

Neural aromatization accelerates the acquisition of spatial memory via an influence on the songbird hippocampus

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Abstract

Circulating estrogens affect the neural circuits that underlie learning and memory in several vertebrates via an influence on the hippocampus. In the songbird hippocampus local estrogen synthesis due to the abundant expression of aromatase may modulate hippocampal function including spatial memory performance. Here, we examined the effect of estradiol, testosterone, and dihydrotestosterone on the structure and function of the songbird hippocampus. Adult male zebra finches were castrated, implanted with one of these steroids or a blank implant, and trained on a spatial memory task. The rate of acquisition and overall performance on this task was recorded by direct observation. The size and density of cells in the hippocampus and its volume were measured. Estradiol-treated birds learned the task more rapidly than any other group. Although testosterone- and blank-implanted birds did learn the task, we found no evidence of learning in dihydrotestosterone-implanted subjects. Cells in the rostral hippocampus were larger in estradiol- and testosterone-treated birds relative to other groups. A corresponding decrease in the density of cells was apparent in estradiol-implanted subjects relative to all other groups. These data suggest that estradiol may accelerate the acquisition of a spatial memory task and increase the size of neurons in the rostral hippocampus. Since testosterone-mediated changes in acquisition and cell size were similar to those of estradiol, but not dihydrotestosterone, we conclude that neural aromatization of testosterone to estrogen is responsible for effects on the structure and function of the songbird hippocampus. © 2004 Elsevier Inc. All rights reserved.

Keywords: Estrogen; Testosterone; Aromatase; Zebra finch; Dihydrotestosterone; Synapse

Introduction

Estrogenic enhancement of memory performance has been reported in several vertebrates including humans (Sherwin, 1996), non-human primates (Bellino and Wise, 2003), and murine rodents (Gibbs, 1998; McEwen, 1994; Woolley et al., 1997). Enhancement of memory performance seems to occur via the potent activational effects of estradiol (E₂) on the hippocampus (HP). More specifically, in rodents, exogenous E₂ increases the number of dendritic spines on HP neurons and the number of synapses onto these dendritic spines (Murphy and Segal, 1996; Woolley and McEwen, 1994; Yankova et al., 2001). Corresponding

changes in HP neuronal function have also been observed including enhancements in synaptic transmission and spatial memory performance (Gibbs, 1998; Woolley et al., 1997). Thus, HP structure and function are sensitive to estrogens in several vertebrates.

In songbirds (order Passeriformes), in addition to peripheral sources, the brain itself synthesizes E₂ due to the abundant expression of aromatase (*estrogen-synthase*) (Schlinger and Arnold, 1991, 1992). Aromatase is especially enriched in the HP, a characteristic well conserved across every songbird species investigated (Fusani et al., 2000, 2001a,b; Metzdorf et al., 1999; Saldanha and Schlinger, 1997; Saldanha et al., 1998, 1999, 2000; Shen et al., 1994, 1995). Notably, HP aromatase is extremely low or undetectable in adult non-songbirds and adult mammals (Roselli and Resko, 1997; Saldanha et al., 1998). Presumably, HP aromatization of testosterone provides high levels of local E₂ available to maintain the integrity and function of HP circuits.

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Songbirds have long been used as excellent models for the study of learning and memory. Although much of this work involves song-learning (Marler, 1997), emerging experimental paradigms have exploited other ecologically relevant behaviors to reveal HP-associated spatial memory for the location of food in black-capped chickadees (*Poecile atricapilla*; Sherry and Vaccarino, 1989), dark-eyed juncos (*Junco hyemalis*; Hampton and Shettleworth, 1996) and zebra finches (Patel et al., 1997), the search for nest-sites in brown-headed cowbirds (*Molothrus ater*; Reboreda et al., 1996), and episodic-like memory in scrub jays (*Aphelocoma coerulescens*; Clayton and Dickinson, 1998; Emery and Clayton, 2001). Indeed, as in other vertebrates, the HP is necessary for spatial memory function in several songbirds (Hampton and Shettleworth, 1996; Patel et al., 1997; Sherry and Vaccarino, 1989), suggesting that mechanisms of HP-dependent memory function may be well conserved across several vertebrate classes.

In the zebra finch (*Taenopygia guttata*), aromatase is expressed at extremely high levels in the HP (Saldanha et al., 1998; Shen et al., 1994, 1995), suggesting that the role of neural E₂ synthesis on brain function may be well studied in these individuals. To test whether steroids such as E₂ play a role in regulating HP structure and function, we determined the effects of E₂, testosterone (T) and the non-aromatizable androgen-dihydrotestosterone (DHT) on: (a) performance on a spatial memory task and (b) HP volume, cell number, and cell size.

Materials and methods

All subjects were housed and treated under the University of California Chancellor's Animal Use Guidelines. Adult male zebra finches (ZFs) were deeply anesthetized (Equithesin) and castrated using previously published protocols (Arnold, 1975b). After removal of the testes, a 10-mm silastic implant containing either estradiol (E₂; Steraloids EO950-000; *N* = 6), testosterone (T; Steraloids A6950-000; *N* = 5), dihydrotestosterone (DHT; Steraloids A2570-000; *N* = 6) or nothing (B; *N* = 7) was dropped into the abdominal cavity. The incisions were closed with Collodion (Mallinckrodt Baker) and subjects allowed to recover for a period of 10 days. During the recovery period, subjects were housed individually under a 14:10 LD cycle. Temperature was maintained at 20°C, and food and water were available ad libitum.

Behavior acquisition of spatial memory

All subjects were trained to perform a spatial memory task before surgery as follows. Birds were housed individually and exposed to the test tray daily. The test tray consisted of seven compartments, all of which were open (no flaps) with the food clearly visible in one compartment. Over successive days, the empty compartments were cov-

ered with flaps beginning with one flap and ending with all six empty compartments covered. Finally, the compartment containing food was also covered and the bird was required to lift off the flaps to find the food.

Following post-surgical recovery, subjects were trained to perform a new spatial memory task, and all behavioral data were collected by an assistant who was blind to the experimental condition (treatment group) of the animal. Importantly, during this phase, the compartment that contained food was different from the training period. Each bird was tested individually in its home cage, once per day. After 2 h of food deprivation, the bird was presented with an experimental tray containing a unique pattern of seven compartments, one of which was filled with seed. Birds were trained to return to the one compartment that contained the seed hidden under one of the seven flaps that covered these compartments. The hidden food was placed in the same location on every trial (Patel et al., 1997). For each trial, we recorded the number of flaps that a bird removed. Each bird was given 5 min to find the food by removing flaps and then allowed to eat for 30 s. If birds search at random, they should lift four flaps on average to find the food, whereas if they use memory to solve the task, they should lift significantly fewer flaps. Memory performance was measured in terms of the number of flaps lifted to find the hidden food across the 20 days and binned into 5-day intervals (see Fig. 1).

Singing behavior was used as an independent measure of the efficacy of steroid implants and castrations. On day 21, all subjects were exposed to stimulus females and the number of song bouts was recorded over a 15-min interval. A song bout was operationally defined as a consecutive series of at least three songs initiated with an introductory series of chirps (see Marler, 1997) and preceded by at least 15 s of no singing.

Anatomy

Following exposure to stimulus females, all subjects were transcardially perfused with 5 ml 0.1 M phosphate buffer (PB) and 30 ml 4% paraformaldehyde (PF; pH = 7.35). At the time of perfusion, the abdominal cavity was opened and the implants visually inspected. Only birds in which the implant was found to yet contain crystalline steroid (except for blank implants) were used in the behavioral and histological analyses.

The brains were removed and post-fixed overnight at 4°C in PF, immersed in 10% sucrose, 20% sucrose, and 25% sucrose (overnight each at 4°C), and gel-embedded in 8% gelatin. The gel blocks were immersed in PF containing 25% sucrose for 48–72 h at 4°C. Coronal sections (50 µm) were cut on a freezing microtome and collected into 0.1 M PB. Every fifth section was mounted onto subbed glass slides, stained with 0.187% thionin, dehydrated, and coverslipped as previously described (Saldanha et al., 2000).

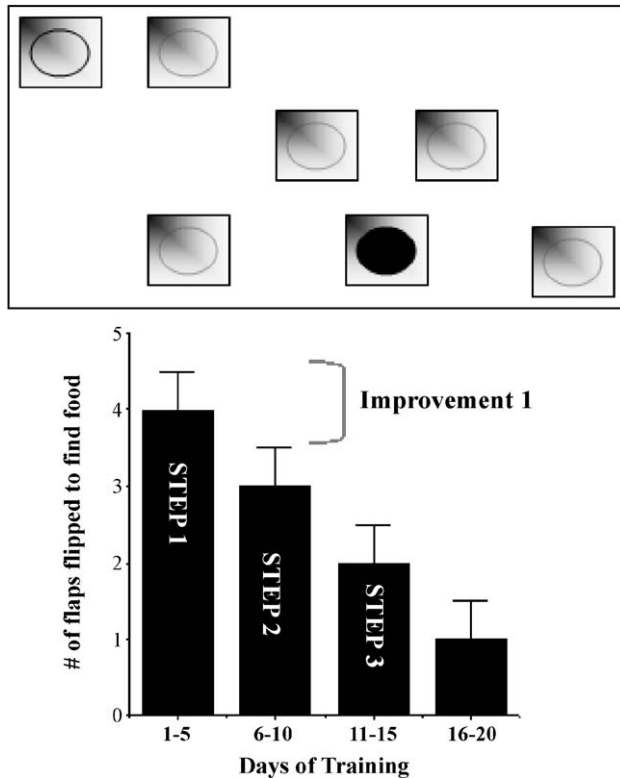


Fig. 1. Schematics depicting the spatial memory task (upper panel) and idealized learning (lower panel) in the current paradigm. Subjects were trained to remember the location of food (black circle) in a tray containing a subject-unique arrangement of seven holes (circles). All holes were covered with flaps (shaded squares) requiring a reliance on memory to locate the food. The number of flaps lifted to find the food is a reflection of memory performance. As shown in B, means over five successive trials were averaged and called “steps” or blocks. The difference between successive steps was called “improvement.”

Measurement of cell size and density

All microscopy and anatomical data collection were performed by an experimenter who was blind to the experimental group of the subjects.

We measured cellular characteristics of approximately 1000 cells per subject. Sections were viewed under a light microscope (Nikon EM 1000) and digitized images collected from the HP as follows. The zebra finch HP is about 4–5 mm in anteroposterior extent. Four to five coronal sections (1 mm apart) were sampled across the extent of the HP. At each of these levels, under high magnification (400 \times), digitized images of thionin-stained cells were collected from the ventral, dorsal, and lateral HP on each side of the brain (total of six pictures per coronal section per bird; Fig. 2). We believe these images comprehensively reflect the changing cytoarchitecture of the avian HP and simultaneously sample multiple areas of this structure by including dorsoventral, mediolateral, and rostrocaudal variations within the HP.

Using NIH Image 4.0, a 64,000- μm^2 sampling area was aligned over one of three portions of the HP in coronal

section. Specifically, we sampled the dorsomedial tip of the HP (dorsal), the ventromedial HP (including the large, principal cells), and the lateral HP. Care was taken so that no ventricular or medial ependymal walls were included in the image. Thus, the images contained exclusively neuropil. Corresponding images from the contralateral hemisphere of the HP were collected. Within each of these images, we measured the area (size) and density (number of cells within the 64,000 μm^2 frame) of individual soma in each image of the HP. To avoid overcounting, only cells with unequivocal, clear nuclei were counted and measured. The resultant data were averaged within each image and the area and density of HP cells were averaged across left and right. Thus, from six images in each of 4–5 rostrocaudal levels per subject, the final data yielded three measures (ventral, dorsal, and lateral) of cell size and density at each of four to five anteroposterior levels of the HP per bird.

Measurements of HP volume

Each coronal section of thionin-stained HP (250 μm apart) was digitized at low power (100 \times) and analyzed using NIH Image. The medial and dorsal edges of the HP are easily discernible as the midline and dorsal margin of the coronal section, respectively. The lateral edge of the HP is less obvious, but is visible based upon a shift in the density of Nissl-stained cell bodies (cells more dense immediately lateral to the border) and an inclusion in the dorsal edge of the parenchyma (see Fig. 2). The HP was outlined and the area of the entire HP was measured on the right and left sides of the brain. The area data was integrated through the third dimension into volume by multiplying it by 250 μm and adding successive sections.

Statistical analyses

Behavior

Measures of memory performance (number of flaps lifted to find the food) were averaged over five successive daily trials yielding four “steps” across the 20-day acquisition period. Typically, subjects began with between 3 and 4 flaps lifted. We computed an index of improvement across the experiment as follows. Improvement 1 = Steps 1–2; improvement 2 = Steps 2–3; and so on (see Fig. 1). As described, this yields a measure of the rate of acquisition across the 20-day experiment. Importantly, an improvement of 0 suggests that the task was not learned or the subject had reached asymptote. The larger the improvement scores, the quicker the subject learned the task. The improvement scores were analyzed using hormonal treatment as a between-subject variable and improvement as a within-subject variable. Fisher LSD was used to examine hormonal effects post hoc, whereas least square means was used to examine effects due to improvement. Finally, 95% confidence intervals were computed for

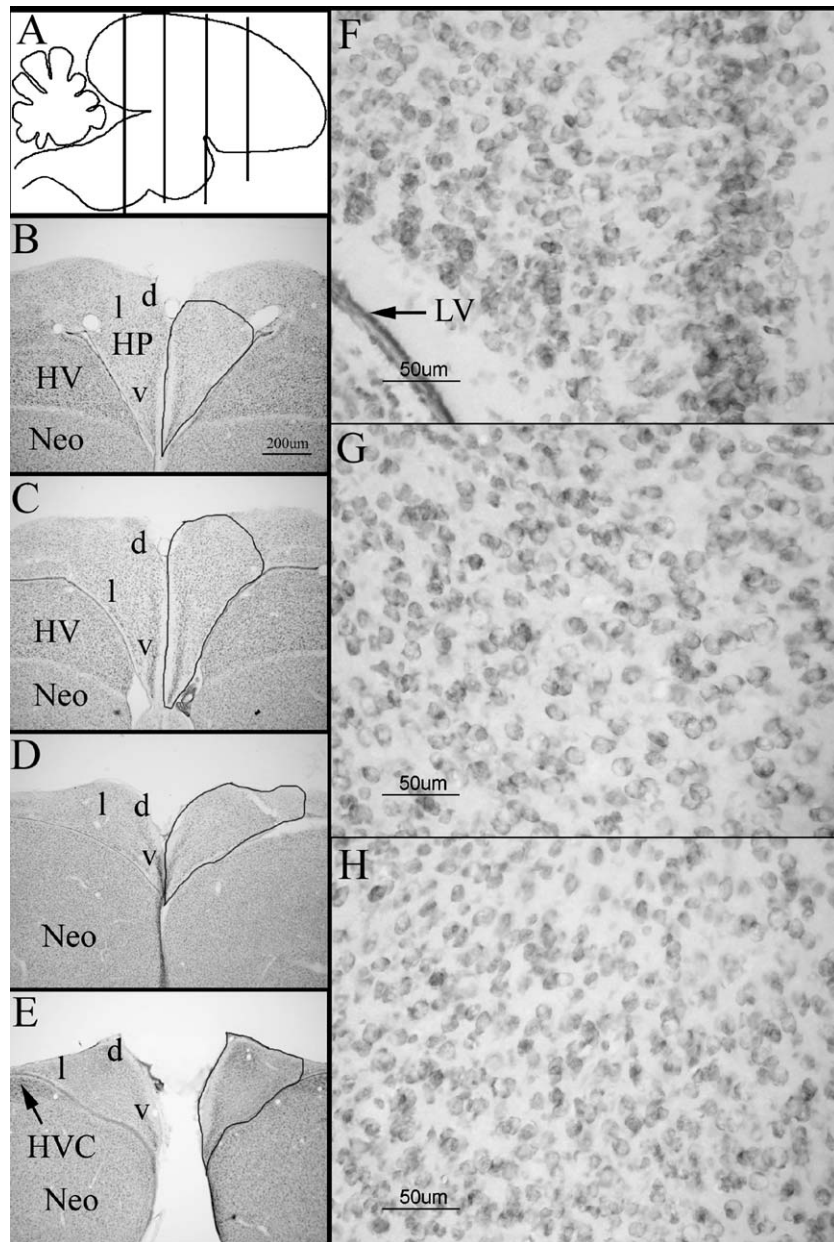


Fig. 2. Photomicrographs depicting the cytoarchitecture of the zebra finch HP in rostrocaudal extent. Panel A depicts a schematic of the songbird brain with vertical lines representing the location of the coronal sections depicted in B–E; the photomicrograph in B corresponds to the most rostral line in A. The changing shape of the HP is depicted in these panels with line-drawings of the sectional area shown on the bird's left (right side of photo). Also depicted is the location of the ventral (v), dorsal (d), and lateral (l) subareas sampled for measurement of cell area and density on the bird's right (left side of photo). These photomicrographs shown in panels F–H are the entire sample area ($64,000 \mu\text{m}^2$) at the rostrocaudal level depicted in C. HV—hyperstriatum ventrale; Neo—neostriatum; HVC—high vocal center; LV—lateral ventricle. Magnification bars are shown.

improvement scores to ask if the change in memory performance was significantly different from zero. The frequency of song bouts was compared across treatment groups by one-way ANOVA followed by Fisher LSD comparisons.

Anatomy

Measures of cell size and density were compared using ANOVA with treatment (hormone) and HP subarea and anteroposterior extent of the HP as main variables

($4 \times 3 \times 4$). Because the interaction between HP subarea and rostrocaudal extent was important to us, subarea was coded as a between-subject variable while rostrocaudal extent was coded as a within-subject variable. The source of significant main effects was evaluated using post hoc Fisher least square difference comparisons. Least-square mean comparisons were used to evaluate significant within-subject effects. Planned comparisons (least-square means) were used to evaluate interaction effects among main variables.

Results

Behavior

As depicted in Fig. 3A, most subjects began performing the task with between 3 and 4 flaps lifted to find the food. In general, subjects learned the task as evidenced by a decrease in the number of flaps lifted to find food over the acquisition period. Statistical analyses revealed that although treatment did not significantly alter the number of flaps lifted to find

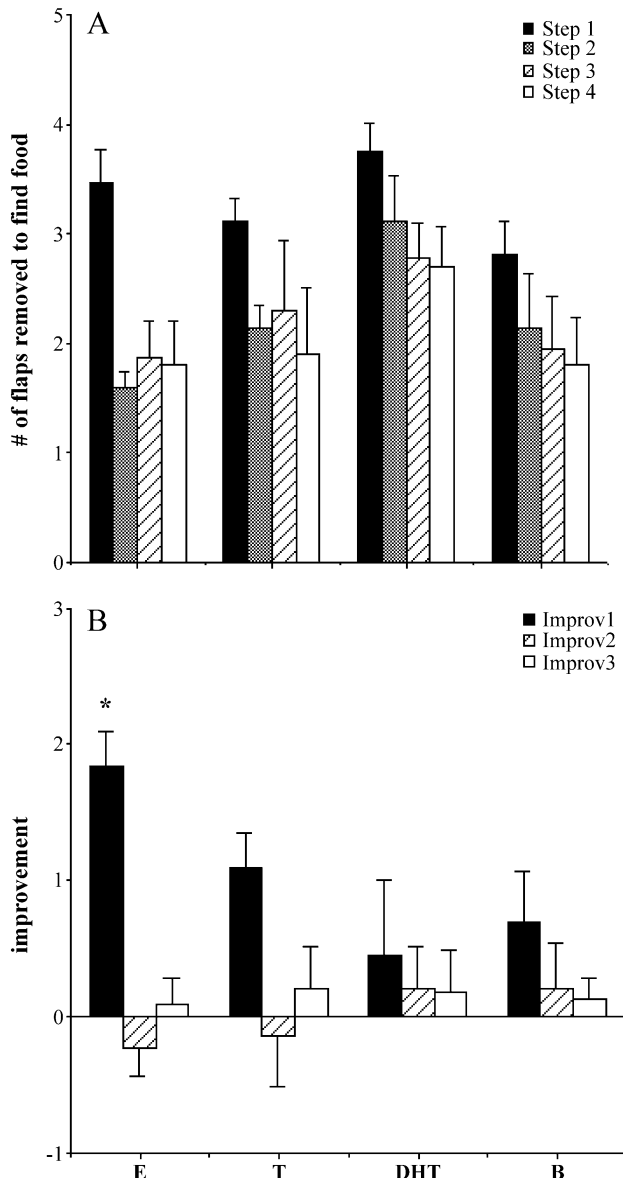


Fig. 3. Histograms depicting acquisition of spatial memory (A) and improvement (B) over the experiment. The number of flaps removed to find the food was measured, and 5-day periods were binned together in (A). The change from step 1 to step 2 in A is represented by improvement 1 in (B), and so on. Improvement 1 of the E₂ group was most rapid ($P = 0.05$). Data are presented as means \pm SEM and were collected by an experimenter who was blind to the experimental condition. Fisher LSD was used post hoc to determine statistical differences between treatments.

food, there was a strong effect of learning ($F_{(3,60)} = 11.486$; $P = 0.0001$) and a significant interaction between treatment and learning ($F_{(9,60)} = 2.604$; $P = 0.013$). Specifically, over successive trials, whereas E₂-, T-, and B-treated birds showed evidence of learning (fewer flaps lifted to find food), the performance of DHT-treated subjects did not diverge from chance over the 20-day acquisition period (Fig. 3A).

The difference between successive scores of memory performance (improvement) was computed (see Fig. 1B). Although there was no effect of treatment overall, significant effects on improvement ($F_{(2,44)} = 4.257$; $P = 0.02$) and a strong trend towards a significant interaction between treatment and improvement was revealed ($F_{(6,44)} = 2.253$; $P = 0.055$). Specifically, E₂-treated subjects showed the most dramatic improvement relative to all other groups (Fig. 3B) and this effect was most clearly demonstrable during the first 10 days of acquisition (Improvement 1).

Notably, there were no overall differences in improvement among groups, but the T- and B-treated birds did show significant improvement and DHT-treated birds did not. Specifically, 95% confidence intervals across the improvement scores revealed that the E₂-treated (1.833 ± 0.52 ; mean ± 1.96 *SEM), T-treated (1.05 ± 0.78), and B-treated (0.65 ± 0.5) birds showed improvements in memory performance in the first 10 days and not thereafter. DHT-treated birds did not improve during this time (0.03 ± 0.55) and showed no evidence of improvement across the entire duration of the experiment (Fig. 3).

There were significant variations across groups in the number of song bouts upon exposure to stimulus females ($P = 0.042$). T-treated birds sang the most (13.8 ± 2.9) followed by E₂-treated (7.6 ± 1.8) or DHT-treated (6.6 ± 2.2) birds that did not differ from each other. Only one of the seven B-treated birds sang during the 15-min data gathering period (0.15 ± 0.15).

Anatomy

Cell size

As depicted in Fig. 4, E₂- and T-affected the anterior HP. There was a significant difference in cell area across treatments ($F_{(3,96)} = 5.286$, $P = 0.0061$). A Fisher LSD post hoc revealed that the E₂- and T-treated groups had larger cells than the DHT- and B-group. Importantly, while E₂ and T groups did not differ from each other, neither did the DHT- and B-treated subjects. There was a significant effect of anterior–posterior orientation ($F_{(4,96)} = 60.186$, $P = 0.0001$). Post hoc least square means analysis revealed that cells in the anterior HP are significantly larger than those in the posterior HP. Notably, there was an interaction between the main variables of treatment and anteroposterior extent ($F_{(12,96)} = 1.919$, $P = 0.0412$), with the larger cells in the E₂- and T-treated groups different from other groups in the anterior but not the posterior HP. More specifically, E₂ and T treatments had more of an effect on the second and

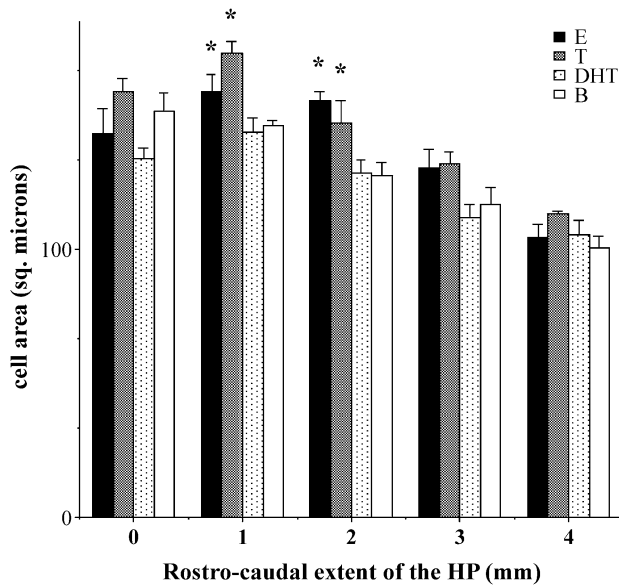


Fig. 4. Effect of treatment on the cell area at five levels of the ZF HP; each level is separated by 1 mm. The cell area in the anterior HP is larger in the E₂- and T groups, which did not differ from each other. Asterisks over T- and E₂-treated subjects in anteroposterior locations 2 and 3 show that these groups were different from DHT and B treated birds at this level.

third anterior–posterior sections. There was no difference between HP subareas. Further, there was no interaction between treatment and HP subarea or between HP subarea and rostrocaudal position.

Cell density

As indicated in Table 1, E₂ affected the cell density of the HP. There was a significant difference of treatment on cell density ($F_{(3,96)} = 10.682, P = 0.0001$). A Fisher LSD post hoc reveals that the T, DHT, and B groups did not differ from each other, but the E₂ group had a lower cell density. There was no significant difference in the cell density between HP subareas (V, D, or L), as well as no significant effect on anteroposterior orientation. There was no interaction be-

tween treatment and HP subarea between anteroposterior orientation and the treatment or between anteroposterior orientation and HP subarea.

HP volume

As depicted in Table 1, there is no effect of treatment on HP volume, telencephalon volume, or the ratio of HP to telencephalon volume.

Discussion

In every songbird species studied thus far, the HP contains some of the highest levels of aromatase activity measured relative to other neural loci (Balthazart et al., 1990; Saldanha et al., 1998, 1999; Soma et al., 1999). Since the songbird HP is critically involved in spatial memory function (Hampton et al., 1995; Krebs et al., 1996; Patel et al., 1997; Sherry and Vaccarino, 1989), aromatization of testosterone to estradiol within the HP may likely support spatial memory function in these species.

This hypothesis is supported by the present results. Castrated males implanted with E₂ demonstrated the most rapid acquisition of spatial memory relative to all other groups. Although T-treated birds showed evidence of learning, DHT-implanted subjects did not appear to learn the task in the 20-day acquisition period (see Fig. 2). A similar pattern of results was obtained in terms of HP structure. E₂ and T increased the size of HP neurons relative to DHT- and B-implanted birds (see Fig. 3, Table 1). These data suggest that the aromatization of T to E₂ potentiates both the structure and function of the songbird HP.

Interestingly, a slow, but significant amount of acquisition was demonstrated by blank-implanted castrates. We believe two processes may account for this observation. Firstly, castration does not necessarily eliminate all circulating steroids in zebra finches (Adkins-Regan et al., 1990), suggesting that these birds may still possess low levels of E₂. Secondly, the low level of learning shown by blank-

Table 1
Means and standard errors of cell area, cell density, and hippocampus volume from thionin sections

	Estradiol	Testosterone	Dihydrotestosterone	Blank	P
Ventral cell area (μm ²)	139.31 ± 8.37 ^a	147.17 ± 7.44 ^a	126.91 ± 4.74 ^b	126.73 ± 3.78 ^b	0.02
Dorsal cell area (μm ²)	138.34 ± 7.09 ^a	139.49 ± 8.72 ^a	123.98 ± 4.48 ^b	130.97 ± 6.07 ^{a,b}	0.01
Lateral cell area (μm ²)	137.52 ± 8.83 ^a	147.17 ± 7.44 ^a	122.6 ± 5.78 ^b	126.82 ± 7.14 ^b	0.01
All subareas cell area (μm ²)	138.39 ± 4.53 ^a	144.61 ± 4.45 ^a	124.5 ± 2.87 ^a	128.17 ± 3.32 ^a	0.01
Ventral cell density	27.2 ± 1.5	33.4 ± 1.3	32.3 ± 1.2	33.9 ± 1.3	0.32
Dorsal cell density	25.9 ± 1.5	30.5 ± 1.4	30.9 ± 1.1	31.4 ± 1.0	0.61
Lateral cell density	26.2 ± 1.2	32.2 ± 1.6	29.7 ± 0.6	32.1 ± 0.8	0.27
All subareas cell density	26.4 ± 0.8 ^a	32.0 ± 0.8 ^b	31.0 ± 0.6 ^b	32.5 ± 0.6 ^b	0.03
Hippocampus volume (mm ³)	3.12 ± 0.02	3.26 ± 0.06	3.23 ± 0.05	3.21 ± 0.02	0.58
Telencephalon volume (mm ³)	415.7 ± 0.4	376.7 ± 0.4	457.4 ± 0.5	439.4 ± 0.4	0.83
Hippocampus per Telencephalon	0.008 ± 0.0003	0.009 ± 0.0002	0.007 ± 0.001	0.008 ± 0.0001	0.74

Data with different superscripts are significantly different.
Density measure = number of cells per 64,000 μm².

implanted birds could be a reflection of removal of androgens, which were observed to be detrimental to spatial memory acquisition. The idea that androgen-removal may be beneficial for spatial memory learning is supported in the present report since we found no evidence of improvement during the acquisition of the spatial memory task in DHT-implanted birds (see Results) and points to a conceptually discrete role for encephalic aromatization. However, this effect needs to be more rigorously tested perhaps by local implantation of DHT near circuits unequivocally involved in memory function. These interesting possibilities warrant further investigation, but do not detract from the steroid-dependent acceleration of memory performance observed in E₂-treated birds.

Estrogen-dependent functional and structural plasticity of the mammalian HP has been the topic of several studies, including neurogenesis (Gould et al., 2000; Tanapat et al., 1999), dendritic spine density (Gould et al., 1990; Pozzo-Miller et al., 1999; Woolley and McEwen, 1994), efficacy of synaptic transmission (Foy et al., 1999; Woolley et al., 1997), synaptic input (Woolley et al., 1997), long-term potentiation and depression (Foy et al., 1999; Gupta et al., 2001; Vouimba et al., 2000), and memory performance (Packard, 1998; Sherwin, 1996; Stephen et al., 1998). The present data add to this list the estrogenic modulation of HP neuron size and function in an additional class of vertebrates. Thus, although the site of steroid synthesis is distinct (peripheral versus central), estrogenic influences on the HP may be a conserved characteristic of the vertebrate brain.

The structural and behavioral alterations observed are likely the effect of implanted steroids affecting neural sites. As an independent test, we measured singing—a well documented, steroid-dependent behavior (Arnold, 1975a,b; Smith et al., 1997). Those with circulating androgens or estrogens sang, while B-implanted birds almost never sang (see Results). These data point to two important interpretations. Firstly, singing behavior was not a result of residual gonadal steroid synthesis, but rather an effect of the implant. Secondly, in excellent agreement with previously published data (Bohner et al., 1992; Walters and Harding, 1988), the highest singing rates were demonstrated by birds with circulating androgens and estrogens (T-implanted birds). These observations suggest that the implanted steroids had access to neural substrates affecting behavior, and indeed, were equally likely to modulate HP structure and function.

The steroidal influence on HP structure included an increase in the soma size of E₂- and T-treated birds (see Fig. 4, Table 1). An increase in somal size is found with an increase in synaptic connections in areas of the avian brain areas such as RA (DeVoogd and Nottebohm, 1981). This increase in synaptic connectivity may be associated with an increased capacity for complex memory (Airey and DeVoogd, 2000; Hauber et al., 1999; Smulders and DeVoogd, 2000).

A potential role for neural aromatization on HP structure and function has been suggested (Schlinger, 1997). In some

songbirds, HP aromatase increases dramatically at times of low circulating steroids (Saldanha et al., 2000; Soma et al., 1999, 2003). This increase in HP aromatization may sustain E₂ provision to circuits during the autumn and winter, times when some songbirds show dramatic plasticity in HP structure and function (Smulders et al., 2000, 1995).

Of particular interest is that E₂ and T influences were limited to the anterior HP. This observation is in good agreement with other studies that have shown seasonal variations in cell number and expression of immediate early genes in the anterior, but not the posterior HP in other songbirds (Smulders and DeVoogd, 2000; Smulders et al., 2000). The physiological basis for this specificity is currently unknown. One possibility is that aromatase expression itself may vary across the rostrocaudal HP resulting in micro-domains of local E₂. This hypothesis is currently being tested.

Alternatively, or in addition, estrogen receptors may vary along the rostrocaudal HP. Indeed, Weiland et al. (1997) reported such a variation in the rodent HP. Although direct comparisons across structures with different hodological characteristics are difficult, the possibility does exist that estrogen-responsive neurons in the songbird HP do vary in rostrocaudal extent. Although estrogen receptors have been documented in the HP of some songbird species (Gahr et al., 1993), any variations of this expression across rostrocaudal extent are currently unknown. This hypothesis is also under scrutiny in our laboratory.

In summary, the results of this study show that the neural aromatization of circulating androgen to estrogen affects both the structure and the function of the songbird HP. These data underlie the importance of neural aromatization in the songbird, where peripheral sources of E₂ are surprisingly sparse (Schlinger and Arnold, 1991, 1992). To the best of our knowledge, this is the first experimental demonstration of a functional role for telencephalic aromatase in the adult songbird. Future studies will investigate estrogenic influences on the ultrastructure of HP neurons in these species.

Acknowledgments

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