Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7006–7007, September 1988
Neurobiology

A critical period for estrogen action on neurons of the song control system in the zebra finch

(brain/gender difference/development)

MASAKAZU KONISHI AND EUGENE AKUTAGAWA

Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Contributed by Masakazu Konishi, May 26, 1988

ABSTRACT **The song nuclei of the male zebra finch (*Poephila guttata*) contain larger neurons than those of the female. This gender difference arises after hatching as a result of cell atrophy in the female and cell growth in the male. Implantation of estrogen in female chicks induces masculine differentiation of neurons in their song nuclei. The effects of estrogen on neuron size decline steeply after posthatching day 35 when neuronal atrophy begins. Estrogen loses its masculinizing effects completely after day 45 when the adult level of neuronal atrophy is reached. Thus, the end and the intensity of hormone action appear to be correlated with the timing of neuronal atrophy.**

Administration of gonadal steroids early in life can induce the development of brain anatomical and behavioral attributes unique to one or the other gender. The inductive action of hormone is generally limited to a short period before or after birth (1). However, the factors controlling the length of the critical period and variation in the intensity of hormone action during the period are not known in most cases. This lack of knowledge is partly due to reliance on descriptive behavioral criteria for hormone action. Sexually dimorphic neurons and neuronal populations are convenient for the establishment of quantitative relationships between the timing and effect of hormone administration (2, 3).

The brain nuclei that control song in the zebra finch (*Poephila guttata*) show marked sexual dimorphism (4). The gender differences do not exist at birth but arise later from a decrease in cell size and cell death in the female nuclei as well as from an increase in cell size in the male (5). An increase in cell number in one of the male nuclei may also contribute to the differences (6). Administration of estrogen to a female chick induces the development of masculine cell size in her song control system (7, 8). This report describes how the time of estrogen implantation affects neuron size in two of the female song nuclei.

MATERIALS AND METHODS

Zebra finch eggs were collected from our own breeding stock and incubated in an incubator so that the exact dates of their hatching could be determined. The chicks were raised by Bengalese finches as foster parents. Estrogen (50 μg of 17β-estradiol) was administered as a slow-release (Silastic) subcutenous implant. Removal of the implant after various lengths of time showed that the induction of full masculinization requires at least 15 days of implantation. In the present study, the implant was left in place from the day of implantation until the bird was killed for histological examination at the age of 90 days or older. The sampling interval for the effect of hormone was 5 days except between day 35 and day 45. We chose for study two of the forebrain song nuclei, the ventral nucleus of hyperstriatum caudal section (HVc) and the robust nucleus of archistriatum (RA). The cross-sectional area of neuronal somata serves as a sensitive measure of their growth and atrophy. Birds were perfused with saline and 10% formalin. Brains were cut into parasagittal sections of 30 μm in thickness and stained with creysl-violet. For the measurement of cross-sectional area, the perikaryal outlines of 50 randomly chosen neurons per bird were drawn with a Zeiss camera lucida and the area within the outlines was calculated by a computer-aided planimetric method.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

RESULTS

Fig. 1*A* shows the somal areas of HVc and RA neurons of adult female zebra finches that received a hormone implant at various early ages. An analysis of variance for the RA shows that despite variance among individuals the mean size of neurons varies with age between day 15 and day 30. The effects of estrogen appear to decline rapidly during the period between day 30 and day 45. The HVc and RA become completely refractory to estrogen treatment at about 45 days of age. Gurney (2), estimating cell number from cell density and nuclear volume, also found day 45 to be the end of the sensitive period for the RA.

One possible factor for the decline of hormonal effects is the hormone/weight ratio, which becomes smaller as the bird grows. The body and brain weights of female zebra finches reach the adult values by 15 days of age so that the hormone/weight ratio of the birds used in the present study was almost constant. Nevertheless, we investigated the effects of a larger dose (250 μg) of estrogen on two females. These birds carried the hormone pellet for a period of 6–8 weeks from days 30 and 35, respectively. The HVc and RA of these birds did not differ from those of birds of similar age treated with 50 μg of estrogen. In another female a 50-μg pellet was left *in situ* from day 35 to day 120. The song nuclei of this bird did not differ from those of birds that retained the hormone pellet from day 35 to day 60. These results indicate that neither an increased hormone/weight ratio nor longer exposure to the hormone affects the end of the hormone-sensitive period or the degree of masculinization. However, these variables may influence the period and the effects of hormone action, if a threshold level of the hormone were to be tested. This level has not been determined.

Comparison of Fig. 1*A* with Fig. 1 *B* and *C* suggests that neuronal growth and atrophy in the normal male and female may be correlated with the degree and end of estrogen action. In the male RA and HVc, neurons attain their maximal size at 35–45 days of age. Neuronal atrophy in the female RA appears to be complete at about 45 days of age; neuron size at that time is not different from the adult size. The end of

Abbreviations: HVc, ventral nucleus of hyperstriatum caudal section; RA, robust nucleus of archistriatum.

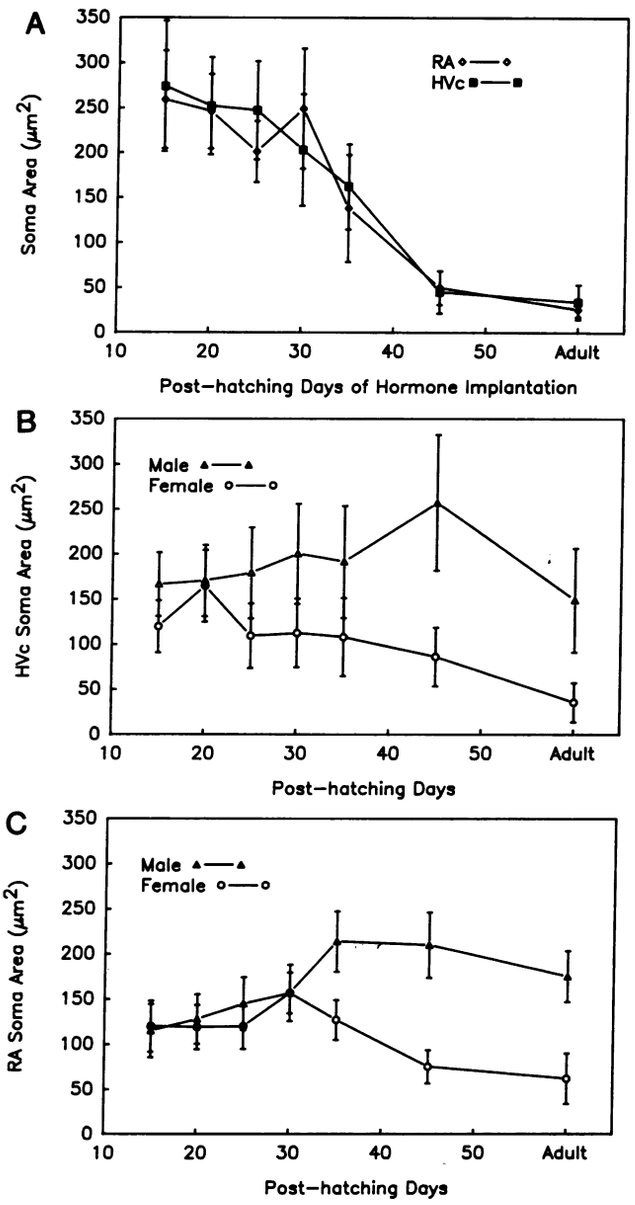

FIG. 1. (*A*) Age-dependent changes in estrogen effects on neuron size. Five females were used in each age class. They received a subcutaneous implant at various early ages, which are plotted on the abscissa. The hormone pellet was left *in situ* until the birds were prepared for histological examination in adulthood. A sample of 50 neurons was selected for the measurement of somal area; means of five means and standard deviations are plotted on the ordinate. The masculinizing effects of estrogen vary somewhat from day 15 to day 30 when they begin to decline sharply. HVc and RA neurons become refractory to estrogen after day 45. (*B* and *C*) Normal time course of neuronal growth and atrophy in the HVc (*B*) and RA (*C*). Neuronal atrophy in the female RA begins at day 30–35 and ends by day 45. The end of cellular atrophy is less clear in HVc. Neuron size in the male HVc and RA reaches a peak at 40–45 days of age, declining slightly thereafter.

atrophy is less distinct in the HVc than in the RA, partly because the obscure borders of the HVc make it difficult to discriminate between cells inside and outside the nucleus.

Female RA and HVc neurons show little change between day 15 and day 30 (Fig. 1 *B* and *C*). This interval corresponds to the period in which the masculinizing effects of estrogen in both nuclei vary less than in the period between day 30 and day 45. The beginning of neuronal atrophy in the RA and HVc is about 35 days of age, and estrogen implantation at this age results in partial growth of neurons in both nuclei (Fig. 1*A*). Thus, the end of the estrogen-sensitive period, particularly in the RA, appears to coincide with the end of neuronal growth in the male and the final stage of neuronal atrophy in the female. Also the degree of estrogen-induced growth of neurons appears to be limited by the extent of their atrophy; estrogen can induce only partial growth after the neurons have started to shrink. When the neurons become as small as those typical of the adult female, they become refractory to estrogen. One possible cause of the refractoriness is a smaller number of estrogen-absorbing cells. The HVc, at least in female chicks, contains estrogen receptor sites, and the number of estrogen-absorbing cells declines sharply after day 40 (9).

Whether the response of RA and HVc neurons to a subcutaneous implant of estrogen simulates the normal process of brain sexual differentiation is unclear. Hutchison *et al.* (10) found a temporary upsurge of estrogen in the serum of 2- to 8-day-old male zebra finch chicks. Although this period is much shorter than the 15 days required for full masculinization of the female song system, the male song system may not require 15 days. Young males also have circulating estrogen throughout their preadult life (11). Whatever the relationship between the natural and experimentally induced differentiation, the masculinized song system of a female zebra finch can generate the signals neccessory for the production of song (12). The recent work of Pohl-Apel (13) suggests that the degree of song development in estrogen-treated females varies with the degree of masculinization of neurons in their song control nuclei.

We thank Drs. Catherine E. Carr, Paul Patterson, and Susan F. Volman for reading early versions of this paper and Dr. Jack Wathey for statistical analysis. This work was supported by a grant for Developmental Biology from the Lucille P. Markey Charitable Trust.

1. Goy, R. W. & McEwen, B. S. (1980) *Sexual Differentiation of the Brain* (Massachusetts Institute of Technology Press, Cambridge, MA).
2. Gurney, M. E. (1980) Dissertation (California Institute of Technology, Pasadena, CA).
3. Konishi, M. & Gurney, M. E. (1982) *Trends Neurosci.* **5**, 20–23.
4. Nottebohm, F. & Arnold, A. P. (1976) *Science* **194**, 211–213.
5. Konishi, M. & Akutagawa, E. (1985) *Nature (London)* **315**, 145–147.
6. Bottjer, S. W., Glaessner, S. L. & Arnold, A. P. (1985) *J. Neurosci.* **5**, 1556–1562.
7. Gurney, M. E. (1981) *J. Neurosci.* **1**, 658–673.
8. Konishi, M. & Akutagawa, E. (1987) *Selective Neuronal Death*, Ciba Foundation Symposium (Wiley, New York) Vol. 126, pp. 173–185.
9. Gahr, M. & Konishi, M. (1988) *Proc. Natl. Acad. Sci. USA*, in press.
10. Hutchison, J. B., Wingfield, J. C. & Hutchison, R. E. J. (1984) *Endocrinology* **103**, 363–369.
11. Proeve, E. (1983) in *Hormones and Behaviour in Higher Vertebrates*, eds. Balthazart, J., Proeve, E. & Gilles, R. (Springer, Berlin), pp. 368–374.
12. Gurney, M. E. (1982) *Brain Res.* **231**, 153–172.
13. Pohl-Apel, G. (1985) *Brain Res.* **336**, 381–383.