Sex and Seasonal Differences in Hippocampal Volume and Neurogenesis in Brood-Parasitic Brown-headed Cowbirds (*Molothrus ater*)

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Received 11 September 2015; revised 14 July 2016; accepted 22 July 2016

ABSTRACT: **Brown-headed cowbirds (*Molothrus ater*) are one of few species in which females show more complex space use than males. Female cowbirds search for, revisit, and parasitize host nests and, in a previous study, outperformed males on an open field spatial search task. Previous research reported a female-biased sex difference in the volume of the hippocampus, a region of the brain involved in spatial memory. Neurons produced by adult neurogenesis may be involved in the formation of new memories and replace older neurons that could cause interference in memory. We tested for sex and seasonal differences in hippocampal volume and neurogenesis of brood-parasitic brown-headed cowbirds and the closely related non-brood-parasitic red-winged blackbird (*Agelaius phoeniceus*) to determine whether there were differences in the hippocampus that reflected space use in the wild. Females had a larger hippocampus than males in both species, but hippocampal neurogenesis, measured by doublecortin immunoreactivity (DCX+), was greater in female than in male cowbirds in the absence of any sex difference in blackbirds, supporting the hypothesis of hippocampal specialization in female cowbirds. Cowbirds of both sexes had a larger hippocampus with greater hippocampal DCX+ than blackbirds. Hippocampus volume remained stable between breeding conditions, but DCX+ was greater post-breeding, indicating that old memories may be lost through hippocampal reorganization following breeding. Our results support, in part, the hypothesis that the hippocampus of cowbirds is specialized for brood parasitism.** © 2016 Wiley Periodicals, Inc. Develop Neurobiol 76: 1275–1290, 2016

Keywords: **hippocampus; neurogenesis; seasonal differences; sex differences; volume**

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Contract grant sponsor: Natural Sciences and Engineering Research Council of Canada; contract grant numbers: 217381 Awarded to S. MacDougall-Shackleton, 105542 Awarded to D. Sherry.

Contract grant sponsor: Ontario Graduate Scholarship with Distinction (awarded to M. Guigueno).

Contract grant sponsor: Animal Behavior Society Student Research Grant (awarded to M. Guigueno).

Contract grant sponsor: Grant-in-Aid of Research (awarded to M. Guigueno) from Sigma Xi, The Scientific Research Society.

Contract grant sponsor: Graduate Thesis Research Award (awarded to M. Guigueno) from the University of Western Ontario.

Additional Supporting Information may be found in the online version of this article.

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Published online 11 August 2016 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/dneu.22421

INTRODUCTION

The brain can be specialized to meet behavioral demands an organism routinely encounters in the wild (Sherry, 2006; Smulders et al., 2010). This specialization has been found in the hippocampus, which plays a central role in spatial memory and orientation. The hippocampus varies in size according to space use in a variety of organisms (Krebs et al., 1989; Sherry et al., 1989; Jacobs et al., 1990), and lesions of the avian hippocampus selectively disrupt spatial memory (Sherry and Vaccarino, 1989; Hampton and

Shettleworth, 1996; Broadbent and Colombo, 2000; Shiflett et al., 2003). In addition, hippocampal neurogenesis may play a role in spatial memory, especially if an individual must encode new information about its surroundings (Barnea and Pravosudov, 2011). Most organisms' environments are subject to change and new neurons may help form new memories and degrade old memories that cause interference (Barnea and Pravosudov, 2011; Frankland et al., 2013). Organisms that rely more on spatial memory might be expected to have evolved both a larger hippocampus and greater levels of neurogenesis.

The hippocampus of food-storing and migratory birds can be larger with greater hippocampal neurogenesis than that of non-food-storing and non-migratory birds, respectively. Birds belonging to families in which most species store food, such as Paridae, Sittidae, and Corvidae, have a larger hippocampus relative to brain size than birds from non-food-storing families (Krebs et al., 1989; Sherry et al., 1989). Phylogenetic comparisons have shown that relative hippocampus size is positively related to food-storing behavior (Garamszegi and Eens, 2004; Lucas et al., 2004). Within a food-storing species, hippocampus size (Roth and Pravosudov, 2009) and hippocampal neurogenesis (Chancellor et al., 2011) are greater in populations that presumably depend more on stored food due to harsher environmental conditions (Roth and Pravosudov, 2009). Migratory sub-species of white-crowned sparrows (*Zonotrichia leucophrys*) have a larger hippocampus (Pravosudov et al., 2006) and greater hippocampal neurogenesis than non-migratory sub-species (LaDage et al., 2011). Hippocampal neurogenesis is also greater in a migratory Old World *Acrocephalus* warbler than in a closely-related non-migratory species (Barkan et al., 2014). In sum, hippocampus size and neurogenesis are closely associated with patterns of space use between and within songbird species.

Although there are consistent patterns of hippocampus size and neurogenesis related to space use among species, seasonal changes in the hippocampus present a more complex picture (Sherry and MacDougall-Shackleton, 2015). Some studies of food-storing birds show patterns of seasonal change in hippocampus size and neurogenesis that are clearly associated with seasonal change in food-storing behavior (Barnea and Nottebohm, 1994; Smulders et al., 1995) while others show seasonal changes that vary from year to year in timing or magnitude (Hoshooley et al., 2007; Hoshooley and Sherry, 2007)

Sex differences in hippocampus size and neurogenesis have been reported in mammals and in avian brood parasites. Meadow voles (*Microtus pennsylvanicus*) are polygynous and males have home ranges that are 4–7 times larger than the home ranges of females, whereas there is no sex difference in space use in monogamous pine voles (*M. pinetorum*; Gaulin and FitzGerald, 1986, 1989). Consistent with these sex differences in space use, the hippocampus is larger in male meadow voles than females, but no sex difference exists in pine voles (Jacobs et al., 1990). In Richardson's ground squirrels (*Urocitellus richardsonii*), which are also polygynous, males have a larger hippocampus with more neurogenesis than females (Burger et al., 2013, 2014). Although the avian hippocampus is organized differently than the mammalian hippocampus (Herold et al., 2014), the hippocampus of mammals is evolutionarily homologous to that of birds (Colombo and Broadbent, 2000; Jarvis et al., 2005). Because ninety percent of birds are socially monogamous and provide bi-parental care (Mock and Fujioka, 1990), we might expect few sex differences in hippocampus size or neurogenesis in birds.

Avian brood parasites have an unusual form of reproduction, however, in which there is no parental care. Although sex differences might not be expected in most bird species, previous work on avian brood parasites has found sex differences in hippocampus size and spatial memory, along with seasonal differences in hippocampus size. In brown-headed cowbirds (*Molothrus ater*), only females repeatedly visit dozens of host nests per breeding season to assess the stage of completion of their clutches, to lay their own eggs, and to remove host eggs (Norman and Robertson, 1975; Rothstein et al., 1986; Gates and Evans, 1998; White et al., 2009; Guigueno and Sealy, 2011). Female cowbirds performed better than males on a spatial task requiring birds to find hidden food in a large room, with performance remaining stable across breeding conditions (Guigueno et al., 2014). Female brown-headed cowbirds also had a larger hippocampus relative to the size of the telencephalon than males in a previous study (Sherry et al., 1993). A sex difference in hippocampus size was also observed in shiny cowbirds (*M. bonariensis*), a South American congener of brown-headed cowbirds, in which only females search for nests (Reboreda et al., 1996). In contrast, no sex differences were found in screaming cowbirds (*M. rufoaxillaris*), a brood parasite in which males assist females in nest searching (Reboreda et al., 1996). A seasonal comparison found that both shiny and screaming cowbirds had a larger relative hippocampus size in the breeding season but a sex difference only existed in shiny cowbirds (Clayton et al., 1997). The sex difference in hippocampus size in favor of females found in shiny and brown-headed cowbirds is thus the reverse of that usually found in mammals.

In the current study, we tested for potential sex differences and seasonal changes in hippocampal volume and neurogenesis in brown-headed cowbirds (hereafter "cowbirds") and a close relative that is not a brood parasite, the red-winged blackbird (hereafter "blackbirds", *Agelaius phoeniceus*). Like most other birds, blackbirds normally provide bi-parental care and females and males have similar sized home ranges (Yasukawa and Searcy, 1995). We hypothesized that the hippocampus of cowbirds is specialized for their brood-parasitic mode of reproduction. Specifically, we predicted that cowbirds would have a larger hippocampus with more neurogenesis than blackbirds and that a sex difference would exist in cowbirds only, with female cowbirds having a larger hippocampus with more neurogenesis than male cowbirds. Our seasonal predictions were less certain. Because spatial performance of brown-headed cowbirds on a navigational task did not vary seasonally (Guigueno et al., 2014), one possibility is that relative hippocampus volume would remain the same between breeding conditions. Alternatively, any female-biased sex difference might be greatest in breeding condition when female cowbirds search for host nests. Indeed, in deer mice (*Peromyscus maniculatus*), a sex difference in spatial performance only occurred in breeding condition (Galea et al., 1996) and similarly, South American cowbirds had a larger hippocampus in breeding condition (Clayton et al., 1997).

METHODS

Subjects

Female and male cowbirds and blackbirds were collected in breeding and post-breeding conditions (Table 1). We collected birds in breeding condition between mid-March and mid-May 2013 and birds in post-breeding condition between mid-September and mid-November 2013. All birds were captured using ground traps and mist nets at sites near Port Rowan, Ontario, Canada. Cowbirds and blackbirds at our field site are partial migrants, leaving the site for one to four months per year between December and March and this can vary between years (George Finney [Bird Studies Canada], personal communication). All birds included in our study were adults; birds in the breeding group were hatched at least the previous summer and birds in the post-breeding condition group were all after hatch-year individuals (i.e., none of them were young of the year). Aging was based on plumage and, in post-breeding condition, also on skull pneumatization (Pyle, 1997). Mean body weights ± SE were: female cowbirds ($n = 22$) 39.62 g ± 0.63; male cowbirds ($n = 23$) 50.33 g ± 0.97; female blackbirds ($n = 16$) 42.49 g ± 0.66; male blackbirds ($n = 23$) 65.29 g ± 0.98. Sample sizes for body weights and sample sizes in Table 1 are not equal because body weight was not measured in the field for three individuals. After capture, birds were transported to the Advanced Facility for Avian Research at the University of Western Ontario where they were housed overnight in individual cages with food and water.

Table 1 Sample sizes for gonad size, circulating androgen concentration, brain weight, telencephalon volume, hippocampus volume, and hippocampal doublecortin immunoreactivity

	Brown-headed cowbird		Red-winged blackbird	
Analysis	Female	Male	Female	Male
Gonad size				
Breeding	16	16	8	16
Post-breeding	8	8	8	8
Circulating androgens				
Breeding	16	16	8	16
Post-breeding	8	8	7	8
Brain weight				
Breeding	15	16	8	16
Post-breeding	8	7	8	7
Telencephalon volume				
Breeding	15	16	8	16
Post-breeding	8	8	8	8
Hippocampus volume				
Breeding	15	16	8	16
Post-breeding	8	8	8	8
Hippocampal doublecortin immunoreativity				
Breeding	15	16	8	14
Post-breeding	8	6	8	8

Brains were collected the day after the birds were captured in the field.

Blood Sampling

We collected blood samples in the field to confirm breeding condition. We punctured the brachial vein of each bird with a 26-gauge needle. All samples were collected within 30 min, except for 14 out of 88 samples that were taken 30–92 min after capture. There was no statistically significant correlation between androgen concentration and time between capture and blood sampling, and we therefore kept these data in the analyses. We collected approximately 400 μL of blood into heparinized capillary tubes and centrifuged the blood for 10 min at 13,000*g*. Finally, we extracted the plasma from the tubes with a Hamilton syringe and froze the plasma at −30°C until the hormone assay.

Androgen Assay

Testosterone increases in the breeding season for both female and male brown-headed cowbirds (Dufty and

Wingfield, 1986a,b; Guigueno et al., 2014). We assayed plasma androgen concentration using a testosterone enzyme immunoassay previously validated for a variety of bird species (EIA; Cat. #1-2402, Salimetrics; [Washburn et al., 2007]). We previously validated the assay in cowbirds (Guigueno et al., 2014). To validate the assay for blackbirds, we used the same protocol as in the work by Newman et al. (2008) and Guigueno et al. (2014), assayed a serial dilution of blackbird plasma, and compared measured levels of testosterone in the dilutions to the standard curve using an analysis of covariance (ANCOVA). A nonsignificant interaction term ($F_{1,10} = 0.01$, $p = 0.94$) indicated that the slopes were similar and that the assay was suitable for blackbirds. Intra-assay variation was 8.85%. Interplate variation, based on a pooled red-winged blackbird plasma sample and low and high controls was 3.79%. The sensitivity of the assay was 5 pg/mL (two standard deviations from the average value of zero on the standard curves). Samples below this level were assigned a value of 2.5 pg/mL for the analyses. Sample sizes for circulating androgen concentrations are shown in Table 1.

Brain Collection and Gonad Size

The day after capture, we deeply anesthetized the birds using isoflurane. We then transcardially perfused the birds with heparinized saline, followed by 4% paraformaldehyde. The brains were removed from the skull and placed in 4% paraformaldehyde for 24 h, followed by 30% sucrose in phosphate-buffered saline (PBS) for 48–72 h (until the brains sunk to the bottom of the vial). Finally, we froze the brains on crushed dry ice and stored them in aluminum foil at −80°C until immunohistochemical processing. Whole brain weight was measured immediately before sectioning (Table 1). After extraction of the brain, we estimated or measured gonad size (Table 1). For follicle size in females, we estimated follicles that were too small to measure to be 0.1 mm and follicles that were visible but hard to measure to be 0.5 mm. We measured the actual diameter of the largest follicle with calipers when there was follicular hierarchy (see MacDougall-Shackleton et al. (2001) for further details on ovary development). For males, we measured the length of the left testis with calipers.

Immunohistochemistry

We sectioned brains into 40 μm coronal sections using a cryostat. Two alternating sets of brain sections, each set ten sections apart, were collected for NeuN and doublecortin (DCX) immunoreactivity. NeuN is a protein expressed in most mature neurons (Mullen et al., 1992) and was used to calculate the volume of the hippocampus. DCX is a microtubule-associated endogenous protein only expressed in migrating and differentiating immature neurons (Francis et al., 1999; Gleeson et al., 1999) and is a reliable marker of neurogenesis in birds (Balthazart and Ball, 2014a,b). Each run consisted of a random sample of brains from different groups (Table 1).

We used the following steps for NeuN immunohistochemistry. First, we washed free-floating sections twice in 0.1 *M* PBS then incubated the sections in 0.5% hydrogen peroxide for 30 min. Next, we washed sections three times in PBS then incubated the sections overnight in 10% normal goat serum (Vector, Burlingame, CA) that was diluted in 0.3% Triton in PBS (PBST). We replaced the diluted goat serum with primary antibody (mouse monoclonal MB377, Millipore, Billerica, MA) diluted 1:2000 in 0.3% PBST and incubated overnight. Next, we washed the sections three times in 0.1% PBST and incubated the sections for 1 h in biotinylated secondary antibody (goat anti-mouse IgG, Vector) diluted 1:250 in 0.3% PBST. Then, we washed the sections three times in 0.1% PBST and incubated the sections for 1 h in avidin–biotin horseradish-peroxidase complex (VectaStain Elite ABC Kit) diluted 1:200. We washed the sections three times in 0.1% PBST and visualized the sections by exposing them to diaminobenzidine solution (SigmaFAST DAB), followed by four washes in PBS. We mounted the sections onto gelatin-coated slides, dehydrated them gradually with increasing ethanol concentrations, cleared them in solvent (Harleco Neo-Clear, EMD Chemicals, Billerica, MA), and cover slipped the slides using Permount (Fisher Scientific, Pittsburgh, PA).

The DCX immunohistochemistry protocol was similar to the NeuN protocol, except for the following differences. First, the sections were incubated in 0.5% hydrogen peroxide for 15 min instead of 30 min. Second, the sections were incubated in 10% normal horse serum (Vector, Burlingame, CA) instead of goat serum and sections were incubated for 1 h instead of overnight. Finally, the primary DCX antibody was goat polyclonal (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:250 and the secondary antibody was biotinylated horse anti-goat IgG (Vector) diluted 1:400.

Microscopy

We used the NeuN-labeled sections to measure the volume of the hippocampus (Fig. 1). NeuN immunoreactivity provides readily discernible boundaries of the hippocampus as well as many song-control brain regions (Newman et al., 2010). We defined the hippocampus as the hippocampus proper plus the parahippocampal area (Sherry et al., 1989; Sherry, 2011). The dorsal boundary of the hippocampus corresponds to the dorsal surface of the brain, the ventral boundary corresponds to the ventricle, and the medial boundary corresponds to the mid-line (Sherry et al., 1989; Sherry, 2011). We identified the lateral boundary of the hippocampus by a marked increase in cell density and a mixture of small and large neurons characteristic of the hyperpallium apicale (HA), a region adjacent and lateral to the hippocampus (Sherry et al., 1989; Sherry, 2011; Fig. 1). The neurons in the hippocampus are characteristically larger and at a lower density than those in the HA (Sherry et al., 1989; Sherry, 2011; Fig. 1). The rostral and caudal ends of the hippocampus were identified by the appearance and disappearance, respectively, of this hippocampus-HA boundary, which begins medially at the rostral point of the

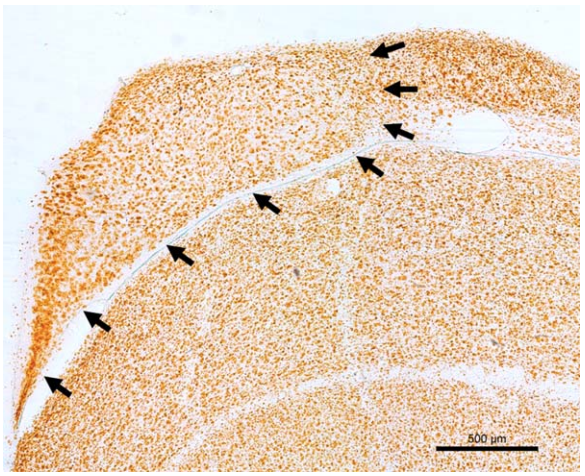

Figure 1 NeuN staining used for volume analyses, with the hippocampus boundary indicated by arrows. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hippocampus, extends laterally at the mid-point of the hippocampus, and returns medially at the caudal end (Sherry et al., 1989; Sherry, 2011).

We captured images of the hippocampus sections with a Spot Idea 5-megapixel digital camera (Diagnostics Instruments) using a ×1.25 objective lens on a Zeiss Axiophot microscope. Only a bird ID was assigned to each photo and images were analyzed blind to sex, species, and season. We traced the perimeter of the hippocampus in every tenth 40-μm thick section in ImageJ software (NIH) to measure its cross-sectional area. To acquire an accurate measurement of the hippocampus volume, we calculated the frustum volume between each area measurement (400 µm section interval) and summed the frusta volumes to estimate the total volume of the hippocampus in both hemispheres (Sherry et al., 1989). We use the same approach and sampling interval to measure the total telencephalon volume in both hemispheres. We captured images of the telencephalon with a high-resolution (2400 dpi) flatbed scanner with a transparency adapter and traced the perimeter of every tenth telencephalon section with ImageJ. The hippocampus and telencephalon volumes used in the analyses for each bird were the mean for both hemispheres. We adjusted the sampling interval and used the next nearest section if a section was damaged or lost. Sample sizes for hippocampal and telencephalon volume analyses are found in Table 1.

We used DCX immunoreactivity to quantify neurogenesis (Fig. 2). We took three types of DCX measurements in each sampling field (see below for details) to quantify neurogenesis: (1) the percentage of each field of view that was covered in doublecortin immunoreactivity (the percent immunoreactive cover; %DCX+), (2) the number of DCX immunoreactive (DCX+) round cells, and (3) the number of DCX+ fusiform cells (Wada et al., 2014). We thus have both cell counts and a measure of immunoreactivity that includes cell processes that are stained but may not have a visible cell body. We captured images of DCX+ round and fusiform cells and projections with a Leica DFC 420C camera mounted on a Leica DM5500B microscope (Fig. 2). We chose three sections (rostral, middle, and caudal) from the hemisphere that was most intact and best stained. The rostral section was located 800–1200 µm posterior to the rostral limit of the hippocampus and the caudal section was located 1200–2000 µm anterior to the caudal limit of the hippocampus. The rostral and caudal limits of the hippocampus were identified by searching for the absence of the boundary described above between the hippocampus and HA. The middle section was chosen approximately equidistant between the rostral and caudal sections, near the coronal plane of the anterior commissure. For each section, we chose the following sampling fields: (1) three fields of view inside the hippocampus; one in the ventral position, the second in the medial position dorsal to the ventral field (similar to Wada et al., 2014), and the third lateral to the medial field (middle section only), (2) two fields of view outside the hippocampus to serve as covariates for the hippocampus analyses: one in HA and one in the telencephalon lateral to the hippocampus ventral field, and (3) three fields of view in the subventricular zone (SVZ) in the ventral, medial, and lateral (middle section only) positions, with half of each subventricular field in the hippocampus and half in the telencephalon [Fig. 2(a,b)]. The SVZ, an area where the production of new neurons occurs at a particularly high rate, is the area lining the dorsal and ventral sides of the lateral ventricle, which is in turn located on the ventral side of the hippocampus (Goldman and Nottebohm, 1983). Our fields of view of the SVZ contained both the hippocampus and telencephalon on either side of the ventricle [Fig. 2(b)]. Data from the half of each subventricular field in the hippocampus was used as the dependant variable in statistical analyses and the half in the telencephalon was used as a covariate. All of the fields of view above were 255 µm × 255 µm and were acquired through the ×40 objective lens (Fig. 2).

For all DCX+ images, we captured z-stack images in 0.63 µm steps through focal planes with a ×40 objective lens. These images were then compiled using the montage mode in the Leica Application Suite software, which produced an image that displayed all DCX+ cells and projections in focus. We then used the threshold feature in ImageJ software to calculate the percent coverage by DCX+ cells and projections [Fig. 2(c)]. Both fusiform and round cells were present, which are normally interpreted as migrating and recently differentiated neurons, respectively [Fig. 2(d); Balthazart and Ball, 2014a,b]. We counted and analyzed these cell types separately. We were not able to quantify DCX+ in some birds due to the quality of labeling and therefore sample sizes for doublecortin analyses differ from those for volume (Table 1).

Data Analysis

All statistical analyses were performed in SAS (version 9.3, SAS Institute Inc., Cary, NC). We used different analyses, depending on the data collected.

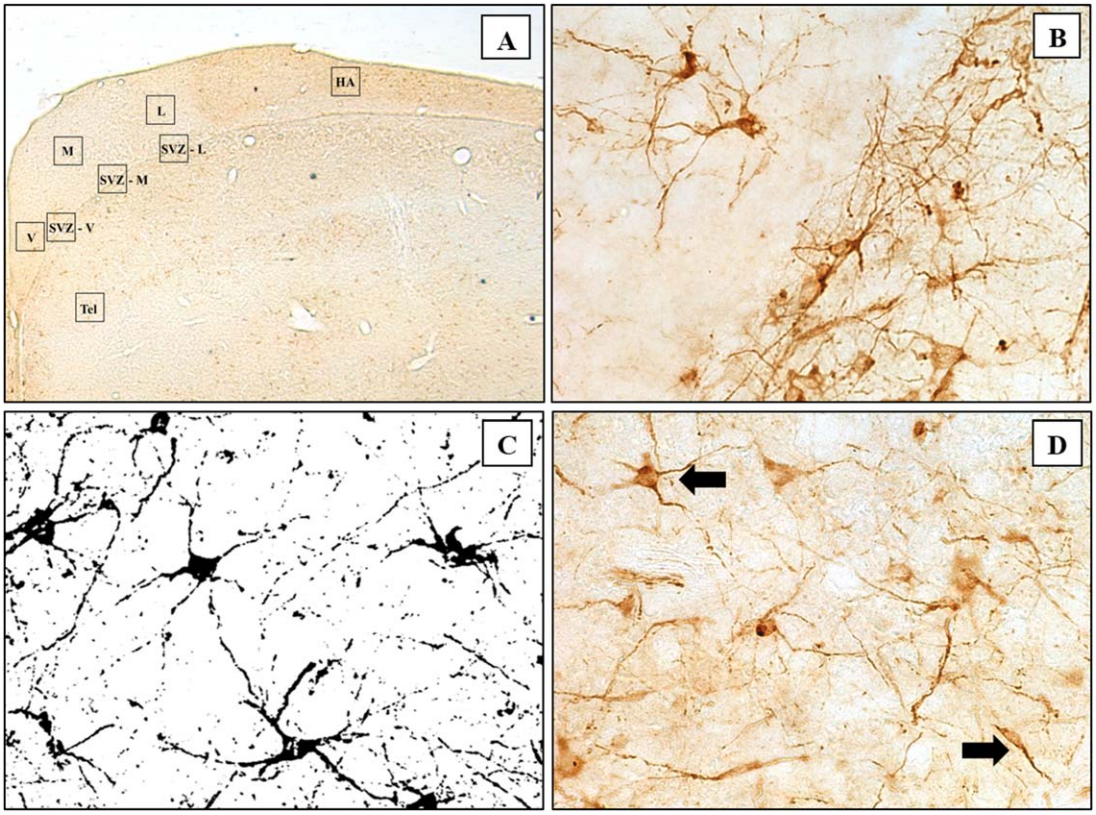

Figure 2 A: Doublecortin staining, used to quantify neurogenesis, with the fields of view used in the analyses. Images were captured inside the hippocampus, with the hyperpallium apicale (HA) and the telencephalon (Tel) as covariates, and in the subventricular zone (SVZ), with the half of each field of view (telencephalon) acting as the covariate. V: Ventral, M: Medial, L: Lateral. B: An example of a field of view in the SVZ, with the hippocampus in the upper half (dependent variable) and the telencephalon (covariate) in the lower half of the image. C: Example of thresholding to measure the % doublecortin immunoreactive cover within a field of view. D: Examples of round (top arrow) and fusiform (bottom arrow) cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

We used general linear models (PROC GLM) for the analyses to confirm breeding condition. For gonadal development, we analyzed females and males separately. We measured follicle diameter in females and the length of the left testis in males and used a general linear model with species, breeding condition, and their interactions as explanatory variables. For the androgen analysis, we used species, sex, breeding condition, and all interactions as explanatory variables and androgen concentration as the dependent variable. We log-transformed androgen concentrations to produce normally distributed residuals.

For the volume analysis, we used a general linear model (PROC GLM) with species, sex, breeding condition, and all interactions as explanatory variables, telencephalon volume (minus the hippocampus) as a covariate, and hippocampus volume as the dependent variable. For the brain weight analysis, we used a general linear model (PROC GLM) with species, sex, breeding condition, and all interactions as explanatory variables. We log-transformed the data to produce normally distributed residuals if they were not already normally distributed.

For the DCX+ analyses, the fields in the hippocampus (ventral, medial, and lateral) were assigned the HA and telencephalon fields as covariates and were analyzed separately from the fields in the SVZ. The covariate (HA or telencephalon) that produced the lowest AIC value was included in the final model. The telencephalon half of the SVZ field of view was the covariate for the SVZ analyses. The explanatory variables were species, sex, breeding condition, field, and all interactions. To analyze % DCX+ cover, we used a linear mixed model (PROC MIXED) because we took multiple measurements from each subject (see details above). We took log arcsine square root transformed percentages from the %DCX+ cover data. For the round cell analyses, we used generalized linear mixed models (PROC GLIMMIX) and specified a Poisson distribution because we had count data, which most frequently fits a Poisson distribution. For fusiform cell analyses, the model

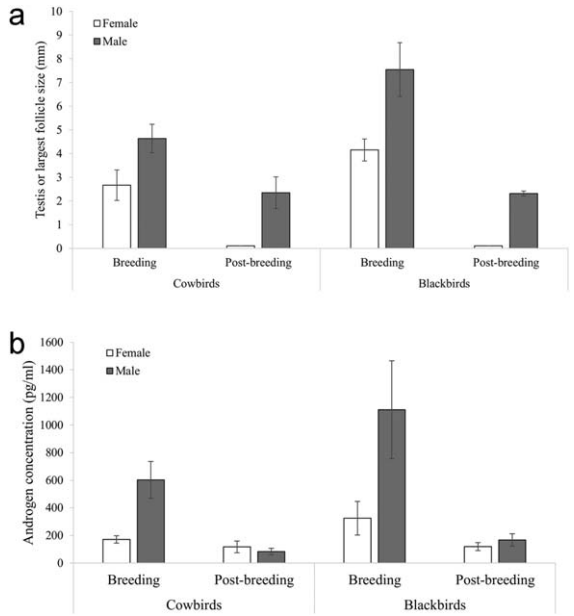

Figure 3 Gonad size (mm) and androgen concentrations (pg/mL) (mean ± SE) in female and male brown-headed cowbirds and red-winged blackbirds in breeding and post-breeding conditions.

did not converge because there were too many zero values for some fields, leading to a standard deviation and mean of zero for certain fields for both sexes and species. We therefore pooled data from all fields and ran a general linear model with a Poisson link function (PROC GENMOD).

Although data were transformed for some analyses, data are not transformed in the figures displaying the means ± SE in the Supporting Information file for ease of visualization. Figures 6 and 7, however, show least squares means ± SE adjusted to the covariate of the transformed data and Figures 8 and 9 show least squares means adjusted to the covariate ± SE. Significant interactions were further analyzed using two-tailed (unless otherwise indicated) Fisher least significance difference (LSD) *post hoc* tests. Results were considered significant if $p \leq 0.05$.

RESULTS

Raw data are available in Supporting Information "Raw Data Spreadsheet."

Gonad Development and Androgens

Gonad development changed with breeding condition. Females had larger follicles in breeding condition than in post-breeding condition [$F_{1,36} = 32.42$, $p < 0.0001$; Fig. 3(a)]. There was neither a significant species difference nor a significant species by breeding condition interaction for follicle size (Supporting Information Table S1). Males had larger testes in breeding condition than in post-breeding condition [$F_{1,44} = 15.71$, $p = 0.0003$; Fig. 3(a)]. There was neither a significant species difference nor a significant species by breeding condition interaction for testis size (Supporting Information Table S1).

Birds in breeding condition had higher androgen levels than post-breeding birds ($F_{1,79} = 24.55$, $p < 0.0001$), confirming breeding condition in these birds, and males had higher androgen levels than females ($F_{1,79} = 3.85$, $p = 0.05$) [Fig. 3(b), Supporting Information Table S1]. There were no significant species differences or interactions (Supporting Information Table S1).

Hippocampal Volume

Cowbirds had a larger hippocampus, relative to the size of the telencephalon, than red-winged blackbirds ($F_{1,78} = 19.10$, $p < 0.0001$), and females had a larger relative hippocampus size than males ($F_{1,78} = 6.17$, $p = 0.01$) (Fig. 4; Supporting Information Table S2). Breeding condition and all interactions were non-significant (Supporting Information Table S2). Once breeding and post-breeding data were merged, female cowbirds had the largest relative hippocampus of all four groups (in rank order: female cowbirds, male cowbirds, female blackbirds, and male blackbirds; Fig. 5). Female cowbirds had a significantly larger hippocampus relative to telencephalon size than male cowbirds (one-tailed Fisher LSD *post hoc* test: $p = 0.05$), but female blackbirds also had a significantly larger hippocampus, relative to telencephalon size, than male blackbirds (one-tailed Fisher LSD post-hoc test: $p = 0.02$; Figs. 4 and 5).

Telencephalon Volume

Breeding birds had a larger telencephalon than post-breeding birds ($F_{1,79} = 21.82$, $p < 0.0001$), males had a larger telencephalon than females ($F_{1,79} = 24.40$, $p < 0.0001$), and red-winged blackbirds had a larger telencephalon than cowbirds ($F_{1,79} = 96.82$, $p < 0.0001$; Fig. 4; Supporting Information Table S2). A significant sex by species interaction ($F_{1,79} = 5.53$, $p = 0.02$) indicated that the effects of sex and species were mainly driven by blackbirds. Male blackbirds had a significantly larger telencephalon than female blackbirds (Fisher LSD *post hoc* test: $p < 0.0001$), but male cowbirds did not differ significantly from female cowbirds (Fisher LSD *post hoc* test: $p = 0.06$).

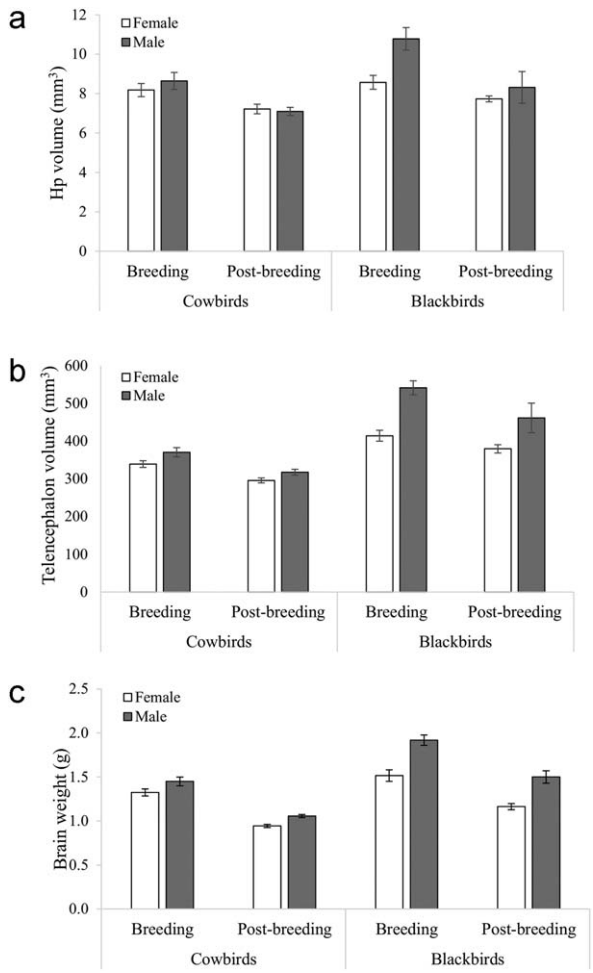

Figure 4 Hippocampus (Hp) volume (A), telencephalon volume (B), and brain weight (C) (mean ±SE) of brown-headed cowbirds and red-winged blackbirds in breeding and post-breeding conditions.

Whole Brain Weight

The whole brain weight results paralleled the data on telencephalon volume. Breeding birds had a heavier brain than post-breeding birds ($F_{1,77} = 68.82$, $p < 0.0001$), males had a heavier brain than females ($F_{1,77} = 37.39$, $p < 0.0001$), and red-winged blackbirds had a heavier brain than cowbirds ($F_{1,77} = 93.72$, $p < 0.0001$; Fig. 4; Supporting Information Table S2). A significant sex by species interaction ($F_{1,77} = 9.94$, $p = 0.002$) indicated that the effects of sex and species were mainly driven by blackbirds. The male-biased sex difference was greater in blackbirds (Cohen's $d = 1.71$; Fisher LSD *post hoc* test: $p < 0.0001$) than in cowbirds (Cohen's $d = 0.58$; Fisher LSD *post hoc* test: $p = 0.03$).

Doublecortin

Detailed data from neurogenesis analyses are presented in the Supporting Information "Supplementary Tables and Figures" (Figs. S1–S6).

Percent DCX+ Cover. Inside the hippocampus, the %DCX+ cover in a field of view ranged from ~1 to 14% (Supporting Information Fig. S1). The seven hippocampal fields of interest (ventral and medial in the rostral, middle, and caudal sections plus lateral in the middle section) differed significantly ($F_{6,449} = 83.86$, $p < 0.0001$), with the ventral fields in the middle and caudal sections having the highest immunoreactivity relative to fields outside the hippocampus (Fig. 6; Supporting Information Table S3). There was a significant species by breeding condition interaction ($F_{1,75} = 8.00$, $p = 0.006$), with immunoreactivity being greater in post-breeding condition in cowbirds (Fisher LSD *post hoc* test: $p = 0.05$), but greater in breeding condition in blackbirds (Fisher LSD *post hoc* test: $p = 0.05$) [Fig. 7(a), Supporting Information Table S3]. There was also a significant species by field interaction ($F_{6,449} = 2.78$, $p = 0.01$, Supporting Information Table S3), with cowbirds having higher immunoreactivity than blackbirds in the ventral field in the middle section (Fisher LSD *post hoc* test: $p = 0.0009$). Finally, there was a significant sex by breeding condition by field interaction ($F_{6,449} = 2.25$, $p = 0.04$, Supporting Information Table S3), with males having higher immunoreactivity than females in breeding condition in the medial field of the rostral section (Fisher LSD *post hoc* test: $p = 0.03$),

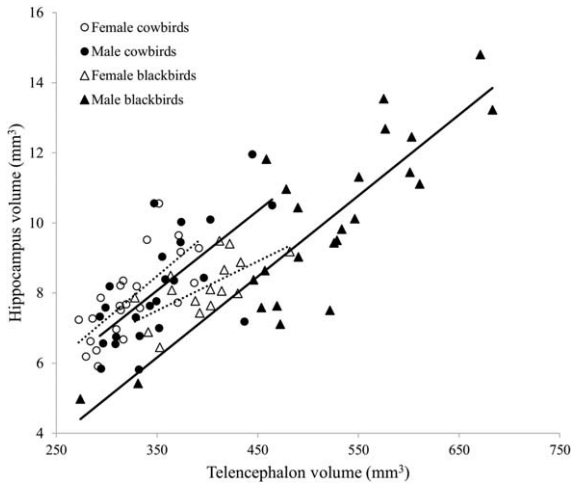

Figure 5 Hippocampus volume relative to telencephalon volume in female and male brown-headed cowbirds and red-winged blackbirds. The trendlines fit the data as follows: upper dotted line—female cowbirds, upper solid line—male cowbirds, lower dotted line—female blackbirds, lower solid line—male blackbirds.

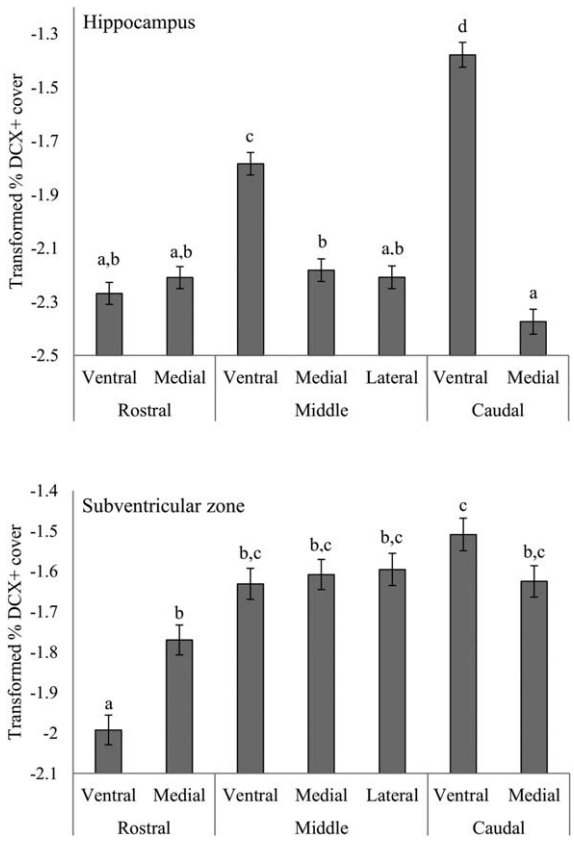

Figure 6 Doublecortin immunoreactivity measured as % DCX+ cover of cell bodies and fibers in fields of view in the hippocampus and subventricular zone in rostral, middle, and caudal sections. Data are arcsine squareroot transformed least square means adjusted to the covariate ± SE with species, sex, and breeding condition combined. Bars that do not share a letter differ significantly by Tukey's *post hoc* test, $p \leq 0.05$.

whereas no sex difference occurred in post-breeding condition (Fisher LSD *post hoc* test: $p = 0.37$).

In the SVZ, the %DCX+ cover in a field of view ranged from ~2 to 22% (Supporting Information Fig. S2). There were multiple significant effects (Supporting Information Table S4). Cowbirds had higher immunoreactivity than red-winged blackbirds ($F_{1,75} = 16.07$, $p = 0.0001$). Percent DCX+ varied significantly between the seven fields of view ($F_{6,449} = 21.00$, $p < 0.0001$; Fig. 6; Supporting Information Table S4). There was a significant species by breeding condition interaction ($F_{1,75} = 10.96$, $p = 0.001$), with greater immunoreactivity in post-breeding condition in cowbirds (Fisher LSD *post hoc* test: $p = 0.007$), but no significant effect of breeding condition in blackbirds [Fig. 7(b); Fisher LSD *post hoc* test: $p = 0.06$]. There was a significant species by sex interaction ($F_{1,75} = 4.35$, $p = 0.04$), with female cowbirds having more immunoreactivity than male cowbirds (Fisher LSD *post hoc* test: $p = 0.03$) and no sex difference in blackbirds [Fig. 7(c); Fisher LSD *post hoc* test: $p = 0.43$]. There was a significant species by area interaction ($F_{6,449} = 2.17$, $p = 0.05$), with cowbirds having more immunoreactivity than blackbirds in the medial field in the rostral section

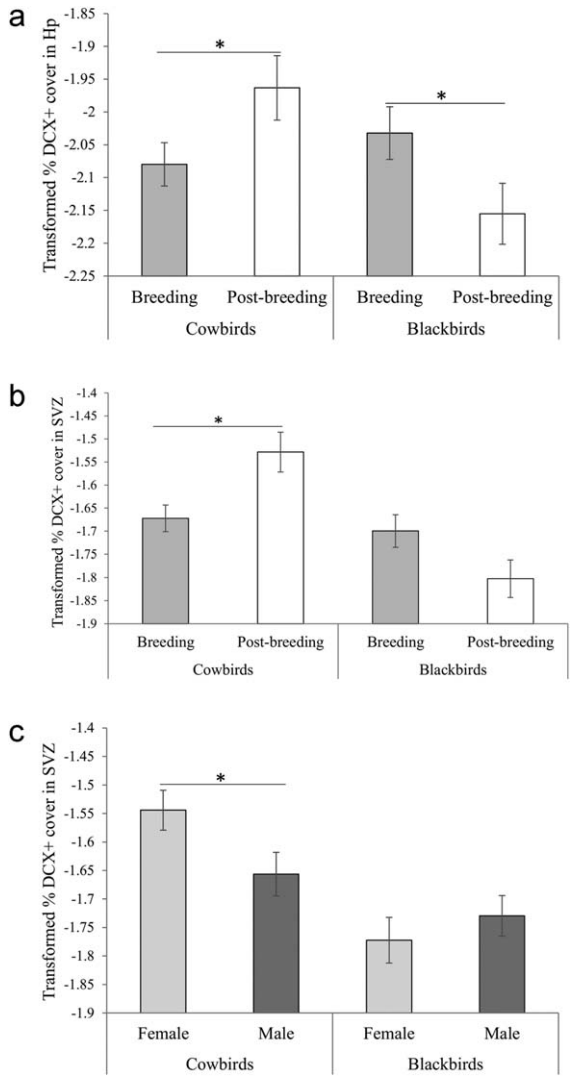

Figure 7 Significant interactions between sex, species, and breeding condition for % doublecortin immunoreactive (DCX+) cover per field of view in the hippocampus (A) and in the hippocampal portion of the subventricular zone (SVZ) (B and C). Data are least square means adjusted to the covariate ± SE. The covariate for (A) was the field of view in the telencephalon in the respective brain section whereas the covariate for (B) and (C) was the respective telencephalon portion of each SVZ field of view. The proportions of DCX+ cover were log arcsine square root transformed for analyses and for the figure. Asterisks indicate $p \leq 0.05$.

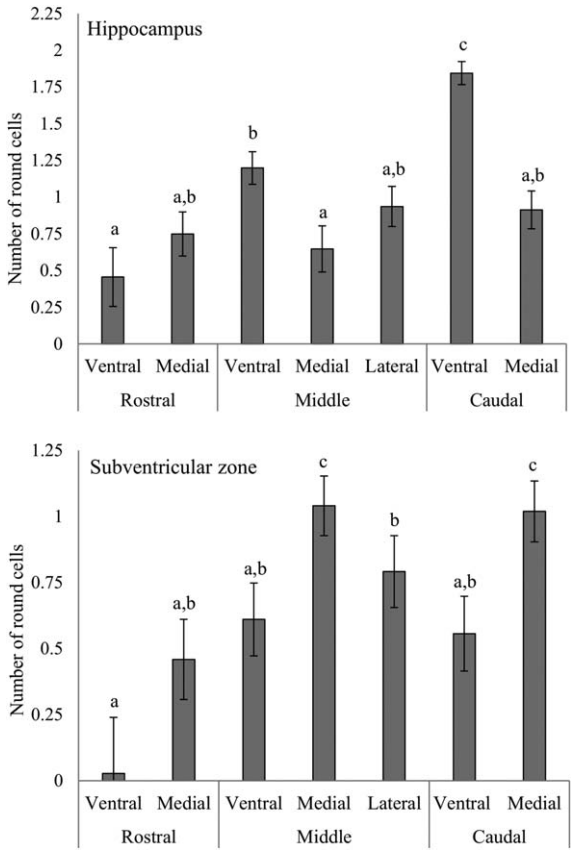

Figure 8 Doublecortin immunoreactivity measured as the number of round cells per field of view in the hippocampus and in subventricular zone in rostral, middle, and caudal sections. Data are least square means adjusted to the covariate ± SE with species, sex, and breeding condition combined. Bars that do not share a letter differ significantly by Tukey's *post hoc* test, $p \leq 0.05$.

(Fisher LSD *post hoc* test: $p < 0.0001$) and the ventral field in the middle section (Fisher LSD *post hoc* test: $p = 0.0009$) (Supporting Information Table S4). All other interactions were not significant (Supporting Information Table S4).

Round Cells. Inside the hippocampus, the number of round cells per field of view ranged from ~1 to 10 (Supporting Information Fig. S3). The number of round cells differed significantly among fields in the hippocampus ($F_{6,449} = 21.17$, $p < 0.0001$), with the ventral fields of the middle and caudal sections having the greatest number of cells (Fig. 8; Supporting Information Table S5). All other effects were not significant (Supporting Information Table S5).

In the SVZ, the number of round cells per field of view ranged from ~1 to 13 (Supporting Information Fig. S4). The number of round cells also differed significantly among the seven fields of view in the SVZ ($F_{6,449} = 5.53$, $p < 0.0001$), with the medial fields in the middle and caudal sections having the highest number of cells (Fig. 8; Supporting Information Table S6). The species by field interaction was also statistically significant ($F_{6,449} = 2.2$, $p = 0.04$), with cowbirds having more cells than blackbirds in the medial field of the rostral section (Fisher LSD *post hoc* test: $p = 0.03$), and in the ventral field of the middle section (Fisher LSD *post hoc* test: $p = 0.04$) (Supporting Information Table S6). All other effects were not significant (Supporting Information Table S6).

Fusiform Cells. Inside the hippocampus, the number of fusiform cells per field of view was small and ranged from ~ 0 to 6 (Supporting Information Fig. S5). Therefore, we merged data from all fields and ran a general linear model with a Poisson link function. The number of hippocampal fusiform cells differed significantly between species ($\chi = 6.54$, df = 1, $p = 0.01$), with cowbirds having more cells per field of view than blackbirds (Supporting Information Table S7). In addition, males had more fusiform cells per field of view than females ($\chi = 5.27$, df = 1,

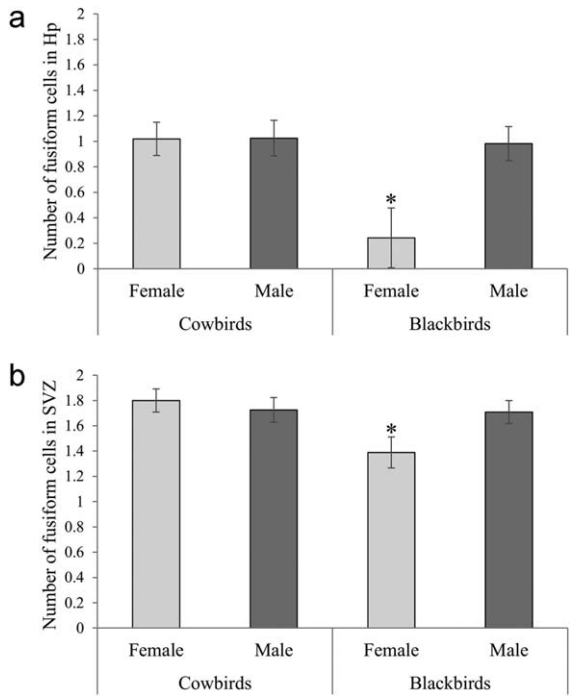

Figure 9 Significant interactions between sex and species in the number of fusiform cells in fields of view in the hippocampus (A) and in the hippocampal portion of the subventricular zone (SVZ) (B). Data are least square means adjusted to the covariate ± SE. Asterisks indicate that female red-winged blackbirds had significantly fewer cells in fields of view in the hippocampus and in the SVZ than any other group ($p \leq 0.05$).

$p = 0.02$) (Supporting Information Table S7). There was also a significant sex by species interaction ($\chi = 5.23$, df = 1, $p = 0.02$). Female blackbirds had significantly fewer cells per field than male blackbirds ($z = 2.72$, $p = 0.006$), whereas no sex difference occurred in cowbirds ($z = 0.03$, $p = 0.98$) [Fig. 9(a)]. Female blackbirds drove the main effects of sex and species in the number of fusiform cells. Female blackbirds had significantly fewer fusiform cells per field than female cowbirds ($z = 2.88$, $p = 0.004$) and male cowbirds ($z = 2.85$, $p = 0.004$) [Fig. 9(a)].

In the SVZ, the number of fusiform cells per field of view was small and ranged from ~0 to 5 (Supporting Information Fig. S6), therefore we merged data from all fields and ran a general linear model with a Poisson link function. In the SVZ, there was a significant main effect of species ($\chi = 4.40$, df = 1, $p = 0.04$), with cowbirds having more fusiform cells than blackbirds, and a significant effect of breeding condition ($\chi = 4.61$, df = 1, $p = 0.03$), with more fusiform cells in post-breeding birds than in breeding birds (Supporting Information Table S8). There was a significant sex by species interaction ($\chi = 3.85$, df = 1, $p = 0.05$), with female blackbirds having fewer cells per field of view than male blackbirds ($z = 2.13$, $p = 0.03$), but there was no sex difference in cowbirds ($z = 0.56$, $p = 0.58$) [Fig. 9(b)]. Female blackbirds also had fewer cells per field than female cowbirds ($z = 2.66$, $p = 0.008$) and male cowbirds ($z = 2.17$, $p = 0.03$) [Fig. 9(b)].

DISCUSSION

Hippocampal Volume

We found that brown-headed cowbirds had a larger hippocampus, relative to the size of the telencephalon, than red-winged blackbirds, and that a sex difference in relative hippocampus size in favor of females occurred in both species (Fig. 5). Although female brown-headed cowbirds had the largest relative hippocampus size in rank order of all four groups examined, there was no interaction between species and sex to indicate that the sex difference found in cowbirds differed from the sex difference in red-winged blackbirds (Fig. 5). Breeding condition had no effect on relative hippocampus size in either species.

Our study replicates previous studies with respect to sex differences in cowbirds but not with respect to sex differences in blackbirds or other control species (Sherry et al. 1993; Reboreda et al., 1996; Clayton et al., 1997). Sherry et al. (1993) found that female brown-headed cowbirds had a larger hippocampus, relative to the size of the telencephalon, than males and that this sex difference was absent in red-winged blackbirds. Reboreda et al. (1996) found that in the shiny cowbird (*Molothrus bonariensis*), a generalist parasite like brown-headed cowbirds in which females search for host nests and males do not, females had a larger hippocampus than males. No sex difference in relative hippocampal size occurred in two other cowbirds, the non-parasitic bay-winged cowbird (*Molothrus badius*, since renamed *Agelaioides badius*) and a specialist parasite the screaming cowbird (*Molothrus rufoaxillaris*), in which males and females search together for host nests. Clayton et al. (1997) also found female shiny cowbirds had a larger hippocampus, relative to telencephalon size, than males. In a subsequent study of all three species, however, no sex differences in relative hippocampal size were detected (Nair-Roberts et al., 2006).

It is unclear why we detected a female-biased sex difference in the hippocampal volume of red-winged blackbirds. The sample sizes in the present study are considerably larger than in any of the previous studies, and we confirmed breeding condition by gonad development and hormone assay (Fig. 3). It is possible that sample size or data from mixed groups consisting of birds of different ages or breeding status in previous studies were responsible for the difference between the present results and previous findings. It is also possible that the sex difference in hippocampal volume found in brown-headed cowbirds is, like many other traits, shared with red-winged blackbirds and perhaps other icterids. Despite some clear differences in behavior due to brood parasitism, red-winged blackbirds and brown-headed cowbird nestlings both show exaggerated begging behavior (Rivers et al. 2013). Female brown-headed cowbirds and red-winged blackbirds also show greater auditory sensitivity compared to males, a sex difference that, according to Gall et al. (2011), is not generally found in passerines. This would suggest that apart from their brood-parasitic behavior, cowbirds may differ only modestly from other icterids (Mermoz and Ornelas, 2004). This conclusion would, however, stand in contrast to neuroanatomical results described earlier on brown-headed cowbirds (Sherry et al., 1993) and South American cowbirds (Reboreda et al., 1996; Clayton et al., 1997). Future research could help to explain the unexpected sex difference in hippocampal volume found in red-winged blackbirds.

Greater hippocampal volume in cowbirds of both sexes, compared to red-winged blackbirds, is notable.

Like the present study, Sherry et al. (1993) found that brown-headed cowbirds had a larger hippocampus than red-winged blackbirds. It is possible this difference is related to a difference in migratory behavior between cowbirds and blackbirds (Healy et al., 1996; Pravosudov et al., 2006) or some aspect of spatial cognition unrelated to brood parasitism (Roth and Pravosudov, 2009). It is also possible, however, that selection acting on female brown-headed cowbirds has caused an increase in the relative hippocampal size of both sexes, compared to blackbirds. Selection acting exclusively on one sex can produce morphological change in both sexes in the absence of sex-specific developmental modifiers (Lande, 1980; Wyman et al., 2013) and the cowbird hippocampus may show the effects of such selection.

Neurogenesis

Although some limitations have been expressed (Vellema et al., 2014), multiple arguments suggest that doublecortin is a reliable endogenous marker of neurogenesis (Balthazart and Ball, 2014a,b). New cells are born in the subventricular zone, from which they migrate as fusiform cells to their final destination where they transform into round immature cells (Balthazart and Ball, 2014b). In canaries, birds were injected daily for five days with the exogenous cell-birth marker BrdU and the percentage of DCX+ fusiform and round cells also labeled with BrdU was noted 10 days and 30 days after the first injection (Balthazart et al., 2008). The percentage of double-labeled fusiform cells decreased from 72% to 31% between the two time points, whereas the percentage of double-labeled round cells increased from 5% to 24%. These results indicated a high turnover in cell type, with many cells surviving only several days to a few weeks.

In the present study, we measured the density of DCX+ fibres and cells in the fields of view within the hippocampus (Wada et al., 2014). We did not measure the number of cells and fibers within the entire hippocampus. Thus, our results presented are not directly comparable to exhaustive sampling in other species.

In the present study, doublecortin labeling for neurogenesis in the subventricular zone of the hippocampus presented a different picture than the results for hippocampal volume. Female cowbirds had significantly more doublecortin labeling than male cowbirds whereas no sex difference occurred in red-winged blackbirds [Fig. 7(c)]. This result was our only observation of an interaction between sex and species in which cowbirds exhibited a sex difference in favor of females that was absent in blackbirds. This sex by species interaction represents the strongest support for the hypothesis of hippocampal specialization in female cowbirds. There were also sex differences in blackbirds that did not occur in cowbirds. Male blackbirds had more migrating fusiform cells in both the hippocampus and subventricular zone than females and this sex difference was absent in cowbirds [Fig. 9(a,b)]. Levels of neurogenesis, as measured by doublecortin immunoreactive cover and doublecortin immunoreactive fusiform cells, were either higher or similar in female cowbirds relative to male cowbirds, but were either similar or lower in female blackbirds relative to male blackbirds (Figs. 7 and 9). Female cowbirds, which rely more on spatial memory than any other group in our study, also had the highest levels of hippocampal neurogenesis (Fig. 7), an association consistent with the food-storing literature in which chickadees that relied more on remembering the location of stored food to survive had higher levels of hippocampal neurogenesis (Chancellor et al., 2011; Freas et al., 2012).

We found several overall differences between species that were unaffected by sex. Cowbirds, both males and females, had more doublecortin labeling than red-winged blackbirds (Fig. 7). Cowbirds had more immature differentiating round cells than red-winged blackbirds in the subventricular zone and more migrating fusiform cells in both the hippocampus and subventricular zone than red-winged blackbirds. Therefore, as with hippocampal volume, selection acting on female cowbirds may have produced enhanced neurogenesis in both sexes, relative to blackbirds (Lande, 1980; Wyman et al., 2013).

We found the greatest level of %DCX+ and the greatest number of DCX+ round cells in the ventral region of the hippocampus (Figs. 6 and 8), as also described for DCX+ cells in the song sparrow hippocampus (Wada et al., 2014). In mammals, the subgranular zone of the dentate gyrus is the neuroproliferative region of the hippocampus. The high level of neurogenesis we found in the ventral region therefore supports the homology proposed by Atoji and Wild (2006) between the ventral avian hippocampus and the mammalian dentate gyrus.

We also found that %DCX+ and the number of DCX+ round cells in the ventral region of the hippocampus increased rostro-caudally (Figs. 6 and 8). In the subventricular zone, we found the most rostral fields to have the lowest DCX+ labeling and the fewest DCX+ round cells (Figs. 6 and 8). Previous studies have found, in contrast, a rostral to caudal decrease in the number of new neurons in the hippocampus of black-capped chickadees and marsh tits (*Poecile palustris*) (Barnea and Nottebohm, 1994;

Patel et al., 1997; Tarr et al., 2009). This could indicate species differences or a different pattern of rostral to caudal neurogenesis in the ventral region of the hippocampus. The difference could also be the consequence of the methods used since previous studies used exogenous markers [^{3}H]-thymidine and BrdU to identify newly divided cells.

Breeding Condition

There was no effect of breeding condition on relative hippocampal size (Fig. 4; Supporting Information Table S2). This absence of a breeding condition effect suggests brown-headed cowbirds differ from other species of cowbirds, notably shiny cowbirds and screaming cowbirds that have been reported to have a larger relative hippocampus in breeding condition (Clayton et al., 1997). However, the breeding and non-breeding groups from Clayton et al. (1997) were processed in two different batches, and the difference could, therefore, be due to a batch effect. Hippocampus volume relative to telencephalon volume in brown-headed cowbirds from the present study remained stable across breeding conditions, suggesting that seasonally variable behavior such as the act of nest searching does not influence the relative size of the hippocampus in free-ranging birds. Our result runs parallel to previous behavioral results on brown-headed cowbirds. We found no effect of photoperiodically induced breeding condition on memory in an open field spatial search task in which female cowbirds performed better than males (Guigueno et al., 2014). In contrast, breeding condition did affect performance on touch screen tasks that did not require cowbirds to move through their environment like the open field spatial search task (Guigueno et al., 2015). Males performed better in breeding condition than in non-breeding condition on a spatial memory touch screen task (in the absence of any breeding condition effect in females) while females performed better in breeding condition than in non-breeding condition on a color memory touch screen task (in the absence of any breeding condition effect in males) (Guigueno et al., 2015). Thus, with regard to seasonal differences, only performance on an ecologically relevant task (Guigueno et al. 2014) parallels the result on relative hippocampus size in the current study.

Breeding condition also affected telencephalon size and overall brain weight. The telencephalon was larger and the whole brain weighed more during breeding condition than in post-breeding condition in both species. Seasonal changes in brain size of many kinds occur widely in birds and mammals and are associated with annual cycles in reproduction, song, food-storing, migration, and other behavior (Yaskin, 1984; Tramontin and Brenowitz, 2000; Smulders, 2002; Barnea and Pravosudov, 2011; Yaskin, 2011; Sherry and MacDougall-Shackleton, 2015).

The level of neurogenesis, measured by %DCX+, was higher in post-breeding condition than in breeding condition in cowbirds. In blackbirds, it was higher in breeding condition than in post-breeding condition in the hippocampus and similar between breeding conditions in the subventricular zone (Fig. 7). The duration of DCX expression in young neurons has not been studied in detail in birds, but in mammals, DCX expression lasts about one month (Balthazart and Ball, 2014b). Thus, it is unlikely that cells and fibers expressing DCX+ in post-breeding condition (Sept–Nov) were born several months earlier in breeding condition (March–May) or vice versa. Because it takes about six weeks for a neuron to mature, however, some of the functional neurons in breeding condition could have been produced in the prior post-breeding condition (Sherry and MacDougall-Shackleton, 2015). The study of seasonal plasticity in the hippocampus of songbirds, unlike the song control system, is relatively recent (reviewed in Sherry and MacDougall-Shackleton, 2015). The song-control system is under strict photoperiodic control mediated by increases in circulating testosterone after photostimulation, which increases the survival of new neurons and leads to an increase in volume (Tramontin and Brenowitz, 2000). The pattern of seasonal change in hippocampal neurogenesis, however, is variable across studies and there may be minimal effects of photoperiod on the hippocampus (Sherry and MacDougall-Shackleton, 2015). Despite this variability, peaks in hippocampal neurogenesis in previous work on food-storing species occurred in the autumn or mid-winter when the photophase is short, sex steroids are low, and melatonin release is greater during long nights, indicating there could be a role for these hormones in mediating seasonal changes in the hippocampus (Sherry and MacDougall-Shackleton, 2015). In addition, the hippocampus of songbirds shows high aromatase activity, further implying that estradiol and testosterone could regulate the hippocampus (Saldanha et al., 1998). Not surprisingly, we recorded in the present study a significant decrease in circulating testosterone in our post-breeding birds (Fig. 3). In sum, a seasonal peak in hippocampal neurogenesis in post-breeding cowbirds may be driven by a decrease in sex steroids and an increase in melatonin, but further research is needed.

What is the role of hippocampal neurogenesis in memory? In addition to producing new neurons to

accommodate new memories, neurogenesis replaces older cells which causes loss of memories (Barnea and Pravosudov, 2011; Frankland et al., 2013). Neurogenesis regulates forgetting in altricial and precocial rodents (Akers et al., 2014). It follows that cowbirds should replace old neurons at a time of the year when they no longer need to rely on them (i.e., post-breeding condition) and replace them with new neurons required to encode new information in the upcoming breeding season (Hoshooley and Sherry, 2007; Barnea and Pravosudov, 2011). Thus, by the time cowbirds enter breeding condition, their hippocampus may be better prepared to acquire new information about their surroundings.

Further research on fine-scale properties of the hippocampus and how they change seasonally is needed. Doublecortin only labels new neurons. Therefore, we can only conclude that more new cells, which may have replaced old cells, were produced in post-breeding condition than in breeding condition. Indeed, the hippocampus of chickadees living in the harsh Alaska climate shows upregulation of several genes related to increases in cell apoptosis (Pravosudov et al., 2013). Future studies should look at seasonal changes in cell apoptosis and other factors related to cellular reorganization in the hippocampus, such as changes in dendritic fields and depletion of synapses.

CONCLUSIONS

Our results, especially our data on neurogenesis, are consistent with the hypothesis that the cowbird hippocampus is specialized for brood parasitism. Female cowbirds generated more new neurons and fibers in the subventricular zone than males, and this difference was absent in blackbirds. There are many hypotheses about the function of adult hippocampal neurogenesis in memory (reviewed in Barnea and Pravosudov, 2011), ranging from the idea that new neurons are needed to form new memories (Barnea and Nottebohm, 1994) to the hypothesis that neurogenesis promotes forgetting of existing memories and improved pattern separation of new memories (Frankland et al., 2013). Higher rates of neurogenesis in the subventricular zone of the hippocampus of female brown-headed cowbirds may be associated with cognitive aspects of spatial search or spatial memory for potential host nests.

The authors thank George and Pat Finney and Stuart Mackenzie from Bird Studies Canada and Emile Vandommele for help in the field. The authors also thank Layla Amer, Amy Cardinal, Marco (Alex) Coto, Adrian Jodzio, Hayden MacDonald, Rutwik Patani, Sammy Shahatto, Lawrence Yip, and Hui (Lily) Zhou for help with immunohistochemistry and image analysis. The authors have no conflict of interest to declare.

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