Inhibition of Hippocampal Aromatization Impairs Spatial Memory Performance in a Male Songbird

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Recent studies have revealed the presence and regulation of aromatase at the vertebrate synapse, and identified a critical role played by presynaptic estradiol synthesis in the electrophysiological response to auditory and other social cues. However, if and how synaptic aromatization affects behavior remains to be directly tested. We have exploited 3 characteristics of the zebra finch hippocampus (HP) to test the role of synaptocrine estradiol provision on spatial memory function. Although the zebra finch HP contains abundant aromatase transcripts and enzyme activity, immunocytochemical studies reveal widespread pre- and postsynaptic, but sparse to undetectable somal, localization of this enzyme. Further, the superficial location of the avian HP makes possible the more exclusive manipulation of its neurochemical characteristics without perturbation of the neuropil and the resultant induction of astroglial aromatase. Last, as in other vertebrates, the HP is critical for spatial memory performance in this species. Here we report that local inhibition of hippocampal aromatization impairs spatial memory performance in an ecologically valid food-finding task. Local aromatase inhibition also resulted in lower levels of estradiol in the HP, but not in adjacent brain areas, and was achieved without the induction of astroglial aromatase. The observed decrement in acquisition and subsequent memory performance as a consequence of lowered aromatization was similar to that achieved by lesioning this locus. Thus, hippocampal aromatization, much of which is achieved at the synapse in this species, is critical for spatial memory performance. **(*Endocrinology* 154: 4707–4714, 2013)**

Steroids such as 17β-estradiol (E_2) have profound influences on the vertebrate central nervous system via endocrine, paracrine, and autocrine delivery. Recently, attention has been focused on the acute, rapid, and targeted provision of E_2 at synapses as evidenced by the identification of aromatase in presynaptic boutons (1–3), the rapid regulation of aromatase activity therein (4), and the nongenomic behavioral effects of de novo E_2 synthesis (5–11).

In the male zebra finch (*Taeniopygia guttata*), the brain is the primary source of local and circulating E_2 (12, 13) via microsomal and synaptic aromatization in neurons of the hippocampus (HP), nucleus taeniae, caudomedial nidopallium (NCM), preoptic area (POA), and other regions (2, 3, 14–16). Aromatase can also be induced in nonneuronal cells by treatments that damage neural tissue (17, 18). Thus, in this species, the brain can be exposed to high levels of local E_2 from neuronal (constitutive) and glial (induced) sources.

The zebra finch HP distinguishes itself from other aromatase-rich brain regions. Despite high levels of aromatase transcripts and activity (16, 19, 20), somal aromatase immunoreactivity is low to undetectable (2, 14, 21). Indeed, ultrastructural examinations reveal an abundance of aromatase in presynaptic boutons and postsynaptic dendrites at this locus (2). These observations point to the possibility that areas sparse or even devoid of somal aromatase, such as the HP, may be capable of E_2 provision by

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Abbreviations: ATD, 1,4,6-androstatriene-3,17-dione; E_2, 17β-estradiol; HP, hippocampus; NCM, caudomedial nidopallium; POA, preoptic area.

aromatization in terminals. This characteristic renders the zebra finch HP ideal for understanding the role of synaptic aromatization.

Songbirds remain excellent models for studies on steroid-dependent development of procedural memory systems such as song behavior (22). Birds also acquire and display robust episodic-like memories, such as spatial memories, which are dependent on the HP (23–28). Importantly, a recent report suggests that aromatization, possibly in the HP, is important for spatial memory retrieval, rather than acquisition (29), strengthening the potential role for locally synthesized E_2 on HP-dependent function. Further, the largely pre- and postsynaptic localization of aromatase therein provides an excellent means for examining the behavioral role of synaptocrine E_2 provision.

The superficial location of the avian HP on the dorsal surface of the brain allows for experimental manipulation of synaptic aromatase without the confound of aromatase induction in nonneuronal cells (30, 31). The latter occurs in response to disruption of the neuropil (17, 32) and is an unfortunate consequence of any technique that perturbs the brain to experimentally manipulate local neurochemical characteristics (8, 32–34). In the current report, we have inhibited aromatization by infiltrating the HP with a lipophilic aromatase inhibitor, 1,4,6-androstatriene-3,17-dione (ATD). This technique, followed by measures of HP-dependent memory function, local E_2 levels, and verification of HP aromatase expression and integrity, allowed us to test 2 hypotheses. The first hypothesis is that local inhibition of the predominantly synaptic aromatase in the zebra finch HP would affect acquisition in a spatial memory task, and the second hypothesis is that the effects of aromatase inhibition would be similar in characteristic and extend to the compromise of HP function achieved via lesions of this brain locus.

Materials and Methods

Experiment 1: Inhibition of presynaptic aromatase in the HP and spatial memory function

Animals and housing

All procedures were approved by the institutional animal care and use committees at St Norbert College and American University. Adult (>100 days posthatch) male zebra finches were obtained from a commercial breeder and housed in communal, same-sex aviaries. Lights were maintained on a 12:12-hour light/dark cycle (lights on at 7:00 AM). Birds had free access to seed, water, grit, and once weekly supplements of hardboiled chicken eggs, enriched bread, and Vita-Sol.

Surgical manipulations

All surgical manipulations were done between 8:30 and 11:00 AM. Each bird was anesthetized with isoflurane (4% in oxygen; 1.5 L/min) and mounted in a stereotaxic frame with anesthesia maintained at 3%. An incision was made over the skull, and the intersection of the lambdoidal and sagittal sutures was measured, as were the relative coordinates for the HP (anterior/posterior, +1.5 mm; lateral, ±0.5 relative to the intersection point). In all groups, bilateral holes were drilled above the HP using a beveled, 0.8-mm diameter bit, and the dura mater was carefully peeled away from the surface of the HP. A bird was then given one of the following manipulations. Silicone pellets soaked in ATD, a lipophilic and highly specific inhibitor of aromatase in zebra finches (35), were placed in the openings of the skull to rest on the surface of the brain. Pellets were made on a glass slide by mixing 100 mg of ATD with 65 mg of aquarium sealant, which was allowed to dry for 2 days at 4°C, covered by foil. When dry, pellets were punched out with the narrow opening of a 1-mL pipette tip and stored covered by foil at 4°C. Before placement on the brain surface, each pellet was trimmed with forceps under a dissecting scope, made malleable to fit snugly in the opening of the skull, and held in place by a small amount of silicone (ATD; n = 9). As a control, another group received silicone alone into the holes made by the craniotomy (SIL; n = 13). In another group, 0.1 μL of ibotenic acid in 0.1 M PBS (pH 7.4) was injected under gentle pressure through 10-μm diameter glass micropipettes lowered bilaterally into the HP to a depth of 0.3 mm relative to the surface of the brain. Micropipettes remained in place for 1 minute to ensure diffusion (LESION; n = 9). As a control, a fourth group was identical to that above but injected with 0.1 μL of PBS (SHAM; n = 8). A final group remained under anesthesia for 5 minutes (CRANIOTOMY; n = 13). After suture and recovery, each bird was returned to the colony but housed in an individual cage with free access to seed and water until behavioral testing. The general health of all birds and the integrity of the incision site were regularly monitored. Three animals died within 24 hours postsurgery (2 in the CRANIOTOMY group and 1 in the ATD-treated group).

Behavioral testing

Three days after surgery, spatial memory was tested in a modified "t-maze" (23), which consisted of a base that led upward into horizontal left and right arms. In the horizontal arms were identically colored and patterned cups with closable flaps, 3 in each arm, arranged at varying heights from the base of each arm and such that each arm was a mirror image of the other (for a description and drawing of the apparatus, see Figure 1 in Ref. 23). Before testing, birds were deprived of seed and water at lights-on for approximately 5 hours; recently, a similar manipulation (6 hours of food deprivation) in zebra finches was shown to decrease levels of E_2 in plasma but not affect E_2 amounts in the HP (36). After this fast, each bird underwent acquisition trials. One of the cups, chosen randomly, was filled with seed, and a bird was removed from the chamber after it ate from the baited cup. The maximum latency for each acquisition trial was 20 minutes, the trials were separated by an intertrial interval of 10 minutes (during which a bird was returned to its individual cage in a room next to the testing room), and an animal "failed to acquire" when it did not meet the predetermined criterion level (arm that contained food was entered within 30 seconds on 3

consecutive trials) at the 10th acquisition trial. Because the minimum number of trials to reach the criterion was 4, additional trials needed to reach the criterion were considered "mistakes" in acquisition. Upon reaching the criterion, birds were returned to their individual cage for 1 hour (retention interval). After the retention period, birds underwent 4 20-minute probe trials, each separated by 10 minutes. Before the probe trials, all cups that did not contain seed were filled and then emptied to obscure any olfactory cues, and probe trials were conducted with no food in any of the containers. Each bird was placed through each of the 4 doors of the chamber at random, and the time to make contact with the baited cup (by beak or by landing on the cup) as well as the number of cups contacted before the baited one ("mistakes") were recorded. All birds were closely monitored throughout the procedure for general aspects of behavior, such as proper flight and the ability to maintain balance on a perch.

Tissue collection and data analysis

Immediately after the last retention trial, birds were euthanized, and brain tissue was immersion-fixed in 5% acrolein in PBS overnight and then in 5% acrolein containing 30% sucrose in PBS for 3 days. For each bird, 3 sets of sections (30-μm coronal) were cut frozen via a microtome into cryoprotectant. After histological confirmation (see below), the number of mistakes in acquisition (number of acquisition trials minus 4, the minimum number) were analyzed via a one-way ANOVA and least squares difference tests post hoc. The latency to contact the baited cup, as well as the number of mistakes made during the probe trials, were analyzed by a repeated-measures ANOVA for the main effects of surgical manipulation (ATD, SIL, LESION, SHAM, and CRANIOTOMY), time (retention trials 1–4), and any interactions.

Histology and immunocytochemistry: lesion extent, HP integrity, and aromatase expression

The integrity of HP tissue in all groups was examined by light microscopy after a thionin stain of one of the alternate sets of tissue from each bird. Damage in LESION birds was qualitatively determined by examining the extent of loss of neuronal cell bodies; only birds in which the lesion was confined to the dorsolateral subdivision of the HP were included in the analysis of the behavioral data (see *Results*).

Another alternate set of sections was processed for aromatase immunocytochemistry to examine, also via light microscopy at a number of magnifications, whether expression of the enzyme in the HP could be observed in glia, as well as to observe the relative amounts of immunoproduct in the NCM and diencephalon. Staining was done as published previously (14) with a few modifications: sections were initially rinsed 9 times for 10 minutes each in PBS to remove cryoprotectant from the tissue, and the rinses after primary, secondary, and avidin-biotin incubations were overnight, 2 hours, and 2.5 hours, respectively. Diaminobenzidine immunoproduct was visualized under light microscopy at magnifications from ×40 to ×1000. Sections processed for thionin and aromatase were mounted onto subbed slides and dehydrated through serial alcohols, and representative photomicrographs were collected using an Olympus BX51 microscope and an Optronics MicroFIRE digital camera connected to an Olympus U-CMAD3 lens.

Experiment 2: Estradiol assay of microdissected brain areas in ATD and SIL birds

Animals, housing, and surgical manipulations

An additional group of adult male zebra finches were housed as described previously and underwent 1 of 2 surgical manipulations described above: ATD (n = 15) or SIL (n = 16).

Tissue collection

Three days after surgery, to correspond with the time that behavioral testing ended in the prior experiment, ATD and SIL birds were euthanized via decapitation, and trunk blood was collected and placed on ice until further separation. The skullcap and dura mater were removed, and brain areas were microdissected as follows under a dissection microscope into microcentrifuge tubes (catalog no. 02682558; Fisher) on dry ice. As described previously (37), to collect HP tissue, angled, superficial cuts were made with a razor blade on the dorsal surface of each hemisphere from the coronal point at which the optic tecta began to a point about 1 mm lateral from the midline at the caudal end; the length of this cut was about 3.1 to 3.2 mm. The HP tissue often adhered to the blade, given the lateral ventricle between the HP and the underlying telencephalic tissue. When this did not occur, forceps were used to remove the HP, avoiding NCM tissue at the caudal portion of the HP. Tissue from the NCM was then collected from beneath the excised HP. Cuts were made about 0.75 mm rostral to the cerebellum, 1 mm lateral, and 1.5 mm deep, and the bilateral protrusions were collected. What remained of the telencephalic lobes were collected with forceps. The diencephalon, revealed below the extracted telencephalon, was removed with sharp forceps inserted at its lateral borders and incised ventrally and then was pulled off by moving the forceps medially along the roof of the optic chiasm. Finally, gonadal tissue was collected from each bird.

The time from the attempt to capture to completion of the dissection was recorded. Blood was spun at 10,000 × *g* for 10 minutes to separate the plasma. Plasma was pipetted into fresh tubes and frozen at −80°C along with the brain tissue samples.

Solid-phase extraction

E_2 was extracted from all samples (brain tissue and plasma) via solid-phase extraction before measurement (38, 39). Brain tissue was homogenized in 960 μL of ice-cold water-methanol (1:5, v/v) by using a bead mill homogenizer (Omni Bead Ruptor 24, no. 19–101; Omni International). The samples were then centrifuged, and the supernatants were diluted with 10 mL of deionized water before loading onto Bond Elut LRC-C18 OH columns (500 mg of sorbent and 10 mL of column volume, no. 12113045; Agilent). The pellets were stored at −20°C until protein assay (see below). Plasma samples (31.5 μL) were directly diluted with 10 mL of deionized water before loading onto C18 columns. Before sample loading, the C18 columns were primed with 3 mL of HPLC-grade methanol and equilibrated with 10 mL of deionized water. After sample loading, columns were washed with 10 mL of 40% HPLC-grade methanol. Steroids were eluted with 5 mL of 90% HPLC-grade methanol and dried at 40°C in a vacuum centrifuge (SPD111V SpeedVac; Thermo Fisher Scientific). Dried extracts were resuspended for RIA in 350 μL of PBS with 0.1% gelatin containing 0.7% absolute ethanol.

E_2 radioimmunoassay

Resuspended samples were then assayed with a modified double-antibody ^{125}I-E_2 RIA (DSL-4800; Beckman Coulter Canada) as described previously (39–42). Because of the low level of E_2 in tissues, samples were measured as singletons to maximize the number of detectable samples. Recovery was determined by spiking known amounts of E_2 to tissue pools before solid-phase extraction and then comparing these measures with unspiked samples from the same tissue pool. The recovery of E_2 was 113.3% in plasma and 109.3% in brain tissue. The intra-assay coefficient of variation was 6.0% and the interassay coefficient of variation was 6.9%.

Protein and tissue mass determination

The remaining tissue pellets from homogenization were resuspended in 0.2 M NaOH using a bead mill homogenizer. Homogenates were further diluted in water, and protein was quantified using a Coomassie Plus Bradford Assay Kit (no. 23236; Fisher). The absorbance was read at 595 nm on a microplate spectrophotometer according to the manufacturer's instructions to determine the protein amounts in samples. We then used the protein amount to calculate tissue mass, using a conversion factor that we had previously determined using tissues of known masses. There is a very tight linear correlation between the measured protein amount in milligrams (x) and brain tissue mass in milligrams (y), yielding an equation of $y = 9.620x$ ($n = 23$, $r^2 = 0.8736$, $P < .01$). For small brain samples that are challenging to weigh directly on a microbalance (eg, 1–2 mg wet weight), measuring protein with calculation of tissue mass based on protein is more accurate and reliable. Further, expressing the data as picograms of E_2 per milligram of wet weight allows a more direct comparison with levels of E_2 per milliliter of plasma (39).

Data analysis

E_2 concentrations in microdissected brain tissue and plasma in ATD- and SIL-treated birds were analyzed via a two-way ANOVA, with a significant interaction of tissue type, and treatment analyzed via a least squares difference post hoc test.

Results

Experiment 1

Spatial memory performance

The minimum number of trials to reach the criterion in the spatial memory task was 4; thus, any number of attempts above this level was considered a "mistake" in acquisition. The type of manipulation significantly affected the number of mistakes birds made during acquisition trials [$F_{(4, 34)} = 4.000$, $P = .0091$] (Figure 1). ATD and LESION birds made a similar number of mistakes and significantly more mistakes than SIL and SHAM birds, which did not differ from each other. CRANIOTOMY birds performed significantly better than ATD birds and marginally better than LESION birds ($P = .07$), but no different from SIL or SHAM birds (Figure 1).

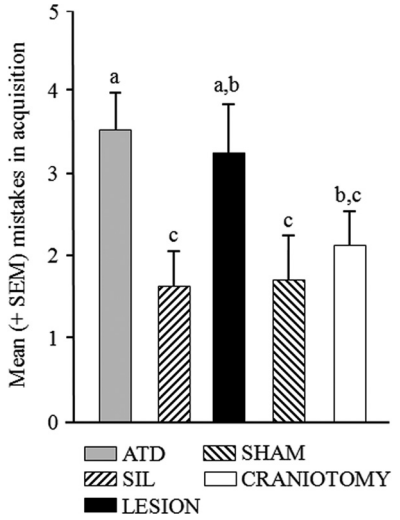

Figure 1. Mean (+SEM) number of mistakes to criterion during acquisition. The numbers of mistakes made by ATD and LESION birds were significantly higher than those made by birds in the SIL and SHAM groups. There was no difference between ATD and LESION birds, and no difference between SIL and SHAM groups. Groups that do not share a letter are statistically different ($P < .05$).

The amount of time to reach the baited cup during the probe trials was used as a measure of the accuracy of memory for the task (Figure 2). The type of manipulation significantly affected the time to reach the baited cup [$F_{(4, 34)} = 9.014$, $P < .0001$], which differed across the trials [$F_{(3, 102)} = 5.897$, $P = .0009$]; there was no interaction of treatment and time [$F_{(12, 102)} = 1.150$, $P = .3297$].

The type of manipulation also affected the number of mistakes birds made over the probe trials [$F_{(4, 34)} = 5.921$, $P = .0010$] (Figure 3). In addition, more mistakes were made in earlier than in later trials [$F_{(3, 102)} = 3.101$, $P = .0300$]. There was no significant interaction between the

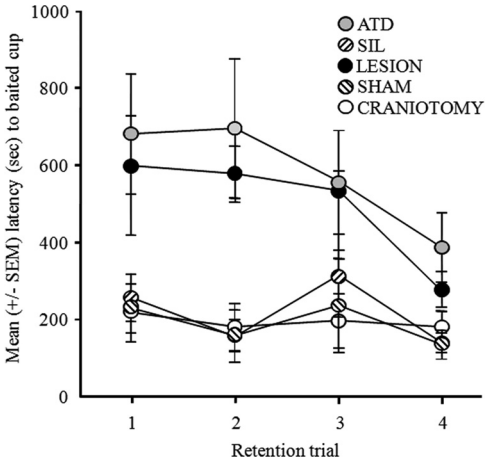

Figure 2. Mean (±SEM) latency in seconds to make contact with the baited cup by birds in the treatment conditions during the 4 retention trials. Overall, the type of manipulation significantly affected latencies. In the first 2 trials, birds treated with ATD or with HP lesions did not differ in their latencies but took significantly longer than the other groups (which did not differ) to make contact with the baited cup.

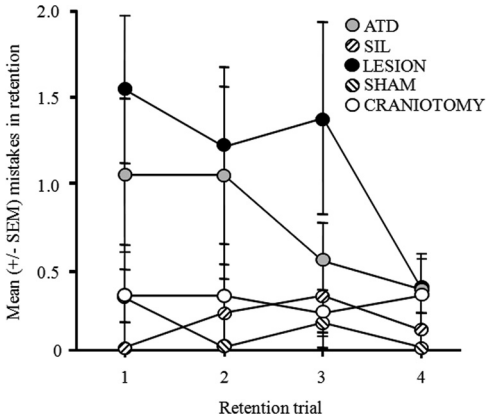

Figure 3. Mean (±SEM) mistakes (contact with a cup other than the baited one) by birds in the treatment conditions during the 4 retention trials. In the first 2 trials, the number of mistakes made by ATD-treated birds and those with HP lesions were not significantly different.

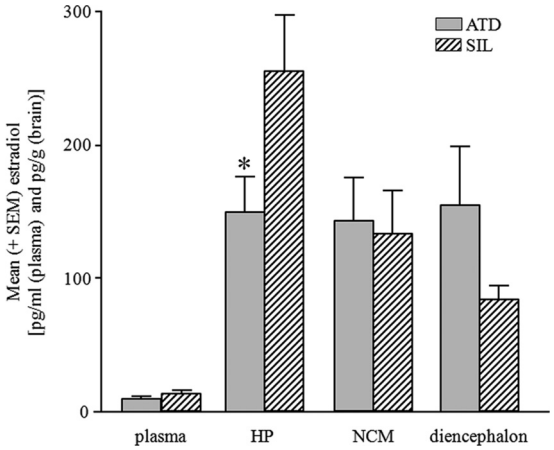

Figure 4. Mean (+SEM) E_2 levels in plasma (picograms per milliliter) and brain (HP, NCM, and diencephalon; picograms per gram) from birds in the ATD and SIL groups. Overall, E_2 levels were highest in the HP and lowest in the plasma, and ATD treatment lowered E_2 levels only in the HP. *, $P < .05$.

type of surgical manipulation and the retention trial [$F_{(12, 102)} = 1.310$, $P = .2243$].

Experiment 2

E_2 levels in brain and plasma

The mean amount of time for removal of a bird and its cage from the animal holding room to euthanasia in an adjacent laboratory was 34.74 ± 0.87 seconds, and the amount of time to collect all of the tissue indicated above was, on average, 156.14 ± 4.51 seconds.

The average concentration of neural E_2 did not differ across treatments [ATD vs SIL; $F_{(1, 27)} = 0.055$, $P = .8161$] but did across brain areas [$F_{(3, 81)} = 23.356$, $P = .0001$]. Levels of E_2 in the HP were significantly higher than those in the NCM and diencephalon, which did not differ. E_2 levels were significantly lower in plasma than in all brain areas. Notably, there was a significant interaction between treatment and brain area [$F_{(3, 81)} = 4.868$, $P = .0037$], with ATD resulting in reduced E_2 concentrations in the HP but not in the NCM, diencephalon, or plasma (Figure 4).

Verification of HP integrity and aromatase expression

Thirteen birds were removed from the analysis for the following reasons. Three LESION birds were removed because damage extended beyond the ventral border of the HP. Given the variability of these lesions in their ventral and lateral extents, the spatial memory behavior of this small group of animals was not analyzed. The rest of the birds in this group had lesions confined to the dorsolateral subdivision of the HP (Figure 5B). In prior work (23), a similar technique produced cell death in up to 23% of the entire HP. Damage in birds in the SHAM group appeared to be restricted to that caused by the needle tract. No obvious compromise to HP integrity was observed in birds in the CRANIOTOMY, ATD, or SIL groups, save for minimal compression of the dorsal surface of the tissue in some ATD and SIL birds as a result of the pellet placement (Figure 5A). One subject each in the ATD, SHAM, and SIL groups was removed and euthanized for difficulty in flight and failure to properly balance on a perch during the initial phase of the spatial memory test. Finally, 2 ATD, 1 LE-

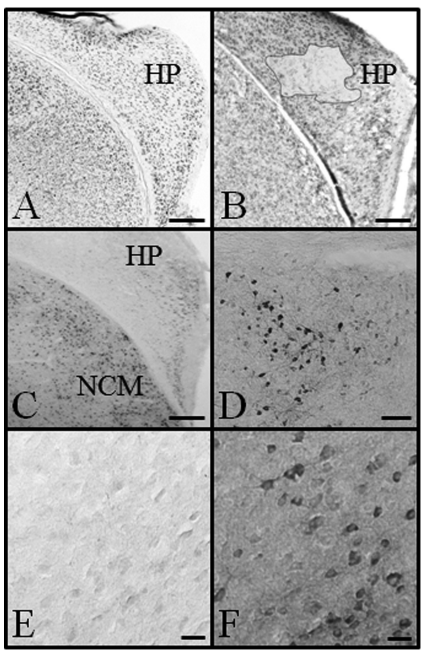

Figure 5. Coronal sections of the Nissl-stained HP in birds in the ATD (A) and LESION (B) (dotted line traced around the lesion zone [23] for coronal reconstructions of a similar lesion) treatment group; C and D, aromatase immunoproduct in the HP and underlying NCM (C) as well as the preoptic area (D) of the hypothalamus in a SIL bird. E and F, immunoreactivity in the HP (E) and NCM (F), respectively, at higher magnification; note the low-intensity somal staining in the HP relative to that in the NCM. Scale bars correspond to 150 μm (A, B, and C), 50 μm (D), and 25 μm (E and F).

SION, 3 CRANIOTOMY, and 2 SIL birds were removed from the analysis because they failed to acquire the behavioral task.

No glial aromatase expression was observed in ATD or SIL birds. In the HP of SIL birds, very low levels of punctate and flocculent immunoreactivity were observed around somata (Figure 5, C and E), in contrast to the robust expression seen in neurons in the underlying NCM (Figure 5, C and F) and POA (Figure 5D). Aromatase expression was of higher intensity in the HP of ATD birds but appeared confined to a pocket of cells in the dorsomedial subdivision of the structure and otherwise resembled that in SIL birds.

Discussion

In the adult zebra finch central nervous system, the HP expresses the highest levels of aromatase activity (19, 43) primarily in extrasomal, presynaptic compartments (2). Local application of the aromatase inhibitor ATD decreased performance of a spatial memory task. Further, in the initial probe trials after the retention interval, ATD increased the number of mistakes made and the duration to successfully complete the trials. Notably, the performance of ATD-treated subjects was indiscernible from that of subjects with HP lesions and significantly worse than that of subjects that received control implants or sham lesions. These data replicate previously published reports establishing the HP-dependent nature of this experimental paradigm (23, 44) and support the hypothesis that the predominantly pre- and postsynaptic aromatization within the passerine HP is a crucial modulator of spatial memory function (19, 23–26).

These neuroendocrine characteristics of the passerine HP are similar to those of the mammalian HP (45), although aromatase activity in the rodent and primate HP is extremely low, especially compared with that in the songbird (46, 47). Notably, in contrast to other aromatase-rich neural sites, the subcellular localization of aromatase in the zebra finch HP is almost exclusively extrasomal and sequestered within and around presynaptic boutons (2, 3, 9).

Synaptic aromatization, although described previously (1, 48), has more recently emerged as an intensely studied aspect of neuroendocrine function. Indeed, recent evidence strongly implicates synaptic aromatization in auditory processing during social interactions (6, 7, 11, 49). Given that aromatase is primarily nonsomal and presynaptic in the zebra finch HP, the decrease in spatial memory performance of ATD-treated birds probably reflects a specific effect on synaptic E_2 synthesis. The current data strongly support the hypothesis that synaptic E_2 synthesis may be important in synaptic events such as those involved in aspects of HP-dependent memory function. However, additional experiments replacing this steroid in subjects treated with an aromatase inhibitor are required to specifically test this hypothesis. The E_2-dependent nature of this effect is strongly suggested, however, by studies that have documented enhanced HP-dependent function in E_2, but not androgen-treated, birds (24).

Several recent studies have measured neural E_2 in songbirds (39–41, 50) and have altered the steroidal milieu around sites of aromatase expression in zebra finches (6, 8). In the current report, aromatase inhibition via implants of ATD placed on the HP significantly lowered local levels of E_2 by approximately 50%. Levels of E_2 in brain were found to be considerably higher than those in plasma, underscoring the considerable contribution of spatially circumscribed neurosteroidogenesis in the vertebrate brain. Further, the ATD implants inhibited concentrations of E_2 in the HP, but not in the neighboring NCM or the diencephalon, supporting the idea of microregulation of neural steroid levels and its potential importance in behavior. We do not know how much of the HP was affected by ATD treatment nor can we expect regional specificity of ATD, given its lipophilic nature and because the implants were placed on top of each lobe of the HP. However, the effect of ATD treatment appears limited to the region, given that E_2 concentrations were lower in the HP than in the other aromatase-rich regions examined. Further, the 50% inhibition in E_2 concentrations within the HP in ATD-treated birds was sufficient to see the behavioral outcome as described, an effect similar to lesions of the structure. These findings are in excellent agreement with previous reports of the spatial and behavioral selectivity of neural E_2 synthesis in the songbird (6, 7).

In the zebra finch, E_2 provision occurs constitutively within neuronal microsomes and synaptosomes (3, 4, 12) but can also be induced in reactive astroglia (17, 33). The latter occurs after perturbation of the neuropil. We analyzed ATD and SIL brains for aromatase immunoproduct and found no evidence of glial aromatase in the HP but readily observable neuronal aromatase in adjacent areas such as the NCM and diencephalon (Figure 5). Correspondingly, local E_2 levels were inhibited in ATD birds relative to SIL controls by approximately 50%. Taken together, these observations suggest that the ATD application did not damage the HP and did not cause any untoward induction of glial aromatase expression. Because HP neuronal aromatase expression is difficult to observe at the light level but is abundantly detectable in presynaptic boutons under an electron microscope (2), we are confident that the observed effects of ATD on HP function are not the consequence of glial aromatization but rather

are very likely a reflection of largely synaptocrine E_2 synthesis and effect.

The impairment of spatial memory acquisition observed in ATD-treated and LESION birds was surmountable. Specifically, although both groups showed a decrement in the rate of acquisition and took longer to complete the task correctly during the probe trial, it must be noted that they were not completely compromised in their spatial memory performance. All groups were able to remember the location of the food cup by the fourth probe trial (see Figure 3), suggesting that the residual HP aromatase (ATD) and circuitry (LESION) was sufficient to demonstrate sustained HP function in this behavioral paradigm. Alternatively, it is also possible that the HP in these birds and the synaptocrine E_2 synthesis therein is more important in the initial trials of memory performance in this behavioral paradigm.

We do not yet know the precise synaptic mechanisms affected by synaptocrine aromatization. In the mammalian HP, E_2 has rapid presynaptic and postsynaptic effects and increases the efficiency of neurotransmission (45, 50–53). In the songbird, recent data strongly suggest that neuronal responsivity is acutely modulated by rapid changes in local E_2 levels via a presynaptic mechanism in the NCM (49). Indeed, rapid changes in aromatase activity within synaptosomes do occur as a function of ionic flux and phosphorylation of the aromatase protein (4). Taken together, these data suggest the possibility that local aromatization in the HP may affect synaptic physiology, but the precise nature of this influence remains to be discovered.

It would be exciting to elucidate the precise component(s) of memory function being compromised by the inhibition of presynaptic aromatization. The present data show that decrements in acquisition clearly translated into impairments in memory performance during the probe trials, but we do not know if this was due to a failure to acquire, consolidate, retain, or recall the spatial task. Indeed, others have shown a context-specific effect of aromatization on retrieval (29), but further work is necessary to specifically test the role of HP aromatization in these effects.

In summary, whereas synaptic aromatization is a well-established form of local steroid provision, its functional role has been poorly understood. The current report supports the hypothesis that inhibition of aromatase at a neural locus rich in synaptic but sparse in somal aromatase expression causes a site-specific decrease in local E_2 concentrations and impairs spatial memory performance. The compromise in aromatase-dependent behavior is comparable to that achieved when the HP is lesioned, underscoring the critical role for synaptic aromatization in HP-dependent spatial memory function in vertebrates.

Acknowledgments

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