Critical Stages in Avian Development: Estrogen Hazards to Altricial and Precocial Birds

Grant Title and Principal Investigator

Critical Stages in Avian Development: Estrogen Hazards to Altricial and Precocial Birds

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Key Findings and Implications

- Post-hatch oral administration of estradiol benzoate (EB) to zebra finches produces similar effects at similar doses to parenterally administered EB in that species.
- Post-hatch EB treatment of female zebra finches produces a dose-related increase in the size of the song-control nuclei in their brains as well as singing behavior, which normally is restricted to males.
- Post-hatch EB treatment of male zebra finches alters adult reproductive behaviors in a dose-related manner, demasculinizing copulatory behavior (i.e., failure to mount females) and increasing nesting behavior.
- The effects of post-hatch EB exposure of both males and females can result in total reproductive failure of mated pairs due to an array of behavioral and physiological changes in both sexes.
- Post-hatch oral administration of selected xenoestrogens (i.e., octylphenol) produced no significant effects at the doses tested

Project Period: November 1996 to October 2000

Project Scope

Exposure to estrogenic chemicals during development may permanently alter the structure and subsequent functioning of the avian brain and reproductive systems. Impairment from post-hatching exposure is more likely for altricial species (e.g., songbirds), in which chicks are incompletely developed at hatching, than precocial species, in which the chicks are fully feathered and can walk and feed on their own within hours of hatching (e.g., chickens and quail, shorebirds). The overall objective of this research was to determine critical exposure periods in the development of precocial and altricial birds for estrogenic disruption of differentiation of aspects of the reproductive system and sexually dimorphic areas of the brain.

Specific objectives of this research were first to examine the zebra finch, an altricial species, to evaluate:

- the efficacy of xenobiotic estrogens in masculinizing the song control nuclei in the brains of females and in demasculinizing mounting behavior in males; and
- the reproductive success of mated pairs of zebra finches exposed to estrogenic substances as chicks.

Zebra finch was selected as the model altricial species because the sexually dimorphic regions of the brain involved in song production have been well studied and mapped, and experiments have indicated their developmental sensitivity to estradiaol benzoate (EB). (Males normally sing to defend territories and to attract mates and learn their songs from their fathers; females normally do not sing.) Whereas earlier studies of the effects of EDCs on birds have focused on disruption of hormonal and anatomical characteristics of males and females, this study also addressed changes in brain structure and associated sex-specific behaviors. Depending on the results with zebra finches, additional studies using the quail as a model precocial species might be conducted.

Project Results and Implications

Groups of zebra finch (*Taeniopygia guttata*) chicks were orally administered estradiol benzoate (EB; positive control) and estrogenic environmental contaminants (i.e., the industrial surfactant 4-octylphenol [4-OP], which sometimes is found in sewage sludge used in soil amendments, and two organochlorine insecticdes, dicofol and methoxychlor) between days 5 and 11 post-hatch.

Neuranatomy and Behavior

To study differentiation of the brain nuclei involved in song production, groups of chicks were orally administered the vehicle control (canola oil), the positive control EB at doses of 1, 10, 100, and 1000

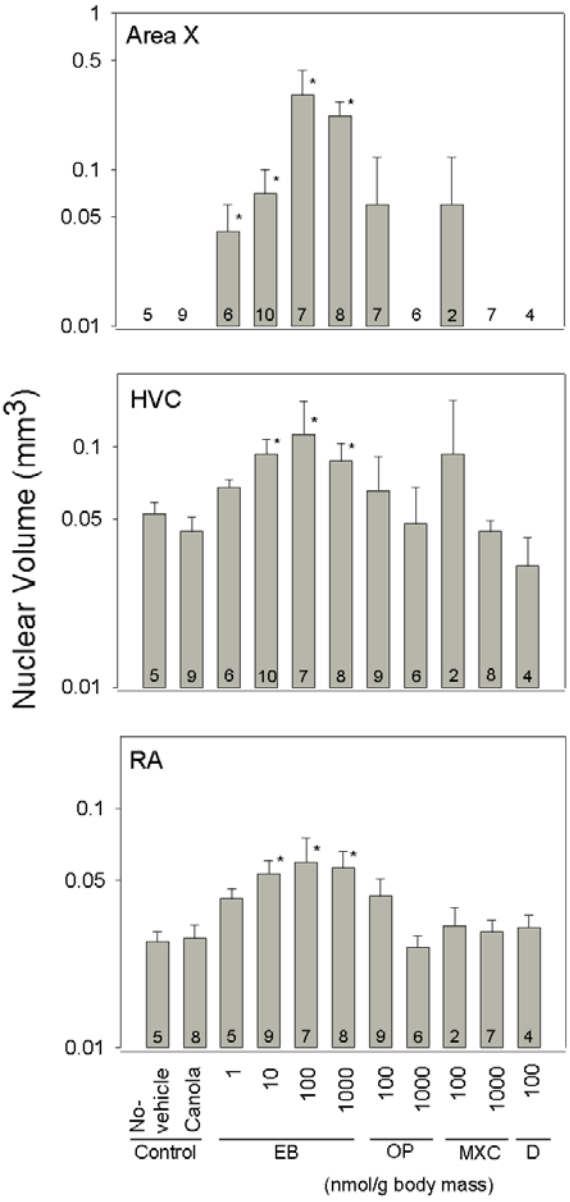

Figure 1. Volumes (mm^3; mean ± SE; log plot; N noted on bar of each treatment group) of female zebra finch song nuclei area X, HVC and nucleus robustus of the archistriatum (RA). Finches were treated as chicks (days of age 5 through 11) orally with the substances denoted under each bar. Canola was used as a control and as a vehicle for estradiol benzoate (EB), octylphenol (OP), methoxychlor (MXC) and dicofol (D). Another control group received no treatment (No vehicle). Asterisks denote groups that are significantly greater than canola-treated controls. Area X was not detectable in groups that lack bars (Quaglino et al., 2001).

nmol/g body weight (bw), or test substances in the following doses: 4-OP at 100 or 1000, dicofol at 100, and methoxychlor at 100 or 1000 nmol/g bw. A no-vehicle control group also was included. The doses selected for the xenoestrogens were within predicted environmental exposure levels estimated from previous studies of these contaminants in the field. All chicks received the same volume of solution (1 uL/g body weight), with the concentration of chemical in the solution adjusted for daily body mass changes of the chicks to produce the intended doses. After 130 days of age (physiologically adult), the birds received testosterone implants to stimulate male-specific reproductive behaviors (e.g., singing). At sacrifice two weeks after behavioral testing (at between 4 and 11 months of age), the bird reproductive tracts and brains were examined for evidence of alterations in sex-specific developmental patterns. The positive control allowed comparison of the test chemicals' potency as an estrogen with that of EB.

Oral administration of EB between days 5 and 11 post-hatch at the three highest doses increased the size of (i.e., masculinzed) the song-control nuclei of the brains of female zebra finches and resulted in female singing in a dose-dependant manner, with the intermediate 100 nmol/g bw dose producing the greatest effect (see Figure 1). This dose-response pattern is similar to those produced by parenteral administration (i.e., silastic implants and intramuscular or subcutaneous injections) of EB documented in other studies. This indicates that oral dosing experiments can be used to directly evaluate the risks to chicks from environmental estrogens found in contaminated foods (e.g., seeds, insects, worms, and plants [i.e., phytoestrogens]) that parent birds feed to their young. Calculations revealed that the dose of EB required to maximally masculinize the brains of zebra finch females via oral administration is approximately one order of magnitude higher than when the dose is provided parenterally.

Post-hatch treatment of male zebra finches with EB feminized their behavior. EB-exposed males exhibited reduced copulatory mounting attempts and increased nesting behavior in a dose-related manner.

None of the three xenoestrogens examined induced a statistically significant growth of song control nuclei in the brains of female finches at the doses tested (see Figure 1). Given the negative results of exposure of the altricial zebra finches to the three xenoestrogens, post-hatch experiments with quail, expected to be less sensitive, were not pursued.

Reproductive Performance

For the reproductive performance study, EB was administered at 10 or 100 nmol/g bw, and 4-OP was given at a dose of 100 nmol/g bw between days 5 and 11 post-hatch. Exposed chicks were reared by their parents until 45 days of age and then transferred to communal cages. Reproductive testing was

conducted starting between 130 and 180 days of age either in individual pair cages or in communal cages that permitted self selection of mates. Several measures of reproduction were assessed, including incidence of pair formation and egg laying, clutch size, eggshell thickness, fertility, hatchability, fledging success, and incidence and severity of altered gonadal morphology. The test pairings included (1) vehicle control males and females, (2) males and females exposed to EB at 10 nmol/g bw, (3) males and females exposed to EB at 100 nmol/g bw, (4) males only exposed to EB (at 100 nmol/g), (5) females only exposed to EB (at 100 nmol/g), and (6) males and females both exposed to 4-OP at 100 nmol/g bw.

Oral administration of EB resulted in sex- and dose-dependent effects on reproductive performance of adult zebra finches. Because reproductive performance also was impaired in the control groups with forced pairings in individual cages, only the results for communal cages were assessed for effects of the test substances. When only females were exposed to EB (at 100 nmol/g bw, their mates were not exposed), impacts on reproductive success included increased incidence of cracked/broken or missing eggs, reduced number of eggs laid, and reduced number of eggs hatched compared with controls (see Figure 2). When only males were exposed (at 100 nmol/g bw EB), candled fertility was significantly reduced compared with the controls. When both sexes were exposed to EB at either 10 or 100 nmol/g bw and mated, there were significant reductions in the number of eggs laid and the number of laid eggs that hatched compared to controls, with a complete failure to hatch eggs at the higher dose. What is not clear is the extent to which the reductions in fertility and hatchability are due to altered reproductive behavior (failure to mate and incubate), reduced gamete viability (reduced sperm number and viability), and/or impaired zygote/embryo viability (due to eggshell thinning). Exposure to 4-OP at 100 nmol/g bw resulted in no significant effects on any of the reproductive parameters measured. These results are consistent with previous experiments by other investigators with 4-OP, which demonstrated that a daily intake of 70 to 200 μg of 4-OP for growing finches between 3.5 and 9 g body mass produced no detectable effect on reproductive parameters; while an equimolar dose of EB produced profound disruption of reproductive parameters. Therefore, the estrogenic potency of 4-OP is clearly less than that of EB.

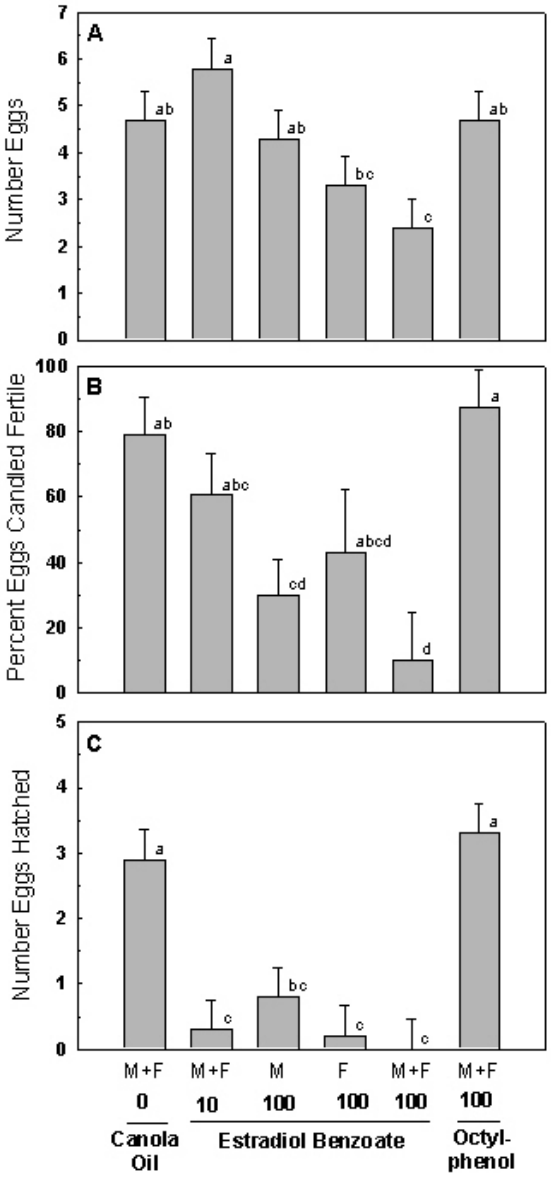

Figure 2. Egg production (A. mean number of eggs per nest box), candled fertility (B. mean percent eggs candled fertile) and hatching success (C. mean number of eggs hatched) of males (M) and/or females (F) housed communally and treated orally, once per day, with 10 or 100 nmol/g body mass on days of age 5 through 11 with estradiol benzoate or octylphenol in canola oil or canola oil alone, as a control. N = 10 per treatment. Error bars are pooled estimates. Means with unlike superscripts are significantly different (P<0.05) (Millam et al., 2001).

Implications

These experiments indicate that post-hatch ingestion of estrogenic substances by atricial passerine species can result in severe adverse effects on reproduction. In the field, the degree of impairment in the population as a whole will depend on dose and the potency of the estrogenic chemical(s). Remaining questions include at what xenobiotic estrogen doses are adverse reproductive effects likely, and whether

there are populations of altricial bird species that could be exposed to sufficiently high concentrations of these chemicals in the environment to impair reproduction and population maintenance.

Relevance to ORD's Multi-Year Research Plan

This project contributes to the first and third long-term goals of the ORD's MYP: (1) to provide a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors; and (3) to support EPA's screening and testing program.

This research demonstrated that oral dosing of a species of songbird (the zebra finch, *Taeniopygia guttata*) with estradiol benzoate (EB) for a short time soon after hatching (days 5 to 11) produced the same pattern of reproductive effects as reported by other investigators for parenteral administration of EB. Oral administration of EB increased the size of (i.e., masculinzed) the song-control nuclei of the brains of the females, which resulted in females singing when they were administered testosterone as adults. Male zebra finches administered EB post-hatch exhibited reduced copulatory mounting attempts and increased nesting behavior as adults. In addition, these treatments severely impaired reproductive performance of mated pairs, with complete reproductive failure for pairs in which both males and females had been treated with EB as chicks. Thus oral dosing paradigms can be used to assess risks to songbirds of exposure to environmental EDCs in their food. Treatment of finches with 4-octylphenol, an industrial chemical with estrogenic activity, at the same doses as EB resulted in no effects, suggesting that it is a less potent estrogen than EB, as has been demonstrated in mammals.

Investigators

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For More Information

http://animalscience.ucdavis.edu/faculty/default.htm

NCER Project Abstract and Reports

http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/725/report/F

References for Figures

Millam J.R., C.B. Craig-Veit, A.E. Quaglino, A.L. Erichsen, T.R. Famula, and D.M. Fry. 2001. Post-hatch oral estrogen exposure impairs adult reproductive performance of zebra finch in a sex-specific manner. *Hormones and Behavior* 40(4):542-549.

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