ELSEVIER

Pharmacology Biochemistry and Behavior, Vol. 53, No. 1, pp. 213–220, 1996

0091-3057/96 $15.00 + .00

0091-3057(95)00183-2

Central DSP-4 Treatment Decreases Norepinephrine Levels and Courtship Behavior in Male Zebra Finches

SHARON R. BARCLAY,[1] CHERYL F. HARDING AND SUSANNA A. WATERMAN

Biopsychology Program, Hunter College, CUNY, 695 Park Ave., New York, NY 10021

Received 21 March 1994

BARCLAY, S. R., C. F. HARDING AND S. A. WATERMAN. *Central DSP-4 treatment decreases norepinephrine levels and courtship behavior in male zebra finches.* PHARMACOL BIOCHEM BEHAV 53(1) 213–220, 1996.—In zebra finches, gonadal steroids activate male courtship, including singing, and also strongly modulate norepinephrine (NE) levels and turnover in brain areas regulating courtship behavior. In a previous study, systemic administration of DSP-4 caused significant decreases in courtship singing. These behavioral decrements were correlated with the degree of NE depletion in several vocal control nuclei. In the present study, we attempted to further decrease brain NE levels while minimizing systemic effects by infusing DSP-4 directly into the third ventricle. DSP-4 treatment significantly reduced NE levels in three of six vocal control nuclei and both hypothalamic nuclei sampled without significantly altering dopamine or serotonin levels in any areas. DSP-4–treated males took longer to begin singing and performed fewer song bouts and courtship displays. Interestingly, behavioral deficits were limited to courtship song displays, other behavior patterns, including female-directed behaviors like approach and follow, were unaffected by DSP-4 treatment. DSP-4 treatment appeared to affect singing behavior by causing deficits in initial attentiveness to females and initiation of singing rather than by affecting song structure.

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

RESEARCH strongly suggests that the catecholamines (CAs) dopamine (DA) and norepinephrine (NE) play a critical 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 can also act directly by modulating the responsiveness of brain areas regulating reproductive behavior (43). Increased CA function is often suggested to alter male reproductive behavior by increasing attentiveness towards females or increasing female-directed behavior (15,34).

Few data are available on the role of CAs in modulating avian behavior. Several studies have implicated NE in modulating male sexual behavior. However, depending on the experimental design, increased CA function has been associated with both increases and decreases in sexual behavior. Barclay et al. (6) demonstrated that noradrenergic neurotransmission was intimately involved in controlling courtship vocalizations in ring doves. Pharmacological manipulations that lowered hypothalamic NE levels increased bow-coos and nest-coos, while treatments that increased hypothalamic NE levels decreased these courtship vocalizations. Similarly, one study in Japanese quail found that decreasing NE function in testosterone-treated castrated males increased the frequency and duration of their sexual behavior, and treatment with an α-adrenergic agonist reversed these effects (5). A second study found that castrated quail that did not receive hormone supplementation responded to NE agonists with increased copulatory behavior, while testosterone-treated castrates responded to a β-adrenoceptor antagonist with decreased sexual behavior (37).

We were interested in how NE might modulate the reproductive behavior of songbirds. Singing is a hormone-dependent behavior, stimulated by the combined actions of androgens and estrogens (24). In zebra finches, we have shown that androgens and estrogens also regulate CA levels and turnover in 9 of 10 brain areas examined [(8); i.e., five of six vocal control nuclei (NIf, RA, DM, X, MAN—nucleus interfacialis, nucleus robustus archistriatalis, dorsomedial portion of nu-

[1] To whom requests for reprints should be addressed.

cleus intercollicularis, area X, and nucleus magnocellularis neostriatum anterior, respectively), an auditory area (field L), and three hypothalamic nuclei (POA, PVM, IN—nucleus preopticus anterioris, nucleus paraventricularis magnocellularis, nucleus infundibularis)]. These steroid-induced changes in CAs were more widespread and of greater magnitude than those previously reported in rats. Interestingly, vocal control nuclei on the efferent motor path controlling singing (NIf, RA), or other vocalizations [DM: (2)] responded to effective hormone treatments with increased CA function, while those involved in song learning [area X: (39,41); MAN: (11); and field L, an area analogous to the auditory cortex], showed decreased function following treatment with hormones which activate courtship behavior.

In a previous study, we administered the neurotoxin DSP-4 to intact male zebra finches and measured its effects on reproductive behavior to determine whether noradrenergic function in vocal control nuclei on the motor pathway is involved in controlling courtship singing in this species. DSP-4 was chosen because in mammals it causes substantial and long-lasting depletion of NE in the telencephalon while leaving the hypothalamus relatively unaffected [e.g., (18,19,21)]. The noradrenergic innervation of much of the telencephalon arises from the locus coeruleus (LC), while about 80% of the hypothalamic innervation originates in the ventral tegmentum (17). DSP-4 acts by entering noradrenergic axons via the NE reuptake mechanism, and it appears that axon terminals of the LC have a higher affinity for this drug than those of the ventral tegmentum, thus explaining the higher vulnerability of areas innervated by the LC to the effects of this toxin (46). DSP-4's effects include irreversible decrements in NE levels (26,32,33), dopamine-β-hydroxylase activity (28), and neuronal uptake of NE (31). The drug causes loss of noradrenergic axons within 2 weeks and profound loss of LC cells by 6 months (20). DSP-4 is more selective than other neurotoxins, reducing NE without causing significant changes in DA, epinephrine, acetylcholine, GABA, glycine, aspartic acid, or glutamic acid levels (25, 27,30). The effects of DSP-4 on 5-HT neurons can be completely prevented by administering the 5-HT reuptake inhibitor, zimelidine, to block its uptake into 5-HT cells (29). Thus, we hoped that DSP-4 treatment of finches would produce substantial depletion of NE levels in telencephalic vocal control areas without having major effects on hypothalamic NE levels and without affecting other neurotransmitters. Systemic administration of DSP-4 did not affect 5-HT or DA levels in the brain areas examined. Systemic DSP-4 treatment lowered brain NE levels in some males but not others, so that the mean NE levels of the DSP-4–treated group did not differ significantly from those of controls. However, DSP-4–treated males showed significant reductions in courtship behavior, taking longer to initiate singing and performing fewer song bouts and courtship displays in tests with females. In DSP-4–treated males, NE levels in specific vocal control nuclei on the motor pathway were highly correlated with courtship singing (i.e., the lower the NE levels in NIf, RA, or DM, the fewer songs a male sang).

In the current study, we administered DSP-4 into the third cerebral ventricle (ICV) to reduce brain NE levels while minimizing adverse systemic side effects. All males received androgen implants at the beginning of the study, to control for the possibility that DSP-4 treatment might decrease androgen secretion. 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), and its effects on reproductive behavior were determined during tests with reproductively active females.

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 L : 10 D cycle, lights on at 0900. The relative humidity was maintained above 50%, because 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 150 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 14 years to monitor the ability of various hormone treatments to activate behavior [see (23,24,44) 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: low, medium, and high. 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 normally 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), nest-building 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.

Initially, all males were tested for 5 min, and any male that did not court at least once was eliminated from the experiment. 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 beginning at least 1 week after cannulation surgery and two postdrug pair tests beginning at least 10 days after DSP-4 treatment.

Hormone Implantation and Cannulation

Birds were anesthetized with a combination of Xylazine : Ketamine (5 mg each/kg body weight) in 0.1 ml saline injected into the pectoral muscle, with additional anesthetic administered as necessary. A silastic capsule of androstenedione was placed subcutaneously under the left wing and the incision sutured. Each implant contained 5 mm of packed crystalline hormone in silastic tubing (0.76 mm i.d., 1.65 mm o.d., Dow-Corning). These implants have previously been shown to elicit the highest levels of reproductive behavior in this species of any hormone treatment tested (24).

Stainless steel guide cannulas (22-gauge) (Plastic Products, Roanoke, VA) were trimmed to 8 mm and their tips beveled to a 45° angle. Birds were placed in a Kopf small animal stereotaxic unit fitted with a songbird headholder as described by Stokes et al. (42). A small hole was drilled through the skull, and a chronic guide cannula stereotaxically implanted (0.3 mm anterior to interaural zero, 0.5 mm off the midline suture, 6 mm below dura). The surface of the skull surrounding the implant was abraded using the drill bit and the implant secured with cranioplastic cement. Once the cement hardened, a 28-gauge dummy cannula extending 1 mm beyond the guide cannula was inserted. Birds were then placed in a heated recovery cage until they had fully recovered from the anesthetic.

Drug Administration

The two drugs used were zimelidine dihydrochloride, a serotonergic reuptake blocker, and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4, generous gifts of Trevor Archer, Astra Pharmaceuticals, Ltd.). Drugs were placed in solution immediately prior to administration. 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, eight males received ICV infusions of 5 μg DSP-4 in saline and eight received control infusions of saline. Microline tubing was filled with DSP-4 solution or saline. One end of the tubing was attached to a 10 μl Hamilton syringe and the other to the 28-gauge injection cannula assembly. The male's dummy cannula was removed, and the injection cannula inserted extending 1 mm beyond the guide cannula. The syringe was placed in a microinfusion pump (Harvard Bioscience, Inc.), which delivered the infusion at 1 μl/min. Two males in each group received infusions of 3 μl, while the remainder received 6 μl to enhance drug diffusion; total drug dose did not vary. After infusion, the injection assembly was left in place an additional 2 min to prevent the infusate from being drawn back into the guide cannula. The injection assembly was then removed, the dummy cannula reinserted, and the male returned to his cage.

Microdissection of Brain Areas

Following the final behavior test, males were quickly caught and sacrificed by decapitation to minimize stress, because handling alters monoamine levels. Anesthesia was not used, because monoamine levels are strongly affected by anesthetization. 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 h before dissection. The location of brain areas was determined using the atlas of Stokes et al. (42), with additions from Nottebohm et al. (36). Tissue was handled as described previously (7,8) and specific areas removed bilaterally using chilled 500 or 1000 μm stainless steel punches as described by Palkovits (38). Cannula position was verified during dissection (see 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 (7,8). Briefly, microdissected nuclei were expelled into 1.5 ml tubes containing 80 μl of 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 (12).

Statistical Analysis

Behavioral data were analyzed nonparametrically (40). Males were assigned randomly to the experimental and control groups. When the data were summarized at the end of the experiment, we found that the pretreatment test means of the two groups differed. Therefore, the behavioral data were analyzed by calculating pretest-posttest difference scores for each male on each measure. The difference scores of the two groups were then compared using Mann–Whitney *U*-tests. Monoamine data were analyzed by two-factor mixed design (factor 1 = treatment: DSP-4 or saline; factor 2 = brain areas) analyses of variance with repeated measures on factor 2, because nine brain areas were taken from each bird, followed by Duncan's post hoc test when a significant *F* value was found (13). Hypothalamic and vocal control monoamine levels were analyzed separately, because hypothalamic levels and turnover are typically much higher. Because area X has exceedingly high DA levels, unlike the other vocal control nuclei, X was included with the hypothalamic nuclei in analyzing effects on DA. Based on our previous results, we predicted that DSP-4 treatment would decrease total courtship displays, total song bouts, and increase latency to sing. The *p*-values given for these measures are based on one-tailed tests. Two-tailed tests were used for all other measures.

RESULTS

The volume of the DSP-4 infusions did not affect the efficacy of DSP-4 treatment. As predicted, DSP-4 treatment sig-

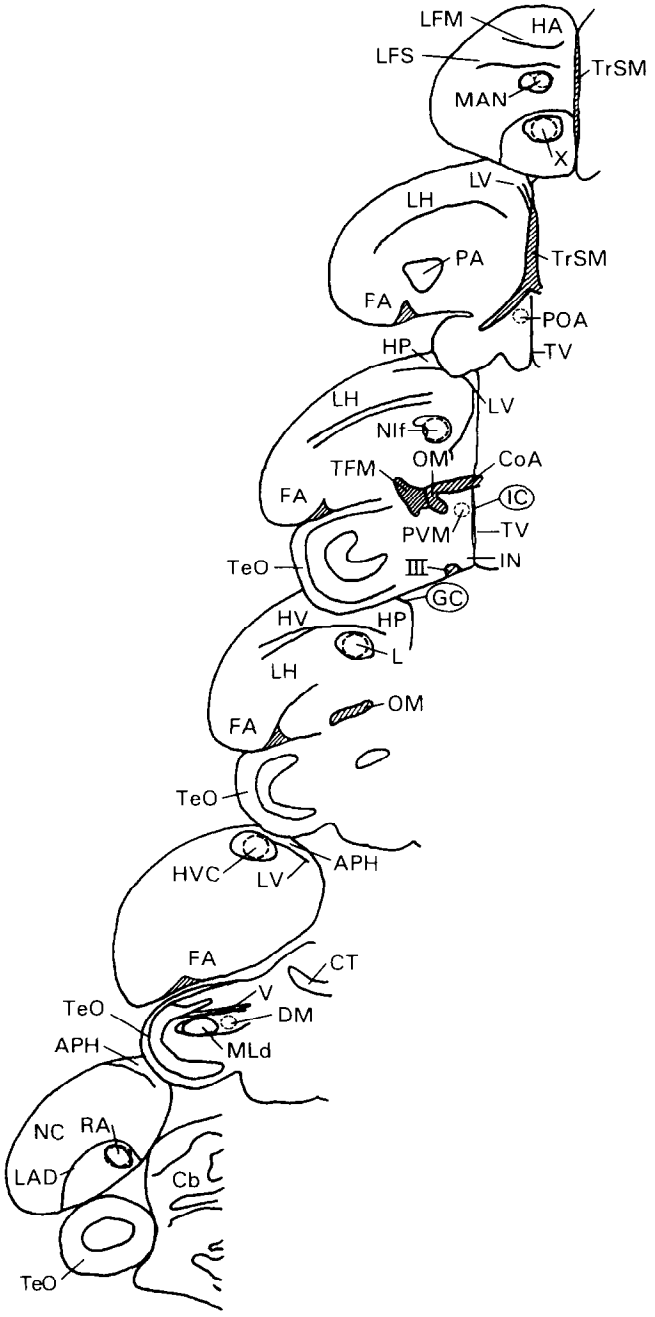

FIG. 1. Schematic drawings of representative frozen brain sections, showing relevant landmarks, microdissected areas, and site of guide cannula (GC) entry into the brain and infusion cannula (IC) placement in third ventricle. AHP, area parahippocampalis; Cb, cerebellum; CT, commissura tectalis; DM, dorsomedial portion of nucleus intercollicularis; FA, tractus frontoarchistriatalis; HA, hyperstriatum accessorium; HP, hippocampus; HV, hyperstriatum ventrale; HVC, high vocal center; III, n. oculomotorius; IN, n. infundibularis; L, field L; LAD, lamina archistriatalis dorsalis; LFM, lamina frontalis suprema; LFS, lamina frontalis superior; LH, lamina hyperstriatica; LMD, lamina medullaris dorsalis; LV, lateral ventricle; MAN, n. magnocellularis of the anterior neostriatum; MLd, n. mesencephalicus lateralis pars dorsalis; NC, neostriatum caudale; NIf, n. interfacialis; OM, tractus occipitomesencephalicus; PA, paleostriatum augmentatum; POA, n. preopticus anterior; PVM, n. paraventricularis magnocellularis; RA, n. robustus archistriatalis; TeO, tectum opticum; TFM, tractus thalamofrontalis et frontalis thalamicus medialis; TrSM, tractus septomesencephalicus; TV, third ventricle; V, ventricle; X, area X.

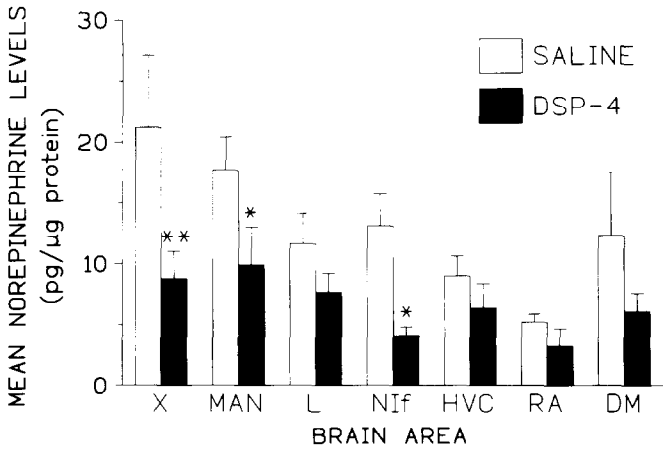

FIG. 2. Mean (± SEM) norepinephrine levels in vocal control nuclei in male finches given ICV DSP-4 (n = 8) or saline (n = 8). See text for abbreviations. *DSP-4 < saline, $p < 0.05$. **DSP-4 < saline, $p < 0.01$.

nificantly lowered NE levels in vocal control [ANOVA, $F(1, 14) = 20.1$, $p < 0.01$, Fig. 2) and hypothalamic nuclei ($F(1, 6) = 78.8$, $p < 0.001$, Fig. 3]. Post hoc comparisons revealed that DSP-4 treatment significantly lowered NE levels in area X, MAN, NIf, POA, and PVM. NE levels varied significantly across brain areas [vocal control: $F(6, 84) = 4.58$, $p < 0.05$; hypothalamic: $F(1, 22) = 37.09$, $p < 0.001$]. For hypothalamic nuclei, there was a significant interaction between treatment and brain area, $F(1, 22) = 16.34$, $p < 0.001$.

Neither DA nor 5-HT levels were significantly affected by DSP-4 administration [ANOVA, DA in vocal control nuclei: $F(1, 14) = 0.69$, NS, Fig. 4; DA in hypothalamic areas: $F(1, 14) = 0.2$, NS, Fig. 3; 5-HT in vocal control nuclei: $F(1, 14) = 0.99$, NS, Fig. 5; 5-HT in hypothalamic areas: $F(1, 14) = 0.51$, NS, Fig. 3]. DA and 5-HT levels both varied significantly across vocal control nuclei [DA: $F(5, 70) = 9.08$, $p < 0.01$; 5-HT: $F(6, 84) = 9.879$, $p < 0.001$]. Only DA levels varied significantly across hypothalamic nuclei [DA: $F(2, 28) = 38.87$, $p < 0.01$; 5-HT: $F(1, 22) = 2.96$, NS]. There were no significant interaction effects between treatment and brain area for either DA or 5-HT levels. There was no significant variation in levels of the 5-HT metabolite 5-HIAA either across brain areas or with treatment. Epinephrine levels were below the limits of detectability in over 60% of the samples from both DSP-4 and control males.

Treatment with DSP-4 caused significant increases in latencies to sing compared to treatment with saline (Mann-Whitney U, U = 8, $p < 0.0044$, Fig. 6). The latencies of DSP-4-treated males rose from an average of less than 13 s to over 68 s. DSP-4 treatment significantly reduced the frequency of courtship displays (Mann-Whitney U, $U = 12$, $p < 0.0137$). This effect was caused by a significant reduction in low-intensity displays (Mann-Whitney U, $U = 7$, $p < 0.0032$); high-intensity displays were not affected (see Fig. 6). DSP-4

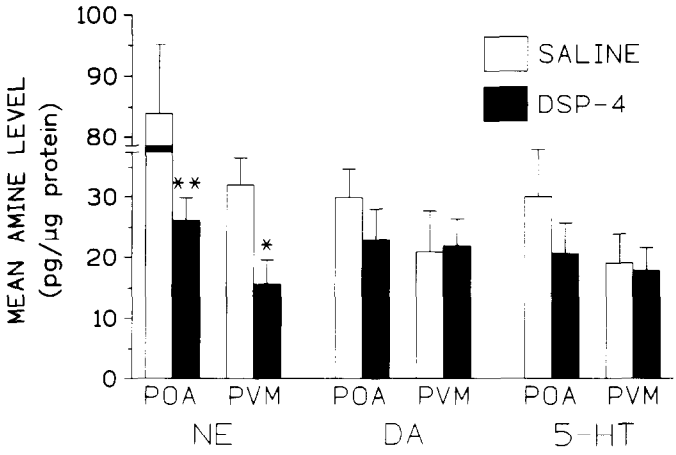

FIG. 3. Mean (± SEM) norepinephrine (NE), dopamine (DA), and serotonin (5-HT) levels in hypothalamic nuclei in male finches given ICV DSP-4 or saline. See text for abbreviations. *DSP-4 < saline, $p < 0.05$. **DSP-4 < saline, $p < 0.01$.

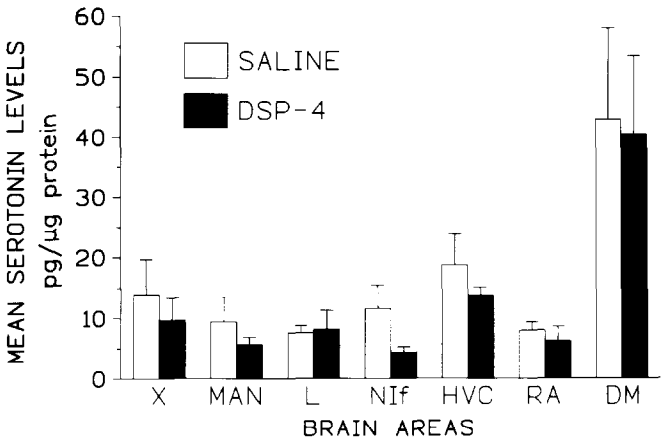

FIG. 5. Mean (± SEM) serotonin levels in vocal control nuclei in male finches given ICV DSP-4 or saline. See text for abbreviations.

treatment also significantly decreased the number of song bouts (Mann-Whitney U, $U = 13$, $p < 0.0178$). DSP-4 treatment had no significant effects on other measures of behavior, such as beak wipes (see Fig. 7), approach, follow, clump, and peck.

To examine whether DSP-4 treatment affected the quality of singing as well as the quantity, 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, and song bout length. DSP-4 treatment had no significant effects on any of these measures (see Fig. 7).

DISCUSSION

In mammals, DSP-4 preferentially destroys terminals of noradrenergic projections originating in the LC, thus eliminating most telencephalic projections while sparing those in hypothalamic areas that arise from the lateral tegmentum (19,21).

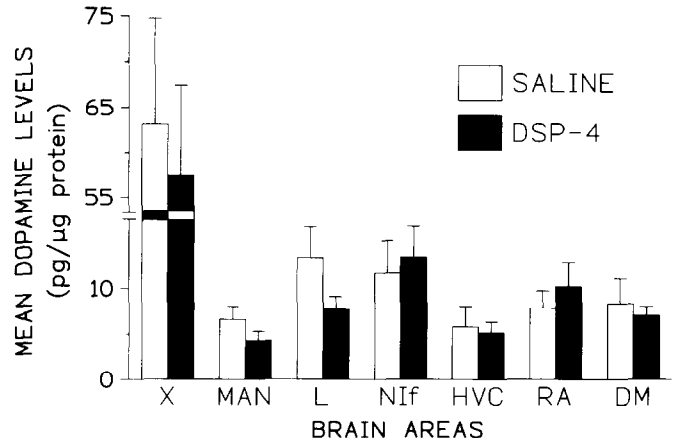

FIG. 4. Mean (± SEM) dopamine levels in vocal control nuclei in male finches given ICV DSP-4 or saline. See text for abbreviations.

The effects of DSP-4 in birds differ from those in mammals. Systemic administration of 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 (9) or quail (4). In finches, increasing the dose of systemically administered DSP-4 resulted in high mortality. Therefore, in the present study we administered DSP-4 centrally. Administration of approximately one tenth the systemically administered dose of DSP-4 depleted NE levels in five of the nine areas examined. Three of six vocal control areas examined showed significant depletions (X = 58%, MAN = 44%, NIf = 69%), but the two hypothalamic nuclei sampled were among the most affected areas (POA = 69%, PVM = 51%). This high degree of hypothalamic depletion may result, in part, from the fact that DSP-4 was infused into the third ventricle in very close prox-

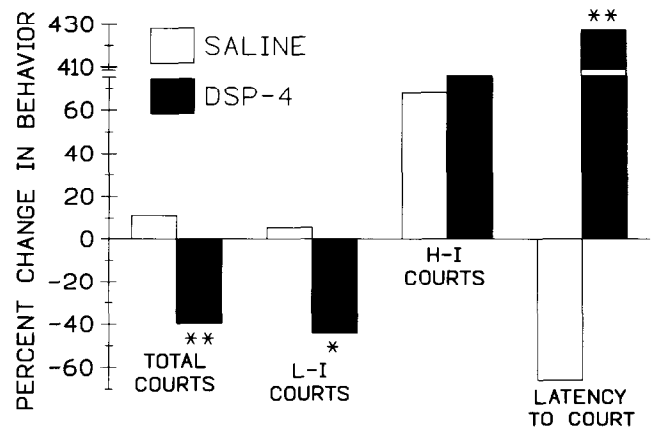

FIG. 6. Mean percent change in behavior from the two pretreatment tests with a female to the two tests following ICV infusion. *DSP-4-treated males significantly different from saline-treated males, $p < 0.02$. **DSP-4-treated males significantly different from saline-treated males, $p < 0.005$. Precise P-values given in the Results section. L-I Courts = low-intensity courtship displays. H-I Courts = high-intensity courtship displays.

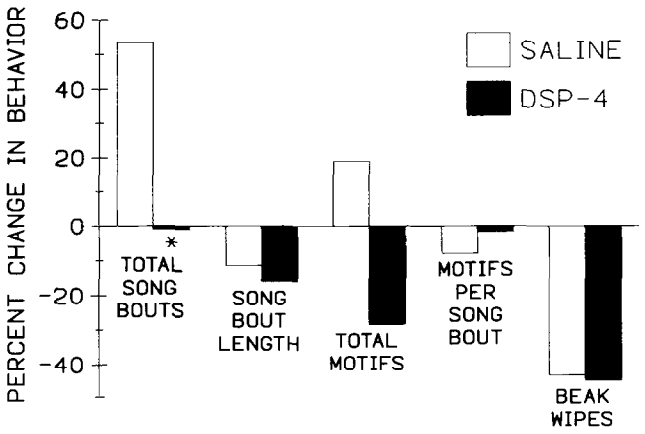

FIG. 7. Mean percent change in behavior from the two pretreatment tests with a female to the two tests following ICV infusion. *DSP-4-treated males significantly different from saline-treated males, $p < 0.02$. Precise p-value given in the Results section.

imity to these areas, exposing them to effectively higher levels of DSP-4 than other brain regions. Two of the four areas in which NE levels were not reduced significantly, HVC and DM, are located immediately adjacent to the ventricular system, so proximity to the ventricular system was not the sole factor influencing the efficacy of DSP-4 treatment. Unlike the five areas with significantly reduced NE levels, the remaining four nuclei were located posterior to the infusion site.

The overall degree of NE depletion found in the brain areas examined in this study, 34-69%, did not approach that seen in mammals given the typical systemic dose of 50 mg/kg (1,10,47). This may result from differences in noradrenergic innervation in avian and mammalian brains. Avian brains have consistently been shown to have higher levels of both DA and NE (7,35,37). Because NE levels in finch brains are about 10 times higher than in comparable areas in rat brains, this factor may explain the inability of the standard dose of systemically administered DSP-4 to induce statistically significant depletions of NE in zebra finches. The much greater tendency of systemically administered DSP-4 to deplete hypothalamic NE in avian brains compared to mammalian brains also needs to be explained. Perhaps the avian hypothalamus receives a greater proportion of its noradrenergic innervation from the LC, or alternatively, axon terminals of the ventral tegmentum in birds may be more vulnerable to DSP-4. This is currently under investigation.

As we previously found with systemic DSP-4 administration, centrally administered DSP-4 caused three striking deficits in courtship behavior. Total number of courtship displays and total number of song bouts decreased, while latencies to first song increased in DSP-4-treated males. Unlike our previous study, in the current study DSP-4 treatment preferentially reduced low-intensity courtship displays without affecting high-intensity displays, which include the visual dance element. Because over 80% of a male's courtship displays were typically low-intensity displays, the total courtship displays of DSP-4-treated birds were significantly reduced. Because all males in the current study had androgen implants, the reductions in courtship behavior in these DSP-4-treated males clearly did not result from indirect effects caused by decreased gonadal hormone secretion.

In previous studies, our laboratory examined the relative contributions of androgens and estrogens in activating courtship singing. Combined stimulation by androgens and estrogens is necessary to increase levels of courtship singing above the baseline shown by castrated males (24). More recently, we showed that singing directed at other birds, both males and females, is stimulated by estrogens (45). 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 (8). Given the evidence from other species that effects of NE projections from the LC on behavior are mediated through changes in attention or arousal (3,14), we hypothesized that perhaps estrogen's effects on attentiveness to other birds were mediated through its effects on NE function. The results of this and our prior study (9) support this hypothesis. DSP-4-induced decreases in NE function resulted in decreased courtship singing, which appeared to result from a deficit in initial attentiveness to the female 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 other species, in which disruption of LC NE function causes deficits in orienting or shifting attention to motivationally relevant environmental stimuli (14,16). In terms of sexual behavior, DSP-4-treated male rats 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 (22). Our methods of assessing motor performance were relatively simple. However, these methods are sufficient to detect changes in the motor patterning of singing caused by lack of estrogen (45). Males treated with an aromatization inhibitor not only sing fewer songs to females, but the courtship songs they sing to females are sung at the distinctly slower rate typical of undirected songs. They also sing fewer songs per courtship bout than untreated males. Thus, while estrogen deprivation causes deficits in singing behavior that appear to be caused by changes in both attention and motor patterning, NE depletion appears to affect only the attentional aspect of song control.

Although DSP-4 treatment decreased courtship singing, it did not affect other behavioral parameters which might be interpreted as indicating interest in the female, such as the frequency of approaching or following the female. Beak wiping is another behavior that indicates interest in the female; levels of beak wiping are typically highly correlated with levels of courtship singing (24). However, beak wiping was also unaffected by DSP-4 treatment. The fact that DSP-4 did not affect these other female-directed behaviors was probably caused by a) the fact that singing is one of a male's earliest and most frequent responses to a female and b) the primary effect of DSP-4 treatment was a short initial delay in responding to the female's presence.

ACKNOWLEDGEMENTS

We thank the anonymous reviewers for their helpful comments on the manuscript. This research was supported by NIMH Grant MH48406, NIMH RSDA Award MH00591, PSC-CUNY Grant 668237 to C.F.H., and NIH Minority Biomedical Research Support Grant RR08176 and NIH Research Centers in Minority Institutions Grant RR03037 to Hunter College.

REFERENCES

1. Archer, T.; Jonsson, G.; Ross, S. B. A parametric study of the effects of the noradrenaline neurotoxin DSP4 on avoidance acquisition and noradrenaline neurones in the CNS of the rat. Br. J. Pharmacol. 82:249-257; 1984.
2. Arnold, A. P.; Nottebohm, F.; Pfaff, D. W. Hormone concentrating cells in vocal control and other areas of the brain of the zebra finch (*Poephila guttata*). J. Comp. Neurol. 165:487-512; 1976.
3. Aston-Jones, G.; Rajkowski, J.; Kubiak, P.; Alexinsky, T. Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. J. Neurosci. 14:4467-4480; 1994.
4. Balthazart, J.; Ball, G. F. Effects of the noradrenergic neurotoxin DSP-4 on luteinizing hormone levels, catecholamine concentrations, α2-adrenergic receptor binding, and aromatase activity in the brain of the Japanese quail. Brain Res. 492:163-175; 1989.
5. Balthazart, J.; Libioulle, J. M.; Sante, P. Stimulatory effects of the noradrenergic neurotoxin DSP4 on sexual behavior in male quail. Behav. Proc. 17:27-44; 1988.
6. Barclay, S. R.; Cheng, M.-F. Role of the catecholamines in the courtship behavior of the ring dove. Pharmacol. Biochem. Behav. 41:739-747; 1992.
7. Barclay, S. R.; Harding, C. F. Androstenedione modulation of monoamine levels and turnover in hypothalamic and vocal control nuclei in the male zebra finch: Steroid effects on brain monoamines. Brain Res. 459:333-343; 1988.
8. Barclay, S. R.; Harding, C. F. Differential modulation of monoamine levels and turnover rates by estrogen and/or androgen in hypothalamic and vocal control nuclei of male zebra finches. Brain Res. 523:251-262; 1990.
9. Barclay, S. R.; Harding, C. F.; Waterman, S. A. Correlations between catecholamine levels and sexual behavior in male zebra finches. Pharmacol. Biochem. Behav. 41:195-201; 1992.
10. Booze, R. M.; Hall, J. A.; Cress, N. M.; Miller, G. D.; Davis, J. N. DSP-4 treatment produces abnormal tyrosine hydroxylase immunoreactive fibers in rat hippocampus. Exp. Neurol. 101:75-86; 1988.
11. Bottjer, S. W.; Miesner, E. A.; Arnold, A. P. Forebrain lesions disrupt development but not maintenance of song in passerine birds. Science 224:901-903; 1984.
12. Bradford, M. M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248-254; 1976.
13. Bruning, J. L.; Kintz, B. L. Computational handbook of statistics. Glenview, IL: Scott, Foresman; 1977.
14. Devauges, V.; Sara, S. J. Activation of the noradrenergic system facilitates an attentional shift in the rat. Behav. Brain Res. 39:19-28; 1990.
15. Everitt, B. J. Sexual motivation – A neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. Neurosci. Biobehav. Rev. 14:217-232; 1990.
16. Everitt, B. J.; Robbins, T. W.; Selden, N. R. W. Functions of the locus coeruleus noradrenergic system: A neurobiological and behavioural synthesis. In: Heal, D. J.; Marsden, C. A., eds. The pharmacology of noradrenaline in the central nervous system. New York: Oxford University Press; 1990:349-378.
17. Fillenz, M. Noradrenergic neurons. New York: Cambridge University Press; 1990.
18. Fritschy, J. M.; Geffard, M.; Grzanna, R. The response of noradrenergic axons to systemically administered DSP-4 in the rat: An immunohistochemical study using antibodies to noradrenaline and dopamine-β-hydroxylase. J. Chem. Neuroanat. 3:309-321; 1990.
19. Fritschy, J. M.; Grzanna, R. Immunohistochemical analysis of the neurotoxic effects of DSP-4 identifies two populations of noradrenergic axon terminals. Neuroscience 30:181-197; 1989.
20. Fritschy, J.-M.; Grzanna, R. Restoration of ascending noradrenergic projections by residual locus coeruleus neurons: Response to neurotoxin-induced cell death in the adult rat brain. J. Comp. Neurol. 321:421-441; 1992.
21. Grzanna, R.; Berger, U.; Fritschy, J. M.; Geffard, M. The acute action of DSP-4 on central norepinephrine axons: Biochemical and immunohistochemical evidence for differential effects. J. Histochem. Cytochem. 37:1435-1442; 1989.
22. Hansen, S.; Köhler, C.; Ross, S. B. On the role of the dorsal mesencephalic tegmentum in the control of masculine sexual behavior in the rat: Effects of electrolytic lesions, ibotenic acid and DSP-4. Brain Res. 240:311-320; 1982.
23. Harding, C. F. The importance of androgen metabolism in the regulation of reproductive behavior in the avian male. Poult. Sci. 65:2344-2351; 1986.
24. Harding, C. F.; Sheridan, K.; Walters, M. J. Hormonal specificity and activation of sexual behavior in male zebra finches. Horm. Behav. 17:111-133; 1983.
25. Heal, D. J.; Butler, S. A.; Prow, M. R.; Buckett, W. R. Quantification of presynaptic α2-adrenoceptors in rat brain after short-term DSP-4 lesioning. Eur. J. Pharmacol. 249:37-41; 1993.
26. Jaim-Etcheverry, G.; Zieher, L. M. DSP-4: A novel compound with neurotoxic effects on noradrenergic neurons of adult and developing rats. Brain Res. 188:513-523; 1980.
27. Jonsson, G. Chemical neurotoxins as denervation tools in neurobiology. Annu. Rev. Neurosci. 3:169-187; 1980.
28. 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. 72:173-188; 1981.
29. Jonsson, G.; Hallman, H.; Sundstrom, E. Effects of the noradrenaline neurotoxin DSP4 on the postnatal development of central noradrenaline neurons in the rat. Neuroscience 7:2895-2907; 1982.
30. Kostrzewa, R. M. Neurotoxins that affect central and peripheral catecholamine neurons. Drugs Tools Neurotransm. Res. 12:1-48; 1989.
31. Lee, C. M.; Javitch, J. A.; Snyder, S. H. Characterization of [^{3}H]-desipramine binding associated with neuronal norepinephrine uptake sites in rat brain membranes. J. Neurosci. 2:1515-1525; 1982.
32. Logue, M. P.; Growdon, J. H.; Coviella, I. L.; Wurtman, R. J. Differential effects of DSP-4 administration on regional brain norepinephrine turnover in rats. Life Sci. 37:403-409; 1985.
33. Lookingland, K. J.; Chapin, D. S.; McKay, D. W.; Moore, K. E. Comparative effects of the neurotoxin N-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride (DSP4) and 6-hydroxydopamine on hypothalamic noradrenergic, dopaminergic and 5-hydroxytryptaminergic neurons in the male rat. Brain Res. 365: 228-234; 1986.
34. Mitchell, J. B.; Stewart, J. Effects of castration, steroid replacement, and sexual experience on mesolimbic dopamine and sexual behaviors in the male rat. Brain Res. 491:116-127; 1989.
35. Muhibullah, M.; Gargiulo, G.; Nistico, G.; Stephenson, J. D. Distribution of monoamine containing neurons in the fowl brain (*Gallus domesticus*). In: Nistico, G.; Bolis, L., eds. Progress in nonmammalian brain research. Boca Raton, FL: CRC Press; 1983:81-112.
36. Nottebohm, F.; Kelley, D. B.; Paton, J. A. Connections of vocal control nuclei in the canary telencephalon. J. Comp. Neurol. 207: 344-357; 1982.
37. Ottinger, M. A.; Cortes-Burgos, L.; Rawlings, C. S. Noradrenergic agonists and LHRH stimulate male reproductive behavior in Japanese quail. Soc. Neurosci. Abstr. 15:529; 1988.
38. Palkovits, M. Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res. 59:449-450; 1973.
39. Scharff, C.; Nottebohm, F. A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: Implications for vocal learning. J. Neurosci. 11: 2896-2913; 1991.
40. Siegel, S. Nonparametric statistics. New York: McGraw-Hill; 1958.
41. Sohrabji, F.; Nordeen, E. J.; Nordeen, K. W. Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. Behav. Neural Biol. 53:51-63; 1990.

42. Stokes, T. M.; Leonard, C. M.; Nottebohm, F. The telencephalon, diencephalon, and mesencephalon of the canary, *Serinus canaria*, in stereotaxic coordinates. J. Comp. Neurol. 156:337–374; 1974.
43. Striker, E. M.; Zigmond, N. T. Brain catecholamines and motivated behavior: Specific or nonspecific contributions? In: Usdin, E.; Carlsson, A.; Dahlstrom, A.; Engel, J., eds. Catecholamines: Neuropharmacology and central nervous system—Theoretical aspects. New York: Alan R. Liss; 1984:259–269.
44. Walters, M. J.; Collado, D.; Harding, C. F. Oestrogenic modulation of singing in male zebra finches: Differential effects on directed and undirected songs. Anim. Behav. 42:445–452; 1991.
45. Walters, M. J.; Harding, C. F. The effects of an aromatization inhibitor on the reproductive behavior of male zebra finches. Horm. Behav. 22:207–218; 1988.
46. Zaczek, R.; Fritschy, J. M.; Culp, S.; Desouza, E. B.; Grzanna, R. Differential effects of DSP-4 on noradrenaline axons in cerebral cortex and hypothalamus may reflect heterogeneity of noradrenaline uptake sites. Brain Res. 522:308–314; 1990.
47. Zahniser, N. R.; Weiner, G. R.; Worth, T.; Philpott, K.; Yasuda, R. P.; Jonsson, G.; Dunwiddie, T. V. DSP-4-induced noradrenergic lesions increase β-adrenergic receptors and hippocampal electrophysiological responsiveness. Pharmacol. Biochem. Behav. 24:1397–1402; 1986.