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Correlations Between Catecholamine Levels and Sexual Behavior in Male Zebra Finches

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BARCLAY, S. R., C. F. HARDING AND S. A. WATERMAN. *Correlations between catecholamine levels and sexual behavior in male zebra finches.* PHARMACOL BIOCHEM BEHAV **41**(1) 195–201, 1992.—In zebra finches, the combined actions of estrogens and androgens activate male courtship, including singing, and also strongly modulate norepinephrine (NE) levels and turnover in brain areas known to be involved in controlling courtship behavior. To determine whether changes in NE levels mediate changes in courtship, we administered DSP-4 to males and measured its effects on monoamine levels and reproductive behavior. DSP-4 treatment did not affect serotonin (5-HT), had small, variable effects on dopamine (DA), and caused moderate, nonsignificant reductions in NE. However, in DSP-4-treated males, NE levels in specific vocal-control nuclei showed high positive correlations with courtship singing. There were no significant correlations between NE levels in hypothalamic nuclei and any behavior or DA or 5-HT levels in any nuclei and any behavior. DSP-4-treated males took longer to begin singing and performed fewer song bouts and courtship displays, but their songs could not be differentiated from those of control males. This suggests that their behavioral deficits resulted from deficits in attention rather than an inability to sing.

Catecholamines Norepinephrine DSP-4 Androgen-dependent behavior Vocal control system Attention

RESEARCH strongly suggests that the catecholamines (CAs), particularly dopamine (DA) and norepinephrine (NE), play a central role in mediating the effects of changing steroid levels on both gonadotropin secretion and reproductive behavior in many vertebrate species. Although CAs may affect reproductive behavior indirectly through their effects on hormone secretion, they also act directly by modulating the responsiveness of brain areas regulating reproductive behavior (35). Studies of male reproductive behavior often discuss CA effects in terms of increasing attentiveness towards females and female-directed behavior (13,26).

Few data are available on the role of CAs in modulating avian behavior, although it has been shown in quail that participating in aggressive interactions increases both DA and NE levels (12). Several studies have implicated NE in modulating male sexual behavior, although results from different studies have been contradictory. Barclay et al. (4) demonstrated that noradrenergic neurotransmission was intimately involved in the control of courtship vocalizations in ring doves. Pharmacological manipulations which lowered hypothalamic NE levels increased bow-coo and nest-coo behavior, while treatments which increased hypothalamic NE levels decreased these courtship vocalizations. Similarly, one study in Japanese quail found that treatments which decreased NE function increased the frequency and duration of sexual behavior in male quail, and treatment with an α-adrenergic agonist reversed these effects (3). However, a second study in quail found precisely the opposite effects (29).

In zebra finches, we have shown that the two classes of steroids which are required to stimulate normal singing behavior, estrogens and androgens, regulate CA levels and turnover in 9 of the 10 brain areas examined [(6); i.e., 5 of 6 vocal control nuclei (NIf, RA, DM, X, MAN), an auditory nucleus (field L) and 3 hypothalamic nuclei (POA, PVM, IN); see Fig. 1 for abbreviations]. These steroid-induced CA changes were more widespread and of greater magnitude than those previously reported in rats. Most brain areas examined (i.e., POA, IN, NIf, RA, DM) responded to effective hormone treatments by increasing CA levels and/or turnover, while the opposite was true for the remaining hormone-sensitive nuclei (i.e., PVM, MAN, area X, field L). Thus, vocal control nuclei on the efferent path controlling singing (NIf, RA) or other vocalizations (DM: 1) responded to effective hormone treatments with increased CA function, while those involved in song learning (area X: 31, 33; MAN: 9; and field L, an area analogous to the auditory cortex) showed decreased function following treatment with hormones which activate courtship behavior.

To elucidate whether CA function in vocal control nuclei on the efferent pathway is involved in controlling courtship singing in zebra finches, we administered the neurotoxin DSP-4 to intact male zebra finches and measured its effects on reproductive behavior. DSP-4's effects on monoamine levels in hypothalamic and vocal control nuclei were quantified by high performance liquid chromatography with electrochemical detection (HPLC-EC). DSP-4 was chosen because numerous reports suggest that this drug produces substantial and lasting depletion of NE in the

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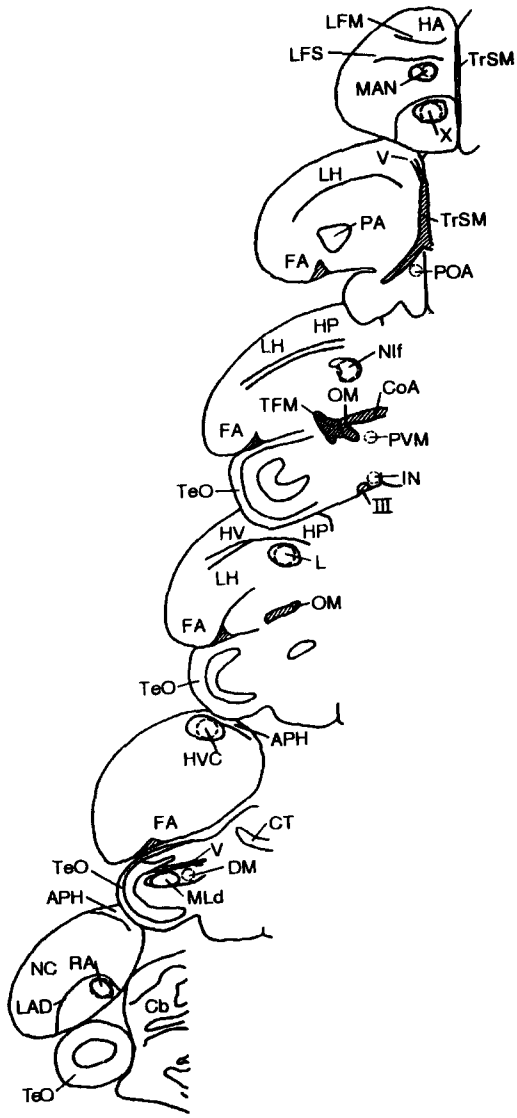

FIG. 1. Schematic drawings of representative frozen brain sections, showing relevant landmarks and microdissected areas. Abbreviations: APH—area parahippocampalis; Cb—cerebellum; CoA—commissura anterior; CT—commissura tectalis; DM—dorsomedial portion of nucleus intercollicularis; FA—tractus fronto-archistriatalis; HA—hyperstriatum accessorium; HP—hippocampus; HV—hyperstriatum ventrale; HVC—high vocal center, previously called hyperstriatum ventrale pars caudale; III—nervus oculomotorius; IN—nucleus infundibularis; L—field L; LAD—lamina archistriatalis dorsalis; LFM—lamina frontalis suprema; LFS—lamina frontalis superior; LH—lamina hyperstriatica; LMD—lamina medullaris dorsalis; MAN—nucleus magnocellularis neostriatum anterior; MLd—nucleus mesencephalicus lateralis, pars dorsalis; NC—neostriatum caudale; NIf—nucleus interfacialis; OM—tractus occipitomesencephalicus; PA—paleostriatum augmentatum; POA—nucleus preopticus anterioris; PVM—nucleus paraventricularis magnocellularis; RA—nucleus robustus archistriatalis; TeO—tectum opticum; TFM—tractus thalamofrontalis et frontalis thalamicus medialis; TrSM—tractus septomesencephalicus; V—ventricle; X—area X.

cortex and hippocampus leaving the hypothalamus relatively unaffected (7,15) and because it appears to be more selective than other neurotoxins, affecting NE without causing profound changes in DA or serotonin (5-HT) levels (21). Thus, we expected DSP-4 to reduce NE levels in vocal control nuclei, leaving hypothalamic levels relatively unaltered. We also expected that DSP-4 treatment would leave DA and 5-HT function relatively unaffected throughout the brain. Based on the results of our previous work on hormonal regulation of catecholamines and sexual behavior in finches, we predicted that decreased NE levels in vocal control nuclei on the efferent motor pathway would be associated with deficits in courtship singing.

METHOD

Subjects

Zebra finches were obtained from our breeding colony. Males and females were housed in large isosexual aviaries until needed. All animal rooms were temperature controlled (24 ± 2°C) with a 14:10 h light:dark cycle, lights off at 20:30. The relative humidity was maintained above 50%, since breeding in this species normally occurs under humid conditions. Birds were fed a vitamin-supplemented (8 in 1, Pet Products) commercial finch seed mix, grit, water and cuttlebone ad lib and received fresh greens and oranges daily. Birds used in the experiment were sexually-naive adults at least 120 days of age. During the experiment, males were housed in individual cages (56 × 56 × 56 cm) and females introduced for behavioral observations.

Behavioral Tests

Our behavioral testing paradigm has been successfully used for the past 10 years to monitor the ability of various hormone treatments to activate behavior [see (18, 19, 36) for additional details]. Most behavior categories are self-explanatory. Courtship, however, merits further description. A courting male sings to the female while showing specific postural displays. The intensity of both the song and the visual display can vary. In this experiment, we recorded three levels of intensity: high, medium and low. In low-intensity displays, the male assumes a fairly relaxed posture, turns toward the female and sings, fluffing the sexually dimorphic throat feathers. In medium-intensity displays, the male stands erect, fluffs the throat feathers, turning his head from side to side as he sings. In high-intensity displays, the male stands very erect, sleeks the feathers on top of his head, fluffs out sexually dimorphic feathers on cheeks, throat and flanks and begins to sing to the female from a distance. He hops down the perch towards her, turning 180° with each hop, always twisting his head and tail towards the female while continuing to sing. High-intensity displays appear more exaggerated, coherent, and vigorous. Although males often beak wipe after feeding to remove food remnants, males also beak wipe when a female is first introduced into their cages and between songs. Levels of beak wiping are highly correlated with levels of courtship singing. In addition to courtship and copulatory behaviors, the occurrence of 45 other common behavior patterns was recorded during these tests. These included patterns with a primarily aggressive function (e.g., peck, pluck, chase, supplant), comfort behavior (e.g., preen, stretch, shake), nestbuilding activities (e.g., mandibulate, transport, build), and other social behavior (e.g., follow, clump, heteropreen). In addition to the standard behavioral observations, a second observer recorded vocalizations on a Uher tape deck for later analysis of song patterns.

For pair tests, a female was placed in a male's cage for 15 min and their behavior observed. The order of observations was counterbalanced from test to test. Each male was tested with a different female on each test and had two predrug pair tests and

one postdrug pair test. Any male which did not court in both predrug tests was dropped from the experiment.

Drug Administration

The two drugs used were zimelidine dihydrochloride, a serotonergic re-uptake blocker, and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4, kind gifts of Trevor Archer, Astra Pharmaceuticals, Ltd.). Males were assigned randomly to control and experimental groups. After the second pair test, 20 mg/kg zimelidine (20 μg/0.01 ml saline/g body weight) was administered IP to all males to protect serotonergic neurons. Thirty minutes later, half the males (final N = 5) received 50 mg/kg DSP-4 IP (50 μg/0.01 ml saline/g body weight) and the other half (N = 5) received control injections of saline. Males remained in their individual cages for ten days until the final pair test. Following this test, males were quickly caught and sacrificed by decapitation to minimize stress, since handling alters monoamine levels. Anesthesia was not used, since monoamine levels are strongly affected by anesthetization.

Microdissection of Brain Nuclei

Brains were quickly removed and frozen in powdered dry ice. Frozen brains were mounted on cryostat chucks with distilled water and stored at −70°C until transferred to a Hacker/Bright cryostat and allowed to equilibrate to −15°C for at least 20 min before cutting sequential coronal sections 180 μm thick. Sections were thaw-mounted onto glass slides and stored at −70°C for 16–72 hours before dissection. The location of nuclei was determined using the atlas of Stokes et al. (34) with additions from Nottebohm et al. (28). Tissue was handled as described previously (5,6), and specific nuclei removed using chilled 500 or 1000 μm stainless steel punches as detailed in Fig. 1.

Measurement of Catecholamines by HPLC-EC

The simultaneous determination of NE, DA, epinephrine (EPI), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) in microdissected samples was carried out by HPLC-EC as previously described (5,6). Briefly, microdissected nuclei were expelled into 1.5 ml tubes containing 80 μl of a sodium acetate buffer with α-methyldopamine (αMDA, gift from Merck, Sharp and Dohme, Inc.) as the internal standard. Tubes were frozen on dry ice, thawed, and centrifuged at 15,000 × g for 10 min at 2°C. After centrifugation, the supernatant was aspirated and 60 μl injected into a Waters chromatographic system with a radially compressed 10 cm C-18 Novapak analytical column (4 μm particles, Waters Associates) and a LC-4B electrochemical detector (Bioanalytical Systems). No prior purification steps were necessary. Separation of compounds employed an aqueous/acetonitrile mobile phase, pH lowered to 3.9 with glacial acetic acid. The system was calibrated by injecting 200 pg of the external standards (arterenol free base, epinephrine sulfate, 3-hydroxytyramine HCl, 5-hydroxytryptamine creatinine sulfate, 5-hydroxyindoleacetic acid, Sigma Chemical Co.) at the beginning, middle and end of each sample run. Standard peak area was determined from mean peak areas of three chromatograms of each standard. The internal standard, αMDA, was injected three times to determine peak area for calculation of percent recovery. Sample pellets were dissolved in 100 μl of 0.2 N NaOH, and the protein content determined using the dye-binding method of Bradford (10).

Statistical Analysis

Behavioral data were analyzed by two-factor mixed design analyses of variance with repeated measures (DSP-4 vs. saline, with 3 trials per animal) followed by Duncan's post hoc test when a significant F value was found (11). Since the latency data were skewed, they were analyzed nonparametrically (32). Each male's latency to sing on the postdrug test was subtracted from his latency to sing on the second predrug test. The difference scores of the two groups were then compared using the Mann-Whitney U-test. Pearson correlation analyses (11) were used to determine if the level of a specific neurotransmitter in a specific nucleus was related to the frequency of a specific behavior. Prior to the experiment, we hypothesized that DSP-4 treatment would cause deficits in singing behavior. The p values given for measures of singing behavior are based on one-tailed tests. Two-tailed tests were used for the catecholamine and correlation analyses.

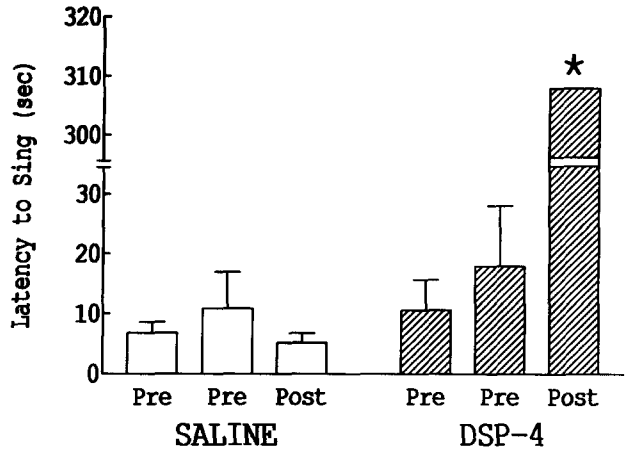

FIG. 2. Mean (± SE) latencies to the first song of saline- and DSP-4-treated male finches in 15-min tests with females. The SE for the DSP-4-treated males' posttreatment test is not shown because it would go off scale. It was 200.1 s. *Significant change from pretreatment latencies compared to saline-treated males, $p<0.008$. See text for description of how latencies were compared.

RESULTS

Treatment with DSP-4 caused significant increases in latencies to sing compared to treatment with saline (Mann-Whitney U, U = 1, $p<0.008$, Fig. 2). The latencies of DSP-4-treated males rose from an average of less than 20 seconds to over 300 seconds. DSP-4 treatment significantly reduced the frequency of courtship displays without differentially affecting the intensity of the displays (see Fig. 3) and significantly decreased the number of song bouts [ANOVA, Treatment × Trials, $F(2,16)=5.82$, $p<0.0125$; $F(2,16)=3.84$, $p<0.025$, respectively]. Duncan's post hoc comparison demonstrated a significant decline in mean song bouts in the DSP-4 group from 13.6 ± 3.7 in their first test to 7.4 ± 3.4 following treatment, while mean bout frequency in the saline group was unaffected (10.2 ± 3.0 in first test and 10.0 ± 2.6 following treatment). DSP-4 treatment had no significant effects on other measures of behavior such as beak wipe, follow, clump and peck.

To examine whether DSP-4 treatment decreased singing by affecting attentional or motor mechanisms, we used several measures to examine whether DSP-4 treatment affected the motor patterning of songs, including total motifs (individual song phrases) per test, total motifs per song bout, song bout length and total song bouts in the five-minute interval after singing be-

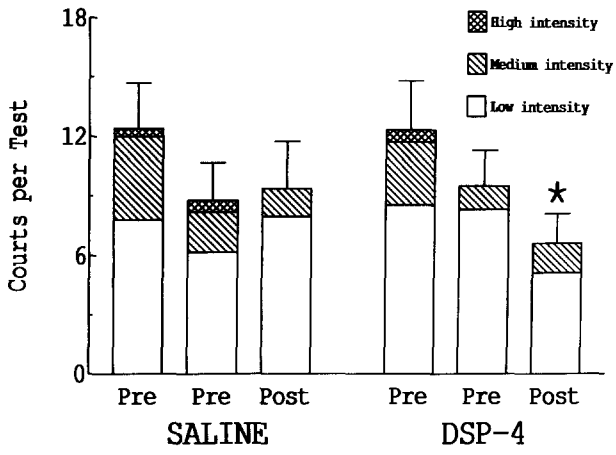

FIG. 3. Total courtship displays (mean ± SE) shown by saline- and DSP-4-treated male finches in 15-min tests with females. *Significantly decreased from pretreatment frequencies of the same males, $p<0.0125$. Intensity of the visual dance display was not significantly affected by drug treatment.

gan. DSP-4 treatment had no significant effects on any of these measures.

5-HT and 5-HIAA levels in the 10 nuclei examined were not significantly affected by DSP-4 administration. The effects of drug treatment on NE and DA levels in the 10 brain areas varied greatly (see Table 1). DA levels were somewhat reduced following DSP-4 treatment in three brain areas examined: RA (34%), PVM (25%), and POA (23%). Substantial reductions in NE levels in drug-treated males were found in five of the areas examined: area X (43%), RA (40%), DM (28%), PVM (22%) and IN (21%). Although the mean DA and NE levels in DSP-4-treated males were not significantly different from those of control males, reductions of NE in vocal control nuclei in DSP-4-treated males were highly correlated with specific reductions in behavior (see Table 2). NE levels in three of the vocal control nuclei on the efferent motor pathway (NIf, RA and DM) were significantly correlated with total courtship displays and total song bouts in DSP-4-treated males. NE levels DM and area X were positively correlated with total motifs and motifs/bout in these males. There were also two significant correlations between NE levels in DM and field L and measures of singing behavior in the saline-treated controls (see Table 2). Only decreases in NE levels in specific vocal control areas were correlated with deficits in male courtship behavior and these NE deficits were only correlated with decreases in singing behavior. There were no significant correlations between NE levels in hypothalamic nuclei and any behavior or DA or 5-HT levels in any nuclei and any behavior.

TABLE 1

MEAN (± SEM) NOREPINEPHRINE AND DOPAMINE LEVELS (pg AMINE/μg PROTEIN) IN VOCAL CONTROL AND HYPOTHALAMIC NUCLEI OF SALINE- AND DSP-4-TREATED MALES

Brain Area	Norepinephrine		Dopamine	
	Saline	DSP-4	Saline	DSP-4
MAN	12.9 ± 3.6	11.4 ± 1.8	6.5 ± 1.0	13.0 ± 3.0
X	32.0 ± 9.3	18.2 ± 9.4	112.8 ± 41.2	105.1 ± 28.2
NIf	6.4 ± 1.3	6.5 ± 1.9	7.7 ± 2.8	11.9 ± 3.7
HVC	4.8 ± 1.5	5.2 ± 2.0	8.2 ± 3.4	8.5 ± 12.3
RA	19.6 ± 7.3	11.7 ± 4.1	11.1 ± 5.2	7.3 ± 1.7
DM	30.6 ± 11.0	21.9 ± 10.8	13.2 ± 4.6	16.0 ± 2.3
L	4.9 ± 1.6	5.7 ± 1.1	4.0 ± 1.2	6.6 ± 2.5
POA	93.1 ± 22.3	84.3 ± 23.5	46.0 ± 16.2	35.5 ± 5.3
PVM	58.3 ± 11.3	45.4 ± 10.4	32.3 ± 11.1	24.2 ± 5.8
IN	61.2 ± 7.5	48.4 ± 9.2	32.7 ± 8.9	29.8 ± 4.0

See Fig. 1 for abbreviations.

DISCUSSION

DSP-4 is a neurotoxin which after systemic injection reportedly passes easily through the blood-brain barrier and exerts its effects preferentially on terminals of noradrenergic projections originating in the locus coeruleus (16, 22, 30). The dose of DSP-4 commonly used in rat studies (50 mg/kg) did not cause the expected degree of NE depletion in either finches or, as reported previously, in quail (2). In neither case did NE depletions reach expected levels in extra-hypothalamic areas, and there was some depletion in the hypothalamus. This may result from differences in noradrenergic innervation in avian and mammalian brains. Two facts support this suggestion. First, the pattern of locus coeruleus projections in pigeons differs from that in rats. In rats, most, if not all, cortical fields receive input from neurons within the locus coeruleus (20). In pigeons, the hyperstriatum accessorium, most of the neostriatum and hyperstriatum ventrale, and all but the dorsal portion of the archistriatum receive little or no input from either the locus coeruleus or subcoeruleus cell groups (23). Unfortunately, a map of noradrenergic projections in the zebra finch is not yet available. If NE innervation in finches is similar to that in pigeons, it would explain why DSP-4 had little effect on MAN, HVC, NIf, and field L which are in the neostriatum and hyperstriatum ventrale. Second, consistently higher levels of both DA and NE are found in avian brains (5, 27, 29). This factor alone may explain the inability of the standard dose of DSP-4 to induce statistically significant depletions of NE in the zebra finch, since NE levels in the finch brain are about ten times higher than in comparable areas of the rat brain. However higher dosages of DSP-4 resulted in unacceptably high mortality levels in zebra finches.

Although NE depletions in even the most affected brain areas in DSP-4-treated finches were under 45%, the resulting decrements in courtship behavior were quite striking. Three measures of behavior were affected: total number of courtship displays and total number of song bouts decreased, while latencies to first song increased. DSP-4 treatment did not affect the intensity of males' postural dance display. Males were more likely to show high intensity displays on the first test because it was their first pairing with an adult female. Our previous work demonstrated that hormone treatments which activated high levels of singing behavior caused significant increases in noradrenergic activity in the efferent pathway controlling vocalizations. The data from the current study suggest that NE mediates some of the hormone-dependent changes in singing in male zebra finches, adding finches to the many species in which NE modulates male reproductive behavior.

The effects of DSP-4 on NE levels varied greatly between individuals in this study. This is not unusual in this species, since finches also show higher variability than rats on many biochemical measurements including catecholamine turnover, cholinergic enzyme activity, and steroid receptor concentrations, probably caused by the lack of inbred stock. This high individual variation may have prevented our small subject group from showing statistically significant depletions of NE when DSP-4

TABLE 2

CORRELATIONS BETWEEN NOREPINEPHRINE LEVELS IN SPECIFIC BRAIN AREAS AND FIVE COURTSHIP BEHAVIORS

Brain Area		Total Courts	Song Bouts	Total Motifs	Motifs/Bout	Beak Wipes
MAN	S	.72	.38	.69	.20	.55
	D	−.46	−.62	−.77	−.67	−.56
X	S	.85	.84	.74	−.34	.65
	D	.66	.84	.99‡	.97‡	.42
NIf	S	.46	.41	.64	.20	−.28
	D	.92†	.96‡	.84	.82	.77
HVC	S	−.04	.16	.28	−.38	−.49
	D	.44	.32	.15	.16	.63
RA	S	.80	.08	.80	.59	.40
	D	.88*	.90*	.82	.79	.87
DM	S	.88*	.79	.81	−.28	.55
	D	.94†	.98‡	.91†	.88*	.84
L	S	.26	−.67	.35	1.00‡	.42
	D	.24	−.03	−.45	−.42	.42

S = saline-treated controls. D = DSP-4-treated males. *$p \leq 0.05$; †$p \leq 0.025$; ‡$p \leq 0.01$.

was administered. Depletions of NE in five areas (DM, RA, area X, PVM and IN) reached levels comparable to those reported in mammals (25) and quail (2). DA levels in 3 nuclei were also somewhat depressed in DSP-4-treated males. However, DA levels were not related to changes in singing behavior. Unlike NE, DA levels were just as likely to rise following DSP-4 treatment as to fall, suggesting that changes in DA levels might not be a direct effect of the neurotoxin on DA neurons. Others have suggested that DSP-4's effects on dopaminergic function are primarily indirect, caused by the loss of NE modulation of DA neurons (8,14).

Correlation analyses revealed that NE levels in each of three vocal control nuclei, NIf, RA, and DM, were significantly correlated with both the total number of courtship displays and the number of song bouts shown. The total number of motifs and motifs/bout was significantly correlated with NE levels in area X and DM. Although the DSP-4-treated group showed greater individual variation in NE levels in the vocal control nuclei than the saline-treated group, and most of the statistically significant differences were found in this group, there were two significant correlations between behavior and NE levels in field L and DM in the saline-treated group. This suggests that even within this smaller range of NE variation, NE levels in these nuclei appear to be related to levels of singing behavior. Other patterns of courtship behavior, such as beak wiping which is normally highly correlated with courtship singing (19), were not correlated with NE levels in any brain areas. In addition, NE levels in the three hypothalamic areas were not correlated with any of the behavioral measures. These data suggest two important conclusions about the effects of DSP-4 on male reproductive behavior in zebra finches. First, these were not indirect effects mediated through decreases in plasma androgens since closely related androgen-dependent behaviors such as beak wiping which is very sensitive to androgen levels (19) were not affected by drug treatment. Second, the decreases in singing behavior seen were mediated through drug effects on the vocal control system.

The relationship between decreased NE levels in NIf, RA, and DM and decrements in singing behavior were expected. Castration reduces NE levels and/or turnover in these nuclei (5) and causes significant reductions in singing (19). Hormone treatments which restore normal levels of singing behavior (e.g., androstenedione; estradiol + dihydrotestosterone) increase NE levels and/or turnover in these three nuclei which are part of the efferent motor pathway controlling singing and other vocalizations (5,6). The relationship between decreased NE levels in area X and decreases in the numbers of motifs per bout and per test are more problematic. First, area X has only been shown to play a role in song learning; there is no evidence implicating it in song production in adult males. These data open the possibility that area X may be involved in the motor patterning of courtship songs in adults since motifs per bout was affected. Second, hormonal modulation of NE in area X in adult males is in the opposite direction from that in areas known to be involved in song production. Castration increases NE levels and turnover in area X, while hormone treatments which increase singing cause significant decreases (5,6). Thus, the correlation between decreases in NE levels in area X and decreased production of motifs was unexpected. Depletions of NE in area X may provide a good measure of the general level of functioning of locus coeruleus NE projections, since area X receives a strong catecholaminergic projection from area ventralis of Tsai (24) which itself receives a direct projection from the locus coeruleus (23). Perhaps the general level of NE functioning rather than something specific to NE function in area X is the factor related to motif production.

In previous studies, our laboratory has examined the relative contributions of androgens and estrogens in activating courtship singing. Stimulation by both classes of hormones is required to raise levels of courtship singing above the baseline shown by castrated males (19). More recently, we have shown that singing directed at other birds, both males and females, is stimulated by estrogens. Treating birds with an aromatization inhibitor to decrease available estrogens significantly reduced courtship singing directed towards females as well as singing directed towards other males, while having no effect on undirected singing (37). Estrogenic stimulation appears to make males more attentive to the presence of other birds. Interestingly, hormonal modulation of NE levels and turnover in hypothalamic and vocal

control nuclei is primarily estrogen dependent (6). Although treatment with estrogen + androgen always restored normal CA function in castrated males, 79% of hormone-induced changes in NE levels and turnover rates could be induced by estrogen alone; the remaining 21% required stimulation by estrogen + androgen. In contrast, DA function was totally androgen dependent in 56%, estrogen + androgen dependent in 22%, and totally estrogen dependent in 22% of cases. Given the evidence in other species that NE's effects on behavior are mediated through changes in attention or arousal, we hypothesized that perhaps estrogen's effects on attentiveness to other birds were mediated through its effects on NE function. The results of the present study suggest this hypothesis may be correct. Using DSP-4 to decrease NE function resulted in decreases in courtship singing which appeared to result from a deficit in attention or arousal rather than an inability to perform the behavior. DSP-4-treated males took longer to begin singing to females, but once they began singing, we could not differentiate their songs from those of control males. These results are similar to those obtained in DSP-4-treated male rats which showed decreases in measures of arousal, such as increased ejaculation latencies and length of postejaculatory intervals, but no drug-induced effects on measures of motor performance such as number of mounts and intromissions (17). Our methods of assessing motor performance were simple. However, these methods are sufficient to detect changes in song performance caused by lack of estrogen (37). Males treated with an aromatization inhibitor not only direct fewer songs to females, but those songs they do sing to females are sung at the distinctly slower rate typical of undirected songs. They also link fewer songs together in a bout than untreated males. Thus, while estrogen deprivation causes deficits in singing behavior which appear to be caused by changes in both attention and motor patterning, NE deprivation appears to affect only the attentional aspect of song control. Estrogen's effects on the motor patterning of song are probably mediated by a different neurotransmitter system.

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