranging males, females show a dramatic decrease in egg laying (S. I. Rothstein, personal communication, March 2007). Wild female cowbirds normally lay 40 eggs or more over an 8-week breeding season (Rothstein et al., 1986), which is far more than the average passerine. This lengthy and robust egg-laying period is associated with longer elevation of estrogen (estradiol, E_2) levels than in most passerines (Dufty & Wingfield, 1986). Although it is unclear exactly how a reduction in egg laying could affect Hp volume, it is likely that CF experience an altered hormonal profile compared to WCF, which in turn could affect Hp volume as discussed in the following section.

Hormones and Hp

Hp volume in female cowbirds may be influenced by changes in E_2 in a manner similar to the that in mammalian species where E_2 is associated with increased neurogenesis, dendritic arborization, and synaptogenesis in the Hp (Galea et al., 2006; Parducz et al., 2006). In birds, we know that testosterone (T) and its metabolites, E_2 and 5α-dihydrotestosterone (DHT), influence the anatomy of the vocal control circuit in adult songbird species (DeVoogd & Nottebohm, 1981; Schlinger, 1997; Tramontin & Brenowitz, 2000). In addition, E_2 appears to regulate apoptosis after Hp damage in the zebra finch (Saldanha, Rohmann, Coomaralingam, & Wynne, 2004). Last, the estrogen synthetic enzyme, aromatase, is abundant in brown-headed cowbird Hp (Saldanah & Schlinger, 1997). Thus, it is possible that differences in E_2 or T levels between WCF and CF affected Hp size. It would not be surprising

Table 1
Range Size and Morphology of Populations of Eastern and Western Cowbird Subspecies

	Western male	Western female	Eastern male	Eastern female
Feeding range	550 ha[a]	220 ha[a]	< Western subspecies[a,b]	
Commute feeding to breeding	7 km or >[a,e]	7 km or >[a,e]	< Western subspecies[a,b] Feeding may overlap breeding[a,b]	
Feeding sites visited	Males > females[a]		Males = females[c]	
Breeding range	56 ha[a]	78 ha[a]	6.6 ha[b] ≥7.9 ha[c] 20.4 ha[d]	4.5 ha[b] 7.9 ha[c] 20.4 ha[d]
Nest searching	No	Yes	No	Yes
Hippocampus	9–10.6 mm^3 M = 10.4 mm^3	10.3–12.4 mm^3 M = 11.0 mm^3	3.5–9.5 mm^3 $M \approx$ 5.5 mm^3[f]	5–10 mm^3 $M \approx$ 6.8 mm^3[f]
Telencephalon	220–276 mm^3 M = 250 mm^3	222–246 mm^3 M = 235 mm^3	110–290 mm^3 $M \approx$ 160 mm^3[f]	125–240 mm^3 $M \approx$ 170 mm^3[f]
Weight	41.3 g	28.3 g	43.9 g	35.5 g

Note. For Western subspecies, behavioral data refer to two western subspecies *M.a. obscurus* and *M.a. artemisiae*, while neuroanatomical data are from *M.a. obscurus* only. All Eastern subspecies data are from *M.a. ater*.
[a] Rothstein et al., 1984, 1986. [b] Darley, 1968. [c] (Darley, 1983. [d] Dufty, 1982. [e] Curson, Goguen, & Mathews, 2000. [f] Measurements approximated from single study presented in Figure 2 (Sherry et al., 1993).

if any housing-induced changes in hormones altered Hp volume in female cowbirds only, given that effects of E_2 on Hp neurogenesis, spinogenesis, and synaptogenesis are low or lacking in male compared to female rats (Galea et al., 2006; Parducz et al., 2006).

Even if E_2 affects Hp morphology similarly in male and female cowbirds, unlike the case in rats (Galea et al., 2006; Parducz et al., 2006), captivity could differentially affect male and female hormone profiles. Although both CM and CF were in apparent breeding condition, it is possible that there were sex-specific effects of housing on hormonal profiles in our cowbirds. As mentioned, there were likely greater differences in reproductive condition between WCF and CF than between WCM and CM as a result of reduced egg laying that could potentially affect female hormonal profiles. In addition, females normally have elevated T levels related to defense of breeding areas (Dufty & Wingfield, 1986). Thus, a lack of this defense could have altered the secretion of T in females. Last, it has previously been shown that peak T levels are similar between WCM and CM during the time that we killed birds (Rothstein et al., 1986). Comparable studies have not been done in female cowbirds. Because hormonal state can alter Hp neuroanatomy, future studies attempting to isolate how captivity alters female Hp while not affecting male Hp should include measurement of levels of T, E_2, and the estrogen synthetic enzyme, aromatase, in blood and brain of males and females. Experiments aimed at determining the direct effects of E_2 on the Hp in cowbirds and other species are also warranted.

Cowbird Population Differences

It is important to acknowledge that, unlike studies in the eastern brown-headed cowbird (*M. a. ater*; Sherry et al., 1993), our study and two others (Uyehara, 1994; Uyehara & Nairns, 1992) that examined the western subspecies (*M. a. obscurus*) did not find that WCF had a significantly larger Hp than WCM. These discrepancies could result from methodological differences. However, if we perform our statistical comparison exactly as Sherry et al. (1993) did, comparing only male to females and including weight as a covariate along with Tel, $p = .13$, and without weight as a covariate, $p = .08$. In Uyehara's (1994) two studies that used statistics similar to Sherry et al. (1993), sex differences for western cowbirds were $p = .25$ and $p = .15$. Thus, at the very least, there appears to be a reduction in the extent of sex differences in the western subspecies compared to the eastern subspecies using identical methods.[1] In addition to decreased sexual dimorphism, Hp size and Tel size appear to be larger in *M. a. obscurus* than *M. a. ater* despite the smaller body size of *M. a. obscurus* compared to *M. a. ater* (see Table 1). Pronounced differences in habitat use, such as the larger ranges in the western than eastern subspecies, could account for the population differences observed in Hp size and extent of sexual dimorphism (see Table 1).

However, if *M. a. obscurus* has a less sexual dimorphic Hp than *M. a. ater* because both males and females have large feeding ranges, why would captivity in the present study not reduce the size of the male Hp given the enormous reduction in home range? Both male and female screaming cowbirds (*M. rufoaxillaris*) show a reduction in Hp volume in the nonbreeding season compared to the breeding season (Clayton & Reboreda, 1997), thus male Hp plasticity is possible in cowbirds. Obviously, more research is needed to isolate why we found sex-specific effects of captivity on Hp volume in *M. a. obscurus* despite a lack of sex-specific Hp size in wild birds of the population we studied. Future studies should consider subspecies and within-subspecies population differences as cowbird space use appears to depend on a number of habitat, habitat use, and population size dimensions (Darley, 1982, 1983;

[1] Using residual analysis or arcsine transformed Hp/Tel as per methods in Reboreda et al. (1996) and Clayton and Reboreda (1997) for shiny cowbirds yields results as per our GLM except WCF versus WCM is significant.

Dufty, 1982; Lowther, 1993; Rothstein, Verner, & Steven, 1984; Rothstein et al., 1986). Precedents for such within-species population differences are found in black-capped chickadees (*P. atricapilla*). A larger Hp in Alaskan chickadees compared to those from Colorado is thought to reflect adaptations to the limited and unpredictable food supply in Alaska (Pravosudov & Clayton, 2002).

Conclusion

As hypothesized, female but not male cowbirds (*M. a. obscurus*) had a smaller absolute and relative Hp compared to wild-caught counterparts when they were prevented from nest searching by being held in large outdoor aviaries for 1 year. This effect did not appear to be general to the whole brain as no reduction in NRot occurred. The effects of an impoverished environment cannot adequately explain results. Males were not affected by captivity, and females placed in isolation chambers did not have a smaller relative Hp volume than CF or WCF. The experiential or physiological mechanism underlying the differences we found remains to be determined. Regardless of the exact mechanism, our results are unique in demonstrating that experimental alteration in behavior can be associated with a sex-specific effect on Hp volume in adult birds.

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