References and Notes

1. F. B. Golley and J. B. Gentry, *Oikos* **15**, 185 (1966); R. Margalef, *Perspectives in Ecological Theory* (Univ. of Chicago Press, Chicago, 1968); C. D. Monk, *Amer. Natur.* **101**, 173 (1967); E. P. Odum, *Science* **164**, 262 (1969); E. C. Pielou, *J. Theor. Biol.* **10**, 370 (1966); R. H. Whittaker, *Science* **147**, 250 (1965).
2. Over 200 publications were examined. The references were drawn from P. T. Haug and G. M. Van Dyne, *Secondary Succession in Abandoned Cultivated Fields: An Annotated Bibliography* (Publ. No. 228, Ecological Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1968); P. T. Haug, thesis, Colorado State University (1970). Most of the sources did not contain sufficient information for us to calculate species turnover rate coefficients as a function of time.
3. The sources of the data used in Fig. 1 are: (a) D. W. Smith, thesis, University of Tennessee (1968); (b) D. W. Johnston and E. P. Odum, *Ecology* **37**, 50 (1956); (c) H. H. Shugart, thesis, University of Arkansas (1968); (d) H. H. Oosting, *Amer. Midl. Natur.* **28**, 1 (1913); and (e) W. A. Reiners, I. A. Worley, D. B. Lawrence, *Ecology* **52**, 55 (1971).

4. Supported by the Eastern Deciduous Forest Biome Project, International Biological Program, funded by the National Science Foundation under interagency agreement AG-199, 40-193-69 with the Atomic Energy Commission–Oak Ridge National Laboratory. Oak Ridge National Laboratory is operated by Union Carbide Corporation for the U.S. Atomic Energy Commission. Contribution No. 82 from the Eastern Deciduous Forest Biome.

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Testosterone Concentration in the Male Chick Brain: An Autoradiographic Survey

Abstract. *Differential uptake of [^{3}H]testosterone in male chick brain was found in periventricular areas of preoptic-hypothalamic continuum. Concentration of silver grains for all decapitation periods was especially high in the medial preoptic area, particularly the nucleus praeopticus paraventricularis magnocellularis. Distribution of testosterone-sensitive cells is in agreement with studies showing neuroanatomical control of reproductive behavior by the avian forebrain.*

Recent studies using the domestic chick have shown the preoptic-anterior hypothalamic continuum to be involved in patterns of reproductive behavior. Specifically, precocial copulation in the male chick has been activated by testosterone implants in the preoptic region (*1*), inhibited by progesterone implants placed in the medial preoptic area (*2*), and disrupted through bilateral electrolytic lesions in the anterior hypothalamus (*3*). These data suggest that hormone-sensitive neurons exist in the male chick brain, possibly similar in neuroanatomical distribution to the male rat, where autoradiographic surveys have shown the presence of androgen-sensitive (*4*) and estrogen-sensitive (*5*) neurons.

The purpose of the present study was to determine the topographical distribution of androgen-sensitive cells in the male chick brain by surveying for the differential uptake of tritiated testosterone. The autoradiographic technique has been a useful method for mapping hormone-sensitive sites in the mammalian brain (*4*, *5*), and recent studies (*6*) have incorporated improvements in order to minimize artifactual data due to diffusion of water-soluble compounds. In the present study, brain tissue was sliced in a microtome-cryostat under safelight and directly mounted on slides precoated with photographic emulsion. The resultant autoradiograms contained adequate cellular resolution for the analysis of silver grain concentrations in various neuroanatomical areas.

Fifteen male Rhode Island Red × White Rock chicks were reared in isolation. At 2 weeks of age, they were subcutaneously injected with 200 μc (1.3 μg) of 1,2-[^{3}H]testosterone (specific activity 45 c/mmole), and decapitated ½ hour ($n = 3$), 2 hours ($n = 4$), or 3 hours ($n = 5$) later. One chick for each of the three decapitation times was injected with unlabeled testosterone. Brains were removed after decapitation and frozen in powdered CO_2. Brain tissue was sliced at 6 μm in a cryostat at −18°C, under safelight conditions (*7*), and the sections were mounted directly on slides precoated with emulsion (Kodak NTB-3). The slides were then packed in lightproof boxes, placed in plastic containers under partial vacuum, and exposed in lead-lined boxes at 5°C. After an exposure period of 4 to 6 months, the autoradiograms were developed and stained with thionin.

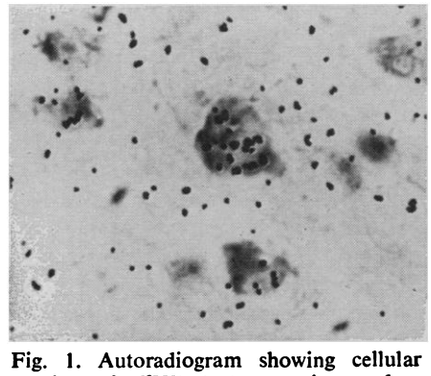

Fig. 1. Autoradiogram showing cellular uptake of [^{3}H]testosterone in nucleus praeopticus paraventricularis magnocellularis (× 450). Exposure 146 days, 3-hour decapitation.

Quantitative analyses of reduced grains beneath brain cells were performed for each of the 18 neuroanatomical areas (*8*) given in Table 1. Fifty cells were counted under bright-field microscopy at × 400 for each brain area in every animal. Radioactivity beneath cell bodies for the three decapitation times is given in Table 1. These data, grouped according to neuroanatomical area, are presented in terms of mean grains per cell body, and also as the mean number of labeled cells, that is, cells which contained 11 or more silver grains. Highest mean uptake over the three decapitation periods was seen in the nucleus praeopticus paraventricularis magnocellularis, which consistently showed the greatest mean concentration of reduced grains per cell, as well as cells with 11 or more grains. In addition, relatively high mean grain counts were found in the nucleus supraopticus, nucleus praeopticus medialis, nucleus hypothalamicus anterior medialis, nucleus paraventricularis magnocellularis, and nucleus hypothalamicus posterior medialis. A labeled neuron in the nucleus praeopticus paraventricularis magnocellularis is shown in Fig. 1.

Generally, reduced grain content decreased as decapitation time increased, although the nucleus praeopticus paraventricularis magnocellularis retained high mean grain counts for all three decapitation periods. Chicks injected with unlabeled testosterone had low concentrations of silver grains throughout the brain, and averaged no higher than 1.03 ± 0.37 grains per cell in any neuroanatomical area. In no instance did an unlabeled control contain a cell with 11 or more grains. Moreover, experimental slides which had been fogged with light showed no fading of the latent image.

In order to determine the highest uptake over the three decapitation times, the chi-square test was used to compare neuroanatomical areas for differences between labeled (11 or more grains) and unlabeled cells. The nucleus praeopticus paraventricularis magnocellularis had scores for mean labeled cells which were higher than all other areas within the 2- and 3-hour decapitation periods ($P < .05$),

Table 1. Quantitative summary of topographical distribution of radioactivity for the three decapitation periods. Uptake of [^{3}H]testosterone is expressed as the mean number of grains per cell (± S.E.M.) for the various neuroanatomical areas, as well as the mean number of cells per brain area which contained 11 or more reduced grains.

Brain area	½ hour ($n = 3$)		2 hours ($n = 4$)		3 hours ($n = 5$)	
	Grains per cell	Cells with 11 or more grains	Grains per cell	Cells with 11 or more grains	Grains per cell	Cells with 11 or more grains
Olfactory structures						
Bulbus olfactorius	3.30 ± .36	2.00	3.16 ± .36	1.50	2.66 ± .33	0.20
Hippocampal complex	3.95 ± .38	2.67	2.69 ± .29	0	2.22 ± .28	.20
Striatal centers						
Neostriatum	3.96 ± .37	2.33	2.59 ± .28	0.25	3.20 ± .35	.80
Paleostriatum primitivum	4.44 ± .40	1.67	2.72 ± .29	.50	2.89 ± .35	.60
Archistriatum	4.31 ± .42	1.67	2.63 ± .30	.25	2.68 ± .33	.20
Preoptic-hypothalamic continuum						
Nucleus supraopticus	7.37 ± .50	8.00	5.58 ± .41	4.50	6.30 ± .45	3.40
Nucleus praeopticus paraventricularis magnocellularis	8.53 ± .54	13.67	8.12 ± .65	13.25	9.77 ± .77	17.20
Nucleus praeopticus medialis	7.88 ± .49	11.33	5.74 ± .44	6.25	6.35 ± .44	4.20
Lateral preoptic region	5.88 ± .47	6.33	3.64 ± .35	0.75	3.65 ± .41	0.80
Nucleus hypothalamicus anterior medialis	7.45 ± .44	9.00	5.60 ± .48	6.00	6.11 ± .51	5.80
Nucleus paraventricularis magnocellularis	7.15 ± .48	9.33	5.32 ± .40	4.50	4.20 ± .42	1.40
Nucleus hypothalamicus posterior medialis	6.94 ± .46	7.33	4.86 ± .42	3.75	5.84 ± .47	5.60
Lateral hypothalamic region	6.17 ± .42	5.67	3.87 ± .36	2.00	2.78 ± .35	0
Other areas						
Nucleus rotundus	7.03 ± .47	8.33	4.78 ± .41	2.50	3.88 ± .43	1.60
Optic tectum	3.47 ± .39	0.33	2.52 ± .28	0	2.46 ± .34	0.40
Nucleus mammilaris medialis	4.46 ± .39	2.67	3.87 ± .35	1.50	1.72 ± .26	0
Cerebellum	3.01 ± .32	0.33	2.08 ± .25	0	1.20 ± .18	0

while in the ½-hour condition the mean number of labeled cells was similar to that found in the nucleus praeopticus medialis, nucleus hypothalamicus anterior medialis, and the nucleus paraventricularis magnocellularis.

Comparisons across decapitation times indicated that the nucleus praeopticus paraventricularis magnocellularis and nucleus hypothalamicus anterior medialis showed no reliable decreases in mean labeled cell content, while other areas such as the nucleus supraopticus, nucleus praeopticus medialis, nucleus paraventricularis magnocellularis, and the nucleus hypothalamicus posterior medialis all showed significant decreases ($P < .05$) in mean labeled cell content from the ½-hour to 2- or 3-hour decapitation times.

The results suggest that the medial preoptic area, especially the nucleus praeopticus paraventricularis magnocellularis, contains neurons which are particularly sensitive to radioactive testosterone. Cellular regions surrounding the third ventricle, and extending from the preoptic area to the medial hypothalamus, showed the highest uptake and retention over the three decapitation periods. It should be noted that some neuroanatomical regions, such as the midbrain and striatal centers, were only cursorily sampled, and the possibility remains that testosterone may have been taken up in these or other brain areas. Autoradiographic studies using the male rat (*4*, *9*) have also shown [^{3}H]testosterone retention within the preoptic-hypothalamic continuum. In addition, [^{3}H]estradiol has also been shown to concentrate in this neuroanatomical region in the female rat (*4*, *5*). For the male ring dove, scintillation studies have shown [^{3}H]testosterone to accumulate in greater quantities in the hypothalamus as compared with the cerebrum (*10*).

Other data are available which indicate that the testosterone-sensitive areas described in this report are directly involved in the various patterns of avian reproductive behavior. For the male chick it has been shown that testosterone implants in the medial preoptic area activate copulation (*1*), while lesions in the anterior hypothalamus disrupt it (*3*). Testosterone implants in the preoptic-anterior hypothalamic continuum have also been shown to activate copulation in male fowl (*11*) and male ring doves (*12*). Thus it appears that a preoptic-hypothalamic periventricular network exists in the bird which is sensitive to radioactive testosterone and is involved in the control of reproductive behavior. This system is at least partially developed at 2 weeks of age, and is similar in neuroanatomical distribution to that found in the rat. Moreover, this network may contain receptors for other sex steroids such as estrogen and progesterone. Whether testosterone receptor-neurons within this network are sensitive to other sex steroids has yet to be established.

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References and Notes

1. J. E. Gardner and A. E. Fisher, *Physiol. Behav.* **3**, 709 (1968).
2. C. C. Meyer, *J. Comp. Physiol. Psychol.* **79**, 8 (1972).
3. ——— and E. A. Salzen, *ibid.* **73**, 365 (1970).
4. D. W. Pfaff, *Science* **161**, 1355 (1968).
5. W. E. Stumpf, *ibid* **162**, 1001 (1968).
6. C. H. Anderson and G. S. Greenwald, *Endocrinology* **85**, 1160 (1969); W. E. Stumpf, *J. Histochem. Cytochem.* **18**, 21 (1970); J. L. Gerlach and B. S. McEwen, *Science* **175**, 1133 (1972).
7. The darkroom is equipped with two Kodak model A safelights, Wratten series, filter No. 2, 15-watt bulb, placed at a working distance of 4 feet (1.2 m). I have found the frost-free microtome-cryostat from International Equipment Co. (model CTF) to be particularly appropriate for slicing brain tissue under safelight conditions. When slicing tissue I use light reflected off the microtome knife. Sections appear dark against the blade, and are cut with the antiroll bar adjusted slightly past the edge of the blade.
8. Nuclei were identified by using the atlas for the adult chicken prepared by A. van Tien-

hoven and L. P. Juhasz [*J. Comp. Neurol.* **118**, 185 (1962)].
9. M. Sar and W. E. Stumpf, *Fed. Proc.* **30**, 363 (1971), abstr.
10. J. M. Stern, *Gen. Comp. Endocrinol.* **18**, 439 (1972); R. E. Zigmond, J. M. Stern, B. S. McEwen, *ibid.*, p. 450.
11. R. J. Barfield, *Horm. Behav.* **1**, 37 (1969).
12. ———, *Endocrinology* **89**, 1470 (1971).
13. I thank W. E. Stumpf of the University of North Carolina and D. W. Pfaff of Rockefeller University for help with the autoradiographic technique. I also thank O. Meyer for histological assistance throughout the study. This research was supported by a grant from the National Institute of Mental Health (MH-17384).

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Control of Their Environment Reduces Emotionality in Rats

Abstract. *Rats reared from birth in a "contingent environment" in which they controlled lighting conditions and the delivery of food and water were compared as adults to rats reared in an environment in which they received the same food, water, and lighting conditions, but without control over their occurrence. Rats reared in the contingent environment were less emotional, as judged by activity and defecation scores in open-field testing.*

Effects of infantile stimulation on adult "emotionality" in rats have been widely investigated. Stimulation has included manipulations such as handling for a few minutes a day (*1*), exposure to mild electric shock (*2*), cooling (*3*), and maternal deprivation (*4*). Application of these procedures to rats prior to weaning has affected their emotional behavior in adulthood, as measured by open-field performance (*5*).

The degree to which the animal has control over its early environment has never been systematically investigated for its effect on adult behavior. Several cognitive theorists (*6*, *7*) have suggested that this variable is important in much human behavior. In particular, Rotter (*7*) devised a rating scale designed to differentiate individuals who perceive a contingency between their behavior and environmental events (internal locus of control) from individuals who perceive that their behavior has little effect on their environment (external locus of control). Rotter theorized that the differences between the two groups of individuals are caused by variations in their early reinforcement histories.

Since controlled long-term manipulation of reinforcement in humans would be impossible, a systematic investigation with rats appeared to be one way to evaluate the importance of this variable for adult behavior. In view of the fact that open-field "emotionality" is sensitive to early environmental manipulations in rats, this test was used. One of the personality traits that seems to be correlated with differences in perceived locus of control in humans is variation in "anxiety" levels (*8*). Therefore, insofar as "anxiety" in humans is similar to "emotionality" in rats, the test appeared appropriate.

In the present study, two groups of animals that had different degrees of control over their early environments were compared on a behavioral measure—open-field behavior—that is susceptible to many other forms of environmental manipulation. In this test, the "emotional" animals defecate more throughout the test and are less active after the first day (*9*).

Animals in the contingent group were housed in cages in which the delivery of food and water and control of lighting were response-contingent. That is, three levers were available; one controlled the delivery of food (one 45-mg Noyes pellet per press) to an adjacent trough, the second controlled the delivery of water (0.05 ml per press), and the third controlled a house light (*10*), switched on and off by alternate presses. Animals in the noncontingent group lived in chambers that were physically identical to those of the contingent group (*11*) but the delivery of food and water and the operation of the light were controlled by lever presses in one of the contingent group cages. Consequently, animals in the noncontingent group had levers available and received the same presentations of food and water and changes in lighting as did the contingent animals, but did not have control over the occurrence of these events.

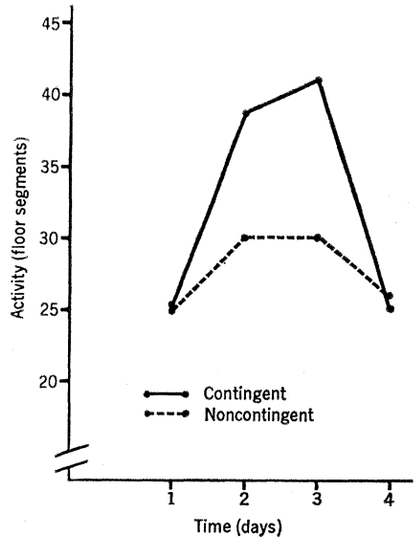

Fig. 1. Activity scores (floor segments entered) during 4 days of open-field testing.

Four chambers were used. Presses on the three levers in chamber 1 produced food, water, or a change in lighting in chambers 1 and 2; lever presses in chamber 4 controlled these conditions in chambers 4 and 3. In other words, chamber 2 was yoked to chamber 1 and chamber 3 was yoked to chamber 4, with control of food, water, and lights available in chambers 1 and 4 only (contingent chambers). Levers were available in chambers 2 and 3 and lever presses in these chambers were recorded, but presses did not affect the delivery of food or water or the lighting (noncontingent chambers).

The chambers were located in a darkened room and were arranged in such a way that lights in one chamber provided very little general room illumination. Electromechanical relay programming and recording equipment were located in an adjacent room. The apparatus was operated continuously from day 1 until the end of adult testing.

At the start of the experiment one pregnant Long-Evans hooded female rat (*12*) was placed in each of the four chambers. The pregnant females in two contingent chambers acquired the lever-pressing response within 2 days and provided adequate amounts of food and water. After the four litters had been delivered 18 to 20 days later, all pups were removed briefly from their mothers, and four males and four females from the pooled litters were assigned randomly to each mother. When the pups were 21 days old, the mothers were removed, the litters were weighed, and each litter was randomly reduced to two male and two female pups. The four males from the two contingent litters were placed in one contingent chamber, and the four females from these litters were placed in the other. The males from the noncontingent litters were placed in the chamber yoked to the contingent males, and the noncontingent females were placed in the chamber yoked to the contingent females. As a result of this rehousing, males were yoked to males and females were yoked to females. The four groups