Estradiol Concentrating Neurons in the Amygdala[1] (35204)

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Evidence has been provided that the amygdala, or certain areas of it, plays an important role in the regulation or modification of sex behavior (1–4) and gonadal function (5–12) in different species. That estrogen levels in the blood affect amygdaloid structures is suggested by local EEG changes during estrus (13) and by the fact that estrogen implantation in the amygdaloid region led to a lactogenic response (14) and inhibition of estrogen synthesis (13). The implication of amygdaloid structures in estrogen-neuron systems (15) can be more directly demonstrated and topographically defined by autoradiography with ^{3}H-estradiol. The technique of dry-mount autoradiography has been applied to provide a precise anatomical definition of estradiol "feedback" areas of the hypothalamus (16). This information was hitherto unobtainable using lesion, electrical stimulation, or implantation techniques. Since the topography of estradiol concentrating neurons in the preoptic region and hypothalamus corresponds to the terminations of stria terminalis fibers (16), estrogen concentrating neurons could be expected in the amygdala (17) at the sites of origin of this fiber tract.

Materials and Methods. This report is based upon observations in nine rats. Four 24-day-old immature, intact female and male, three mature ovariectomized (48 hr prior to experiment) and two intact mature male Sprague-Dawley rats were injected subcutaneously with a dose of 0.1 to 0.2 μg/100 g of body weight, of 6,7-^{3}H-estradiol-17β, (sp act, 208 μCi/μg) dissolved in isotonic saline, and sacrificed 1 hr afterwards. The low dose of 0.1 μg of ^{3}H-estradiol was selected in order to remain in a physiologic range; this however, required photographic exposure times of the autoradiograms between 6 and 8 months. The exposure times required in order to demonstrate concentration of radioactivity in neurons of the amygdala are the same as for neurons of the preoptic and basal tuberal region of the hypothalamus. In comparable radioassay studies radioactivity has been reported to be retained and concentrated in the hypothalamus and in the amygdala and has been identified as ^{3}H-estradiol-17β in the hypothalamus (18). This, in connection with the similarity in exposure times, strongly suggest that the silver grains over the nuclei of neurons of the amygdala represent estradiol. The autoradiograms were prepared at 1 hr after the injection of ^{3}H-estradiol since maximal uptake exists in target tissues at this time. Two to 3 mm^3 pieces of brain containing amygdala were excised, placed on a tissue holder, and frozen in liquified propane. Two micron serial frozen sections were cut in a cryostat (Harris Mfg. Co., Cambridge, Mass.) and freeze-dried (Thermovac Industries Corp., Copiague, N.Y.). The freeze-dried, unfixed and unembedded sections were dry-mounted on photographic emulsion coated slides for autoradiographic exposure. The dry-mount technique, which excludes the use of solvents and therefore avoids known sources of diffusion artifacts, has been described in detail (15).

Results. In the amygdala of all of the animals studied, similar patterns of distribution of ^{3}H-estradiol concentrating neurons (estrogen-neurons) existed. No qualitative differences could be recognized between female and male rats. Radioactivity was found to be concentrated in nuclei of certain neurons (Fig. 1), not in others. Relatively few silver grains were found in the cytoplasm of the labeled neurons and in the neuropil. Glia cells and ependyma cells were not labeled.

[1] This study was supported by PHS Grant AM-12,649.

The topography of estrogen-neurons in the amygdala is depicted in the schematic drawings (Fig. 2 a–h). In the anterior area of the amygdala very few labeled neurons were found. The nucleus (n.) of the lateral olfactory tract was unlabeled. In serial coronal sections from frontal to caudal the first concentrations of radioactively labeled neurons appeared in a ventral portion of the n. corticalis, in the pars anterior of the n. lateralis and in the n. centralis. In the n. centralis, labeled neurons were scattered among unlabeled neurons and the labeling index was low when compared with the heavily labeled n. medialis and n. corticalis. As can be seen from the schematic drawings, the n. centralis, n. medialis, and n. corticalis are not labeled throughout their entire course. This suggests functional inhomogeneity of what has been characterized by descriptive neuroanatomists as an entity. A similar observation had been made for the n. paraventricularis magnocellularis and the n. ventromedialis hypothalami (15, 16).

The most intense neuronal labeling was observed in the n. medialis, the ventrolateral n. corticalis, including the zona transitionalis, and the n. basalis anterior. The neurons of the nucleus corticalis parvocellularis, described by Uchida (19), could be identified and were unlabeled. In the central amygdala it appeared that the labeled neurons are continuous throughout different nuclei, including neurons of the n. medialis, n. corticalis, and n. basalis medialis, being interrupted only by stria terminalis fibers and unlabeled cells of the massa intercalata. The massa intercalata, although unlabeled, was surrounded by labeled neurons. In the piriform cortex (area 51 of Krieg), in the claustrum and in the putamen, no radioactively labeled neurons were found except for the rare observation of single cells in internal layers of the piriform cortex. Scattered labeled neurons existed in the subiculum.

The neurons of the dorsal hippocampus were unlabeled with the exception of a few isolated labeled neurons. In the ventral hippocampus, in the vicinity of the lateral ventricle and the amygdala, an accumulation of weakly labeled neurons has been observed. The concentration of radioactivity in nuclei of these neurons is on the average about 10 to 15 times less than in labeled neurons of the amygdala or hypothalamus. A precise definition of the radioactively labeled structures in the hippocampus as well as the elucidation of the presumed differences in binding affinity require further investigation.

Discussion. The topography of estrogen-neurons in the amygdala encompasses areas which seem to be connected with the origin of the stria terminalis and, perhaps, the ventral amygdalofugal pathway (20, 21). A more detailed correlation between fiber tracts and estrogen-neurons is, however, at present not possible since the precise sites of origin of these fiber tracts are still debated. The results from microlesion experiments in conjunction with sensitive degeneration studies, will have to be awaited. The autoradiographic experiments demonstrate estrogen-neurons at both the origin as well as the termination of stria terminalis fibers and, most likely, of fibers of the ventral amygdalofugal pathway. This suggests the existence of estrogen-neuron systems (15)—both circuitous and open—involving certain amygdaloid nuclei, preoptic nuclei, and hypothalamic nuclei. An additional estrogen-neuron system may exist combining the ventral hippocampus—subiculum at the one end and the nucleus arcuatus and nucleus periventricularis at the other end, linked by the medial corticohypothalamic tract (20). The localization of estrogen concentrating neurons in the hippocampal regions supports previous physiologic data which suggested involvement of the hippocampus in the regulation of gonadotropin secretion (22–24).

The localization of estrogen concentrating neurons in the diencephalon and amygdala seem to make up a major portion of the areas of the brain which are involved in the regulation of sex behavior and gonadotropin secretion (1–14, 25). Thus, in view of our findings and the cited evidence in the literature, the concept of "sex center" or "sex centers" seems no longer satisfactory (15).

The subcellular nuclear concentration of ^{3}H-estradiol may be attributable to uptake into receptors or into binding sites in the immediate vicinity of receptors. This nuclear uptake may reflect a genomic effect of estra-

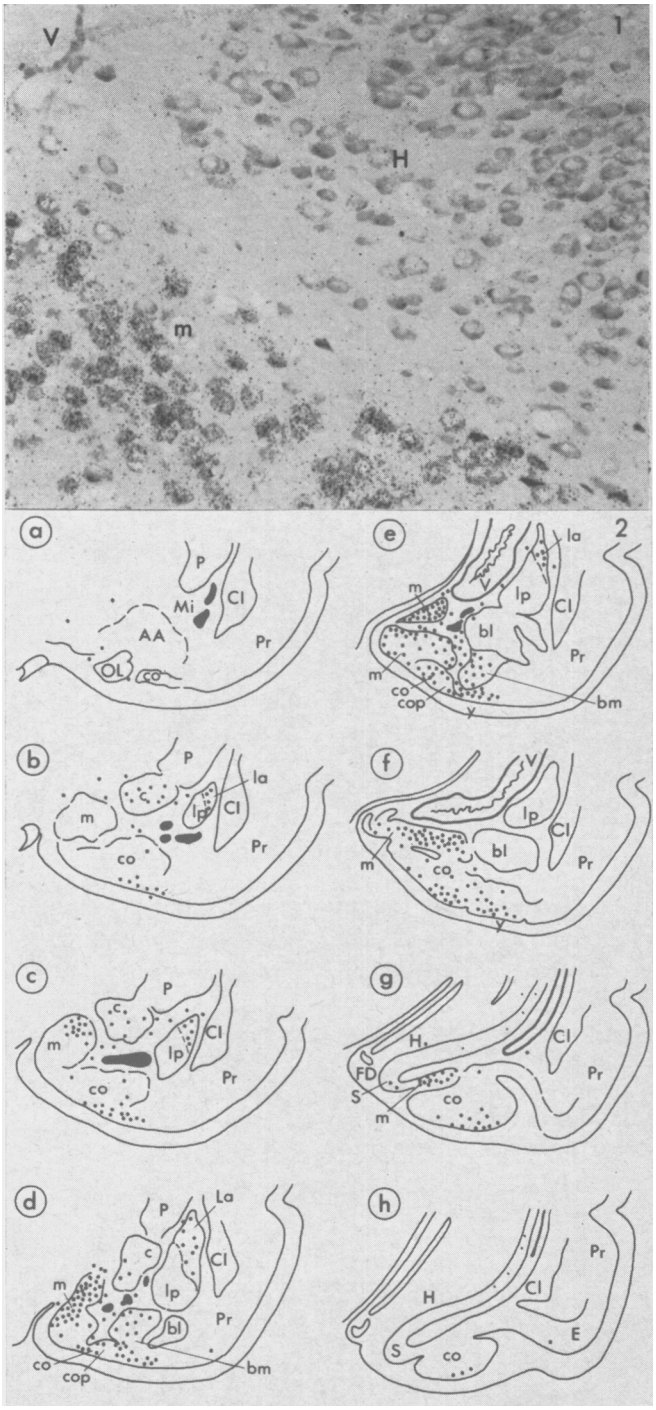

FIG. 1. Dry-mount autoradiogram showing the amygdaloid-hippocampal contact zone and the lateral ventricle (V) with heavy radioactive labeling of neurons of the nucleus medialis of the amygdala (m), while the neurons of the hippocampus (H) are relatively devoid of radioactivity. One hr after the injection of ^{3}H-estradiol. Two μ freeze-dried, unfixed, unembedded sagittal section. Exposure time 199 days. Stained with methylgreen pyronin. 200×.

FIG. 2a-h. Schematic demonstration of the localization of estrogen-neurons in the amygdala of immature intact female, male and mature ovariectomized rats prepared after the distribution of radioactivity in frontocaudal serial section dry-mount autoradiograms at 1 hr after the injection of 0.1 μg of 6,7-^{3}H-estradiol-17β/100 g of body weight. The intensity of the stippling represents the respective accumulation of estradiol concentrating neurons. Cells of the massa intercalata were unlabeled and are indicated in black. The schematic drawings were prepared according to the description of the amygdala by Brodal (27). AA, area amygdala anterior; bl, n. amygdaloideus basalis, pars lateralis; bm, n. amygdaloideus basalis, pars medialis; c, n. amygdaloideus centralis; CL, clautrum; co, n. amygdaloideus corticalis; cop, n. corticalis parvocellularis; E, entorhinal area, area 28; FD, fascia dentata; H, hippocampus; la, n. amygdaloideus lateralis, pars anterior; lp, n. amygdaloideus lateralis, pars posterior; m, n. amygdaloideus medialis; MI, massa intercalata; Pr piriform cortex, area 51; S, subiculum; V, ventriculus lateralis; Y, zona transitionalis.

diol in these labeled neurons with stimulation of RNA and protein synthesis, similar to peripheral target tissues. Fluctuations of RNA concentration in different brain areas depending on the state of the estrous cycle have been described (11) and actinomycin D has been shown to nullify the suppressive effect of estrogen on the plasma LH surge in ovariectomized animals (26). The autoradiographic data in conjunction with evidence from lesion and hormone implant experiments suggest that the estrogen-neurons are sites for the production of hormonal messenger, probably releasing or inhibiting factors relevant to gonadal (5–13, 25) and mammary function (14) as well as sex behavior (1–4). It is noteworthy that the concentrations of estrogen-neurons are in close proximity to ventrally located portions of the ventricles. This encourages reinvestigation of the question of whether or not neuronal secretion occurs into the ventricular lumen and whether or not the cerebrospinal fluid is involved in the transport of hormonal messengers. While the physiologic significance of the autoradiographic findings remains to be further elucidated, the detailed anatomical definition of the topography of estrogen-neurons will provide a basis for better defined biochemical, electrophysiological, and behavioral studies.

Summary. After injection of a physiologic dose of ^{3}H-estradiol into female and male rats, radioactive labeling was found in certain neurons of the amygdala, while not in others. Labeled neurons were concentrated in the nucleus medialis; nucleus centralis; nucleus lateralis, pars anterior; nucleus basalis, pars medialis; and nucleus corticalis. In the ventroanterior portion of the hippocampus, close to the lateral ventricle, weakly labeled neurons were found. The stria terminalis, the ventral amygdalofugal pathway, and the medial corticohypothalamic tract are considered interlinking pathways for estrogen-neuron systems in the amygdala and diencephalon. Estrogen-neuron systems seem to constitute a major component of what has been regarded the "sex center" or "sex centers" in the brain.

We thank Christiane Baerwaldt for technical assistance.

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Received Sept. 2, 1970. P.S.E.B.M., 1971, Vol. 136.