Published in final edited form as:

Brain Res Bull. 2013 January ; 90: 132–136. doi:10.1016/j.brainresbull.2012.10.010.

Norepinephrine inhibition in juvenile male zebra finches modulates adult song quality

Juli Wade^{a,b}, Jennifer Lampen^b, Linda Qi^b, and Yu Ping Tang^{a,b}

^aMichigan State University, Department of Psychology, East Lansing MI 48824

^bMichigan State University, Neuroscience Program, East Lansing MI 48824

Abstract

During development, male zebra finches learn a song that they eventually use in courtship and defense of nest sites. Norepinephrine (NE) is important for learning and memory in vertebrates, and this neuromodulator and its receptors are present throughout the brain regions that control song learning and production. The present study used the neurotoxin N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP4) to reduce brain levels of NE in juvenile males. This manipulation inhibited the development of quality songs, with some birds producing syllables that were unusually long and/or contained frequencies that were predominantly higher than normal. These results suggest that NE is important for the acquisition of typical song.

Keywords

Songbird; development; DSP4; catecholamine

1. Introduction

Norepinephrine (NE) plays important roles in vertebrate learning and memory [10, 19]. Courtship song in passerine birds is a learned behavior. Juveniles acquire these vocalizations from adult tutors, in many cases their fathers [22]. The timing of this process and the degree of plasticity that exists as animals mature varies among species. However, across songbirds two key phases exist – a period of template formation in which a memory of tutor song is stored and phase of sensorimotor integration in which young animals match their developing vocalizations to the template [14, 16].

Zebra finches are classic examples of ‘closed-ended’ or ‘age-limited’ learners. Memory acquisition begins at approximately 20 days post-hatching and continues to about day 60. Vocal practice overlaps with this period, beginning at about 35 days of age and completing around 3 months after hatching with the formation of crystallized song. After this point, the song remains stable throughout adulthood, in contrast to ‘open-ended’ learners in which songs may change in adulthood [16].

© 2012 Elsevier Inc. All rights reserved.

Corresponding Author: Juli Wade, Michigan State University, Neuroscience Program, 108 Giltner Hall, 293 Farm Lane, East Lansing MI 48824-1101, wadej@msu.edu, Phone: 517-432-8301.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Song is controlled by two interconnected circuits in the forebrain. An anterior pathway including Area X and the lateral magnocellular nucleus of the anterior nidopallium (LMAN) is critical for song learning. A motor pathway important for the production of song consists of HVC (proper name; [17]) projecting to the robust nucleus of the arcopallium (RA), which innervates the motoneurons of the vocal organ (syrinx; reviewed in [16]). The HVC and RA are substantially larger in males, who sing, compared to females who do not; Area X is not visible in females [26, 27].

The locus coeruleus (LoC) is a prominent source of NE in the vertebrate brain. It has widespread projections in the forebrain of birds, including the song control nuclei. These brain regions contain high levels of NE, as well as α 2- and β -adrenergic receptors. This pattern in adults is consistent with a role of NE in singing, and experimental studies in adults suggest roles for NE in song production and perception (all reviewed in [5]). In developing zebra finches, NE function in the song nuclei declines after 25-30 days post-hatching [9, 18]. This pattern is suggestive of a role for NE in template formation. However, it is possible that activity of this neuromodulator peaks earlier, as measurements were not taken prior to day 25.

To more directly test the role of NE in song system development, we treated birds with N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP4). This drug selectively targets axon terminals originating from the LoC and causes rapid and substantial decreases in NE from this source [11]. Our goal was to determine effects on development of both singing behavior (see above) and morphology of the forebrain song control nuclei. These brain regions first become visible around 5-10 days after hatching [7, 12, 13]. Maturation, which includes substantial growth of these brain regions in males compared to females, occurs through approximately day 50; sexual differentiation occurs at a high rate between days 20-30 [13]. We modified the approach that was effectively used on adult male zebra finches by Waterman and Harding [28] and gave a first injection on post-hatching day 10 and a second on day 18.

2. Materials and Methods

Animals

Experimental zebra finches were raised in adjacent walk-in aviaries at Michigan State University, each containing roughly 5-7 breeding pairs and their offspring, with 100 or more birds present in the room at any time. Animals were exposed to a 12:12 light:dark cycle, and provided *ad libitum* access to drinking water, seed (Kaytee Finch Feed; Chilton, WI), gravel and cuttlebone. Their diets were supplemented weekly with hard-boiled eggs, bread, spinach and oranges. Genetic sex of the birds was determined by PCR [1] on DNA extracted from toe clips used as unique identifiers on the day of hatching. Only males were used in the present experiment. All procedures were approved by the Michigan State University IACUC and followed NIH guidelines.

2.1 Treatment

On post-hatching days 10 and 18, birds were administered DSP4 (25 μ g/g in saline, IP, Sigma-Aldrich, St. Louis MO) or one of two control manipulations, randomly assigned across nests and aviaries. Because DSP4 can also be taken up by serotonergic cells, the selective reuptake blocker zimelidene dihydrochloride was injected one hour prior to prevent degradation of serotonergic neurons ([3, 28], 10 μ g/g, IP, Sigma) in DSP4-treated animals. Controls consisted of injections on day 10 and 18 of either zimelidene followed by saline one hour later, or two injections of saline an hour apart.

2.2 Behavior

Birds were left to reach sexual maturity in their home aviaries, until 94-110 days of age. To acclimate subjects prior to behavioral testing, each male was then housed in an individual cage in the same room as the colony aviaries for two days. They were able to see and hear other birds within the room. The animals were then taken to a sound-proof room and placed into an individual cage containing a sexually mature female that they had not previously encountered. Sound was recorded for 1 hour using a Sennheiser ME 62 omnidirectional microphone, Sound Devices USBPre2 amplifier, and Adobe Audition software at a 44.1kHz sampling rate, with 16-bit resolution. Because audio but not video was recorded we cannot be sure, but these conditions most likely resulted in the collection of female directed songs.

To obtain a rough estimate of relative levels of song learning, vocalizations of experimental males were compared to those of their fathers using Sound Analysis Pro 2011 software [25] by an observer blind to treatment group. Fathers were unambiguously determined by repeated observations of individuals who shared the nest containing each hatchling. For father-son pairs, a representative motif from each bird was compared. To assess song quality, a spectrogram of this bout of song for each animal was analyzed based on the work of Simpson and Vicario [20]. This method of selection was used because a few birds sang a single bout, and because within birds that sang multiple bouts, the content was very similar in content and structure (accuracy scores for each pairwise comparison for across motifs within a bout from one randomly selected bird per group ranged from 86.0 to 97.1, average = 91.4; accuracy scores across two full bouts = 83.9 to 92.3, average = 88.7). Specifically, we determined whether unusual aspects of the song existed based on definitions of Simpson and Vicario, including whether the bout contained fewer than three distinct song elements (syllables), and syllables longer than 300ms or predominantly higher than 1.5 kHz. In addition, the total number of syllables in the bout was counted. Finally, syllable duration and inter-syllable interval were determined for the bout using Adobe Audition software. Average values were calculated for each individual on these measures.

2.3 Tissue Collection and Processing

If the male sang, it was rapidly decapitated within 30 minutes of the end of the recording, and its brain was removed and flash frozen in methyl butane. If the male did not sing, it was returned in its individual cage to the colony room and recorded again two days later. This process was repeated for a maximum of 5 trials. Any birds that did not sing were euthanized at the end of the fifth recording session. Brains were stored at -80°C until further tissue processing. The song of the father of each of the subjects was also recorded using the same protocol. Two of the fathers did not sing and were excluded from analysis along with their sons, leaving a total of 22 subjects on which behavioral and brain data were analyzed: 7 treated with DSP4 and zimelidine hydrochloride (hereafter identified as the 'DSP4' group), 7 with zimelidine hydrochloride plus saline ('zimelidine'), and 8 with two injections of saline ('saline').

Brains were coronally sectioned at 20 μm and thaw-mounted in 6 series onto SuperFrost Plus slides (Fisher Scientific, Hampton, NH). Tissue was stored at -80°C with dessicant until processing.

One series of slides from each animal was stained with thionin to facilitate identification of brain regions and allow analysis of song system morphology. Another set was used for dopamine beta-hydroxylase (DBH) immunohistochemistry to identify potential NE-containing cell bodies and fibers. DBH is the rate limiting enzyme for the conversion of dopamine to NE. All tissue was reacted simultaneously. After warming to room temperature, it was rinsed in 0.1 M phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde for

15 min, and washed 3 times in PBS (5 min each). Slides were treated with 0.9% H₂O₂/methanol for 30 min and incubated for 1 hr in 10% normal goat serum in PBS with 0.3% Triton X-100. The tissue was then incubated in a DBH rabbit polyclonal antibody (0.2 µl/ml; Cat# 22806, ImmunoStar, Hudson, WI) in 0.1 M PBS containing 0.3% Triton X-100, and 10% NGS overnight at 4°C. This primary antibody was validated for use in songbirds [4, 23]. A biotin-conjugated goat anti-rabbit secondary antibody (0.5 µg/ml; Vector Labs, Burlingame, CA) was then used for 1.5 hours at room temperature, followed by treatment with *Elite* ABC reagents (Vector Labs) and diaminobenzidine (DAB) with 0.0024% hydrogen peroxide to produce a brown reaction product. Slides were then rinsed in PBS to be sure the reaction was terminated, dehydrated, and coverslipped with DPX (Sigma-Aldrich, St. Louis MO).

2.4 Quantification of Neural Features

Analysis of brain tissue was completed by an observer blind to treatment group. Cross-sectional areas of HVC, RA and Area X were determined in each thionin-stained section in which it was visible using Image J software (National Institutes of Health). Brain region volume was calculated by summing the areas and multiplying by the sampling interval. Measurements were taken on both sides of the brain, and an average was calculated, except in the few cases in which one side of the tissue was damaged. In those instances, only the intact side was used.

DBH labeling was also quantified using Image J as a marker for relative NE exposure at the time behavior and morphology were evaluated. A 0.05 mm² box was placed over the center of the LoC. Three or four locations were randomly selected from the rostro-caudal extent of the nucleus and across the two sides of the brain, depending on tissue quality and availability (in a few animals the caudal-most portion of the region was not collected). Immunohistochemical labeling was highlighted using the ‘threshold’ function (Huang setting), and the area covered by cell bodies and fibers was calculated. Values were averaged within individuals. Fibers within the song nuclei were not as abundant, and were classified on a 4 point scale: 0 = none visible; 1 = very few fibers; 2 = moderate number of distinct fibers; 3 substantial labeling of fibers.

2.5 Statistics

Analyses reflected our primary interest of determining whether limiting exposure of the developing song system to NE modulates development of singing behavior and/or song system morphology. One-way ANOVAs within each brain area were used to determine whether DSP4 treatment affected the volumes of HVC, RA and/or Area X. They were also used to evaluate the indices of song quality and comparisons to the father that were represented by continuous variables. We used a Chi-square test to assess effects on the proportion of individuals with unusual syllables across the groups. Finally, a one-way ANOVA was used to evaluate DBH labeling in the LoC, and a Kruskal-Wallis test was employed to determine whether the extent of DBH fibers differed across groups in adulthood. All analyses were computed using SPSS version 19.

3. Results

Of the 22 sons that were analyzed, 16 sang during the period of 5 trials. The 6 that did not were from each of the three groups: 2 from the DSP4 group, 1 from the zimelidine treatment, and 3 administered just saline. Thus, juvenile treatment did not appear to affect *whether* an individual sang under these testing conditions. However, it did affect song quality. Of the 16 individuals that sang, only birds treated that were administered DSP4 produced unusual syllables (as defined by [20]). Three of 5 sang long notes and one of these

also sang notes with unusually high frequencies (Figure 1). These results are in contrast to 0 of 6 in the zimelidine and 0 of 5 saline groups producing these types of unusual syllables ($\chi^2 = 8.12$, $p=0.017$).

All birds that sang, with the exception of one in the zimelidine group, produced more than 3 distinct song elements. The total number of syllables in the bouts did not differ across the groups ($F_{2,19} = 0.62$, $p = 0.548$ if zeros are included for birds that did not sing; $F_{2,13} = 1.88$, $p = 0.191$ if these animals are excluded from the analysis). Syllable duration ($F_{2,13} = 2.40$, $p = 0.130$) and inter-syllable interval ($F_{2,13} = 0.44$, $p = 0.656$) were also consistent across our manipulations (Table 1). Finally, treatment did not affect any of the variables evaluated across the father and son songs in Sound Analysis Pro: similarity ($F_{2,13} = 0.34$, $p = 0.715$), accuracy ($F_{2,13} = 0.32$, $p = 0.732$), sequential matches ($F_{2,13} = 0.28$, $p = 0.759$), pitch ($F_{2,13} = 1.04$, $p = 0.380$), wiener entropy ($F_{2,13} = 1.90$, $p = 0.188$), frequency modulation ($F_{2,13} = 0.81$, $p = 0.467$), amplitude modulation ($F_{2,13} = 2.18$, $p = 0.153$), goodness of pitch ($F_{2,13} = 0.12$, $p = 0.883$).

The general pattern of DBH labeling across the brain paralleled previous work [15]. Qualitatively, Area X consistently contained fewer DBH+ fibers than HVC and especially RA (Figure 2), but relative levels of expression within these song nuclei did not differ across the treatment groups (Kruskal-Wallis all $p > 0.300$). Similarly, LoC labeling was consistent across treatments ($F_{2,18} = 0.43$, $p = 0.656$). These results are consistent with the idea that the DSP4 manipulation during development did not have permanent effects on catecholamine levels either at their source or in the forebrain song control regions.

Juvenile treatment with DSP4 had no effect on the adult volume of HVC, RA or Area X (all $F_{2,16-19} < 1.40$, $p > 0.293$; Figure 3).

4. Discussion

DSP4 treatment of juvenile male zebra finches inhibited the development of typical song in some individuals; it induced a deficit in the quality of songs produced in adulthood. This result suggests that early exposure to NE is important for the establishment of normal vocalizations. The DSP4 manipulation did not affect the volume of the song control nuclei, which indicates either that NE is not critical for maturation of this feature or that the size of these regions can still reach control values even if development was temporarily inhibited while NE levels were low. We believe the latter is more likely, as pilot data indicated that DSP4 caused a reduction in the size of song control regions. These birds were treated in a manner similar to the present study, with injections of zimelidine hydrochloride and DSP4 on post-hatching days 8 and 18. They were euthanized on post-hatching day 25, one week following the second set of injections. Quantification of Nissl-stained sections in birds treated from DSP4 ($n=3$) and unmanipulated males ($n=4$) indicated a 40% decrease in the volume of HVC ($t_5=13.3$, $p<0.001$) and a 15% decrease in the volume of RA ($t_5=2.98$, $p=0.031$) due to treatment. Area X was 25% smaller in DSP4-compared to saline-treated birds, but this effect did not reach statistical significance, perhaps because tissue was damaged in one control animal so the sample size was only 3 ($t_4=2.22$, $p=0.091$). In contrast to controls, DBH labeling in the LoC was limited in two and not detectable in the other one of these DSP4-treated birds.

We do not know exactly how long our manipulation was effective, although NE projections appeared to have recovered by adulthood, as indicated by equivalent DBH labeling in the song nuclei across the groups. Prior to the present study, DSP4 in zebra finches had only been used in adults. Intracerebroventricular injections resulted in inhibition of NE compared to control treatment in several regions of the forebrain, including Area X and LMAN, 10

days later[3]. Systemic injections also induced substantial reductions in NE in regions including Area X and RA (40-43%), but these were not significantly different from controls [2]. Using manipulations that more closely paralleled those in the present study (two injections 10 days apart), DBH-labeled neurons in the LoC were decreased by 60% quantified 10 days following the second injection. This DSP4 treatment caused significant reductions in the percent area with DBH labeling in HVC, RA and LMAN [28]. Studies in rodents have produced variable results from peripheral injections of DSP4, but generally document inhibition of a duration that would cover much, if not all, of song system development. These include effects in the cortex of projections beginning to recover at 3-4 weeks and becoming comparable to controls at 4-6 months [6], NE concentration significantly diminished at 8 months and taking a year for full recovery [31], and a reduction of about 70-80% within 6 hours and as much as 95% at two weeks, with effective reduction for at least a month [8, 11].

We also do not know where in the brain reduced NE may have affected song development. In addition to song control nuclei, NE is present in auditory areas of the forebrain, including the caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM) where song templates may be stored, as well as the preoptic area which is involved in motivation to sing (all reviewed in [5]). Work in brain slices from juveniles suggests that NE modulates LMAN input to RA [21], circuitry critical for vocal learning.

It is difficult to draw conclusions about song learning specifically from this study. Songs might have been more alike if we had isolated father/son pairs [24]. However, we felt it was important to test the effects of NE depletion in the more natural social environment of a colony[32]. Under these types of conditions in the laboratory, males learn from multiple tutors [29]. Thus, it is not necessarily surprising that similarity scores were highly variable (ranges: DSP4 = 22-88, zimelidine = 14-68, saline 18-57). However, these and all other comparative measures between father and son were equivalent across the groups. These results suggest that inhibition of NE during development may not specifically inhibit the potential for a male to copy features of his father's song, but more likely influences the addition of unusual elements.

While our temporary inhibition of NE did not affect development of typical masculine volumes of the song control nuclei, the neuromodulator does play a role in sexual differentiation of the rodent brain. NE mediates the masculinizing effects of estradiol on the preoptic area, including increasing the size of the sexually dimorphic nucleus of the preoptic area (SDN-POA), which is larger in males than females. It may also be involved with defeminization or feminization of behavior, depending on the receptors at which it acts [reviewed in 30].

In conclusion, the present study provides the first *in vivo* experimental evidence that NE is involved in song development in the zebra finch. This work complements a body of descriptive evidence on the distribution of NE, DBH, and relevant receptors, as well as functional evidence of roles of NE in adult song production and perception [5]. Future work should utilize specific agonists and antagonists across key stages of morphological and functional development to assess the roles of NE in more depth.

Acknowledgments

We are grateful to the undergraduates in the lab who helped with animal care, to Camilla Peabody for sexing the birds, and Chelsea Hatcher and Sara Weiss for assistance with song recordings. This work was supported by NIH grant MH55488.

References

- [1]. Agate RJ, Perlman WR, Arnold AP. Cloning and expression of zebra finch (*Taeniopygia guttata*) steroidogenic factor 1: Overlap with hypothalamic but not with telencephalic aromatase. *Biol. Reprod.* 2002; 66:1127–1133. [PubMed: 11906934]
- [2]. Barclay SR, Harding C, Waterman SA. Correlations between catecholamine levels and sexual behavior in male zebra finches. *Pharm. Biochem. Behav.* 1992; 41:195–201.
- [3]. Barclay SR, Harding CF, Waterman SA. Central DSP-4 treatment decreases norepinephrine levels and courtship behavior in male zebra finches. *Pharm. Biochem. Behav.* 1996; 53:213–220.
- [4]. Castelino CB, Diekamp B, Ball GF. Noradrenergic projections to the song control nucleus Area X of the medial striatum in male zebra finches (*Taeniopygia guttata*). *J. Comp. Neurol.* 2007; 502:544–562. [PubMed: 17394158]
- [5]. Castelino CB, Schmidt MF. What birdsong can teach us about the central noradrenergic system. *J. Chem. Neuroanat.* 2010; 39:96–111. [PubMed: 19686836]
- [6]. Fritschy J-M, Grzanna R. Restoration of ascending noradrenergic projections by residual locus coeruleus neurons: Compensatory response to neurotoxin-induced cell death in the adult rat brain. *J. Comp. Neurol.* 1992; 321:421–441. [PubMed: 1506478]
- [7]. Gahr M, Metzdorf R. The sexually dimorphic expression of androgen receptors in the song nucleus hyperstriatalis ventrale pars caudale of the zebra finch develops independently of gonadal steroids. *J. Neurosci.* 1999; 19:2628–2636. [PubMed: 10087076]
- [8]. Grzanna R, Berger U, Fritschy J-M, Geffard M. Acute action of DSP-4 on central norepinephrine axons: Biochemical and immunohistochemical evidence for differential effects. *J. Histochem. Cytochem.* 1989; 37:1435–1442. [PubMed: 2768812]
- [9]. Harding CF, Barclay SR, Waterman SA. Changes in catecholamine levels and turnover rates in hypothalamic, vocal control, and auditory nuclei in male zebra finches during development. *J. Neurobiol.* 1998; 34:329–346. [PubMed: 9514523]
- [10]. Harley CW. Norepinephrine and dopamine as learning signals. *Neur. Plast.* 2004; 11:191–204.
- [11]. Jonsson G, Hallman H, Ponzio F, Ross S. DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) – a useful denervation tool for central and peripheral noradrenaline neurons. *Eur. J. Pharmacol.* 1981; 72:173–188. [PubMed: 6265244]
- [12]. Kim Y-H, Perlman WR, Arnold AP. Expression of androgen receptor mRNA in zebra finch song system: Developmental regulation by estrogen. *J. Comp. Neurol.* 2004; 469:535–547. [PubMed: 14755534]
- [13]. Kirn JR, DeVoogd TJ. Genesis and death of vocal control neurons during sexual differentiation in the zebra finch. *J. Neurosci.* 1989; 9:3176–3187. [PubMed: 2795159]
- [14]. Marler P. Three models of song learning: evidence from behavior. *J. Neurobiol.* 1997; 33:501–516. [PubMed: 9369456]
- [15]. Mello CV, Pinaud R, Ribeiro S. Noradrenergic system of the zebra finch brain: Immunocytochemical study of dopamine- β -hydroxylase. *J. Comp. Neurol.* 1998; 400:207–228. [PubMed: 9766400]
- [16]. Nordeen KW, Nordeen EJ. Anatomical and synaptic substrates for avian song learning. *J. Neurobiol.* 1997; 33:532–548. [PubMed: 9369458]
- [17]. Reiner A, Perkel DJ, Bruce LL, Butler AB, Csillag A, Kuenzel W, Medina L, Paxinos G, Shimizu T, Striedter G, Wild M, Ball GF, Durand S, Gütürkün O, Lee DW, Mello CV, Powers A, White SA, Hough G, Kubikova L, Smulders TV, Wada K, Dugas-Ford J, Husband S, Yamamoto K, Yu J, Siang C, Jarvis ED. Revised nomenclature for avian telencephalon and some related brainstem nuclei. *J. Comp. Neurol.* 2004; 473:377–414. [PubMed: 15116397]
- [18]. Sakaguchi H, Saito N. The acetylcholine and catecholamine contents in song control nuclei of zebra finch during song ontogeny. *Dev. Brain Res.* 1989; 47:313–317. [PubMed: 2743564]
- [19]. Sara SJ. The locus coeruleus and noradrenergic modulation of cognition. *Nat. Rev. Neurosci.* 2009; 10:211–223. [PubMed: 19190638]
- [20]. Simpson HB, Vicario DS. Early estrogen treatment alone causes female zebra finches to produce learned, male-like vocalizations. *J. Neurobiol.* 1991; 22:755–776. [PubMed: 1765782]

- [21]. Sizemore M, Perkel DJ. Noradrenergic and GABA_B receptor activation differentially modulate inputs to the premotor nucleus RA in zebra finches. *J. Neurophysiol.* 2008; 100:8–18. [PubMed: 18463188]
- [22]. Slater PJB, Mann NI. Do male zebra finches learn their fathers' songs? *Trends Ecol. Evol.* 1990; 5:415–417. [PubMed: 21232404]
- [23]. Sockman KW, Salvante KG. The integration of song environment by catecholaminergic systems innervating the auditory telencephalon of adult female European starlings. *Dev. Neurobiol.* 2008; 68:656–668. [PubMed: 18278799]
- [24]. Tchernichovski O, Nottebohm F. Social inhibition of song imitation among sibling male zebra finches. *Proc Natl Acad Sci U S A.* 1998; 95:8951–8956. [PubMed: 9671785]
- [25]. Tchernichovski O, Nottebohm F, Ho CE, Pesaran B, Mitra PP. A procedure for an automated measurement of song similarity. *Anim. Behav.* 2000; 59:1167–1176. [PubMed: 10877896]
- [26]. Wade J. Zebra finch sexual differentiation: The aromatization hypothesis revisited. *Microsc. Res. Tech.* 2001; 54:354–363. [PubMed: 11668648]
- [27]. Wade J, Arnold AP. Sexual differentiation of the zebra finch song system. *Ann. Rev. N.Y. Acad. Sci.* 2004; 1016:540–559.
- [28]. Waterman SA, Harding CF. Neurotoxic effects of DSP-4 on the central noradrenergic system in male zebra finches. *Behav. Brain Res.* 2008; 188:271–280. [PubMed: 18160108]
- [29]. Williams H. Models for song learning in the zebra finch: fathers or others? *Anim. Behav.* 1990; 39:745–757.
- [30]. Wilson CA, Davies DC. The control of sexual differentiation of the reproductive system and brain. *Reprod.* 2007; 133:331–359.
- [31]. Wolfman C, Abo V, Calvo D, Medina J, Dajas F, Silveira R. Recovery of central noradrenergic neurons one year after the administration of the neurotoxin DSP4. *Neurochem. Int.* 1994; 25:395–400. [PubMed: 7820072]
- [32]. Zann, RA. *The zebra finch: A synthesis of field and laboratory studies.* Oxford University Press; Oxford: 1996.

Highlights

- !! Norepinephrine (NE) inhibition in juvenile zebra finches affects adult song quality
- !! DSP4 in developing male zebra finches does not permanently affect song nucleus size
- !! Future work should elucidate specific roles of NE in song system development

Figure 1.

Representative spectrograms from each treatment group. Images depict two seconds of song from animals administered DSP4 (top), zimelidine (middle), and saline only (bottom). In the saline-treated bird, this clip contains 2 clearly identifiable motifs. In the one administered zimelidine, roughly 2.5 motifs are included. The pattern of notes in the DSP4 was less consistent, and the long notes (>300ms) combined with relatively limited breaks made it somewhat difficult to define discrete motifs. At least 4 notes in this clip (arrows) had elements that were predominantly higher than 1.5kHz.

Figure 2.

DBH immunohistochemistry in the song control nuclei (RA, Area X and HVC) and the locus coeruleus (LoC) of a saline-treated bird. The inset diagrams the location of the LoC at the level of the photograph, indicated with the arrow. Substantial labeling of cell bodies and fibers was observed in the LoC. Punctuate labeling typical of fibers was visible in each of the song control regions, with qualitatively more apparent in the HVC, and particularly RA, compared to Area X. Cb= cerebellum, FLM = Fasciculus longitudinalis medialis, TeO = optic tectum.

Figure 3.

Volumes of song control nuclei across the three treatment conditions. Values are means $\pm$ standard errors. Sample sizes are noted within the bars; they are reduced in some groups due to tissue damage that prevented accurate tracing of the borders in some sections. No significant effects were detected.

Table 1

Song characteristics across treatment groups. Values represent means (standard error) calculated in ms from averages per individual.

Treatment	Syllable Duration	Inter-Syllable Interval
DSP4	142.8 (8.7)	30.2 (2.6)
Zimelidine	134.4 (10.5)	32.4 (4.9)
Saline only	112.4 (9.7)	36.9 (6.5)