Corticosterone and Dehydroepiandrosterone Have Opposing Effects on Adult Neuroplasticity in the Avian Song Control System

Amy E.M. Newman,[1,2]* Scott A. MacDougall-Shackleton,[3,4] Yong-Seok An,[3] Buddhamas Kriengwatana,[4] and Kiran K. Soma[1,2,5]

[1]Department of Psychology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4
[2]Graduate Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4
[3]Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5C2
[4]Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C2
[5]Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

ABSTRACT

Chronic elevations in glucocorticoids can decrease the production and survival of new cells in the adult brain. In rat hippocampus, supraphysiological doses of dehydroepiandrosterone (DHEA; a sex steroid precursor synthesized in the gonads, adrenals, and brain) have antiglucocorticoid properties. With male song sparrows (*Melospiza melodia*), we examined the effects of physiological doses of corticosterone, the primary circulating glucocorticoid in birds, and DHEA on adult neuroplasticity. We treated four groups of nonbreeding sparrows for 28 days with empty (control), corticosterone, DHEA, or corticosterone + DHEA implants. Subjects were injected with BrdU on days 3 and 4. In HVC, a critical song control nucleus, corticosterone and DHEA had independent, additive effects. Corticosterone decreased, whereas DHEA increased, HVC volume, $NeuN^+$ cell number, and $BrdU^+$ cell number. Coadministration of DHEA completely reversed the neurodegenerative effects of chronic corticosterone treatment. In an efferent target of HVC, the robust nucleus of the arcopallium (RA), DHEA increased RA volume, but this effect was blocked by coadministration of corticosterone. There were similar antagonistic interactions between corticosterone and DHEA on $BrdU^+$ cell number in the hippocampus and ventricular zone. This is the first report on the effects of corticosterone treatment on the adult song control circuit, and HVC was the most corticosterone-sensitive song nucleus examined. In HVC, DHEA is neuroprotective and counteracts several pronounced effects of corticosterone. Within brain regions that are particularly vulnerable to corticosterone, such as the songbird HVC and rat hippocampus, DHEA appears to be a potent native antiglucocorticoid. J. Comp. Neurol. 518:3662–3678, 2010.

INDEXING TERMS: adult neurogenesis; BrdU; cortisol; DHEA; hippocampus; NeuN; neurosteroid; songbird; stress

Neurogenesis continues during adulthood across vertebrates and is profoundly influenced by steroids. In mammals, cells proliferate in the subventricular zone and dentate gyrus and migrate to the olfactory bulb and granular layer of the hippocampus, respectively (Cameron et al., 1993; Alvarez-Buylla and Garcia-Verdugo, 2002). In birds, neuronal progenitor cells migrate from the lateral ventricular zone (VZ) to many areas in the telencephalon (Alvarez-Buylla and Kirn, 1997; Goldman, 1998; Nottebohm, 2002). Songbirds have high neuronal turnover in HVC and area X, two nuclei of the song control system (Nottebohm, 1981; Alvarez-Buylla and Kirn, 1997).

Glucocorticoids, which are secreted by the adrenal glands during stress and bind to receptors that act as transcription factors, are potent steroidal modulators of adult neurogenesis. In adult male rats, corticosterone

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

Grant sponsor: Natural Sciences and Engineering Research Council of Canada (NSERC; to S.A.M.-S., A.E.M.N.); Grant sponsor: Canadian Institutes of Health Research (CHIR; to K.K.S.); Grant sponsor: Michael Smith Foundation for Health Research (MSFHR; to K.K.S., A.E.M.N.).

*CORRESPONDENCE TO: Amy E.M. Newman, Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 2W1. E-mail: newman01@uoguelph.ca

Received July 14, 2009; Revised February 23, 2010; Accepted March 31, 2010
DOI 10.1002/cne.22395
Published online April 14, 2010 in Wiley InterScience (www.interscience.wiley.com)

treatment in vivo decreases cell proliferation and survival of new neurons in the dentate gyrus (Gould et al., 1992; Karishma and Herbert, 2002; Wong and Herbert, 2004). In adult male zebra finches (*Taeniopygia guttata*), corticosterone treatment in vitro decreases cell proliferation in the VZ (Katz et al., 2008). In adult chickadees (*Poecile atricapillus*), corticosterone treatment in vivo does not affect hippocampus size or cell number or cell proliferation in the VZ (Pravosudov and Omanska, 2005). Effects of corticosterone treatment on HVC in adult songbirds have not been reported.

Dehydroepiandrosterone (DHEA) is a precursor to sex steroids. Like glucocorticoids, DHEA can be increased by acute stress or adrenocorticotropic hormone (ACTH) in the rat brain (Corpechot et al., 1981; Torres and Ortega, 2003) and human plasma (Oberbeck et al., 1998; Arvat et al., 2000). DHEA has numerous effects on the brain (Maninger et al., 2009) and, for example, increases HVC size in nonbreeding adult song sparrows (*Melospiza melodia*) by ~50% (Soma et al., 2002). DHEA has been described as a native antiglucocorticoid in the nervous system (Kalimi et al., 1994; Maninger et al., 2009). For example, DHEA prevents corticosterone-induced translocation of stress-activated protein kinase 3 to the nucleus in rat hippocampal cells in vitro (Kimonides et al., 1999). In vivo, corticosterone suppresses recruitment of new cells into the adult male rat dentate gyrus, and this corticosterone effect is prevented by DHEA treatment (Karishma and Herbert, 2002). Together these studies suggest that DHEA is regulated by stress and modulates the effects of glucocorticoids on the brain. However, because rats have very low levels of circulating DHEA, the effects of high DHEA doses in this species are difficult to interpret.

Songbirds have higher levels of circulating DHEA than traditional model systems such as laboratory rodents (Soma and Wingfield, 2001; Hau et al., 2004; Newman et al., 2008b; Newman and Soma, 2009), and a physiological DHEA dose has robust effects on behavior and neuroanatomy (Soma et al., 2002; Goodson et al., 2005a). In song sparrows, DHEA levels in jugular plasma (exiting the brain), but not in brachial plasma, are affected by acute restraint stress (Newman et al., 2008b). Also, DHEA levels are much higher in song sparrow brain than plasma, and DHEA concentrations are highest in the hippocampus (Newman and Soma, 2009), a brain region that is particularly sensitive to glucocorticoids, at least in rats (McEwen, 2001). Together these data raise the hypothesis that DHEA reduces the effects of glucocorticoids on the brain. Here, with adult male song sparrows, we assessed the effects of corticosterone and DHEA treatments on 1) neuroanatomy of and 2) new cell recruitment into the song control system and hippocampus.

MATERIALS AND METHODS

Animals

Wild adult male song sparrows (*Melospiza melodia melodia*) were used for experiments 1B and 2. Subjects were captured using mist nets and conspecific playback and were then transferred to the University of Western Ontario (for specific capture locations, see descriptions of experiments 1B and 2 below). Subjects were housed individually with ad libitum access to water, food (Mazuri small bird maintenance diet + white millet seeds), and grit and kept on a natural photoperiod. Subjects were gradually shifted to a short photoperiod (8L:16D) and nonbreeding condition. Subjects were maintained on 8L:16D for at least 1 month prior to treatment. All song sparrows were used in accordance with the Canadian Council on Animal Care following procedures approved by the University of Western Ontario Animal Care and Use Committee.

Steroid implants

Implants were made from silastic tubing (for corticosterone: i.d. 1.47 mm, o.d. 1.96 mm; for DHEA: i.d. 0.76 mm, o.d. 1.65 mm). For corticosterone, silastic implants with effective lengths of 10–15 mm have been used previously in songbirds to elevate corticosterone moderately above baseline levels (Astheimer et al., 2000; Breuner and Hahn, 2003; Martin et al., 2005), so we used an effective length of 12 mm. Implants were packed with crystalline corticosterone (catalog No. C2505; Sigma-Aldrich, Oakville, Ontario, Canada) and sealed with liquid silastic. In previous studies, corticosterone release from silastic implants was facilitated by making a small hole in the implant (Silverin, 1998; Astheimer et al., 2000). We used a 22-gauge needle to make one hole in each corticosterone implant near the end of the effective length (i.e., not through the liquid silastic plug). For DHEA, we used an effective length of 7 mm, which clearly and consistently elevates plasma DHEA levels in song sparrows within the physiological range (Soma et al., 2002; Goodson et al., 2005b). Implants were packed with crystalline DHEA (catalog No. A8500-000; Steraloids, Newport, RI) and sealed with liquid silastic.

Experiment 1: validation of corticosterone implants

In experiment 1A, the corticosterone implants were tested in vitro. To examine the release of corticosterone from silastic implants over 28 days, we incubated corticosterone implants with zero, one, three, or six needle holes per implant ($n = 5$ per group) in microcentrifuge tubes containing 550 μl assay buffer at ~40°C. Implants were incubated for 3 days, then transferred to fresh

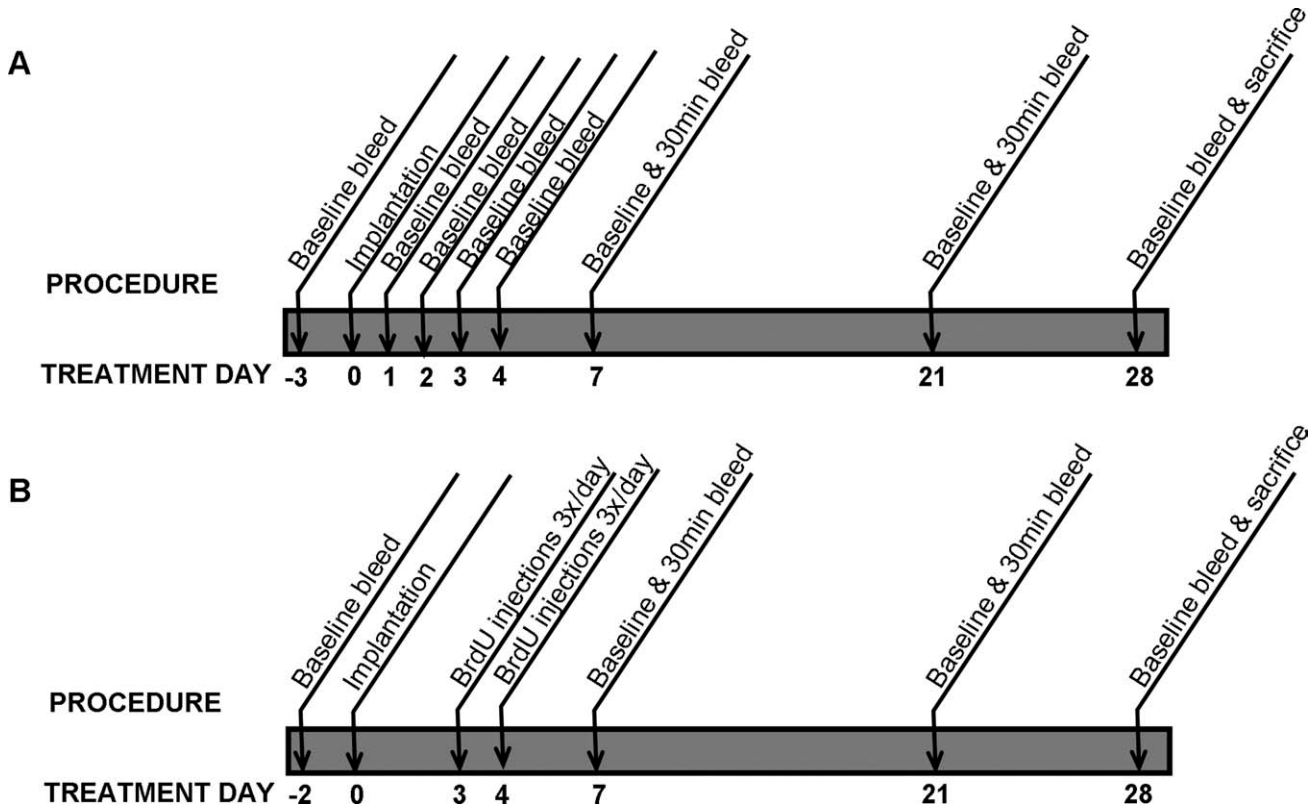

Figure 1. Timelines for experiment 1B (**A**) and experiment 2 (**B**). Experiments 1B and 2 were similar, but BrdU was injected three times per day on days 3 and 4 only in experiment 2. In experiment 1B, no BrdU was injected, and baseline blood samples were collected on days 1–4. After day 4, the timelines for experiments 1B and 2 were identical.

medium and incubated for 4 days, then transferred to fresh medium and incubated for 14 days, then transferred to fresh medium and incubated for 7 days. Empty implants were incubated for 3 days (n = 5) or 28 days (n = 5). Samples were stored at −20°C. At day 28, all corticosterone implants still contained steroid.

In experiment 1B, the corticosterone implants were tested in vivo. Subjects (n = 12) were captured in August from around Guelph, Ontario, Canada (43°33′N, 80°15′W) and the University of Western Ontario (43°0.5′N, 81°16.8′W). On day 0, subjects received corticosterone implants (n = 6) or empty implants (control, n = 6; Fig. 1A). Three subjects in the control group and one subject in the corticosterone group either continued or began molting feathers during the experiment, and these molting subjects were excluded because molt dramatically alters stress physiology in song sparrows and other songbirds (Romero, 2006; Newman et al., 2008b; Newman and Soma, 2009).

Three days prior to implantation (day −3), a baseline blood sample (~50 μl) was collected from the brachial vein within 3 minutes of entering the room. Baseline blood samples were also collected on days 1, 2, 3, and 4 (~25 μl on each day). On days 7 and 21, a baseline blood sample (~50 μl) was collected, and another blood sample (~50 μl) was collected after 30 minutes of restraint in an opaque bag. On day 28, a final baseline blood sample was collected. Blood was kept on ice until centrifuged, and plasma was stored at −20°C. On day 28, all implants were present, and all corticosterone implants still contained steroid.

Subjects were treated for 4 weeks because 1) over 3 weeks is required for the migration, maturation, and integration of neurons in the adult song system (Nottebohm, 2002); 2) prior studies in rats on the effects of corticosterone treatment or chronic stress use a similar timeline (Wong and Herbert, 2004; Joels et al., 2007); and 3) removing the implants during the middle of the experiment would have introduced an additional stressor. Finally, as demonstrated in Results (Fig. 2A), plasma levels of corticosterone were elevated only for the first few days of treatment, suggesting that the corticosterone dose and treatment duration were physiological.

Experiment 2: effects of corticosterone and DHEA treatment on adult neuroplasticity

Subjects

Subjects were wild adult male song sparrows (n = 36 total) captured at the Queens University Biological Station

(44°34′N, 76°19.5′W; n = 12) or near the University of Western Ontario (n = 24; June 4 to July 6, 2007). Subjects were housed in individual cages in four separate rooms (n = 9 per room) under conditions identical to those of experiment 1B. Subjects were gradually shifted to a short-day photoperiod (8L:16D). A short photoperiod was used in this experiment because 1) in the songbird brain, the rate of incorporation of new neurons into HVC is highest during the nonbreeding season (Tramontin and Brenowitz, 1999, 2000; Nottebohm, 2002); 2) individual variation in both circulating corticosterone and DHEA is lowest during the nonbreeding season (Newman et al., 2008b); 3) DHEA treatment has a large effect on song production and HVC volume in the nonbreeding season (Soma et al., 2002); and 4) neural DHEA metabolism is highest during the nonbreeding season (Pradhan et al., 2010). We randomly assigned subjects to one of four treatment groups: empty implant + empty implant (control group), corticosterone implant + empty implant (corticosterone group), DHEA implant + empty implant (DHEA group), or corticosterone implant + DHEA implant (corticosterone + DHEA group). Steroid treatments started from 3 November to 7 December, 2007, depending on the room. There were birds from each treatment group in each room. On day 0 (Fig. 1B), we anesthetized subjects (1.5% isoflurane, 2 liters/minute O_2) and inserted silastic implants (see above) s.c. on the back. Implants were inserted through a small incision in the skin, which was then sealed with veterinary skin adhesive (Nexaband S/C; Closure Medical Corporation, Raleigh, NC).

Experimental timeline

On day 3 and day 4 (Fig. 1B), all subjects received three intramuscular injections (at 0900, 1300, and 1700 hours) of the thymidine analogue bromodeoxyuridine (BrdU; catalog No. B9285; Sigma). BrdU is incorporated into cells that are synthesizing DNA (in the S-phase of mitosis) at the time of BrdU exposure (Thompson and Brenowitz, 2009). BrdU was dissolved in 0.1 M phosphate-buffered saline (PBS; pH 7.4, 100 μl volume, 0.015 g/ml), which resulted in a BrdU dose of 65 mg/kg, similar to that used in previous songbird studies (Wang et al., 2002; Hoshooley et al., 2007).

Baseline blood samples were collected from the brachial vein within 3 minutes of entering the room. Two days prior to implantation (day −2), a baseline blood sample (~100 μl) was collected. On days 7 and 21, a baseline blood sample (~100 μl) was collected, and subjects were restrained in an opaque paper bag for 30 minutes. After this restraint, another blood sample was collected (~100 μl). To minimize stress, blood was not collected on treatment days 1–4 as in experiment 1B. On day 28, a baseline blood sample (~100 μl) was collected prior to death. Blood was kept on ice until centrifuged, and plasma was stored at −20°C. Subjects were weighed, and fat score (on a scale of 0–5) was recorded on days −2, 7, and 21. Subjects were not weighed immediately prior to death (day 28).

After blood collection on day 28, subjects were deeply anaesthetized with ketamine and xylazine and transcardially perfused with cold heparinized saline followed by buffered 4% paraformaldehyde (pH 8.5, as in previous songbird studies; see, e.g., MacDonald et al., 2006). The brain was removed from the skull (completely pneumatized in all subjects, which is indicative of adulthood), postfixed in 4% paraformaldehyde for 24 hours, and then cryoprotected in 30% sucrose until saturated (~48 hours). Brains were then frozen on pulverized dry ice and stored at −80°C. We also collected the spleen and syrinx as positive controls because these are known targets for corticosterone and DHEA, respectively (Soma et al., 2002; Shini et al., 2008). These tissues were postfixed in 4% paraformaldehyde for 24 hours and then weighed, and tissue mass was corrected for day 21 body mass. Testes were regressed in all subjects (mean volume of one testis ± SEM: 0.83 ± 0.1 mm^3), consistent with nonbreeding condition. Finally, we checked the silastic implants. All implants were present, and all corticosterone and DHEA implants contained steroid.

Steroid measurement

Corticosterone levels were measured in plasma and in medium without an extraction step (as in Newman et al., 2008b), with a sensitive and specific corticosterone radioimmunoassay (ImmuChem 07-120103; MP Biomedicals, Orangeburg, NY). The lowest point on the corticosterone standard curve was 3.12 pg corticosterone per tube. Intraassay variation was 5.9%, and interassay variation was 9.5% (low control) and 7.6% (high control; n = 7 assays).

DHEA levels were measured in plasma after extraction with dichloromethane (as in Newman et al., 2008b) with a sensitive and specific DHEA radioimmunoassay (DSL 8900; Diagnostic Systems Laboratories, Webster, TX). The lowest point on the DHEA standard curve was 2 pg DHEA per tube. Intraassay variation was 9.5%, and interassay variation was 5.2% (low control) and 11.7% (high control). Recovery of 50 pg exogenous DHEA from a pool of song sparrow plasma (n = 6 replicates) was 143%. Plasma concentrations were corrected for recovery. Both the corticosterone and the DHEA radioimmunoassays have been validated extensively for song sparrow plasma (Newman et al., 2008a,b; Newman and Soma, 2009).

Immunocytochemistry

Brains were sectioned in the coronal plane at 30 μm thickness on a cryostat. Two sets of sections were

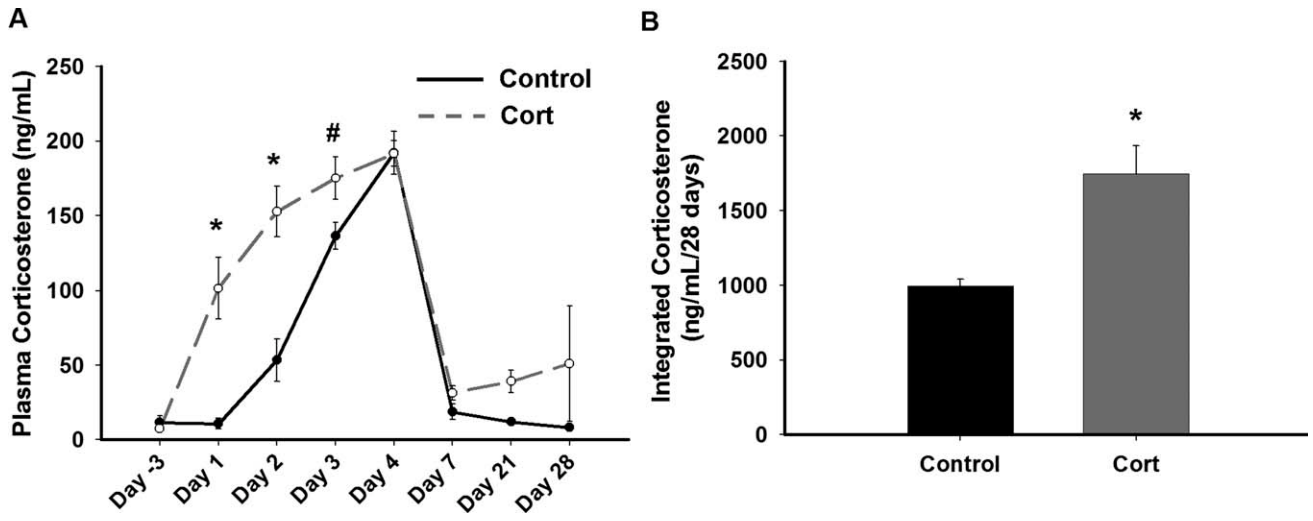

Figure 2. Effects of corticosterone implants (Cort) on baseline plasma corticosterone levels (**A**) and integrated corticosterone levels (**B**) compared with subjects that received empty silastic implants (Control). Significant differences between treatment groups ($^{*}P < 0.05$ and $^{\#}P = 0.06$).

collected into PBS to examine BrdU and NeuN immunoreactivity, and a third set was collected into cryoprotectant and stored at −20°C.

To detect BrdU^{+} cells (see Fig. 3A), free-floating sections were washed in PBS and then immersed in 2 N HCl for 40 minutes. Sections were washed in 0.1 M sodium borate (with 0.5% HCl) for 10 minutes and then washed in PBS. Sections were then incubated in 0.5% H_2O_2 for 30 minutes at room temperature and then washed in PBS. Sections were blocked in 10% normal goat serum (Vector) for 30 minutes and then exposed to an anti-BrdU/IdU monoclonal primary antibody raised in mouse (immunogen: 5-iodo-2′-deoxyuridine-5′-monophosphate conjugated through the phosphate group to albumin; clone IU-4; purified immunoglobulin; Caltag MD5000, Burlington, Ontario, Canada; Vanderlaan et al., 1986). Sections were exposed to this anti-BrdU antibody (diluted 1:500 in PBS with 0.3% Triton) for 20 hours at room temperature. This antibody has been used previously in chick brain at the same dilution (Carnahan et al., 1994), and we tested a range of dilutions on song sparrow brain sections. In pilot studies, we also tested various BrdU antibodies (from BD Pure and BD Pharmingen, San Diego, CA; Chemicon, Billerica, MA; Caltag) and chose the antibody that gave the highest signal to noise ratio, similarly to Leuner et al. (2009). Next, sections were washed in PBS with 0.1% Triton (3 × 5 minutes) and then exposed to the biotinylated secondary antibody (1:250 in PBS with 0.3% Triton; goat anti-mouse IgG; Vector, Burlingame, CA) for 1 hour. Sections were washed in PBS with 0.1% Triton (3 × 5 minutes), and then treated with avidin-biotin-peroxidase reagent (Vector Elite ABC kit) for 1 hour. BrdU labelling was visualized by exposing the sections to a diaminobenzidine solution (Sigma FastDAB). Sections were then washed thoroughly in PBS, mounted on glass slides, dehydrated through a series of graded ethanols, and cleared in solvent (Harleco Neo-Clear; EMD Chemicals, Mississauga, Ontario, Canada), and then coverslips were affixed with Neo-Mount (EMD Chemicals). As a negative control, 12 brain sections from one subject that had not been injected with BrdU were processed for BrdU staining; staining was absent in these sections. As an additional negative control, 20 brain sections from different subjects that had been injected with BrdU were processed for BrdU staining with the primary antibody omitted; staining was absent in these sections.

We also examined NeuN, a neuron-specific protein that is expressed in the large majority of neuronal cell types (Mullen et al., 1992). To detect NeuN^{+} cells (Fig. 3B–E), sections were processed similarly; however, sections were not exposed to HCl or sodium borate and were exposed to an anti-NeuN monoclonal primary antibody raised in mouse (immunogen: purified cell nuclei from mouse brain; clone A60; purified immunoglobulin; Chemicon MAB377; Mullen et al., 1992). Sections were exposed to this anti-NeuN antibody (diluted 1:2,000 in PBS with 0.3% Triton) for 20 hours at room temperature, as in Phillmore et al. (2006). NeuN immunoreactivity is present in cytoplasm and nucleus in birds in most neuronal cell types (Mullen et al., 1992; Hoshooley and Sherry, 2007). NeuN immunoreactivity is absent in a few neuronal cell types, such as cerebellar Purkinje cells (Mullen et al., 1992). Thus, as negative control, we examined NeuN immunoreactivity in Purkinje cells of song

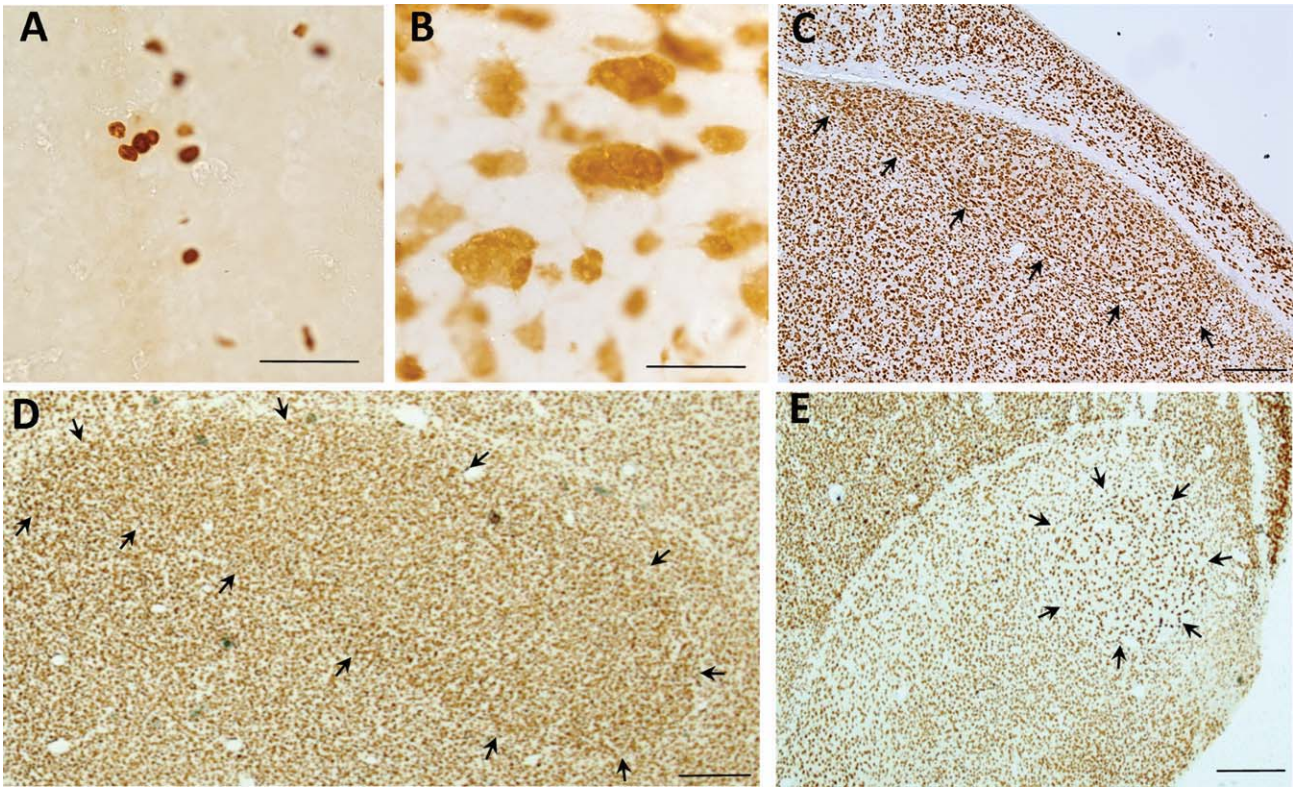

Figure 3. Representative immunocytochemical staining for BrdU and NeuN. **A:** $BrdU^+$ cells in the lateral ventricular zone (VZ). **B:** $NeuN^+$ cells in HVC. **C:** $NeuN^+$ cells in HVC; the dorsal edge of HVC is defined by the lateral ventricle and the ventral edge is indicated by arrows. Dorsal is upward, and medial is to the left. **D:** $NeuN^+$ cells in area X; perimeter is indicated by arrows. **E:** $NeuN^+$ cells in RA, perimeter is indicated by arrows. Scale bars = 25 μm in A,B; 250 μm in C–E.

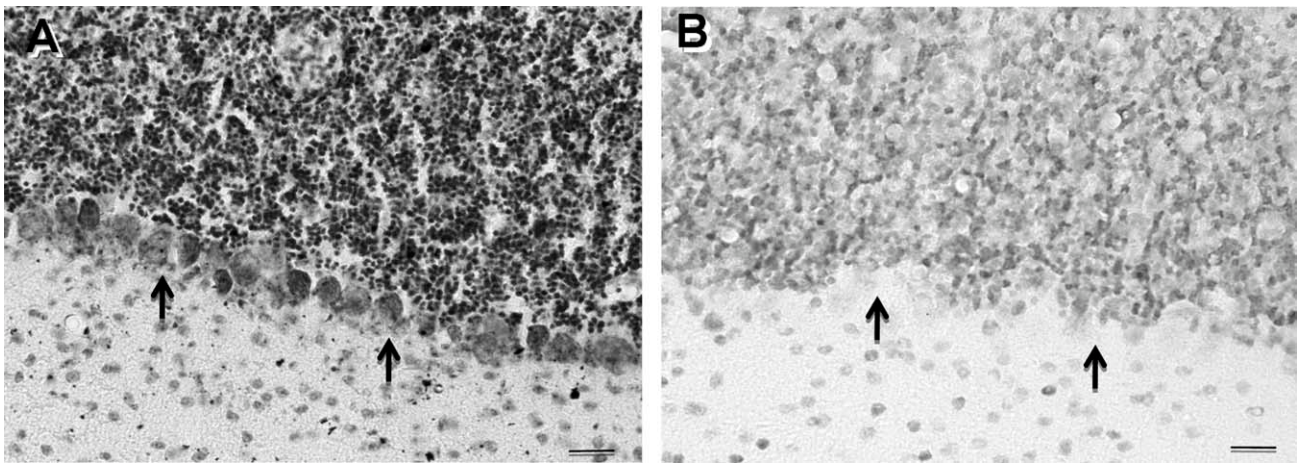

Figure 4. Comparison of Nissl (thionin) staining (**A**) with NeuN immunoreactivity (**B**) in the cerebellum. Although NeuN is specific to neurons, NeuN is known to be absent in some neuron types, such as cerebellar Purkinje neurons (Mullen et al., 1992). The lack of NeuN immunoreactivity in song sparrow Purkinje neurons thus serves as a negative control and demonstrates the specificity of the NeuN antibody. Scale bars = 25 μm.

sparrows. Purkinje cells were also examined using Nissl staining. As expected, cerebellar Purkinje neurons were detectable in the Nissl-stained sections but were absent in the NeuN-stained sections (Fig. 4). As a second negative control, six sections of song sparrow intestine were processed for NeuN, and staining was absent in these

sections. As a third negative control, 20 brain sections from different subjects were processed for NeuN staining with the primary antibody omitted; staining was absent in these sections. Note that, because a few neuronal cell types are not NeuN immunoreactive, we refer to "$NeuN^+$ cell number" rather than "neuron number."

Microscopy

All brain measurements were made by an observer blind to the treatment group. To obtain estimates of total telencephalon volume, slides with NeuN-labelled sections were scanned at 2,400 dpi into a computer using a high-resolution (2,400 dpi) flatbed scanner. The left and right telencephalon area was measured from every twelfth section in NIH ImageJ software (Wayne Rasband, National Institutes of Health, Bethesda, MD). Telencephalon volume was calculated by multiplying the area of each section by the sampling interval (360 μm). We also used NeuN immunoreactivity to measure the volume of three nuclei in the song control system: HVC, RA and area X. HVC is critical for both song production and learning and projects to both RA and area X. RA is primarily involved in song production, and area X is involved in song learning and auditory feedback. Finally, NeuN immunoreactivity was used to measure hippocampus volume, as in Hoshooley and Sherry (2004). We captured images from at least 10 sections per subject per brain region, with the distance between sections dependent on the size of the regions of interest. We captured images from every NeuN-labelled section containing the HVC, RA, and area X (90 μm apart, ~12 sections per subject) and from every twelfth section containing hippocampus (360 μm apart, ~20 sections per subject) using a Leica Digital CCD camera mounted on a Leica DM5000 B light microscope (Leica Microsystems Inc., Richmond Hill, Ontario, Canada) through a ×10 objective lens. Leica Application Suite software was used to make area measurements of HVC, RA, area X, and hippocampus in one hemisphere; volume was calculated by multiplying the area of each brain region in each section by the sampling interval (90 μm or 360 μm). Values were then multiplied × 2 to obtain an estimate of total volume (both hemispheres). Song nuclei, hippocampus, and telencephalon volumes do not differ between the left and right hemispheres (Tramontin et al., 2000; Pravosudov et al., 2006).

To estimate the number of HVC neurons (i.e., $NeuN^+$ cell number in HVC), we used the stereological optical fractionator method (Glaser et al., 2007) on sections labelled for NeuN. $NeuN^+$ cells were counted in HVC, because HVC is known to be sensitive to both androgens and glucocorticoids (see, e.g., Soma et al., 2002; Buchanan et al., 2004). In one hemisphere, $NeuN^+$ cells were counted in every ninth section that contained HVC (i.e., 270 μm apart; n = ~10 sections per subject). With a ×20 objective, we used the Leica Application Suite software driving a motorized stage to cover HVC with a 270 × 270-μm sampling grid. Then, under a ×63 oil objective, the section was moved to the first square in the sampling grid. At each point in the sampling grid (270 μm apart), the counting frame was 30 × 30 μm. Moreover, we measured the thickness of the tissue section by focusing on its top and bottom edges in three counting frames per section. We did not count cells in the top and bottom 1 μm of the section (guard zones) except in a few cases where the section was ≤7 μm thick, in which case we counted all of the cells. Because of shrinkage during processing, the section thickness was 7.7 ± 0.3 μm (range 6–11 μm). We focused through the section by 1-μm intervals and counted all of the cells that came into focus within the 5 μm- × 30-μm^2 section. We confirmed that the NeuN stain completely penetrated the 30-μm tissue section. In three subjects from each treatment group, we counted the number of separate $NeuN^+$ cells that came into focus at each 1-μm interval in 10 counting frames per subject (n = 120 frames of view). There was no statistically significant effect of section depth or treatment group on $NeuN^+$ cell number (data not shown). The total population of cells in one-half of HVC was then estimated using the following formula:

$$\text{Total population} = n \times (1/ssf) \times (1/asf) \times (1/hsf),$$

where n = total number of cells counted, ssf = section sampling frame (i.e., 1/9, because we counted every ninth section), asf = area sectioning frame (30 μm^2/270 μm^2), and hsf = optical dissector height (5 μm)/tissue section thickness. The total was multiplied by 2 to obtain an estimate of total HVC $NeuN^+$ cell number (both hemispheres).

To estimate the number of $BrdU^+$ cells in HVC, in the hippocampus, along the VZ, and in the telencephalon (not including HVC and hippocampus), $BrdU^+$ cells were counted exhaustively in one hemisphere of the brain. Exhaustive sampling was used rather than a stereological approach because $BrdU^+$ cells were relatively infrequent. In such cases, exhaustive sampling is more accurate than sampling from a small subset of sections (Hoshooley and Sherry, 2004). For all four regions, $BrdU^+$ cells were counted in all sections containing HVC (90 μm apart). In the rest of the brain (rostral and caudal to HVC), $BrdU^+$ cells were counted in every twelfth section (360 μm apart). The number of counted $BrdU^+$ cells was then multiplied by the section interval (i.e., 12 or 3) and subsequently multiplied by 2 to obtain an estimate for both hemispheres. Also, we checked for an effect of corticosterone and DHEA treatments on the size of $BrdU^+$ cell nuclei for a subset of 400 $BrdU^+$ cells along the VZ

(n = 50/treatment group) and in the telencephalon (n = 50/treatment group). There was no effect of treatment on $BrdU^+$ cell nucleus diameter along the VZ (control: 5.49 ± 0.11 μm; corticosterone: 5.84 ± 0.14 μm; DHEA: 5.85 ± 0.13 μm; corticosterone + DHEA: 5.89 ± 0.15 μm; one-factor ANOVA: $F_{3,199} = 1.93$, $P = 0.13$) or in the telencephalon (control: 5.53 ± 0.13 μm; corticosterone: 5.56 ± 0.11 μm; DHEA: 5.50 ± 0.11 μm; corticosterone + DHEA: 5.77 ± 0.13 μm: one-factor ANOVA: $F_{3,199} = 1.01$, $P = 0.39$). Thus, $BrdU^+$ profile size did not differ systematically across groups. For both NeuN and BrdU photomicrographs, images were adjusted for contrast and brightness in Leica Application Suite software.

Statistical analysis

In experiment 1, in vitro results were analyzed using one-factor ANOVAs at each time (days 3, 7, 21, 28) to analyze the effect of the number of needle holes on corticosterone levels in media. As a result of small sample sizes for the in vivo validation, the effect of corticosterone implants on plasma corticosterone levels was analyzed using Welch's *t*-tests. The effects of acute restraint stress on days 7 and 21 were calculated using difference scores (stressed − baseline). The effect of corticosterone treatment on the difference scores was analyzed on days 7 and 21 by using Welch's *t*-tests. Area under the curve was calculated with the trapezoid rule (Breuner et al., 1999; Cockrem et al., 2008) to estimate the total integrated amount of corticosterone present in the plasma, and a Welch's *t*-test was used to test the effect of corticosterone treatment on integrated corticosterone levels.

In experiment 2, the effects of corticosterone and DHEA treatments on telencephalon volume, telencephalon $BrdU^+$ cell number, HVC volume, HVC $NeuN^+$ cell number, HVC $BrdU^+$ cell number, RA volume, area X volume, hippocampus volume, hippocampus $BrdU^+$ cell number, VZ $BrdU^+$ cell number, brain mass, syrinx mass, and spleen mass were assessed using two-factor ANOVAs. We tested for main effects of corticosterone treatment and DHEA treatment, as well as an interaction between corticosterone and DHEA treatments. Because each factor in the ANOVA had only two levels, significant main effects without a significant interaction were interpreted without further post hoc analysis. However, significant interactions were explored by comparing groups using Tukey's honestly significant difference (HSD) post hoc tests. The effects of corticosterone and DHEA treatment on body mass and fat score were also evaluated using two-factor ANOVA on each measurement day (days 7 and 21). The effects of steroid treatments and treatment duration on plasma corticosterone and DHEA levels were analyzed by using two-factor mixed-design ANOVAs in experiment 2, where treatment day was a within-subjects factor and treatment group was a between-subjects factor. A two-factor mixed-design ANOVA was used to analyze the effect of acute restraint stress on plasma steroid levels, where stress was a within-subjects factor and treatment group was a between-subjects factor. The effects of 30 minutes of acute restraint stress were measured on day 7 and day 21.

For all experiments, data were log transformed if necessary to achieve normality and homogeneity of variance. All data were analyzed with JMP 7 (SAS, Cary, NC). Data are presented as mean ± SEM.

RESULTS

Steroid levels

In vitro corticosterone levels

In experiment 1A, corticosterone-filled silastic implants continued to release corticosterone over the course of 28 days in vitro. A serial dilution of media samples revealed that the samples required a 1:100,000 dilution (0.0005 μl per RIA tube) prior to analysis. Corticosterone levels in medium containing empty silastic implants for 3 days (n = 5) or 28 days (n = 5) were below detection (<3.12 pg/tube). Corticosterone levels in medium containing corticosterone implants were extremely high, even when the corticosterone implants had no needle holes (Supp. Info. Fig. 1). On days 3 and 7, there was no significant effect of number of needle holes (day 3: $F_{3,19} = 2.18$, $P = 0.13$; day 7: $F_{3,19} = 0.29$, $P = 0.83$). On day 21, when implants had been incubating for the longest period of time (14 days), there was a significant effect of needle hole number ($F_{3,19} = 6.52$, $P = 0.005$), and post hoc tests revealed that corticosterone levels were lowest in media containing corticosterone implants with zero holes. On day 28, there was no significant effect of needle hole number ($F_{3,19} = 2.56$, $P = 0.10$).

Baseline plasma corticosterone levels

To validate corticosterone implants in vivo in experiment 1B, we started collecting plasma on day 1 (Fig. 1A), and baseline plasma corticosterone levels were significantly higher in the corticosterone group than in the control group on days 1 and 2 and tended to be higher on day 3 (Fig. 2A). Baseline plasma corticosterone levels were not significantly different on subsequent days. Furthermore, the integrated amount of corticosterone was significantly higher in the corticosterone group (Welch's *t*-test: $t = 3.85$, $P = 0.02$; Fig. 2B, Table 1). In experiment 2, the first measurement of baseline plasma corticosterone after implantation was on day 7 (Fig. 1B) to avoid stressing the subjects soon after BrdU administration.

Baseline plasma corticosterone levels (Table 2) were not significantly affected by treatment ($F_{3,138} = 0.99$, $P = 0.40$) or sampling day ($F_{3,138} = 1.87$, $P = 0.14$), and there was no interaction ($F_{9,138} = 1.25$, $P = 0.27$).

TABLE 1.

Effects of Corticosterone and DHEA Treatments on NeuN Measurements and $BrdU^+$ Cell Number[1]

Two-factor ANOVA	df	F ratio	P
Tel volume			
Corticosterone	1,30	1.16	0.29
DHEA	1,30	0.003	0.96
Corticosterone × DHEA	1,30	0.06	0.82
Tel $BrdU^+$ cell number			
Corticosterone	1,26	0.72	0.41
DHEA	1,26	4.36	0.05
Corticosterone × DHEA	1,26	6.50	0.02
HVC volume			
Corticosterone	1,31	7.95	0.008
DHEA	1,31	12.26	0.002
Corticosterone × DHEA	1,31	0.001	0.97
HVC NeuN+ cell number			
Corticosterone	1,31	6.14	0.02
DHEA	1,31	15.69	0.0005
Corticosterone × DHEA	1,31	1.32	0.26
HVC $BrdU^+$ cell number			
Corticosterone	1,25	5.80	0.02
DHEA	1,25	13.00	0.002
Corticosterone × DHEA	1,25	2.49	0.13
RA volume			
Corticosterone	1,29	6.74	0.02
DHEA	1,29	20.58	0.0001
Corticosterone × DHEA	1,29	4.79	0.04
Area X volume			
Corticosterone	1,24	3.33	0.09
DHEA	1,24	2.35	0.14
Corticosterone × DHEA	1,24	0.31	0.59
Hp volume			
Corticosterone	1,28	1.07	0.31
DHEA	1,28	0.32	0.58
Corticosterone × DHEA	1,28	0.06	0.81
Hp $BrdU^+$ cell number			
Corticosterone	1,26	3.29	0.08
DHEA	1,26	5.90	0.02
Corticosterone × DHEA	1,26	4.61	0.04
VZ $BrdU^+$ cell number			
Corticosterone	1,26	1.42	0.25
DHEA	1,26	7.99	0.01
Corticosterone × DHEA	1,26	18.05	0.0003

[1]Tel, telencephalon; RA, robust nucleus of arcopallium; Hp, hippocampus; VZ, lateral ventricular zone.

Baseline plasma DHEA levels

DHEA treatment significantly increased plasma DHEA levels throughout the course of experiment 2 (DHEA was not measured in experiment 1). For baseline plasma DHEA levels (Table 3), there was a significant interaction between treatment and sampling day ($F_{9,138} = 3.46$, $P = 0.0008$). Post hoc tests revealed that plasma DHEA levels were higher in the DHEA and corticosterone + DHEA groups on days 7, 21, and 28, relative to control and corticosterone groups. In the DHEA-treated groups, plasma DHEA levels remained within the physiological range for this song sparrow population, insofar as baseline plasma DHEA levels before implantation ranged from 1.2 to 17 ng/ml.

Stressed plasma corticosterone levels

In experiments 1B and 2, the effect of acute restraint stress was measured on day 7 and day 21. In experiment 1B on day 7, the stress-induced increase in plasma corticosterone levels was significantly greater in controls than in the corticosterone group (stress – baseline corticosterone levels: control group = +171.1 ± 38.5 ng/ml; corticosterone group = −8.9 ± 4.6 ng/ml; $t = 3.14$, $P = 0.04$). Similarly, on day 21, the stress-induced increase in plasma corticosterone levels was significantly greater in controls than in the corticosterone group (stress – baseline corticosterone levels: control group = +23.4 ± 6.1 ng/ml; corticosterone group = −10.0 ± 10.4 ng/ml; $t = 2.59$, $P = 0.05$). In experiment 2 on day 7, there was a significant interaction between stress and treatment ($F_{3,61} = 4.50$, $P = 0.007$). Post hoc tests revealed that acute restraint stress increased plasma corticosterone levels in all groups except the corticosterone group. In experiment 2 on day 21, there was also significant interaction between stress and treatment ($F_{3,67} = 2.96$, $P = 0.04$). Post hoc tests revealed that acute restraint stress increased plasma corticosterone levels in all groups and that stressed plasma corticosterone levels were greater in the controls than in the corticosterone group.

Stressed plasma DHEA levels

There was no main effect of stress on DHEA levels on day 7 and day 21 and no interaction with treatment;

TABLE 2.

Effects of Corticosterone and DHEA Treatments on Plasma Corticosterone Levels (ng/ml; Experiment 2)

	Treatment day					
Treatment group	Day −2 (baseline)	Day 7 (baseline)	Day 7 (stressed)	Day 21 (baseline)	Day 21 (stressed)	Day 28 (baseline)
Control	6.4 ± 1.5	6.5 ± 1.7	58.1 ± 28.3	6.7 ± 1.8	74.0 ± 24.9	6.8 ± 1.5
Corticosterone	6.3 ± 0.9	11.2 ± 6.7	18.7 ± 1.6	6.7 ± 1.0	23.3 ± 5.0	4.5 ± 0.8
DHEA	7.9 ± 2.3	9.3 ± 1.5	36.7 ± 4.7	6.7 ± 0.6	33.7 ± 4.0	5.6 ± 0.9
Corticosterone + DHEA	7.2 ± 1.5	7.1 ± 1.6	30.4 ± 4.5	7.6 ± 1.9	27.8 ± 4.6	8.7 ± 2.3

TABLE 3.

Effects of Corticosterone and DHEA Treatments on Plasma DHEA Levels (ng/ml; Experiment 2)

Treatment group	Treatment day					
	Day −2 (baseline)	Day 7 (baseline)	Day 7 (stressed)	Day 21 (baseline)	Day 21 (stressed)	Day 28 (baseline)
Control	3.4 ± 1.3	1.4 ± 0.5	1.0 ± 0.5	2.6 ± 1.3	0.8 ± 0.3	1.0 ± 0.3
Corticosterone	2.2 ± 1.1	1.4 ± 0.3	0.7 ± 0.3	1.1 ± 0.2	0.7 ± 0.1	2.6 ± 1.8
DHEA	2.1 ± 1.3	8.7 ± 1.8	12.6 ± 1.8	10.2 ± 1.9	11.0 ± 2.5	7.6 ± 1.5
Corticosterone + DHEA	3.8 ± 1.2	12.9 ± 1.9	13.5 ± 2.5	15.4 ± 3.7	20.6 ± 5.5	10.3 ± 3.1

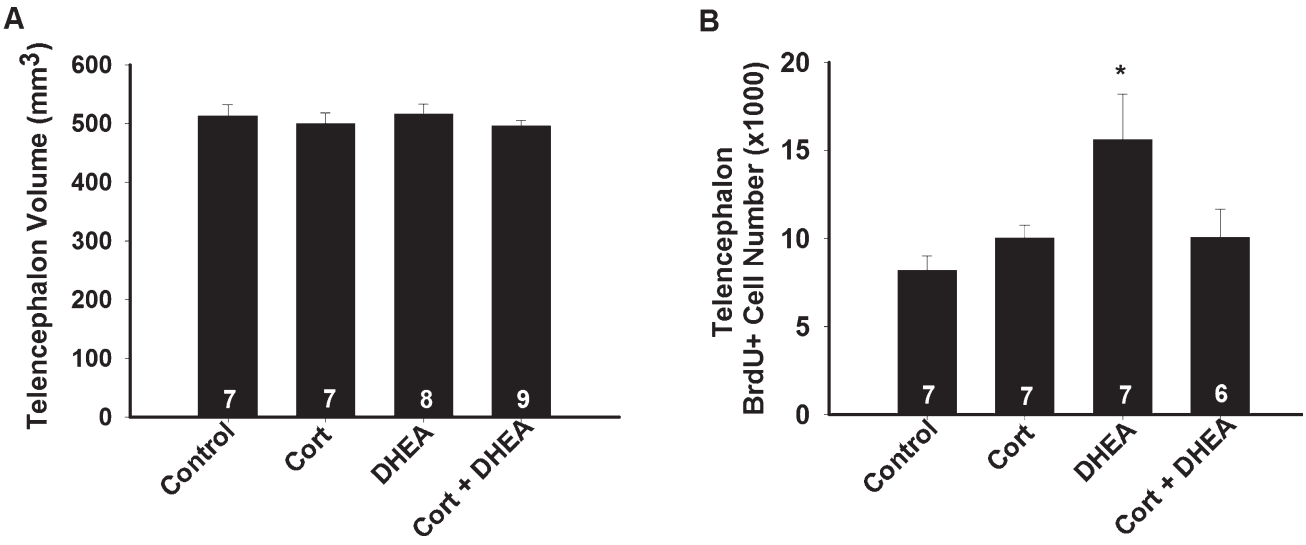

Figure 5. Effects of corticosterone (Cort) and DHEA treatments on telencephalon volume (A) and $BrdU^+$ cell number in the telencephalon (excluding HVC, hippocampus and VZ; B). Numbers in bars indicate sample sizes, and the asterisk indicates a significant difference from all other treatment groups.

however, there was a main effect of treatment (day 7: $F_{3,67} = 51.29$, $P < 0.0001$; day 21: $F_{3,67} = 24.96$, $P < 0.0001$). Post hoc tests revealed that, on both days, plasma DHEA levels were greater in the DHEA and corticosterone + DHEA groups compared with the control and corticosterone groups.

Neural attributes

Telencephalon

For the volume of the entire telencephalon, there were no main effects of corticosterone treatment or DHEA treatment and no interaction (Table 1, Fig. 5A). Furthermore, neither corticosterone nor DHEA treatment affected brain mass, and there was no interaction (data not shown). For telencephalon $BrdU^+$ cell number, there was no main effect of corticosterone, but there was a main effect of DHEA and a significant interaction effect. $BrdU^+$ cells were more abundant in the telencephalon of the DHEA group than in all other groups (Tukey's HSD, $P < 0.05$; Fig. 5B). DHEA treatment increased recruitment of new cells into the telencephalon, but only in the absence of corticosterone treatment. Stated another way, corticosterone treatment had no effect on recruitment of new cells into the telencephalon, except in the presence of DHEA treatment.

HVC

Corticosterone and DHEA treatment had significant effects on HVC (Table 1). For HVC volume, HVC $NeuN^+$ cell number, and HVC $BrdU^+$ cell number, there were main effects of both corticosterone and DHEA treatment, but there was no interaction effect. Corticosterone significantly decreased, whereas DHEA significantly increased, HVC volume, HVC $NeuN^+$ cell number, and HVC $BrdU^+$ cell number (Fig. 6).

RA

For RA volume, there were main effects of both corticosterone and DHEA treatment but also a significant interaction effect between corticosterone and DHEA treatment (Table 1). RA volume was greatest in the DHEA

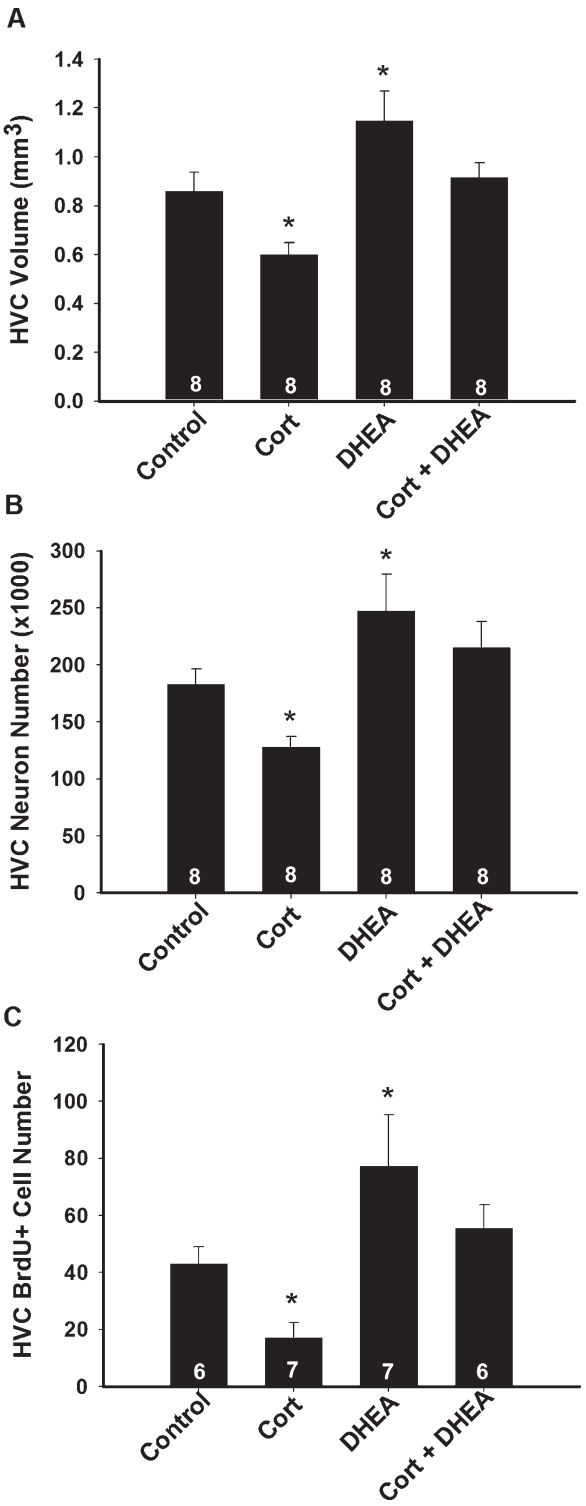

Figure 6. Effects of corticosterone (Cort) and DHEA treatments on HVC volume (**A**), HVC NeuN$^+$ cell number (**B**), and HVC BrdU$^+$ cell number (**C**). Data were analyzed using a two-factor ANOVA with two levels (presence or absence of corticosterone and DHEA); asterisks indicate significant main effects of corticosterone and DHEA. Numbers in bars indicate sample sizes.

group compared with all other groups (Tukey's HSD, $P < 0.05$; Fig. 7A). Thus, DHEA treatment increased RA volume, but only in the absence of corticosterone treatment.

Area X

For area X volume, there were no main effects of corticosterone or DHEA treatment and no interaction effect (Table 1, Fig. 7B).

Hippocampus

For hippocampus volume, there were no main effects of corticosterone or DHEA and no interaction effect (Table 1, Fig. 8A). For hippocampal BrdU$^+$ cell number, there was no main effect of corticosterone, but there was a main effect of DHEA and a significant interaction effect (Table 1, Fig. 8B). As in the telencephalon, BrdU$^+$ cells were more abundant in the hippocampus of the DHEA group than in all other groups (Tukey's HSD, $P < 0.05$).

Lateral VZ

For BrdU$^+$ cell number along the VZ, there was no main effect of corticosterone treatment but a significant main effect of DHEA treatment and a significant interaction (Table 1). BrdU$^+$ cells were more abundant along the VZ in the DHEA group (Tukey's HSD, $P < 0.05$; Fig. 9), as in the telencephalon and hippocampus.

Peripheral measures

To assess the physiological effects of corticosterone and DHEA treatment in experiment 2, we took several peripheral body measurements. For body mass on days 7 and 21, there were no main effects of corticosterone or DHEA treatments and no interaction (data not shown). For fat score on days 7 and 21, there was no main effect of corticosterone, but there was a main effect of DHEA, and there was no interaction. On both days 7 and 21, DHEA treatment decreased the fat score (as in Soma et al., 2002; data not shown). For spleen mass, there was a significant main effect of corticosterone treatment ($F_{1,35} = 4.63$, $P = 0.04$), where corticosterone significantly decreased spleen mass, but there was no main effect of DHEA ($F_{1,35} = 2.58$, $P = 0.12$) and no interaction ($F_{1,35} = 0.23$, $P = 0.64$). For syrinx mass, there was no significant effect of corticosterone treatment ($F_{1,35} = 1.10$, $P = 0.30$) and no interaction ($F_{1,35} = 0.01$, $P = 0.91$), but there was a significant main effect of DHEA ($F_{1,35} = 33.82$, $P \leq 0.0001$), where DHEA significantly increased syrinx mass (as in Soma et al., 2002).

DISCUSSION

This is the first study to report the effects of corticosterone on the adult song system and the opposing effects

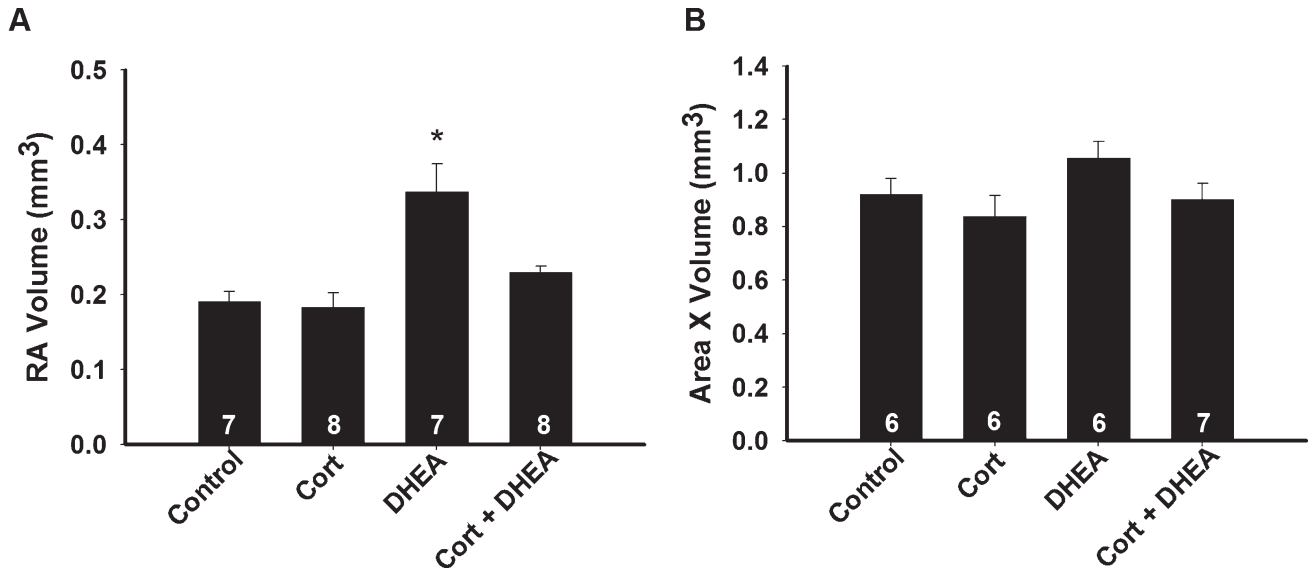

Figure 7. Effects of corticosterone (Cort) and DHEA treatments on RA (A) and area X (B) volume. Numbers in bars indicate sample size, and the asterisk indicates a significant difference from all other treatment groups.

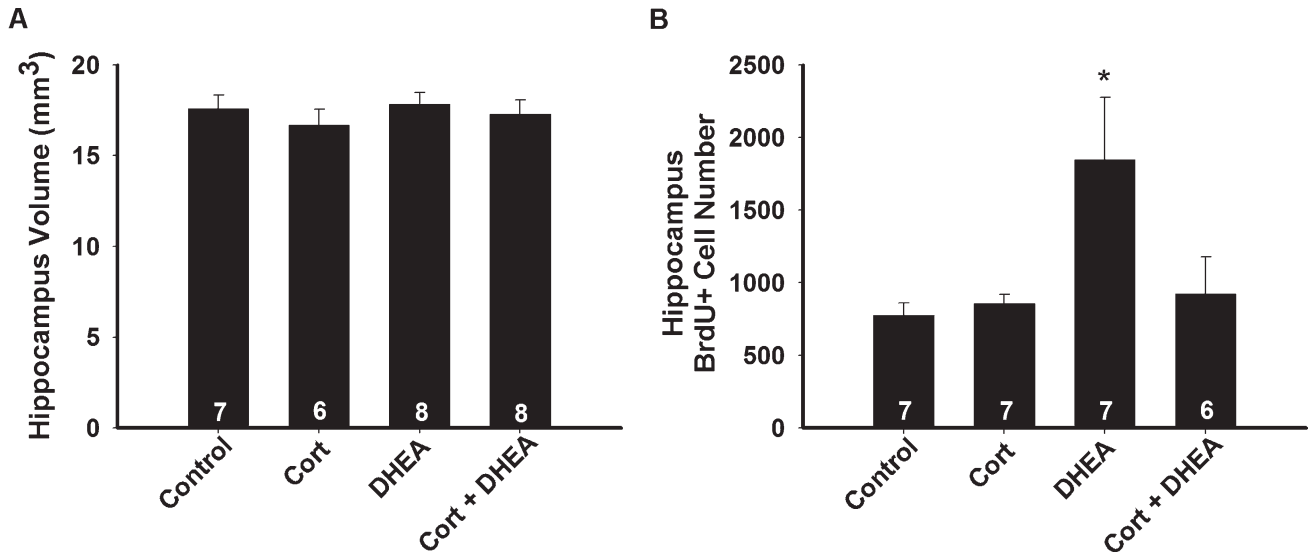

Figure 8. Effects of corticosterone (Cort) and DHEA on hippocampus volume (A) and hippocampal $BrdU^+$ cell number (B). Numbers in bars indicate sample sizes, and the asterisk indicates a significant difference from all other treatment groups.

of corticosterone and DHEA in the songbird brain. We demonstrate for the first time that a physiological, rather than pharmacological, DHEA dose can counteract the effects of chronic corticosterone treatment in the brain. Specifically, corticosterone treatment decreased, whereas DHEA treatment increased, HVC volume, $NeuN^+$ cell number, and $BrdU^+$ cell number. Corticosterone and DHEA had additive effects in HVC, and DHEA coadministration completely blocked the neurodegenerative effects of chronic corticosterone treatment. These data add to a growing literature on antiglucocorticoid neuroprotection. For example, recent evidence suggests that the neurodegenerative effects of stress and corticosterone on the adult brain can also be attenuated by estradiol treatment (Takuma et al., 2007) or voluntary exercise (Chang et al., 2008).

Plasma corticosterone levels

In recent avian studies, long-term corticosterone treatment via implants increased baseline plasma corticosterone levels only during treatment days 1–4 (Angelier et al.,

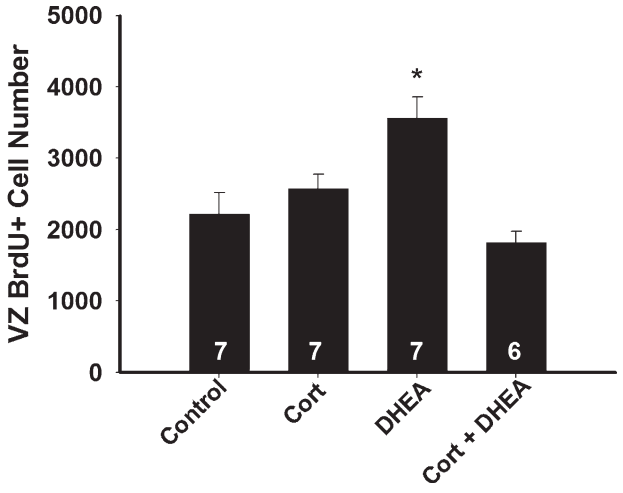

Figure 9. Effects of corticosterone (Cort) and DHEA on BrdU$^+$ cell number along the lateral ventricular zone (VZ). Numbers in bars indicate sample sizes, and the asterisk indicates a significant difference from all other treatment groups.

2007; Müller et al., 2009). Thus, in experiment 1, we examined the effects of corticosterone implants during days 1–4 and then on days 7, 21, and 28. Baseline plasma corticosterone levels were elevated in the corticosterone group on days 1 and 2, but, by day 4, corticosterone levels were elevated in both treatment groups, likely because of daily handling and blood collection. On day 7, corticosterone levels in both groups had declined, and baseline corticosterone levels remained low throughout the rest of the experiment. Transient increases in circulating corticosterone in response to corticosterone treatment are similar to those reported for corticosterone-implanted mice (Herrmann et al., 2009) and rats (Bodnoff et al., 1995) and for young rats given corticosterone in water (Enkel and Koch, 2009). By day 7, the corticosterone implants may have 1) stopped releasing corticosterone, 2) suppressed endogenous corticosterone secretion, and/or 3) increased metabolism or clearance of circulating corticosterone. Consistent with the idea that endogenous corticosterone secretion was suppressed, acute restraint stress on days 7 and 21 had less or no effect on plasma corticosterone levels in the corticosterone group. Similarly, in kestrels (*Falco tinnunculus*), acute restraint stress did not increase plasma corticosterone levels after 8 days of corticosterone treatment (Müller et al., 2009). Furthermore, the results from experiment 1 suggest that, in vitro, the corticosterone implants did not stop releasing corticosterone during the 28 days of treatment. In fact, corticosterone levels in the media were extremely high, even during the last 7 days of incubation in fresh medium (equivalent to days 21–28 in vivo). Although it is possible that, in vivo, the corticosterone implants are encapsulated and isolated from blood vessels, the same would presumably be true for the DHEA implants, which were directly adjacent to, and in contact with, the corticosterone implants in the CORT + DHEA group; however, DHEA levels in this group were high throughout the treatment period.

Corticosterone and DHEA effects on neural morphometry and NeuN$^+$ cell number

Corticosterone treatment decreased HVC volume and HVC NeuN$^+$ cell number by ~30%. In contrast, compared with controls, corticosterone did not affect RA, area X, hippocampus, or overall telencephalon volumes. These results suggest that HVC is particularly sensitive to glucocorticoid exposure in adult songbirds. Zebra finches treated with corticosterone during early development have reduced HVC volume but unaffected RA volume in adulthood (Buchanan et al., 2004). Moreover, stressors during early development, such as parasite infection and food restriction, result in reduced HVC volume in adults (Spencer et al., 2005) and fledglings (MacDonald et al., 2006). How corticosterone affects HVC volume remains unclear. Glucocorticoid receptors are present in the songbird brain (Breuner and Orchinik, 2001; Hodgson et al., 2007; Katz et al., 2008), but whether they are expressed in HVC is unknown. These effects of corticosterone on HVC are similar to the effects of corticosterone on the rat dentate gyrus, where corticosterone decreases neuron survival (Sapolsky et al., 1985; Karishma and Herbert, 2002).

DHEA treatment increased HVC volume and NeuN$^+$ cell number by ~35%, similar to Soma et al. (2002). Although there is no known classical intracellular steroid receptor for DHEA (Widstrom and Dillon, 2004), DHEA is converted to androgens and estrogens in the songbird brain (Soma et al., 2004a,b; Schlinger et al., 2008; Pradhan et al., 2010), and these potent metabolites of DHEA can bind to receptors in HVC (Soma et al., 1999; Ball et al., 2003). Testosterone or estradiol treatment increases HVC volume in song sparrows and white-crowned sparrows (Tramontin et al., 2003; Soma et al., 2004a,b), and estradiol treatment increases HVC neuron number in canaries (*Serinus canaria*; Hidalgo et al., 1995). Also, HVC volume and neuron number show dramatic seasonal changes in adult song sparrows and are maximal during the breeding season, when plasma levels of testosterone and estradiol are also maximal (Smith et al., 1997; Soma et al., 1999). DHEA treatment also increased RA volume by ~77%, but did not affect area X or hippocampus volumes. The effect of DHEA on RA differs from that described by Soma et al. (2002), in which treatment for only 14 days did not significantly increase RA; however, there was a trend in the same direction.

Androgen receptors are highly expressed in HVC and RA, with lower levels of expression in area X and hippocampus (Soma et al., 1999; Kim et al., 2004). Therefore, DHEA might affect HVC and RA directly, or changes in HVC might have downstream effects on RA (Brenowitz, 2008). Furthermore, HVC also expresses high levels of estrogen receptors in some songbird species (Bernard et al., 1999). Thus, regional differences in DHEA metabolism and steroid receptors may govern the regional specificity of DHEA effects.

In HVC, corticosterone and DHEA treatments had independent additive effects, rather than interactive effects. Corticosterone and DHEA may act via separate mechanisms or converge on the same mechanism. For example, corticosterone and DHEA could regulate neurotrophic factors. Brain-derived neurotrophic factor (BDNF) increases neuron survival in HVC (Rasika et al., 1994). In rat hippocampus, BDNF is decreased by corticosterone (Jacobsen and Mørk, 2006) but is not affected by DHEA (Gubba et al., 2004; Pinnock et al., 2009). Alternatively, corticosterone and DHEA may converge on the same mechanism, such as the phosphatidylinositol-3 kinase/Akt pathway. In cultured rat neurons, corticosterone reduces phosphorylation of Akt and increases apoptosis (Nitta et al., 2004), whereas DHEA increases phosphorylation of Akt and decreases apoptosis (Zhang et al., 2002).

Corticosterone and DHEA effects on $BrdU^+$ cell number

We also examined the number of new cells incorporated into the adult song control system and hippocampus using BrdU. In HVC, corticosterone decreased $BrdU^+$ cell number by ~60%, whereas DHEA increased $BrdU^+$ cell number by ~80%. These effects of corticosterone and DHEA are similar to, but more pronounced than, their effects on HVC volume and $NeuN^+$ cell number. Again, the effects of corticosterone and DHEA on $BrdU^+$ cell number in HVC were additive and could be mediated by mechanisms similar to those mentioned above.

In contrast to HVC, the effects of corticosterone and DHEA on $BrdU^+$ cell number in the hippocampus, along the VZ, and in the rest of the telencephalon were interactive, rather than additive. In these three regions, corticosterone treatment alone did not affect $BrdU^+$ cell number, but corticosterone coadministration completely prevented the DHEA-induced increase in $BrdU^+$ cell number. The effects of corticosterone treatment on cell proliferation in the brain are not consistent across species. Our results are consistent with data from chickadees, in which corticosterone treatment for 51 days did not affect hippocampal volume or hippocampal $BrdU^+$ cell number (Pravosudov and Omanska, 2005), even though corticosterone receptors are expressed in the songbird hippocampus (Hodgson et al., 2007). However, our results differ from those in zebra finch brain slices, in which, in vitro, corticosterone decreased $BrdU^+$ cell number along the VZ (Katz et al., 2008). Furthermore, corticosterone treatment reduces $BrdU^+$ cell number in the dentate gyrus of rats (Cameron and Gould, 1994; Wong and Herbert, 2004), although repeated acute restraint stress did not affect cell proliferation in the mouse dentate gyrus (Snyder et al., 2009). Thus, it is possible that there are species differences in the sensitivity to corticosterone in these brain areas. Alternatively, corticosterone treatment could alter the proportions of proliferating cells that mature into neurons, thus altering the phenotype of new cells in these brain areas without affecting the total number of new cells.

In the absence of exogenous corticosterone, DHEA might increase $BrdU^+$ cell number by decreasing local levels of corticosterone via modulation of 11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes 1 and 2. 11β-HSD1 regenerates active glucocorticoids from inactive metabolites, whereas 11β-HSD2 catalyzes the inactivation of glucocorticoids. Recent evidence suggests that 11β-HSD2 is expressed in the developing songbird brain (Katz et al., 2010). 11β-HSD1-mediated synthesis of local glucocorticoids is reduced by 7-hydroxy-DHEA derivatives, because these DHEA derivatives are the preferred substrates for 11β-HSD1 (Müller et al., 2006a,b). Also, in peripheral tissues, DHEA decreases expression of 11β-HSD1 (Apostolova et al., 2005) and increases the activity of 11β-HSD2 (Balazs et al., 2008). Alternatively, DHEA may act via the serotonergic system. In adult male rats, DHEA potentiates fluoxetine-induced cell proliferation in the dentate gyrus, and this effect is blocked by corticosterone treatment (Pinnock et al., 2009).

Corticosterone might block the effects of DHEA on $BrdU^+$ cell number by reducing DHEA metabolism or by inactivating DHEA metabolites. First, corticosterone might decrease DHEA metabolism by 3β-hydroxysteroid dehydrogenase (3β-HSD). In the zebra finch brain, acute corticosterone effects on cell proliferation were not affected by 3β-HSD inhibition (Katz et al., 2008), but chronic corticosterone treatment might decrease cell survival over the long term. Second, in rodents, expression of hydroxysteroid sulfotransferase 2, which converts free hydroxysteroids (e.g., estradiol) to their inactive sulfated forms, is induced by a synthetic glucocorticoid (Runge-Morris et al., 1999; Gong et al., 2008). Future studies should determine whether chronic corticosterone treatment alters DHEA metabolism or estrogen inactivation in the songbird brain.

DHEA as a native antiglucocorticoid

In conclusion, this study demonstrates region-specific effects of corticosterone and DHEA in the adult song sparrow brain. In HVC, corticosterone decreased, whereas DHEA increased, HVC volume, $NeuN^+$ cell number, and $BrdU^+$ cell number. If corticosterone-induced neurodegeneration in HVC, a critical part of the song control system, is associated with behavioral changes within and across seasons, then periods of prolonged stress during adulthood could substantially affect song production and, subsequently, fitness. DHEA treatment blocked the neurodegenerative effects of chronic corticosterone treatment in HVC. These data suggest that DHEA may be useful in the treatment of glucocorticoid-related neurodegeneration and stress-related psychiatric illness. DHEA is an endogenous steroidal prohormone that can be easily administered orally or percutaneously (Labrie et al., 1996), readily crosses the blood–brain barrier, and is well tolerated in humans (Maninger et al., 2009). The present data provide crucial experimental evidence that DHEA, at physiological concentrations, can act as a native antiglucocorticoid in the adult brain.

ACKNOWLEDGMENTS

We thank Dr. David Sherry for insight into microscopic analysis; Zachary Hall, Kelly Tse, and Lindsay Wittman for help with immunocytochemistry and microscopy; Drs. Liisa Galea and Joanne Weinberg for helpful comments on the manuscript; and Drs. Joanne Weinberg and Elizabeth Hampson for technical support.

LITERATURE CITED

Alvarez-Buylla A, Garcia-Verdugo JM. 2002. Neurogenesis in adult subventricular zone. J Neurosci 22:629–634.

Alvarez-Buylla A, Kirn JR. 1997. Birth, migration, incorporation, and death of vocal control neurons in adult songbirds. J Neurobiol 33:585–601.

Angelier F, Clément-Chastel C, Gabrielsen GW, Chastel O. 2007. Corticosterone and time–activity budget: an experiment with black-legged kittiwakes. Horm Behav 52:482–491.

Apostolova G, Schweizer RAS, Balazs Z, Kostadinova RM, Odermatt A. 2005. Dehydroepiandrosterone inhibits the amplification of glucocorticoid action in adipose tissue. Am J Physiol Endocrinol Metab 288:957–964.

Arvat E, DiVito L, Lanfranco F, Maccario M, Baffoni C, Rossetto R, Aimaretti G, Camanni F, Ghigo E. 2000. Stimulatory effect of adrenocorticotropin on cortisol, aldosterone, and dehydroepiandrosterone secretion in normal humans: dose–response study. J Clin Endocrinol Metab 85:3141–3146.

Astheimer LB, Buttemer WA, Wingfield JC. 2000. Corticosterone treatment has no effect on reproductive hormones or aggressive behavior in free-living male tree sparrows, *Spizella arborea*. Horm Behav 37:31–39.

Balazs Z, Schweizer RAS, Frey FJ, Rohner-Jeanrenaud F, Odermatt A. 2008. DHEA induces 11β-HSD2 by acting on CCAAT/enhancer-binding proteins. J Am Soc Nephrol 19: 92–101.

Ball GF, Riters LV, Balthazart J. 2002. Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones. Front Neuroendocrinol 23: 137–178.

Bernard DJ, Bentley GE, Balthazart J, Turek FW, Ball GF. 1999. Androgen receptor, estrogen receptor α, and estrogen receptor β show distinct patterns of expression in forebrain song control nuclei of European starlings. Endocrinology 140:4633–4643.

Bodnoff SR, Humphreys AG, Lehman JC, Diamond DM, Rose GM, Meaney MJ. 1995. Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. J Neurosci 15:61–69.

Brenowitz EA. 2008. Plasticity of the song control system in adult birds. In: Zeigler HP, Marler P, editors. Neuroscience of birdsong. Cambridge: Cambridge University Press. p 332–349.

Breuner CW, Hahn TP. 2003. Integrating stress physiology, environmental change, and behavior in free-living sparrows. Horm Behav 43:115–123.

Breuner CW, Orchinik M. 2001. Seasonal regulation of membrane and intracellular corticosteroid receptors in the house sparrow brain. J Neuroendocrinol 13:412–420.

Breuner CW, Wingfield JC, Romero LM. 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. J Exp Zool 284:334–342.

Buchanan KL, Leitner S, Spencer KA, Goldsmith AR, Catchpole CK. 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. Proc R Soc Lond B Biol Sci 271:2381–2386.

Cameron HA, Gould E. 1994. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. Neuroscience 61: 203–209.

Cameron HA, Woolley CS, McEwen BS, Gould E. 1993. Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. Neuroscience 56:337–344.

Carnahan J, Patel D, Miller J. 1994. Stem cell factor is a neurotrophic factor for neural crest-derived chick sensory neurons. J Neurosci 14:1433–1440.

Chang Y, Chen Y, Wu C, Yu L, Chen H, Jen CJ, Kuo Y. 2008. Glucocorticoid signaling and exercise-induced downregulation of the mineralocorticoid receptor in the induction of adult mouse dentate neurogenesis by treadmill running. Psychoneuroendocrinology 33:1173–1182.

Cockrem JF, Potter MA, Barrett DP, Candy EJ. 2008. Corticosterone responses to capture and restraint in emperor and adelie penguins in antarctica. Zool Sci 25:291–298.

Corpechot C, Robel P, Axelson M, Sjovall J, Baulieu E. 1981. Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. Proc Natl Acad Sci U S A 78: 4704–4707.

Enkel T, Koch M. 2009. Chronic corticosterone treatment impairs trace conditioning in rats with a neonatal medial prefrontal cortex lesion. Behav Brain Res 203:173–179.

Glaser J, Greene G, Hendricks S. 2007. Stereology for biological research: with a focus on neuroscience, 2nd ed. Williston, VT: MBF Press.

Goldman SA. 1998. Adult neurogenesis: from canaries to the clinic. J Neurobiol 36:267–286.

Gong H, Jarzynka MJ, Cole TJ, Lee JH, Wada T, Zhang B, Gao J, Song W, DeFranco DB, Cheng S, Xie W. 2008. Glucocorticoids antagonize estrogens by glucocorticoid receptor-mediated activation of estrogen sulfotransferase. Cancer Res 68:7386–7393.

Goodson JL, Evans AK, Soma KK. 2005a. Neural responses to aggressive challenge correlate with behavior in nonbreeding sparrows. Neuroreport 16:1719–1723.

Goodson JL, Saldanha CJ, Hahn TP, Soma KK. 2005b. Recent advances in behavioural neuroendocrinology: insights from studies on birds. Horm Behav 48:461–473.

Gould E, Cameron H, Daniels D, Woolley C, McEwen B. 1992. Adrenal hormones suppress cell division in the adult rat dentate gyrus. J Neurosci 12:3642–3650.

Gubba EM, Fawcett JW, Herbert J. 2004. The effects of corticosterone and dehydroepiandrosterone on neurotrophic factor mRNA expression in primary hippocampal and astrocyte cultures. Brain Res Mol Brain Res 127:48–59.

Hau M, Stoddard ST, Soma KK. 2004. Territorial aggression and hormones during the non-breeding season in a tropical bird. Horm Behav 45:40–49.

Herrmann M, Henneicke H, Street J, Modzelewski J, Kalak R, Buttgereit F, Dunstan CR, Zhou H, Seibel MJ. 2009. The challenge of continuous exogenous glucocorticoid administration in mice. Steroids 74:245–249.

Hidalgo A, Barami K, Iversen K, Goldman SA. 1995. Estrogens and non-estrogenic ovarian influences combine to promote the recruitment and decrease the turnover of new neurons in the adult female canary brain. J Neurobiol 27:470–487.

Hodgson ZG, Meddle SL, Roberts ML, Buchanan KL, Evans MR, Metzdorf R, Gahr M, Healy SD. 2007. Spatial ability is impaired and hippocampal mineralocorticoid receptor mRNA expression reduced in zebra finches (*Taeniopygia guttata*) selected for acute high corticosterone response to stress. Proc R Soc Lond B Biol Sci 274:239–245.

Hoshooley JS, Sherry DF. 2004. Neuron production, neuron number and structure size are seasonally stable in the hippocampus of the food-storing black-capped chickadee. Behav Neurosci 118:345–355.

Hoshooley JS, Sherry DF. 2007. Greater hippocampal neuronal recruitment in food-storing than in non-food-storing birds. Dev Neurobiol 67:406–414.

Hoshooley JS, Phillmore LS, Sherry DF, MacDougall-Shackleton SA. 2007. Annual cycle of the black-capped chickadee: seasonality of food-storing and the hippocampus. Brain Behav Evol 69:161–168.

Jacobsen JPR, Mørk A. 2006. Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. Brain Res 1110:221–225.

Joels M, Karst H, Krugers HJ, Lucassen PJ. 2007. Chronic stress: implications for neuronal morphology, function and neurogenesis. Front Neuroendocrinol 28:72–96.

Kalimi M, Shafagoj Y, Loria R, Padgett D, Regelson W. 1994. Anti-glucocorticoid effects of dehydroepiandrosterone (DHEA). Mol Cell Biochem 131:99–104.

Karishma KK, Herbert J. 2002. Dehydroepiandrosterone (DHEA) stimulates neurogenesis in the hippocampus of the rat, promotes survival of newly formed neurons and prevents corticosterone-induced suppression. Eur J Neurosci 16: 445–453.

Katz A, Mirzatoni A, Zhen Y, Schlinger BA. 2008. Sex differences in cell proliferation and glucocorticoid responsiveness in the zebra finch brain. Eur J Neurosci 28:99–106.

Katz A, Oyama RK, Feng N, Chen X, Schlinger BA. 2010. 11β-Hydroxysteroid dehydrogenase type 2 in zebra finch brain and peripheral tissues. Gen Comp Endocrinol 166:600–605.

Kim Y, Perlman WR, Arnold AP. 2004. Expression of androgen receptor mRNA in zebra finch song system: developmental regulation by estrogen. J Comp Neurol 469:535–547.

Kimonides VG, Spillantini MG, Sofroniew MV, Fawcett JW, Herbert J. 1999. Dehydroepiandrosterone antagonizes the neurotoxic effects of corticosterone and translocation of stress-activated protein kinase 3 in hippocampal primary cultures. Neuroscience 89:429–436.

Labrie C, Flamand M, Belanger A, Labrie F. 1996. High bioavailability of dehydroepiandrosterone administered percutaneously in the rat. J Endocrinol 150:S107–118.

Leuner B, Glasper ER, Gould E. 2009. Thymidine analog methods for studies of adult neurogenesis are not equally sensitive. J Comp Neurol 517:123–133.

MacDonald IF, Kempster B, Zanette L, MacDougall-Shackleton SA. 2006. Early nutritional stress impairs development in a song-control brain region in both male and female juvenile song sparrows (*Melospiza melodia*) at the onset of song learning. Proc R Soc Lond B Biol Sci 273:2559–2564.

Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. 2009. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Front Neuroendocrinol 30:65–91.

Martin LB II, Gilliam J, Han P, Lee K, Wikelski M. 2005. Corticosterone suppresses cutaneous immune function in temperate but not tropical house sparrows, *Passer domesticus*. Gen Comp Endocrinol 140:126–135.

McEwen BS. 2001. Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. Ann N Y Acad Sci 933:265–277.

Mullen RJ, Buck CR, Smith AM. 1992. NeuN, a neuronal specific nuclear protein in vertebrates. Development 116: 201–211.

Müller C, Hennebert O, Morfin R. 2006a. The native anti-glucocorticoid paradigm. J Steroid Biochem Mol Biol 100:95–105.

Müller C, Pompon D, Urban P, Morfin R. 2006b. Inter-conversion of 7α- and 7β-hydroxy-dehydroepiandrosterone by the human 11β-hydroxysteroid dehydrogenase type 1. J Steroid Biochem Mol Biol 99:215–222.

Müller C, Almasi B, Roulin A, Breuner CW, Jenni-Eiermann S, Jenni L. 2009. Effects of corticosterone pellets on baseline and stress-induced corticosterone and corticosteroid-binding-globulin. Gen Comp Endocrinol 160:59–66.

Newman AEM, Soma KK. 2009. Corticosterone and dehydroepiandrosterone in songbird plasma and brain: Effects of season and acute stress. Eur J Neurosci 29:1905–1914.

Newman AEM, Chin EH, Schmidt KL, Bond L, Wynne-Edwards KE, Soma KK. 2008a. Analysis of steroids in songbird plasma and brain by coupling solid phase extraction to radioimmunoassay. Gen Comp Endocrinol 155:503–510.

Newman AEM, Pradhan DS, Soma KK. 2008b. Dehydroepiandrosterone and corticosterone are regulated by season and acute stress in a wild songbird: jugular versus brachial plasma. Endocrinology 149:2537–2545.

Nitta A, Zhen WH, Quirion R. 2004. Insulin-like growth factor 1 prevents neuronal cell death induced by corticosterone through activation of the PI3k/Akt pathway. J Neurosci Res 76:98–103.

Nottebohm F. 1981. A brain for all seasons: cyclical anatomical changes in the song control nuclei of the canary brain. Science 214:1368–1370.

Nottebohm F. 2002. Neuronal replacement in adult brain. Brain Res Bull 57:737–749.

Oberbeck R, Dahlweid M, Koch R, van Griensven M, Emmendorfer A, Tscherne H, Pape HC. 2001. Dehydroepiandrosterone decreases mortality rate and improves cellular immune function during polymicrobial sepsis. Crit Care Med 29:380–384.

Phillmore LS, Hoshooley JS, Sherry DF, MacDougall-Shackleton SA. 2006. Annual cycle of the black-capped chickadee: seasonality of singing rates and vocal-control brain regions. J Neurobiol 66:1002–1010.

Pinnock SB, Lazic SE, Wong HT, Wong IHW, Herbert J. 2009. Synergistic effects of dehydroepiandrosterone and fluoxetine on proliferation of progenitor cells in the dentate gyrus of the adult male rat. Neuroscience 158:1644–1651.

Pradhan DS, Newman AEM, Wacker DW, Wingfield JC, Schlinger BA, Soma KK. 2010. Aggressive interactions rapidly increase androgen synthesis in the brain during the nonbreeding season. Horm Behav 57:381–389.

Pravosudov VV, Omanska A. 2005. Prolonged moderate elevation of corticosterone does not affect hippocampal anatomy or cell proliferation rates in mountain chickadees (*Poecile gambeli*). J Neurobiol 62:82–91.

Pravosudov VV, Kitaysky AS, Omanska A. 2006. The relationship between migratory behaviour, memory and the hippocampus: an intraspecific comparison. Proc R Soc Lond B Biol Sci 272:2641–2649.

Rasika S, Nottebohm F, Alvarez-Buylla A. 1994. Testosterone increases the recruitment and/or survival of new high vocal center neurons in adult female canaries. Proc Natl Acad Sci U S A 91:7854–7858.

Romero LM. 2006. Seasonal changes in hypothalamic-pituitary-adrenal axis sensitivity in free-living house sparrows (*Passer domesticus*). Gen Comp Endocrinol 149:66–71.

Runge-Morris M, Wu W, Kocarek TA. 1999. Regulation of rat hepatic hydroxysteroid sulfotransferase (SULT2–40/41) gene expression by glucocorticoids: Evidence for a dual mechanism of transcriptional control. Mol Pharmacol 56:1198–1206.

Sapolsky R, Krey L, McEwen B. 1985. Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. J Neurosci 5:1222–1227.

Schlinger BA, Pradhan DS, Soma KK. 2008. 3β-HSD activates DHEA in the songbird brain. Neurochem Int 52:611–620.

Shini S, Kaiser P, Shini A, Bryden WL. 2008. Biological response of chickens (*Gallus gallus domesticus*) induced by corticosterone and a bacterial endotoxin. Comp Biochem Physiol B Biochem Mol Biol 149:324–333.

Silverin B. 1998. Territorial behaviour and hormones of pied flycatchers in optimal and suboptimal habitats. Anim Behav 56:811–818.

Smith GT, Brenowitz EA, Beecher MD, Wingfield JC. 1997. Seasonal changes in testosterone, neural attributes of song control nuclei, and song structure in wild songbirds. J Neurosci 17:6001–6010.

Snyder JS, Glover LR, Sanzone KM, Kamhi JF, Cameron HA. 2009. The effects of exercise and stress on the survival and maturation of adult-generated granule cells. Hippocampus 19:898–906.

Soma KK, Wingfield JC. 2001. Dehydroepiandrosterone in songbird plasma: seasonal regulation and relationship to territorial aggression. Gen Comp Endocrinol 123:144–155.

Soma KK, Hartman VN, Wingfield JC, Brenowitz EA. 1999. Seasonal changes in androgen receptor immunoreactivity in the song nucleus HVc of a wild bird. J Comp Neurol 409:224–236.

Soma KK, Wissman AM, Brenowitz EA, Wingfield JC. 2002. Dehydroepiandrosterone (DHEA) increases territorial song and the size of an associated brain region in a male songbird. Horm Behav 41:203–212.

Soma KK, Tramontin AD, Featherstone J, Brenowitz EA. 2004a. Estrogen contributes to seasonal plasticity of the adult avian song control system. J Neurobiol 58:413–422.

Soma KK, Alday NA, Hau M, Schlinger BA. 2004b. Dehydroepiandrosterone metabolism by 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase in adult zebra finch brain: sex difference and rapid effect of stress. Endocrinology 145: 1668–1677.

Spencer KA, Buchanan KL, Leitner S, Goldsmith AR, Catchpole CK. 2005. Parasites affect song complexity and neural development in a songbird. Proc R Soc Lond B Biol Sci 272: 2037–2043.

Takuma K, Matsuo A, Himeno Y, Hoshina Y, Ohno Y, Funatsu Y, Arai S, Kamei H, Mizoguchi H, Nagai T, Koike K, Inoue M, Yamada K. 2007. 17β-Estradiol attenuates hippocampal neuronal loss and cognitive dysfunction induced by chronic restraint stress in ovariectomized rats. Neuroscience 146: 60–68.

Thompson CK, Brenowitz EA. 2009. Neurogenesis in an adult avian song nucleus is reduced by decreasing caspase-mediated apoptosis. J Neurosci 29:4586–4591.

Torres JM, Ortega E. 2003. DHEA, PREG and their sulphate derivatives on plasma and brain after CRH and ACTH administration. Neurochem Res 28:1187–1191.

Tramontin AD, Brenowitz EA. 1999. A field study of seasonal neuronal incorporation into the song control system of a songbird that lacks adult song learning. J Neurobiol 40: 316–326.

Tramontin AD, Brenowitz EA. 2000. Seasonal plasticity in the adult brain. Trends Neurosci 23:251–258.

Tramontin AD, Hartman VN, Brenowitz EA. 2000. Breeding conditions induce rapid and sequential growth in adult avian song control circuits: a model of seasonal plasticity in the brain. J Neurosci 20:854–861.

Tramontin AD, Wingfield JC, Brenowitz EA. 2003. Androgens and estrogens induce seasonal-like growth of song nuclei in the adult songbird brain. J Neurobiol 57:130–140.

Vanderlaan M, Watkins B, Thomas C, Dolbeare F, Stanker L. 1986. Improved high-affinity monoclonal antibody to iododeoxyuridine. Cytometry 7:499–507.

Wang N, Hurley P, Pytte C, Kirn JR. 2002. Vocal control neuron incorporation decreases with age in the adult zebra finch. J Neurosci 22:10864–10870.

Widstrom RL, Dillon JS. 2004. Is there a receptor for dehydroepiandrosterone or dehydroepiandrosterone sulfate? Semin Reprod Med 289–298.

Wong EYH, Herbert J. 2004. The corticoid environment: a determining factor for neural progenitors' survival in the adult hippocampus. Eur J Neurosci 20:2491–2498.

Zhang L, Li Bs, Ma W, Barker JL, Chang YH, Zhao W, Rubinow DR. 2002. Dehydroepiandrosterone (DHEA) and its sulfated derivative (DHEAS) regulate apoptosis during neurogenesis by triggering the akt signaling pathway in opposing ways. Brain Res Mol Brain Res 98:58–66.