Autoradiographic Localization of Radioactivity in Rat Brain after Injection of Tritiated Sex Hormones

Abstract. *Radioactivity was found in cell bodies of neurons and glial cells throughout brains of male and female rats that had been injected with either testosterone-H³ or estradiol-H³. Uptake by limbic and hypothalamic structures was higher and longer lasting than that in nonlimbic structures. In all brains, the preoptic area, prepiriform cortex, olfactory tubercle, and septum had particularly high, long-lasting uptake of both hormones.*

Steroid sex hormones affect release of gonadotropic hormones from the pituitary (*1*) and elicit mating behavior (*1, 2*) in many species, including rats. Testosterone is very effective in males, and estradiol is very effective in females. However, testosterone can also affect sexual behavior when injected into female rats (*3*), and estrogens can affect male rats (*4*).

Testosterone and estradiol seem to affect pituitary function and mating behavior by acting on the brain, particularly in the preoptic area and hypothalamus (*1, 2*). In males, the brain takes up testosterone-H³ from the bloodstream (*5*); in females, the brain takes up estradiol-H³ (*6*). These findings were demonstrated by scintillation counting of extracts of homogenized brain tissue from rats given previous intravenous or subcutaneous injections. However, this technique does not afford high spatial resolution; different brain structures, different cell types, and different parts of cells are grouped together. The spatial resolution given by autoradiography is high enough that these problems can be solved, and this method has been useful in the description of hexoestrol-H³ uptake by the brain of the female cat (*7*). I have used autoradiography to determine the uptake of testosterone-H³ and estradiol-H³ in the brains of male and female rats.

Nineteen rats, about 90 days old, were used. All rats were gonadectomized about 2 weeks before the experiment to reduce endogenous amounts of sex hormones. Each rat was anesthetized briefly with ether, and a radioactive hormone dissolved in 0.05 ml of 25 percent ethanol was injected into the femoral vein. Six males and two females were injected with testosterone-1, 2-H³ (150 or 200 μc; specific activity, 46.5 c/mmole) and were killed ½, 2, or 3 hours later. Six females and two males were injected with estradiol-17β-6, 7-H³ (110, 150 or 200 μc; specific activity, 42.4 c/mmole) and were killed ½ or 2 hours later. Brains from three uninjected rats were prepared as above so that the presence of autoradiographic artifacts could be determined.

At the end of the designated period after injection, the rats were decapitated, and the brains were removed quickly and frozen in dry ice. Frozen sections were cut at 8 μm in a cryostat at −15° to −20°C and mounted directly from the microtome knife onto microscope slides, which were then placed immediately on a 60°C hot plate to dry. The mounted sections were stored over calcium chloride in a 60°C oven. The tissue was then fixed by immersion in 1 percent osmium tetroxide for 10 minutes, and then in 10 percent neutral formalin for 10 minutes. After drying overnight in a well-ventilated, 80°C oven, the slides were dipped in Kodak NTB-3 nuclear emulsion, dried in the air, and packed in lightproof boxes with drying agent. After exposure for 9 months at about 5°C, the autoradiograms were developed for 7 minutes in Kodak D19 at 19°C and fixed in Kodak fixer. Alternate autoradiograms were stained in cresyl violet or Mayer's hematoxylin just dark enough so that cell bodies could be located in the sections; prolonged staining often stained or loosened the emulsion.

Reduced grains over cell bodies were counted in 20 regions in each brain. Only those grains in the same plane of focus as the top of the section were counted. For each brain region in each animal, about 100 cells were counted.

Table 1. Distribution of radioactivity over cell bodies in the brains of castrated male and ovariectomized female rats after injection of testosterone-H³ or estradiol-H³. Amount of uptake is expressed in average number of reduced grains per cell with the standard error of the mean.

Brain structures	Testosterone-H³				Estradiol-H³			
	Male		Female		Male		Female	
	½ hour	3 hours	½ hour	2 hours	½ hour	2 hours	½ hour	2 hours
Olfactory bulb								
Granule cell layer	8.1 ± 0.6	8.0 ± 0.9	22.9 ± 1.8	12.0 ± 1.0	14.0 ± 1.2	3.0 ± 1.9	21.4 ± 2.8	9.8 ± 0.9
Mitral cell layer	7.9 ± 1.0	6.1 ± 0.7	15.0 ± 1.1	7.2 ± 1.2	25.1 ± 3.9	8.8 ± 0.9	22.9 ± 2.4	10.5 ± 1.7
Limbic and hypothalamic structures								
Preoptic area	14.9 ± 1.3	8.4 ± 1.0	12.3 ± 1.4	15.5 ± 1.7	9.7 ± 2.1	15.8 ± 1.8	21.6 ± 2.7	20.5 ± 2.6
Prepiriform cortex	8.8 ± 0.8	6.7 ± 0.9	18.0 ± 1.3	15.6 ± 2.3	19.0 ± 1.0	12.7 ± 2.0	22.7 ± 1.7	19.0 ± 3.0
Septum	11.9 ± 0.8	8.3 ± 0.9	7. ± 1.4	11.3 ± 1.6	7.0 ± 0.9	10.0 ± 2.1	21.8 ± 1.6	20.1 ± 2.1
Olfactory tubercle	14.6 ± 1.6	7.5 ± 1.2	12.9 ± 1.6	13.2 ± 2.1	9.8 ± 1.5	12.8 ± 2.0	21.9 ± 2.1	20.2 ± 2.5
Amygdala	12.0 ± 0.8	0.4 ± 0.3	29.4 ± 5.1	7.2 ± 0.9	12.2 ± 2.1	10.2 ± 1.5	20.7 ± 1.6	18.5 ± 2.1
Hippocampus, dentate gyrus	11.3 ± 1,0	0.4 ± 0.2	16.1 ± 1.4	8.4 ± 1.1	8.9 ± 1.7	3.4 ± 1.2	19.8 ± 1.5	17.8 ± 2.4
Hippocampus, Ammon's horn	15.5 ± 0.7	0.5 ± 0.3	18.3 ± 1.3	8.7 ± 1.0	9.5 ± 1.8	3.5 ± 0.9	22.2 ± 1.9	22.5 ± 2.4
Cingulate gyrus	10.0 ± 0.7	2.8 ± 0.9	13.8 ± 2.3	11.6 ± 1.1	21.0 ± 1.4	15.1 ± 2.1	21.3 ± 2.6	20.3 ± 2.0
Ventromedial hypothalamus	13.1 ± 0.9	0.8 ± 0.4	18.5 ± 3.0	8.0 ± 0.9	21.3 ± 2.5	9.8 ± 1.1	26.7 ± 2.2	20.7 ± 2.6
Nonlimbic structures								
Sensory-motor cortex	7.2 ± 0.6	1.0 ± 0.5	15.1 ± 2.9	6.8 ± 1.2	16.3 ± 2.1	5.0 ± 2.3	17.9 ± 3.0	11.0 ± 1.4
Prefrontal cortex	7.8 ± 0.8	3.1 ± 1.2	15.3 ± 2.1	7.7 ± 1.4	8.1 ± 1.9	4.2 ± 1.0	18.7 ± 2.1	13.9 ± 2.3
Cerebellar granule cells	10.8 ± 0.6	0.3 ± 0.2	10.6 ± 1.7	6.1 ± 0.8	4.9 ± 0.5	2.8 ± 0.9	13.1 ± 1.1	3.2 ± 0.9
Caudate	9.1 ± 1.3	2.5 ± 0.7	10.0 ± 1.5	8.6 ± 1.2	4.8 ± 1.1	4.5 ± 0.9	11.8 ± 1.8	9.4 ± 1.1
Ventrobasal thalamus	4.1 ± 0.7	0.1 ± 0.2	6.3 ±0.8	4.9 ± 1.1	7.7 ± 1.2	4.2 ± 1.2	11.8 ± 1.2	7.8 ± 1.3
Superior colliculus	6.2 ± 0.5	0.1 ± 0.2	7.8 ± 1.2	3.9 ± 0.8	8.9 ± 1.3	2.4 ± 1.2	19.9 ± 2.0	9.8 ± 1.5
Superior central nucleus (Bechterew)	6.3 ± 0.9	0.3 ± 0.2	7.5 ± 1.1	4.2 ± 1.1	8.6 ± 1.4	3.8 ± 1.3	20.3 ± 2.8	10.5 ± 1.8
Medial habenula	8.2 ± 0.7	0.7 ± 0.4	7.4 ± 1.3	6.0 ± 1.2	7.9 ± 1.1	4.7 ± 1.0	16.5 ± 1.4	11.4 ± 1.2
Spinal cord	5.9 ± 0.8	0.1 ± 0.1	6.9 ± 0.9	7.9 ± 1.0	10.4 ± 1.6	1.8 ± 1.6	16.0 ± 2.2	5.1 ± 1.0

Care was taken to insure a uniform sampling method in all animals. In many other structures throughout the brain, amount of uptake was estimated qualitatively.

Evidence of both testosterone and estradiol uptake throughout brains from both males and females was seen at short (½ hour) and at longer (2 or 3 hour) times after injection. In all animals injected with radioactive hormone, reduced grains were found regularly over diverse types of neurons and glial cells throughout the brain. No evidence was seen for exclusive uptake by or absence of uptake from any particular type of nerve cell or glial cell. Almost all grain reduction was located over the cell body. No concentrations of reduced grains were seen in the brains of the control animals.

One-half hour after injection of radioactive hormone, the average number of reduced grains over cell bodies in most limbic and hypothalamic structures was greater than that in most nonlimbic structures. For testosterone, the ratio of the number of reduced grains over cell bodies in limbic and hypothalamic structures to that in nonlimbic structures was 1.75 in male brains and 1.73 in female brains. For estradiol, the ratio was 1.42 in male brains and 1.37 in female brains. Uptake was highest throughout the brains of females injected with estradiol.

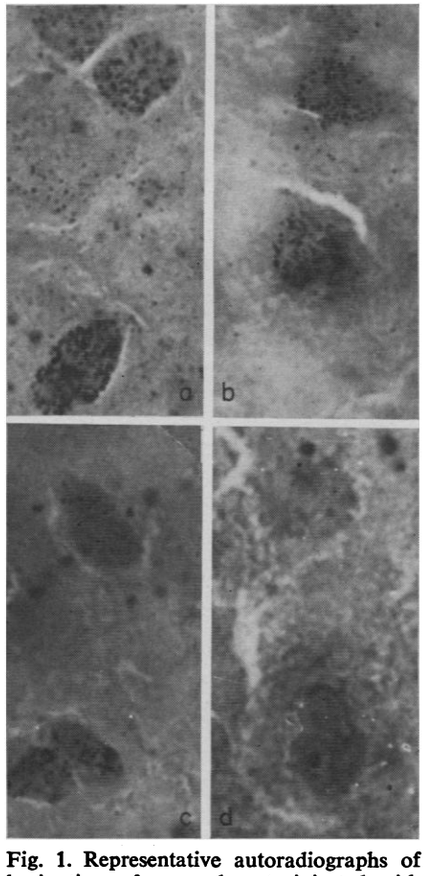

Fig. 1. Representative autoradiographs of brain tissue from male rats injected with testosterone-H^3. Uptake by cells in the preoptic area is high ½ and 3 hours after injection; cortical cells show very little uptake 3 hours after injection. Cell bodies are lightly stained with cresyl violet: (a) three preoptic area cells ½ hour after injection (×1300); (b) two cortical cells ½ hour after injection (×1200); (c) three preoptic area cells 3 hours after injection (×1300); (d) two cortical cells 3 hours after injection, showing virtually no grain reduction (×1300).

Radioactive testosterone and estradiol disappeared at a faster rate from nonlimbic structures than from limbic and hypothalamic structures (Fig. 1). By 2 or 3 hours after injection, the ratio of limbic and hypothalamic uptake to nonlimbic uptake had increased from the value at ½ hour. For testosterone, the ratio was 4.15 in male brains and 1.81 in female brains. For estradiol, the ratio was 3.00 in male brains and 2.03 in female brains. This difference between limbic and hypothalamic, and nonlimbic uptake was statistically significant in all four groups of animals.

Among limbic and hypothalamic structures 2 or 3 hours after injection, uptake of testosterone and estradiol in both male and female brains was highest in anteriorly placed structures in the ventral forebrain, such as the preoptic area, prepiriform cortex, septum, and olfactory tubercle (Table 1). The pattern of long-lasting (2 or 3 hour) uptake among other limbic and hypothalamic structures and in the olfactory bulb depended on the hormone injected and the sex of the recipient rat. Estradiol uptake in female brains was relatively high in posteriorly placed structures such as the hippocampus, ventromedial hypothalamus, and amygdala, but was low in the olfactory bulb compared to limbic and hypothalamic uptake. Testosterone uptake in male brains was low in posterior limbic structures, but was as high in the olfactory bulb as in anterior limbic structures. That is, the ratio of uptake in the olfactory bulb to that in posterior limbic structures was high for testosterone in males and low for estradiol in females. It was intermediate for testosterone in females and for estradiol in males.

A major problem in the preparation of autoradiograms is diffusion of the radioactive chemical from its original site of uptake. In this experiment, fixation of tissue with osmium tetroxide and formalin was used to retain radioactive testosterone in the tissue sections. There was apparently little or no diffusion, for the ratio of the number of grains concentrated over cell bodies to the numbers spread between cell bodies was very high in all brain structures examined (Fig. 1). The method of fixation did not cause artifactual grain reduction, for the autoradiograms prepared from the brain sections of the uninjected control animal were free of grain accumulations. Finally, the fixation technique did not lead to excess variability in measurements of uptake, for the standard errors of the means in Table 1 are acceptably low.

Thus, anteriorly placed limbic and hypothalamic structures in the ventral forebrain took up and retained either sex hormone in brains from either sex of rat. The time course of uptake in these structures resembled that of estradiol uptake in target organs such as the uterus (*8*). High uptake in these structures may be specific for sex steroids, for the uptake of cholesterol (*9*), certain drugs (*10*), and norepinephrine (*11*), by these structures is not particularly high. The type of physiological effects of sex hormones on these structures may differ from the type of effects elsewhere in the brain. However, the functional significance of uptake of sex steroids in these structures must be investigated with other experimental techniques.

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References and Notes

1. G. W. Harris, in *The Pituitary Gland*, G. W. Harris and B. T. Donovan, Eds. (Univ. of California Press, Berkeley, 1966), vol. 2, p. 99; B. T. Donovan, *ibid*, p. 49.
2. F. A. Beach, *Hormones and Behavior* (Hoeber, Harper, New York, 1948); C. H. Sawyer, in *Handbook of Physiology*, J. Field, Ed. (American Physiological Society, Washington, D.C., 1960), vol. 2, p. 1225; W. C. Young, in *Sex and Internal Secretions*, W. C. Young, Ed. (Williams and Wilkins, Baltimore, 1961), vol. 2, p. 1173.
3. F. A. Beach, *Endocrinology* **31**, 673 (1942); J. Ball, *J. Comp. Psychol.* **29**, 151 (1940).
4. F. A. Beach, *Endocrinology* **31**, 679 (1942); J. Ball. *J. Comp. Psychol.* **28**, 273 (1939).
5. J. Resko, R. Goy, C. Phoenix, *Endocrinology* **80**, 490 (1967).
6. A. Eisenfeld and J. Axelrod, *J. Pharmacol. Exp. Therap.* **150**, 469 (1965); J. Kato and C. Villee, *Endocrinology* **80**, 567 (1967).
7. R. Michael, *Brit. Med. Bull.* **21**, 87 (1965).
8. E. Jensen and J. Jacobson, *Rec. Progr. Hormone Res.* **18**, 387 (1962).
9. F. Chevallier and L. Petit, *Exp. Neurol.* **16**, 250 (1966).
10. L. Roth and C. Barlow, *Science* **134**, 22 (1961).
11. M. Reivich and J. Glowinski, *Brain* **90**, 629 (1967).
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