Estrogen-Topographical Localization of Estrogen-Concentrating Cells in the Rat Spinal Cord Following ^{3}H-Estradiol Administration[1] (37333)

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It has been well established by lesion and hormone-implant studies that specific neuronal groups in the brain control reproductive functions and, in turn, can respond to changing levels of sex hormones (1). These areas largely correspond to the topographical localization of estrogen-concentrating neurons (2).

While there is accumulating evidence that some sexual responses are conducted at the spinal level there is but little evidence that sex hormones mediate responses at this level (3, 4, 12). This report is the first to show that neurons capable of concentrating radioactivity after ^{3}H-estradiol injection are distributed in the spinal cord in a specific topographical arrangement.

Materials and Methods. A total of five female and two male Sprague–Dawley rats, weighing 210–270 g, were injected subcutaneously with 6,7-^{3}H-estradiol-17β, sp act 208 μCi/μg. Six of the animals received 0.1 μg and one male received 1.0 μg per 100 g body weight dissolved in isotonic saline. Four female and two male rats were castrated 3–21 days before the experiment to lower endogenous estrogens. Two female and one male were decapitated 60 min after injection, three females 30 min after and one male 15 min after injection.

In addition, four short-term castrated female rats were administered 0.4 μg ^{3}H-estradiol in a competition experiment. Two received 4.0 μg unlabeled estradiol-17β intravenously 5 min before the ^{3}H-estradiol. The remaining two received 4.0 μg unlabeled corticosterone in the same manner. All four were decapitated 30 min after administration of the label.

Segments of spinal cord 2–3 mm long were excised from the cervical, thoracic and/or lumbar levels, placed on tissue holders, frozen in liquid propane at —150 to —180° and stored in liquid nitrogen. The tissues were cut in 2–3 μm thick sections in a cryostat at —40° and freeze-dried in a cryo-pump. The freeze-dried, unfixed and unembedded

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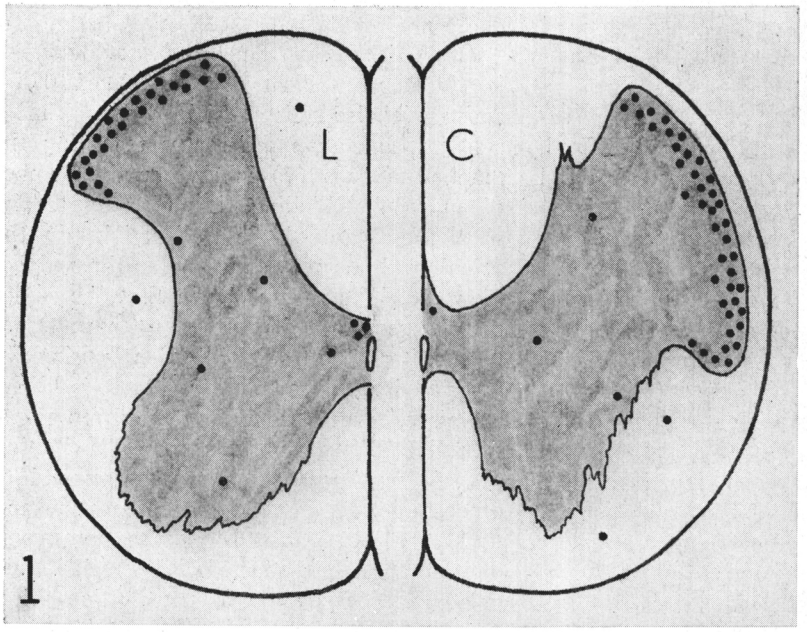
FIG. 1. Schematic diagram of rat lumbar (left) and upper cervical (right) spinal cord. Dots represent the relative number of radioactively labeled cells seen in autoradiograms after ^{3}H-estradiol injection.

FIGS. 2–5. Represent autoradiograms of rat spinal cord of 2–3 μm freeze-dried sections stained with methylgreen-pyronin. Exposure time 375 days. Fig. 2. Cervical dorsal horn showing dorsolateral fasciculus (d), labeled cell nuclei in laminae I and II and a portion of lamina III. ×1300. Fig. 3. Labeled cells in dorsal portion of lumbar lamina X. This accumulation is half way between the central canal and dorsal fasciculus. ×1150. Fig. 4. Labeled cell in cervical lateral fasciculus proprius. Ventral horn is toward bottom of picture. Note the perinucleolar ring of silver grains with the nucleolus devoid of label. ×1480. Fig. 5. Labeled cell with nuclear concentration of radioactivity in lumbar lamina II. ×2050.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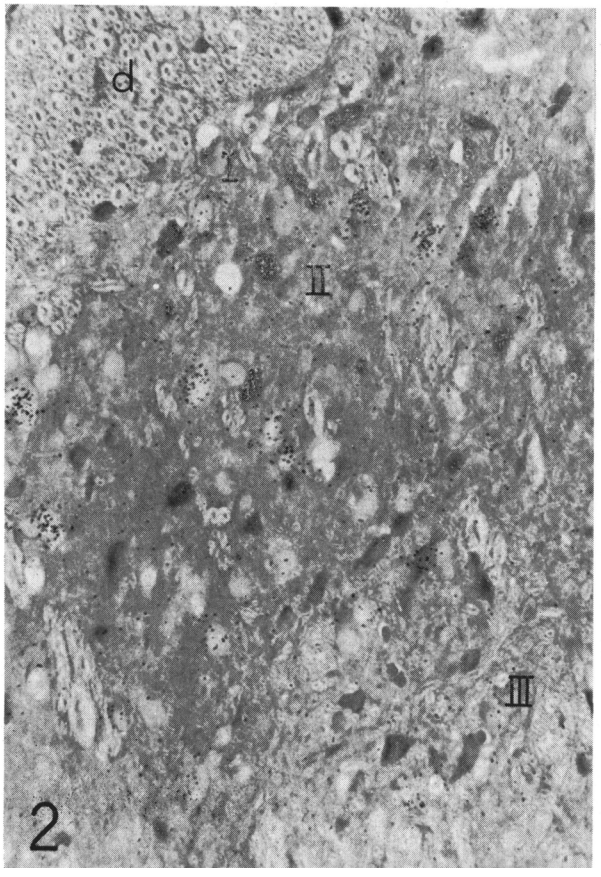
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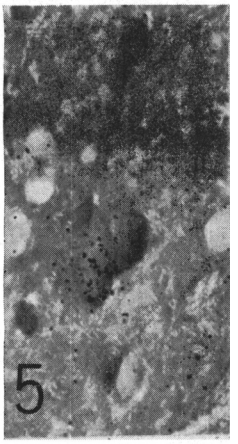
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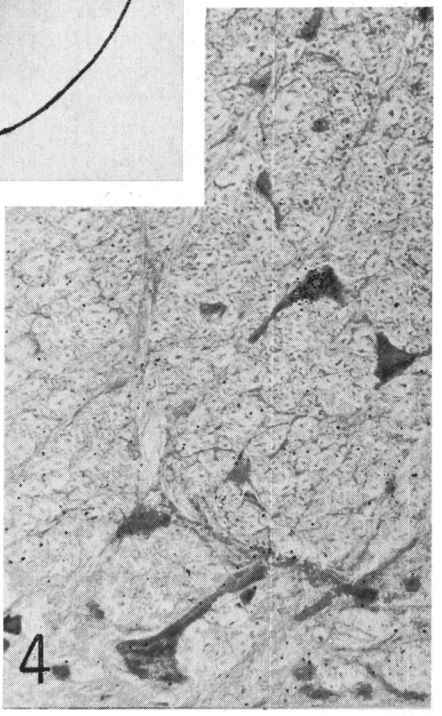
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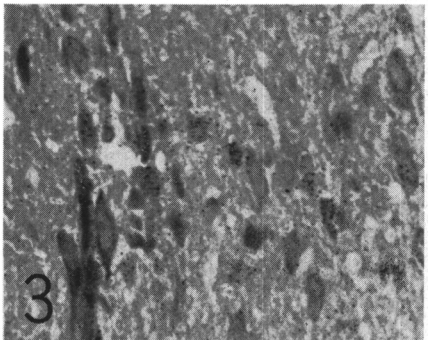
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sections were then mounted on desiccated photographic emulsion-coated slides and stored in light-proof desiccator boxes at —15° for exposure. After 3–12 months exposure, the slides were developed, fixed, stained with methyl green-pyronin, air dried and mounted with a cover glass (5).

Results. Silver grains are concentrated over nuclei of spinal cord cells with selective distribution (Fig. 1). The greatest density of labeled cells exists in the distal region of the dorsal horn (Fig. 2) corresponding to laminae I and II as described by Rexed (6). Under these conditions, no consistent morphological differences are apparent between labeled and unlabeled cells within lamina I and II (Fig. 5). Approximately one-fourth of this cell population was labeled and the mean nuclear diameters of labeled and unlabeled cells were 5.57 ± 1.29 μm and 5.35 ± 2.31 μm, respectively.

A second concentration of labeled cells appears in the dorsal part of the substantia grisea centralis, corresponding to the dorsal portion of lamina X, with a few labeled cells existing also in its lateral part (Fig. 3). The density of labeled cells here is most pronounced in the lumbar region, being comparable to that of lamina I and II. Thoracic and cervical segments show only occasional labeled cells in dorsal lamina X. Ependymal cells of the central canal do not show labeling. In addition to these two accumulations of labeled cells in lamina I, II, and X, occasional labeled cells are seen throughout the remaining lamina and the white matter. The giant ventral horn cells are unlabeled. Single labeled cells in the lateral fasciculus proprius appear to be associated with fiber projections from the gray matter (Fig. 4). Some small labeled cells are seen especially in the cervical dorsal funiculus, and occasional small cells are noted within the dorsal median septum. No qualitative topographical differences are apparent among the rats injected 15, 30, and 60 min before sacrifice or between male and female rats.

Preadministration of cold estradiol-17β prevents the nuclear concentration of label. However, competition with cold corticosterone has no apparent effect on the nuclear uptake pattern.

Discussion. Even though no sex differences in the topographical distribution of radioactively labeled cells could be observed, more subtle quantitative differences regarding the number of cells (7) labeled or the number of subcellular binding sites may exist. The chemical nature of the radioactivity in the spinal cord has not, as yet, been determined. However, it has been shown that the radioactivity seen in cell nuclei of estrogen target tissue after ^{3}H-estradiol injection is primarily associated with estradiol (8, 9). It is likely, therefore, than the radioactivity represents estradiol since the subcellular distribution as well as times of exposure correspond to other target tissues studied in the same and similarly treated animals. Furthermore, competition of the injected label with cold estradiol blocked nuclear localization while cold corticosterone did not. This again attests to the estrogenic nature of the localized product in the spinal cord.

Estrogen uptake in the rat central nervous system, including the spinal cord, has been previously described with labeled cells existing throughout the central nervous system (10), but with no mention, however, of specific topographical arrangement in the spinal cord. This observation that nerve cells and glial cells throughout the brain as well as ependymal cells concentrated the radiochemical, could not be confirmed by other investigators but could be attributed to technique-linked artifacts (11).

The distinct topographic pattern of these radioactively labeled cells may help to elucidate the hitherto obscure roles of laminae I, II, and X. The change in topographic pattern as the spinal cord merges with the medulla is, as yet, undescribed although an earlier report showed "estrogen neurons" in the nucleus commissuralis tractus solitarii and the myelencephalic substantia grisea centralis, pars lateralis (2).

Functionally, it has been shown that major components of the lordosis and ejaculatory responses are organized within the spinal cord (3). Currently, it is thought that sex hormones act primarily upon specific areas of the brain to modulate their inhibition of

the spinal organization. However, Hart and Haugen (4) have shown that testosterone acts at the spinal level to modulate sexual reflexes in the male rat and estradiol appears to have a comparable effect in the female dog (12). If such evidence is forthcoming for estradiol, the cells described in this report may represent those target cells.

Summary. After ^{3}H-estradiol injection, radioactivity was found to concentrate in certain cells in the rat spinal cord. The topography was determined by dry-mount autoradiography. The greatest density at all levels occurred in laminae I and II of the dorsal horn. In the lumbar cord, a second group of labeled cells appeared in the dorsal portion of lamina X. Occasional scattered labeled cells appeared throughout the remaining gray and white matter. It is suggested that the radioactivity-concentrating cells are estradiol target sites involved in sex-related sensation and reflexes.

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