GENERAL AND COMPARATIVE ENDOCRINOLOGY 33, 496–505 (1977)

Autoradiographic Localization of Sex Steroid-Concentrating Cells in the Brain of the Teleost *Macropodus opercularis* (Osteichthyes: Belontiidae)

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Accepted July 5, 1977

Tritiated estradiol or testosterone was administered to gonadectomized male paradise fish, *Macropodus opercularis*, to investigate the neuroanatomical location of sex steroid-retaining cells. Each male was sacrificed 2 hr following intraperitoneal injection of the labeled hormone. Autoradiograms were prepared, and sections were taken from the entire brain, the anterior spinal cord, and the pituitary. Following 4 to 12 months of exposure, the distribution of labeled cells was seen to be the same for males which had received the estradiol as those which received testosterone, but estradiol resulted in a greater number of labeled cells. Steroid-concentrating cells were located in the ventral telencephalon, preoptic area, lateral tuberal nucleus, nucleus of the lateral recess of the third ventricle, and caudal portion of the posterior periventricular nucleus. In addition, the caudal pars distalis of the pituitary contained many labeled cells. No steroid-retaining cells were seen in the mesencephalon, rhombencephalon, or anterior spinal cord.

Brain mechanisms underlying reproductive behavior and neuroendocrine processes are poorly understood in fishes. Gonadal steroids are widely known to participate in the regulation of some aspects of reproduction in fishes (Hoar, 1969; Liley, 1969). Biochemical and histological experiments reveal that the estrogen and androgen levels in the gonad and other organs vary with the reproductive season (Hoar, 1969; Schreck and Hopwood, 1974). Sex steroids influence the development of external morphological secondary sex characteristics which occurs at puberty and, in some species, prior to the breeding season (Yamamoto, 1969; Liley, 1969). Androgen-dependent, or male-positive, secondary sex characteristics predominate in fishes but estrogen-dependent, female-positive, characteristics also occur. Relatively little information exists, however, on the role of sex hormones in the regulation of reproductive behaviors, or of the mode of action of sex hormones on the brain and pituitary gland. Castration results in decreased sexual behavior in some species, but in others sexual response is manifestly unaffected (Liley, 1969). Administration of methyl testosterone restores sexual behavior in castrated male sticklebacks (Wai and Hoar, 1963) and blue gouramis (Johns and Liley, 1970).

Investigations of the brain mechanisms of reproductive behavior in fishes are increasing. Schreck (1973) demonstrated uptake and retention of ^{3}H-labeled testosterone in brown trout brain and pituitary suggesting that steroid-concentrating cells are present in these structures. Additional, detailed information on the neuroanatomical location of estrogen- and androgen-concentrating cells is needed for neuroendocrine and be-

ISSN 0016-6480

havioral investigations. The purpose of the present autoradiographic study in *Macropodus* was to examine the entire brain, anterior spinal cord, and pituitary of a teleost for cells which concentrate hormone after injection of [^{3}H]-estradiol or [^{3}H]-testosterone. The results of this investigation have previously been reported in preliminary form (Morrell *et al.*, 1976; Davis *et al.*, 1976).

METHODS

Ten adult male *Macropodus opercularis* (L.), weighing from 3.6 to 5.7 g, were gonadectomized 1 week prior to the experiment. Each male was anesthetized in a 0.4% solution of ethyl *m*-aminobenzoate methanesulfonate (Finquel, Ayerst) prior to aspiration of the testes through a vertical incision in the posterior abdominal wall. The wound was closed with one or two sutures which were removed several days later. The males resumed feeding within 24 hr and remained healthy.

Five of the males were administered 4 μCi/g body weight of 17β-[2,4,6,7-H^3] estradiol, specific activity 91 Ci/mmol, in isotonic saline containing 15% ethanol by intraperitoneal injection. Another five males received 4 μCi/g of [1, 2, 6, 7-H^3] testosterone, specific activity 85 Ci/mmol. The steroids were obtained from New England Nuclear Corp. several days prior to the experiment. The injection was made with a 27-gauge needle on a 50-μl Hamilton syringe. Two hours following the injection, the male was sacrificed by brief immersion in ice water and kept on crushed ice while the brain was removed. The brain was blocked for horizontal or transverse sectioning and quickly frozen onto a cryostat specimen holder in powdered dry ice (Pfaff and Keiner, 1973). Control sections to test for negative or positive chemography were similarly prepared from brain of additional castrated males which had received no hormone (Morrell and Pfaff, 1977).

Sectioning of the brain was carried out in a darkroom under a single safelight as previously described (Anderson and Greenwald, 1969; Pfaff and Keiner, 1973; Morrell and Pfaff, 1977). Serial frozen sections were cut 6 μm thick in a Harris Equipment Co. cryostat, Model CTD, at $-19°$. The sections were lifted from the microtome blade with a microscope slide that had been previously coated with Kodak NTB-3 emulsion, dried, and stored at room temperature. Every 18 to 30μm, a section of an individual block of brain tissue was picked up on an emulsion-coated slide. Slides were kept in dry, light-tight boxes at 4° for 4, 6, 9, and 12 months prior to development. The slides were developed, fixed, and stained with cresyl violet acetate (Pfaff and Keiner, 1973).

The brain sections were systematically scanned with a light microscope to locate labeled cells. A cell was designated labeled when the concentration of reduced silver grains over a clearly visible cell body was five times the reduced grains over a similar, cell-sized area of the adjacent neuropil. Every section containing labeled cells was drawn with the aid of a microprojector, in sufficient detail to identify the anatomical location in the brain. Atlases of the brain of the goldfish, *Carassius auratus* (Peter and Gill, 1975), and the killifish, *Fundulus heteroclitus* (Peter *et al.*, 1975), were used for the nomenclature and as neuroanatomical references.

The control slides revealed no indication of either positive or negative chemography. No fading of the latent image occurred in emulsion which had been fully exposed to light prior to being stored for 4 to 12 months. Brain sections from the uninjected control fish did not result in reduced silver grains in the emulsion.

RESULTS

Labeled cells were found in the ventral telencephalon, peroptic area, hypothalamus, and pituitary. Systematic inspection of every section from other brain areas, including the rostral spinal cord, medulla, cerebellum, optic tectum, thalamus, and dorsal telencephalon revealed no cell labeling. The number of labeled cells was greatest in the males that had received [^{3}H]estradiol, but the locations of the labeled cells were similar for both hormones. Representative sections from the brains of two males which had received [^{3}H]estradiol were selected to illustrate the anatomical locations of the labeled cells. The planes of the selected sections are shown in Fig. 1.

Ventral telencephalon. Labeled cells occurred in the area ventralis telencephali pars ventralis (Vv),[1] with most cells in its

[1]Abbreviations used: AC, anterior commissure; CB, cerebellum; CM, corpus mammillare; Dc, area dorsalis telencephali pars centralis; Dd, area dorsalis telencephali pars dorsalis; Dl, area dorsalis telencephali pars lateralis; Dm, area dorsalis telencephali pars medialis; HOC, horizontal commissure; IL, inferior lobe of the hypothalamus; ML, medial lobe of the hypothalamus; NAT, nucleus anterior tuberis; NDLI, nucleus diffusus lobi inferioris; NDTL, nucleus diffusus tori lateralis; NE, nucleus entopeduncularis;

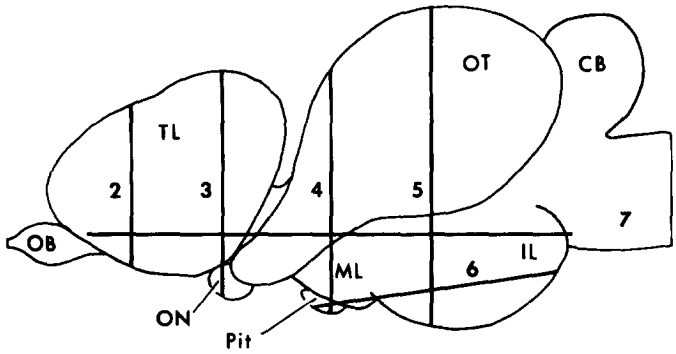

FIG. 1. The brain of *Macropodus opercularis* shown in side view to illustrate the planes of the sections which are presented in Fig. 2 through 7.

dorsal portion (Figs. 2 and 7). Area Vv consists of relatively small, closely packed cells located rostral to the anterior commissure and adjacent to the ventricle. A grooved ependymal thickening at the level of the dorsal boundary of Vv extends dorsocaudally from the rostral pole of the telencephalon and terminates above the anterior commissure. Estradiol- and testosterone-retaining cells were similarly distributed in the Vv. Estradiol cells were more numerous and generally more intensely labeled than testosterone-labeled cells. Area Vv is replaced posteriorly, over the anterior commissure, by a small area, ventralis telencephali pars supracommissuralis (Vs). Dorsal to Vv, the area ventralis telencephali pars dorsalis (Vd) extends the full anterior–posterior extent of Vv. No labeled cells were seen in Vs or Vd, or elsewhere in the area ventralis or area dorsalis of the telencephalon.

Preoptic area. The most rostral portion of the preoptic nucleus, consisting of the nucleus peropticus periventricularis (NPP) and, in addition, the nucleus preopticus pars parvocellularis (NPOp), contained many estradiol- and testosterone-concentrating cells (Figs. 3 and 7). Labeled cells occurred throughout NPP, which in *Macropodus* is located ventral and anterior to NPOp, and in the ventral, anterior region of NPOp. The great majority of the labeled cells in the NPO are small cells, although occasionally larger labeled cells, possibly in the NPO pars magnocellularis, are seen dorsally and just posterior to the region illustrated in Fig.3.

Nucleus lateral tuberis. In the medial lobe of the hypothalamus, a high concentration of labeled cells was identified cells in the nucleus lateral tuberis, pars posterior (NLTp), and pars inferior (NLTi) (Figs. 4–6). The caudal part of nucleus lateral tuberis pars anterior (NLTa) contained a few labeled cells, in the area where it merges with pars posterior which contained many labeled cells. In the plane of the transverse section shown in Fig. 5, NLTi is continuous with the heavily labeled ventromedial portion of NRL.

Nucleus recessus lateralis. Labeled cells were seen in the nucleus recessus lateralis (NRL) following administration of [^{3}H]-

NG, nucleus glomerulosus; NH, neurohypophysis; NLTa, nucleus lateral tuberis pars anterior; NLTi, nucleus lateral tuberis pars inferioris; NLTp, nucleus lateral tuberis pars posterioris; NPG, nucleus preglomerulosus; NPOp, nucleus preopticus pars parvocellularis; NPP, nucleus preopticus periventricularis; NPPv, nucleus posterioris periventricularis; NRL, nucleus recessus lateralis; NRP, nucleus recessus posterioris; NVM, nucleus ventromedialis thalami; OB, olfactory bulb; OC, optic chiasm; ON, optic nerve; OT, optic tectum; Pit, pituitary; PD, pars distalis; TL, telencephalon; Vd, area ventralis telencephali pars dorsalis; Vp, area ventralis telencephali pars postcommissuralis; Vv, area ventralis telencephali pars ventralis; III, third ventricle.

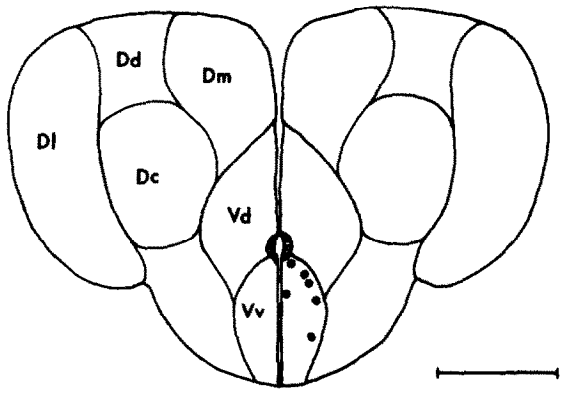

FIG. 2. A representative transverse section through the anterior portion of the telencephalon following administration of [^{3}H]estradiol. Dorsal is at the top of the figure. The distribution of labeled cells is denoted on the right hand side of the figure by black dots (see Methods). Each dot represents a labeled cell. Brain structures are identified on the left of the figure. The same procedure is followed in Fig. 3 to 7. The crosshatched structure dorsal to Vv represents the grooved ependymal thickening which is discussed in the text. The horizontal bar scale shown in this figure and in Fig. 3 through 7 represents 1 mm.

estradiol and of [^{3}H]testosterone. The third ventricle opens into the lateral recess immediately posterior to the nucleus anterior tuberis (NAT). Most of the estradiol- or testosterone-concentrating cells in NRL were located in the ventral and anterior wall of the rostral portion of the recess (Figs. 5 and 9). The recess projects caudoventrally into the inferior lobe of the hypothalamus. The NRL surrounding the caudoventral portion of the recess contained a few estradiol- or testosterone-concentrating cells (Fig. 6) as far posterior as it extends into the inferior lobe. A few labeled cells occurred in nucleus posterioris periventricularis in the plane of the section illustrated in Fig. 4. Further caudally, additional cells were labeled as shown in Fig. 5.

Systematic inspection of all sections revealed no estradiol or testosterone cells in other portions of the diencephalon including nucleus lateral tuberis pars lateralis,

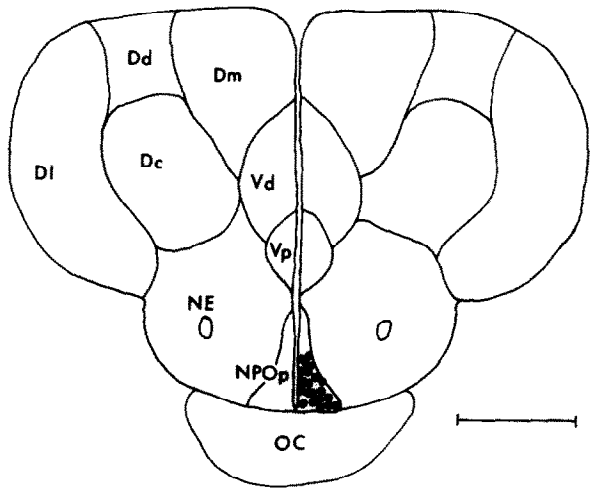

FIG. 3. A representative transverse section showing the location of cells in the preoptic area following estradiol administration.

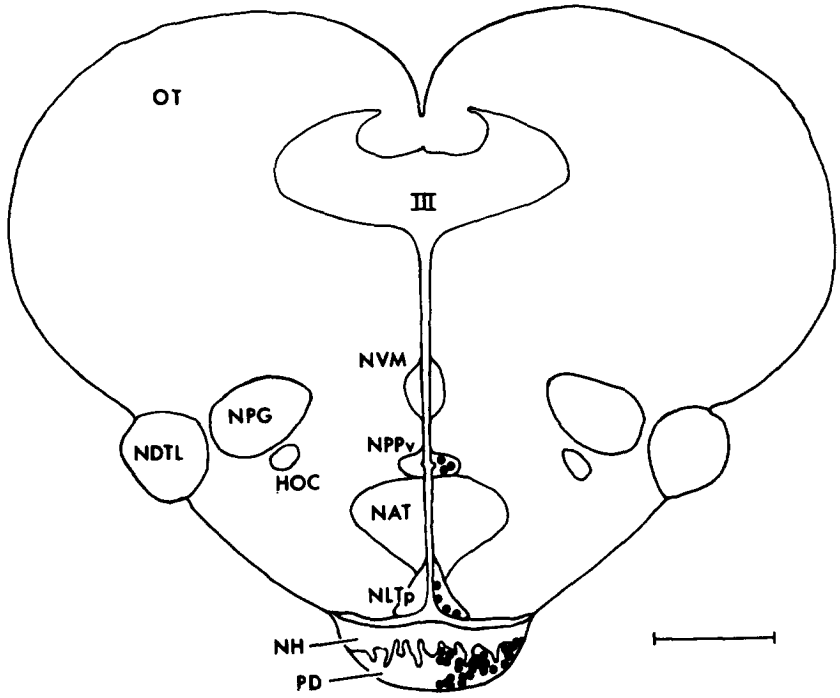

FIG. 4. A representative transverse section through the medial hypothalamus showing the distribution of estradiol-retaining cells.

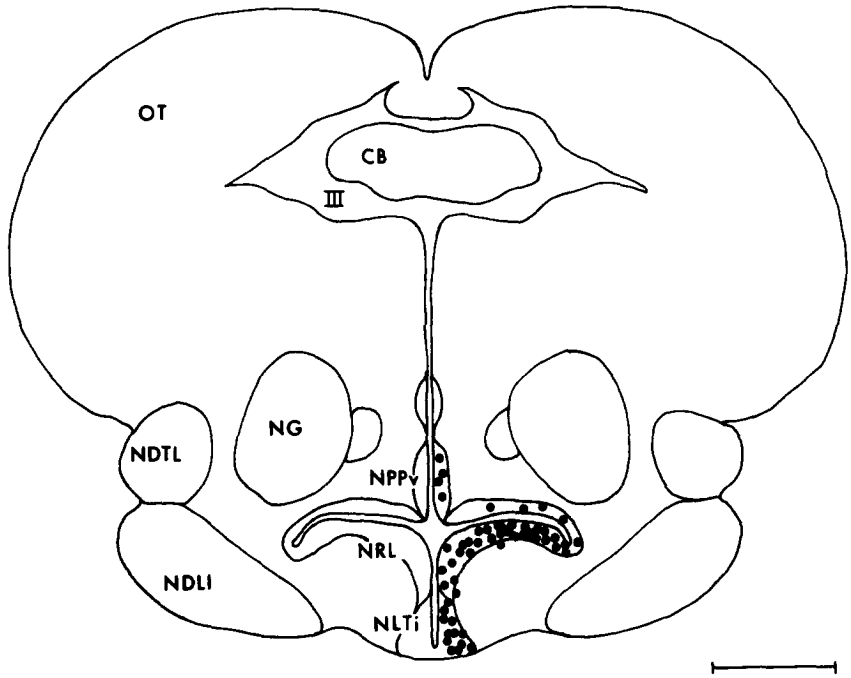

FIG. 5. Estradiol-retaining cells in a representative transverse section through the posterior hypothalamus at the level of the diverticulation of the lateral recess of the third ventricle.

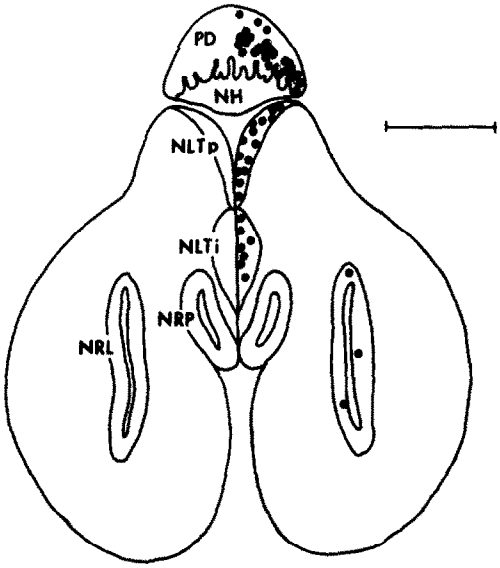

FIG. 6. A representative horizontal section through the pituitary gland and the ventral portion of the hypothalamus showing the distribution of estradiol-retaining cells in the pituitary and the caudal portion of the tuberal area. Anterior is at the top of the figure.

nucleus recessus posterioris, nucleus anterior tuberis, nucleus diffusus lobi inferioris, and corpus mamillare (Fig. 4, 5 and 7). Further, no labeled cells were found in the mesencephalon, rhombencephalon, or anterior spinal cord.

Pituitary. The *Macropodus* pituitary gland is a flattened disk-shaped structure attached to the ventral surface of the medial lobe of the hypothalamus (Fig. 1). Many small, darkly stained cells in the caudal portion of the pars distalis were labelled following [^{3}H]testosterone or [^{3}H]estradiol administration (Figs. 4 and 10), most of them grouped in a particular region. Thus in particular horizontal sections, many of the labeled cells were seen to be grouped in a transverse band (Fig. 6).

DISCUSSION

In the male *Macropodus*, the same areas of the brain show cellular retention of estradiol and testosterone. The hormone-labeled cells are restricted to specific areas

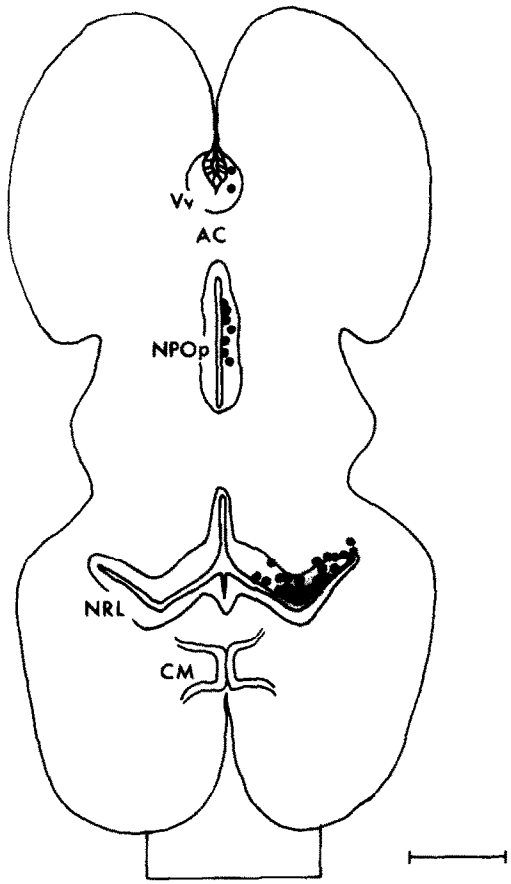

FIG. 7. A representative horizontal section showing the placement of estradiol-retaining cells in the anteroventral telencephalon, preoptic area, and wall of the lateral recess. Anterior is at the top of the figure.

of the ventral telencephalon and diencephalon and the anterior pituitary. Extensive sampling of the mesencephalon, rhombencephalon, and anterior spinal cord revealed no estradiol- or testosterone-retaining cells. The steroid-sensitive cells are located in the ventral telencephalon, the preoptic area, the caudal region of the posterior periventricularis nucleus, the lateral recess nucleus, and the anterior, and inferior areas of the lateral tuberal nucleus (Fig. 8).

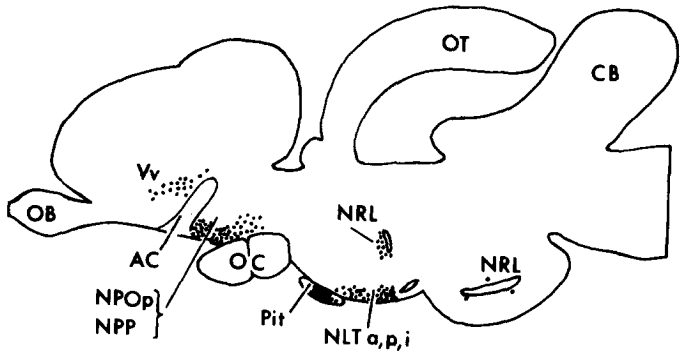

FIG. 8. A schematic drawing of a parasagittal section based on data which were obtained from transverse and horizontal brain sections. This is an abstract representation of the neuroanatomical sites of cellular uptake of [^{3}H]estradiol or [^{3}H]testosterone in the male *Macropodus* brain. The black dots denote groups of labeled cells, not individual cells. Many of the steroid cells in the NRL were located lateral to the plane of this section and are not represented in the figure.

Brain lesion and electrical stimulation experiments provide additional data which indicate that some of the estradiol- and testosterone-concentrating brain areas mediate reproductive functions. Electrical stimulation of the preoptic area evokes components of nest building, sexual behavior, and sperm release in *Lepomis* (Demski and Knigge, 1971; Demski, *et al.*, 1975). If the ventral telencephalon, area Vv, contains steroid cells in *Lepomis*, those neurons might also be activated by stimulation in the preoptic nucleus which is nearby. Selective lesioning of the preoptic area virtually obliterates the spawning reflex in killifish (Macey *et al.*, 1974). In *Macropodus*, reproductive behavior is greatly impeded following ablation of the telencephalon and varying amounts of the preoptic nucleus (Davis *et al.*, 1976; Kassel *et al.*, 1976; Schwagmeyer *et al.*, 1977; Kassel and Davis, 1977). Finally, in the goldfish, lesioning of the nucleus lateral tuberis pars posterior and pars anterior results in gonadal atrophy. This suggests that these tuberal areas contain neuroendocrine cells which regulate gonadotropin release (Peter, 1970).

In fish pituitary, gonadotropin cells have been identified by histochemical changes which occur during sexual development, during seasonal reproductive cycles, and following gonadectomy (Sage and Bern, 1971; Schreibman *et al.*, 1973). These cells are located mainly in a particular region of the caudal pars distalis. In addition, in some fishes, scattered gonadotropin cells occur in the rostral pars distalis. Our results show that in *Macropodus* most of the pituitary cells which concentrate sex steroid are located in the caudal pars distalis.

Sex steroid-retaining cells have previously been localized in the forebrain and pituitary gland of the green sunfish, *Lepomis cyanellus*. Cells labeled with [^{3}H]testosterone were seen in the preoptic area, nucleus lateral tuberis, and pars distalis of the pituitary (Morrell *et al.*, 1975; Morrell and Pfaff, 1977). The loci of sex hormone cells in male *Macropodus* include those found previously in male *Lepomis*, and they are similar to sites which have been discovered in males and females of other vertebrates as well. The preoptic area and the tuberal hypothalamus contain sex steroid-concentrating cells in every species studied, including several mammals and birds and the clawed frog, *Xenopus laevis* (Morrell *et al.*, 1975). The occurrence of populations of sex hormone cells in these brain areas would appear to be a fundamental vertebrate characteristic. The mesen-

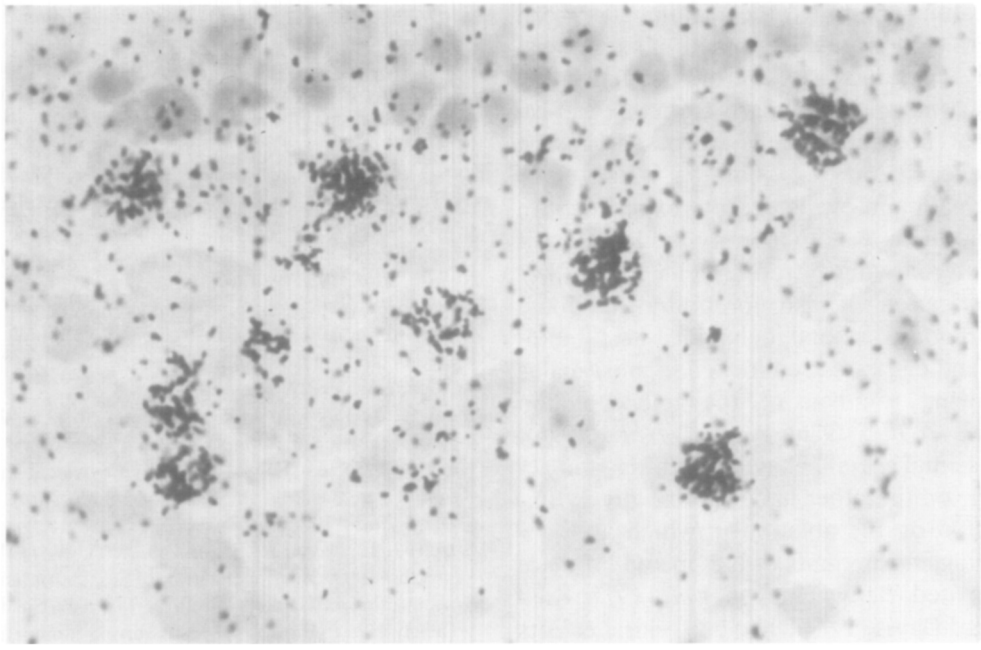

FIG. 9. Photomicrograph of an autoradiogram showing concentration of labeled steroid by cells in the nucleus of the lateral recess following administration of [^{3}H]estradiol. The reduced silver grains are concentrated over cells with large cell bodies which were lightly stained with cresyl violet. The small linearly arranged cell bodies are the ependyma of the ventricle. The section was cut in the horizontal plane. 1750×.

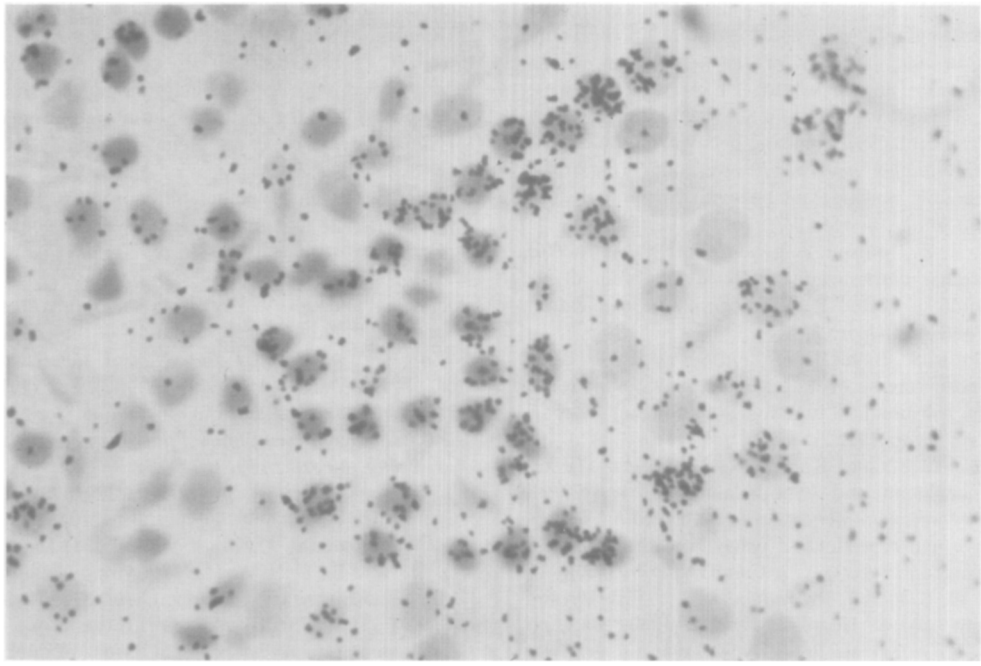

FIG. 10. Photomicrograph of an autoradiogram showing cells in the anterior pituitary which concentrated steroid after [^{3}H]estradiol injection. 1970×.

cephalon, ventral to the tectum, has in all other vertebrates examined also consistently contained sex steroid-concentrating cells. *Macropodus* departs from this pattern in that no labeled cells were seen in the mesencephalon.

A main aim of the present experiment in *Macropodus* was to identify the location of sex steroid-concentrating cells for further investigation of fish forebrain functions. *Macropodus* sexual behavior, nest building, and egg care are blocked in most males following removal of the telencephalon (Davis *et al.*, 1976; Kassel *et al.*, 1976). Nonsexual social behavior, feeding, and activity, on the other hand, are relatively unaffected by the ablation in which area Vv and varying amounts of NPOp and NPP are extirpated (Schwagmeyer *et al.*, 1977; Kassel and Davis, 1977). The behavioral effects of localized lesions in Vv and the preoptic area are under investigation.

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