Sexual Dimorphism of Brain Aromatase Activity in Medaka: Induction of a Female Phenotype by Estradiol

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In this study we identified sex-dependent dimorphism of brain aromatase in the teleost medaka and examined its regulation by sex steroids. We first investigated differential distribution of brain aromatase activity in sexually mature male and female medaka in serial coronal sections of the brain and identified the hypothalamic nuclei contained in each section using the brain atlas of medaka. In the brain of male medaka, high levels of activity are localized in sections containing the preoptic (POA) and suprachiasmatic nuclei (SC) (63–75 fmol/hr) and low levels in the nuclei periventricular dorsalis (HD), ventralis (HV), and caudalis (HC), nuclei diffusus of lobulus inferiores (NDIL), and nuclei tuberi anteriores (TA) and posteriores (TP) (<25 fmol/hr). In the brain of female medaka high aromatase activity is localized in sections containing the HD, HV, HC, NDIL, TA, and TP (85–80 fmol/hr) and highly variable levels in the POA and SC (23–70 fmol/hr). The concentration and time dependency of the exposure of male medaka to estradiol on the total brain aromatase activity and morphologic sex characteristics were determined next. Estradiol increased the activity of brain aromatase in a concentration-dependent manner at 2.5 and 25 µg/L, but the increase was lower at higher concentrations of the hormone. The effect was time dependent, gradually increasing up to the fifth day of exposure, after which it reached a plateau. Estradiol induction of brain aromatase analyzed using Lineweaver-Burke plots of saturation assays revealed a non-first-order reaction. The results indicate that a positive feedback mechanism regulates brain aromatase and imply that the sexual dimorphic distribution of aromatase may be highly sensitive to physiologic cues and environmental perturbations in fish. Key words: aromatase activity, endocrine disruption, medaka, sexual dimorphism. Environ Health Perspect 109:257–264 (2001). [Online 1 March 2001]

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Several classes of environmental contaminants interact with endocrine targets and may disrupt sex determination and differentiation and impair reproductive success (1). The consequences of endocrine disruption include reduced fertility in mammals, abnormal sexual behavior, deformities, and lowered survival of hatchling birds (2), alteration of population sex ratios in reptiles with temperature-dependent sex determination (3), and feminization/masculinization in fish, both in the adult (4) or during sexual differentiation (5).

Levels of circulating hormones constitute important regulatory signals during development and in biological functions. These signals involve mechanisms of feedback loops between the central nervous system and effectors—namely, the hypothalamic-pituitary-gonadal loop of the reproductive function. The synthesis of estrogens in the brain (neuroestrogens) plays a critical role in the differentiation of sex during development (6,7) and in sex-specific reproductive behavior (8), mediating both organizational and activational effects of the circulating hormones.

In this study, we investigated sexual dimorphism in the distribution of the activity of aromatase (estrogen synthase) in the brain of the teleost medaka and its regulation by sex steroids. Cytoschrome P450 aromatase (aromatase) catalyzes the committed step of the synthesis of endogenous estrogens from androgens. In a cascade of reactions, aromatase uses the reductive equivalents from nicotinamide adenine dinucleotide (NADPH) to convert androgen (C19) to estrogen (C18) by removal of the methyl group (C19) and aromatization of the steroid A ring (9). Aromatase is the product of the gene family CYP19. Studies in mammals, including humans (9), birds (10), and medaka (11), support the existence of a single locus of the gene, although the presence of multiple alleles has been reported in pigs (12) and goldfish (13). The regulatory complexity of aromatase expression is achieved by use of alternative gene promoters that are spliced in a tissue-specific manner and hormonally regulated (14,15). Aromatase is evolutionarily conserved among the classes of vertebrates studied (16), and it is found in the brain, gonads, and other peripheral tissues including the placenta and the adipose tissue in mammals (17). In teleosts, levels of aromatase in the brain are 100-1,000-fold higher than in the brain of mammals (18). The adaptive significance of the higher levels of brain aromatase in the evolution of teleosts suggest an important role in regulating brain aromatase activity are poorly understood. The high activity levels of aromatase in the brain of teleosts suggest an important role in regulatory pathways and constitute a potential target for disruption of the endocrine system by xenobiotics. The characterization of the activity of brain aromatase and its regulation are necessary to understand the mechanisms of control of reproductive function and to provide a tool for assessing endocrine disruption. In previous studies, we reported that the levels of aromatase activity in the brain of medaka were significantly higher than those in the gonads and that activity was specifically localized to areas of the brain that contained the hypothalamus (25).
In the present study, we characterized the sexual dimorphism of brain aromatase in medaka and its regulation by sex steroids. Our goal was to investigate sexual dimorphism in the activity of brain aromatase and to characterize the effect of exogenous sex steroids on that activity and on the reversal of sex phenotype. The organism used was the drR strain of the medaka (O. latipes). Medaka are widely used in environmental toxicological assays and were the first teleost in which sex reversal was obtained by exposure to sex steroids (26, 27). We provide evidence that exposure to exogenous estradiol leads to a feminization of the localization of aromatase activity of male medaka. That modification included an increased activity in specific areas of the brain that are usually higher in the female than in the male. The effect of estradiol on the brain aromatase activity is both concentration- and time-dependent. Exposure to the aromatizable androgen methyltestosterone inhibited the activity of the enzyme to levels not measurable by our methods. This decrease occurred in all the areas of the brain that had been shown to have aromatase activity.

**Materials and Methods**

**Test organism.** Cultures of the drR strain of Japanese medaka (O. latipes) were maintained in our laboratory in a balanced salt solution (27) at 25°C and a 16 hr light:8 hr dark cycle. Under these conditions animals breed throughout the year, starting as young as 2.5 months of age. Sexually mature individuals (>5 months) with a body length 25-30 mm were used in our experiments.

**Localization of brain aromatase activity.** We used serial sections in the transverse (coronal) plane of the brain to localize areas with higher levels of aromatase activity. To identify sexual dimorphism, we obtained coronal sections from three individuals of each nontreated male and female fish. To study the effects of sex steroids, we obtained sections from males treated with 250 µg/L estradiol or 250 µg/L methyltestosterone. Males treated with DMSO (vehicle) were used as experimental controls. The experiment was performed with three animals per treatment. Individuals were quickly anesthetized in ice water and decapitated. The whole head was immediately frozen (–10°C) and the hypothalamic nuclei were present in a given coronal section by correspondence with morphologic features using a dissecting microscope.

**Aromatase assay.** We quantified aromatase activity using an in vitro assay, following the tritiated water method adapted from Thompson and Silteri (29) as described by Melo et al. (25). Briefly, the stereospecific release of tritiated water during the conversion of the tritiated substrate 3H-androst-4-ene-3,17-dione (3H-A) to estrone is used as an indicator of aromatase activity. Brain homogenates were incubated for 3 hr at 25°C with 3.0 nM 3H-A in the presence of 1.0 mM NADPH in a phosphate buffer (described above), in 200 µL total volume of reaction. The reaction was terminated by immersion in ice water and addition of 100 µL of 30% trichloroacetic acid (TCA). The solution was then incubated in a mix of 5% active charcoal (Norit A; Sigma, St. Louis, MO) with 0.5% dextrose (Fisher Scientific, Pittsburgh, PA) in double-distilled water, for sterol stripping for 30 min at 4°C. The mixture was then centrifuged twice at 4°C and supernatants collected. Levels of tritiated water produced were determined using 200 µL of the final supernatant by liquid scintillation counting (1211 RackBeta, Gaithersburg, MD).

To localize the activity of aromatase in the brain, we used the tritiated water assay to measure the enzyme activity separately in the homogenate of each serial section of the coronal plane of the brain. To determine the concentration- and time-dependency of the effect of sex steroids on the activity of the enzyme, we used whole-brain homogenates from individuals exposed in the in vivo bioassay as a source of enzyme in the tritiated water method. We investigated the kinetics of brain aromatase by performing saturation aromatase assays using different concentrations of 3H-A (0.25, 0.5, 0.75, 1.5, 2.5, 4.0, and 5.5 nM). Whole-brain homogenates of male medaka exposed in vivo to 2.5 and 25 µg/L estradiol were used and aromatase activity was compared to control animals. We analyzed the results graphically using the double-reciprocal plot (Lineweaver-Burke plot).

**In vivo bioassay.** We evaluated the effect of sex steroids on the activity of aromatase by exposing 5-7-month-old male medaka to an estrogen and an androgen. The estrogen 1,3,5(10)-estratriene-3,17β-diol (E2) (Sigma), commonly designated estradiol, and the androgen 17α-methyl-4-androsten-17β-ol-3-one (MT) (Sigma), designated methyltestosterone, were dissolved in stock solutions in DM SO (Sigma). Stock solution (100 µL) was diluted in 200 mL of aquarium water to obtain the required concentrations of sex steroids in the test vessels, each holding one animal. Control animals were exposed to the equivalent volume of the vehicle (DMSO) obtaining a dilution factor of 1:1,000. Exposure lasted for 10 days (except in the time-dependency experiment, described below), with water changed every 24 hr. Animals were fed twice a day, alternating brine shrimp or flake food (VividColor, Hayward, CA). At the end of the bioassay, animals were quickly anesthetized in ice water and decapitated. For aromatase activity localization, animals (three replicates) were exposed to 20 µg/L of estradiol or methyltestosterone. At the end of the assay, the head was frozen and coronal sections were obtained as described. For the concentration- and time-dependent evaluation, we dissected whole brains with the aid of a dissection microscope and homogenized them in 300 µL of phosphate buffer; five animals per treatment were used. We evaluated the concentration dependency of the effect of estradiol on brain aromatase activity over three log concentrations of 2.5, 25, 75, and 250 µg/L of the sex steroid, and five animals per treatment were used. We investigated the time dependency using 20 µg/L of estradiol and five animals per time point. Animals were killed after 1, 3, 5, and 10 days of exposure. Control individuals were killed at the longest (10 days) sampling time. The exposure concentrations used in this study were pharmacological concentrations 1,000 times greater than those found in surface waters. To identify changes in the secondary sexual characteristics, we photographed each animal at both times, just before the start of the exposure assay and after decapitation at the end of the assay (see description below).

**Effects of sex steroids on sex phenotype.** To identify the effects of estrogens and androgens on the sex phenotype, we determined localization of brain aromatase activity in coronal sections as described above. We then compared the aromatase activity to alterations of secondary sexual characteristics. These characteristics included the genital papilla, the presence of papillary processes on the anal fin, and the notch on the dorsal fin (26, 27). The urogenital papilla is enlarged in females and small in males. Both the presence of papillary processes at the posterior region of the anal fin and a notch in the dorsal fin between the last and second last fin rays occur only in males. Enlargement of the urogenital papilla and a decrease in the notch size and in the...
Results

We examined sexual dimorphism of brain aromatase by measuring aromatase activity in serial coronal frozen sections in male and female fish. The two-factorial ANOVA showed that the activity of aromatase is significantly different between males and females, and it also is different between sections of different areas of the brain, showing an interaction between the effect of these two factors ($F_{0.05(1),1.68} = 10.91, p = 0.001$; $F_{0.05(1),16.68} = 10.4, p = 1.17 	imes 10^{-12}$; and $F_{0.05(1),16.68} = 4.15, p = 1.74 	imes 10^{-5}$, respectively). Considering the inequality of the number of levels between the two factors [17 levels (sections) for the localization factor and 2 levels (male and female) for the other factor], we used a subsample of brain sections (sections 4, 5, 9, 10, and 11), corresponding to areas of the brain that contain hypothalamic nuclei of interest in this study, in a two-factor ANOVA.

The results of this analysis showed that the activity of brain aromatase in these sections is significantly different between males and females and detected a contribution of the localization to that difference ($F_{0.05(1),1.62} = 10.52, p = 0.004$; $F_{0.05(1),4.20} = 2.37, p = 0.08$; and $F_{0.05(1),4.20} = 11.26, p = 5.98 	imes 10^{-5}$, respectively). Brain aromatase was highest in sections 4–6 of male and in sections 6–11 of female. One-way ANOVA performed on individual sections showed that the activity of brain aromatase in section 4 was significantly higher in the male (mean $\pm$ SE, 63 $\pm$ 9.4 fmol/hr) than in the female (24 $\pm$ 4.4 fmol/hr; $F_{0.05(1),1.4} = 14.09, p = 0.019$; Figure 1). In sections 9 and 10 brain aromatase activity was significantly higher in the female (80 $\pm$ 10.5 and 54.6 $\pm$ 16.3 fmol/hr, respectively) than in the male (6.1 $\pm$ 1.2 and 2.8 $\pm$ 0.3 fmol/hr; $F_{0.05(1),1.4} = 48.34, p = 0.002$; $F_{0.05(1),1.4} = 48.3, p = 0.002$, respectively for sections 9 and 10). These sections correspond to an area of the brain that contains specific hypothalamic nuclei: the preoptic nuclei (POA) and suprachiasmatic nuclei (SC) in sections 4 through 7, and the nuclei periventricularis dorsalis (H.D), ventrais (H.V), caudalis (H.C), nucleus lateralis (LH), tuberis anteriores (TA), and tuberis posteriores (TP) in sections 8 through 11 (Figure 2).

These results reveal sexual dimorphism in the localization of brain aromatase activity and suggest the involvement of specific hypothalamic nuclei, which are contained in those sections, in the regulation of male or female functions. When male medaka were exposed to 250 µg/L estradiol, the localization of higher levels of aromatase activity was statistically different. The application of the two-factorial ANOVA showed that at the level of confidence of 5% ($\alpha = 0.05$), the difference between the treatments did not depend on the localization of the activity in the brain sections ($F_{0.05(1),1.69} = 41.9, p = 1.24 	imes 10^{-9}$; $F_{0.05(1),16.68} = 18.15, p = 1.55 	imes 10^{-18}$; $F_{0.05(1),16.68} = 1.53, p = 0.113$, respectively, for estradiol treatment, sections, and interaction). To address the possible occurrence of a type I error due to the inequality of levels in each factor (2 for hormone
Brain aromatase activity was significantly increased (ANOVA, F(0.05(1),1.20 = 16.45, p = 0.0006; F(0.05(1),4.20 = 4.72, p = 0.0076; F(0.05(1),4.20 = 0.82, p = 0.5, respectively, for hormone treatment, sections, and interaction). The activity of aromatase was increased in sections 8–11 relative to the control (Figure 3) with a statistically significant increase in section 9 (12.7 ± 4.3, and 55.0 ± 12.3 fmol/hr in control and estradiol treated fish, respectively, F(0.05(1),1.4 = 10.4, p = 0.03) suggesting a feminization of the brain due to the higher levels of estradiol. Exposure to methyltestosterone inhibited the activity of aromatase in all the brain sections of male medaka to levels near the lower limit of sensitivity of our assay (Figure 4) (F(0.05(1),2.102 = 85.29, p = 1.69 × 10–22; F(0.05(1),16.102 = 19.9, p = 1.85 × 10–24; F(0.05(1),32.102 = 4.2, p = 1.6 × 10–8, respectively for hormone treatment, sections, and interaction). To determine the concentration-dependent effects of estradiol on the activity of brain aromatase and on the reversal of the secondary sex characteristics, we used log dilutions of 250 µg/L of estradiol (E2) to perform in vivo assays with male adult medaka. Brain aromatase activity was significantly increased (ANOVA, F(0.05(1),4.23 = 10.65, p = 0.0001; Tukey-Kramer, df = 0.05 = 2.297, p < 0.05) following treatments with 2.5 and 25 µg/L E2 when compared to controls (Figure 5). However, the higher concentrations tested (75 and 250 µg/L), were not statistically different from those of controls. In parallel with the study of the effect of estradiol on brain aromatase, the reversal of the morphologic secondary sex characteristics of the treated animals was next investigated with the aid of a dissection microscope. Of three separate sex-dependent markers—the genital papilla, the papillary processes, and the dorsal fin notch—only the genital papilla exhibited the female phenotype. The reversal of the sex phenotype of the genital papilla to female was observed in 100% of the males in all the concentrations of estradiol that were tested and 0% in the control animals (Figure 5).

The time dependency of the effect of estradiol on the activity of brain aromatase and on the reversal of the secondary sex characteristics was investigated next. In vivo bioassays were performed using 20 µg/L of estradiol. Time points were 1, 3, 5, and 10 days. The activity of brain aromatase in fish exposed to 20 µg/L estradiol increased progressively with time up to day 5, after which a plateau was observed (Figure 6). The
increase in aromatase activity was not significantly different from that of controls at day 1, but it was significantly different at all other time points (3, 5, and 10 days), with aromatase activity significantly higher on day 5 relative to day 3 (ANOVA, F(0.05)1,4,24 = 19.44, p = 1.15 × 10−6; Tukey-Kramer, q0.05,20,5 = 2.297, p < 0.05). Of the three sexually dimorphic characteristics observed, only the genital papilla was affected by estradiol in a time-dependent manner in our assays. There was no change in morphologic sex phenotype after 1 day of exposure to each of the test concentrations. In the fish exposed for 3 days, there was a reversal to female phenotype in 40% of the animals in the assay with 20 µg/L estradiol. At days 5 and 10 there was 100% reversal to female phenotype of the genital papillae (Figure 6). The papillary processes were affected transiently (20% change on day 3, but no change in samples of day 5 and day 10). There was no observable effect of estradiol on the dorsal fin notch in these experiments. In the experiments testing a range of concentrations of estradiol, the genital papilla was affected in 100% of the animals at every concentration tested.

To investigate the enzyme kinetics of brain aromatase in estrogen-treated medaka, we performed Lineweaver-Burke analysis of enzyme saturation assays. Saturation curves obtained with brain homogenates of control fish and fish exposed to 2.5 and 25 µg/L estradiol in an in vivo assay were compared. First-order Michaelis-Menten (Km) type of curve was observed with the homogenates of control fish, with an apparent Kmean of 1.0 nM (Figure 7). The saturation curves obtained with the brain homogenates from estradioltreated fish failed to reach a plateau. An apparent Kmean of about 5nM, different from that obtained with the control fish, was extrapolated. This indicates that the enzyme is not following a first-order type of kinetics. The effect of estradiol implies the activity of mechanisms other than a simple increase of enzyme concentration.

**Discussion**

We identified sexual dimorphism in brain aromatase activity of medaka. The male phenotype of brain aromatase activity is characterized by the presence of high levels of activity confined to the coronal sections of the brain that contain the POA and the SC. Low levels of activity existed in all the other brain areas, including those containing the HD, HV, and Hc. The female phenotype is characterized by the presence of high aromatase activity in coronal sections of the brain that contain the periventricular nuclei HD, HV, and Hc, the NDIL, and the TA and TP. Aromatase activity in sections 6 and 7 of the female is highly variable (range 23 to 70 fmol/hr).

The organizational structure of the hypothalamus of teleosts comprises functional nuclei as discrete structures distributed along the longitudinal axis of the brain, in a medial–ventral localization. An experimental advantage of this organization is the ability to analyze groups of nuclei by sectioning the brain along the longitudinal axis. Using this approach, the activity of brain aromatase and morphologic changes in the secondary sexually dimorphic characteristics—were obtained in the same experiment and from the same animals. Aromatase activity is expressed in femtomoles of converted tritiated estradiol to estrone, as indicated by the measurement of tritiated water released in the reaction. Sexually mature male medaka were treated with 250 µg/L methyltestosterone for 10 days and then were killed. Brains were immediately sectioned, homogenized, and stored at -80°C until use in aromatase assay. Sections 1–17 are in sequence from the fore- to the hindbrain. Each bar is the mean ± SE of each section from three individuals.

**Figure 4.** Effect of methyltestosterone on the brain aromatase activity of male medaka. Aromatase activity is expressed in femtomoles of converted tritiated estradiol to estrone, as indicated by the measurement of tritiated water released in the reaction. Sexually mature male medaka were treated with 250 µg/L methyltestosterone for 10 days and then were killed. Brains were immediately sectioned, homogenized, and stored at -80°C until use in aromatase assay. Sections 1–17 are in sequence from the fore- to the hindbrain. Each bar is the mean ± SE of each section from three individuals.

**Figure 5.** Concentration-dependent effect of estradiol on the activity of brain aromatase and effect on the sexual phenotype of the genital papillae. The results corresponding to these two independent variables—brain aromatase activity and morphologic changes in the secondary sexually dimorphic characteristics—were obtained in the same experiment and from the same animals. Aromatase activity is expressed in femtomoles of converted tritiated estradiol to estrone, as indicated by the measurement of tritiated water released in the reaction. Male medaka were treated for 10 days with 2.5, 25, 75, or 250 µg/L of estradiol and killed, and homogenates of the dissected whole brain were obtained. Each bar is the mean ± SE of the activity of brain aromatase of five individuals of each treatment. The genital papillae were photographed in a dissecting microscope (magnified 30x) before and after treatment with estradiol. Control animals were treated with DMSO, in volume equivalent to that used to dilute the steroid (< 1/1,000).

*Tukey-Kramer q0.05,20,5 = 2.297, p < 0.05.*
microscope (magnified 30×) before and after the treatment with estradiol. Control animals were treated with DMSO, in volume equivalent to that used to dilute the steroid (< 1/1000).

Fig. 6. Time dependency of the effect of estradiol on the activity of brain aromatase and on the sexual phenotype of the genital papilla. The results corresponding to these two independent variables—brain aromatase activity and morphologic changes in the secondary sexual characteristics—were obtained in the same experiment and from the same animals. Aromatase activity is expressed in femtomoles of tritiated water released in the reaction. Male medaka were treated with 20 µg/L estradiol for 1, 3, 5, or 10 days and killed, and whole-brain homogenates were used in the aromatase assay. Each bar is the mean ± SE of brain aromatase activity of five individuals in each treatment. The genital papillae were photographed in a dissecting microscope (magnified 30×) before and after the treatment with estradiol. Control animals were treated with DMSO, in volume equivalent to that used to dilute the steroid (<1/1000).

*Tukey-Kramer q0.05,0.5 = 2.297, p < 0.05.
Estrogen exposure has two actions on brain aromatase in male medaka: elevated total levels of aromatase activity and localized expression in the medial hypothalamic nuclei. The total levels of brain aromatase activity increased with 2.5 and 25 µg/L estradiol; yet at higher doses (75 and 250 µg/L), significant increases were not found. Alternative regulatory pathways of brain aromatase are probably triggered by levels of estrogens above a certain threshold, which was achieved in our experiment with the exposure to 75 µg/L of exogenous estradiol. Induction of aromatase in the male hypothalamus was observed at 250 µg/L, a concentration where a total brain aromatase activity 100% of sex phenotype reversal after 5 days of exposure was not observed. Whether this mediodiscal distribution of brain aromatase occurs at lower doses of estradiol has not yet been determined.

The sexual phenotype of the genital papilla seems to be a direct effect of estradiol on that tissue, leading to cellular and physiologic processes that increase the volume of that tissue, probably via estrogen receptor. The presence of estrogen receptors in tissues of accessory organs (placenta, mammary glands) and other tissues (adipose, bone) is well known in mammals but has not been investigated in teleosts.

The increase of aromatase activity by estradiol implies a positive feedback in which the enzymatic activity is upregulated by the reaction product. This positive feedback has been suggested (37), although the mechanisms are still not well understood. Enzyme induction is a common biological mechanism to increase total enzymatic activity. We investigated the enzyme kinetics of aromatase by performing Lineweaver-Burke plots to analyze saturation assays. We determined that the brain aromatase of medaka has an apparent K_m of 1 nM. The apparent K_m of brain aromatase of males treated with estradiol showed a different value (5 nM).

We conclude that the induction of genetic expression of aromatase is not the only mechanism regulating the activity of brain aromatase. Neurotransmitters such as dopamine may also modulate the neurogenesis of estrogens by aromatase. It has also been suggested that dopamine may interact directly with aromatase (38). The catechol metabolites of estrogen can compete with dopamine for degradation by catechol-O-methyl transferase, increasing the concentration of dopamine. Cell volume increase and the increase of the number of cells can also increase the activity of aromatase in the brain.

In summary, we identified sexual dimorphism of brain aromatase activity in medaka. Estradiol alters the activity of brain aromatase in adult male medaka by inducing an anterior–posterior profile of aromatase activity distribution in the medial hypothalamic characteristic of the female. A positive regulation of estradiol on the rate-limiting enzyme for its synthesis comprises a positive feedback loop. This positive feedback of estradiol on the activity of aromatase is a mechanism that can lead to very high levels of estrogens in response to a small stimulus and is a potential target that will lead to endocrine disruption in the presence of small alterations of estrogen levels.

References and Notes

1. Colborn T, Dumanoski D, Myers J P. Our Stolen Future. New York: Plume/Penguin, 1997.
2. Giesy JP, Ludwig P, Tillity DL. Deformities in birds of the Great Lakes region - assigning causality. Environ Sci Technol 28:128–138A (1994).
3. Guillette LJ Jr, Crain DA, Rooney AA, Pickford DB. Organization versus activation: the role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. Environ Health Perspect 103(suppl 7):157–164 (1995).
4. Cody RP, Bortone SA. Masculinization of mosquitofish as an indicator of exposure to kraft mill effluent. Bull Environ Contam Toxicol 58:429–436 (1997).
5. Gimeno S, Gerritsen A, Bowmer T. Feminization of male carp. Nature 384:221–222 (1996).
6. Maccusky NJ, Naftolin F. Sexual differentiation in the central nervous system. Science 211 (1981).
7. MCEwen BS. Hormone actions in the brain. In: Endocrinology: Basic and Clinical Principles (Conn PM, Melmed S, eds.). Totowa, N.J: Humana Press Inc., 1997:63–78.
8. Hutchinson JB. Aromatase: neuromodulator in the control of behavior. J Steroid Biochem Mol Biol 44:509–520 (1993).
9. Simpson ER, Michael MD, Agarwal VR, Hinshelwood MM, Bulun SE, Zhao Y. Cytokines and interleukins: expression of the CYP19 (aromatase) gene: an unusual case of alternative promoter usage. FASEB J 11(1):29–36 (1997).
10. Matsunima H, Herbst MA, Ou SH, Wilson JD, McPhaul MJ. Aromatase mRNA in the extragonadal tissue of chickens with the henny-feathering trait is derived from a distinctive promoter structure that contains a segment of a retroviral long terminal repeat. J Biol Chem 266:13900–13907 (1991).
11. Tanaka M, Fukuda S, Matsuyama M, Nagahama Y. Structure and promoter analysis of the cytochrome P-450 aromatase gene of the teleost fish, medaka (Oryzias latipes). J Biochem 117:719–725 (1995).
12. Choi I, Troyer DL, Cornwell D, Kirby-Dobbs KR, Collante WR, Simmen FA. Closely related genes encode developmental and tissue isoforms of porcine cytochrome P450 aromatase, DNA Cell Biol 16:769–777 (1997).
13. Tchoudakova A, Callard GV. Identification of multiple CYP19 genes encoding different cytochrome P450 aromatase isoforms in brain and ovary. Endocrinology 139:2179–2189 (1998).
14. Mahendroo MS, Mendelson CR, Simpson ER. Tissue-specific and hormonally controlled alternative promoters regulate aromatase cytochrome P450 gene expression in human adipose tissue. J Biol Chem 268:19463–19470 (1993).
15. Simpson ER, Zhao Y, Agarwal VR, McPhaul MJ, Bulun SE, Hinshelwood MM, Graham-Lorence S, Sun T, Fisher CR, Qin K, Mendelson CR. Aromatase expression in health and disease. Rec Prog Horm Res 52:185–215 (1997).
16. Callard GV, Petro Z, Ryan KJ. Phylogenetic distribution of aromatase and other androgen-converting enzymes in the central nervous system. Endocrinology 103:2283–2290 (1978).

17. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Corbin CJ, Mendelson CR. Tissue-specific promoters regulate aromatase cytochrome P450 expression. J Steroid Biochem Mol Biol 44:321–330 (1993).

18. Callard GV, Petro Z, Ryan KJ. Estrogen synthesis in vitro and in vivo in the brain of a marine teleost (Mysxoxocephalus). Gen Comp Endocrinol 43:243–255 (1981).

19. Godwin J, Crews D, Warner RR. Behavioural sex change in the absence of gonads in a coral reef fish. Proc R Soc Lond 263:1683–1688 (1996).

20. Bass AH. Shaping brain sexuality. Am Sci 84:352–363 (1996).

21. Parsons B, Rainbow TC, McEwen BS. Organizational effects of testosterone via aromatization on feminine reproductive behavior and neural progestin receptors in rat brain. Endocrinology 115:1412–1417 (1984).

22. Schlinger BA, Callard GV. Aromatization mediates aggressive behavior in quail. Gen Comp Endocrinol 79:39–53 (1990).

23. Pasmanik M, Schlinger BA, Callard GV. In vivo regulation of aromatase and 5α-reductase in goldfish brain and pituitary. Gen Comp Endocrinol 71:175–182 (1996).

24. Lephart ED. A review of brain aromatase cytochrome P450. Brain Res Rev 22:1–26 (1996).

25. Melo AC, Edmunds J SG, Ramsdell J S. Localization and sex-steroid regulation of brain aromatase activity: identification of a positive feedback mechanism that may be triggered by environmental cues. In: Environmental Toxicology and Risk Assessment: Standardization of Biomarkers for Endocrine Disruption and Environmental Assessment, Vol 8 (Henshel DS, Black MC, Harrass MC, eds). West Conshohocken, PA: American Society for Testing and Materials, 1999;226–238.

26. Yamamoto T. Artificially induced sex reversal in genotypic males (Oryzias latipes). J Exp Zool 123:571–594 (1953).

27. Yamamoto T. Artificially induced sex reversal in genotypic females (Oryzias latipes). J Exp Zool 137:227–263 (1958).

28. Anken R, Bourrat F. Brain Atlas of the Medakafish: Oryzias latipes. Paris: INRA Editions, 1998.

29. Thompson EA, Siiteri PK. Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstenedione. J Biol Chem 249:5364–5372 (1974).

30. Gorski RA, Harlan RE, Jacobson CD, Shryne JE, Southam AM. Evidence for the existence of a sexual dimorphic nucleus in the preoptic area of the rat. J Comp Neurol 193(2):529–539 (1980).

31. Balthazart J, Foidart A. Brain aromatase and the control of male sexual behavior. J Steroid Biochem Mol Biol 44:521–540 (1993).

32. Roselli CE. Synergistic induction of aromatase activity in the rat brain by estradiol and 5-alpha-dihydrotestosterone. Neuroendocrinology 53:79–84 (1991).

33. Panzica GC, Viglietti-Panzica C, Balthazart J. The sexually dimorphic medial preoptic nucleus of quail: a key brain area mediating steroid action on male sexual behavior. Front Neuroendocrinol 17:51–125 (1996).

34. Hutchinson JB, Steimer TJ, Hutchinson RE. Formation of behaviorally active estrogen in the dove brain: induction of preoptic aromatase by intracranial testosterone. Neuroendocrinology 43:416–427 (1986).

35. Roselli CE, Abdelgadir SE, Ronnekleiv OK, Klosterman SA. Anatomical distribution and regulation of aromatase gene expression in the rat brain. Biol Reprod 58:79–87 (1998).

36. Lephart ED, Simpson ER, Ojeda SR. Effects of cyclic AMP and androgens on in vitro brain aromatase enzyme activity during prenatal development in the rat. J Neuroendocrinol 4:39–46 (1992).

37. Callard GV, Schlinger BA, Pasmanik M, Corina K. Aromatization and estrogen action in the brain. Prog Comp Endocrinol 342:105–111 (1990).

38. Balthazart J, Ball GF. New insights into the regulation and function of brain estrogen synthase (aromatase). Trends Neurosci 21:243–249 (1998).