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ULTRASTRUCTURAL AND HRP STUDIES OF REGIONAL VARIATION AND MATURATION OF NEUROSECRETORY CELLS AND GLIA OF THE GOLDFISH PREOPTIC AREA

By

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ABSTRACT

ULTRASTRUCTURAL AND HRP STUDIES OF
REGIONAL VARIATION AND MATURATION OF
NEUROSECRETORY CELLS AND GLIA OF
THE GOLDFISH PREOPTIC AREA

By

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The goldfish preoptic area (PA) was examined to determine how the preoptic neurosecretory system changes as body size and presumably demand for neurosecretory hormones increase through life. Intraperitoneal (IP) administration of horseradish peroxidase (HRP) was used to label neurons projecting beyond the blood-brain barrier, including neurosecretory cells. In 2-8g fish, lightly labeled 5μm diameter periventricular neurons bordered the preoptic recess. More caudally, cell size gradually increased, and labeled cells of the caudal ("magnocellular") PA reached 10-15μm diameter. Further caudal, similar cells were labeled in the periventricular ventral thalamus (PVT). Lightly labeled 5μm cells were still found in the rostral PA in larger (48.1-75.0g) fish. A gradation of cell size was maintained, with labeled cells reaching 50μm in the caudal PA. Labeling was again found in the PVT. Even larger (70μm) cells were found in the caudal PA of larger (105.6-283.1g) fish. HRP applied to the transected spinal cord labeled neurons in the PA and PVT in 3.3-44.5g fish. Regional variations in ultrastructural features of the preoptic neurosecretory region were assessed in 1.7-283.1g fish, some of which had received IP HRP injections.

In small (≤6.6g) fish, neurosecretory cells of the caudal PA were 10-15μm in diameter and contained dense core vesicles (DCVs). More rostral and ventral smaller cells contained few DCVs but were labeled following IP HRP injections. Cells in both areas were progressively larger, with larger nuclei and more granular reticulum in larger fish. In large fish, a contiguous population of DCV-containing neurons ranged from 10μm cells in the rostral PA to 70μm multinuclear caudal cells. Some intermediate and large cells and neurites contained vacuoles. A few neurosecretory cells were surrounded by perineuronal glia, but most were involved in extensive soma-somatic membrane apposition, especially in fish removed from cold water in late fall. Gap junctions were present on the somata of preoptic neurosecretory cells of fall fish. PVT labeling favors the consideration of the PA as an extension of ventral thalamus. Although neuronal hypertrophy is the simplest interpretation of maturational changes, continued formation of neurons, cell death and neuronal division may also occur.

This dissertation is dedicated
to my parents, without whose
guidance and support this work
would not have been attempted.

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LIST OF ABBREVIATIONS

AC	Anterior Commissure
DCV	Dense Core Vesicle
EM	Electron Microscopy
Ep	Ependyma
gbw	Gram Body Weight
H	Habenula
HC	Horizontal Commissure
HRP	Horseradish Peroxidase
IL	Inferior Lobe
IP	Intraperitoneal
OC	Optic Chiasm
ON	Optic Nerve
OT	Optic Tract
OVLT	Organum Vasculosum of the Lamina Terminalis
PA	Preoptic Area
PC	Posterior Commissure
PVT	Periventricular Ventral Thalamus
SCN	Suprachiasmatic Nucleus
SL	Standard Length
Tel	Telencephalon
TH	Tuberal Hypothalamus
TL	Torus Longitudinalis
VC	Valvula Cerebelli

INTRODUCTION

The central nervous systems of "lower" vertebrates are useful for the study of vertebrate developmental and related evolutionary, morphological and physiological events. While adult patterns of neuronal morphology may be nearly attained early in mammalian life, homologous regions of fish brains continue to show extensive maturational changes throughout life. Thus, new neurons appear, hypertrophy and form and/or change synapses into "adult" life in several regions of the central nervous system of fish (Polenov, Chetverukhin and Jakovleva, 1972; Johns, 1977; Johns and Easter, 1977; Kock and Reuter, 1978; Leonard, Coggeshall and Willis, 1978; Meyer, 1978; Easter, 1979; Birse, Leonard and Coggeshall, 1980; Easter, Rusoff and Kish, 1981; Johns, 1981). Furthermore, radial glia are retained as such through life in fish (Stevenson and Yoon, 1982) but not in mammals, which is consistent with the postulated roles of radial glia in neural pattern formation (Rakic, 1971, 1972).

The developing and functioning mammalian nervous system is normally relatively protected in utero, and thereafter by the organism's own homeostatic mechanisms, against thermal and chemical variation in the neuronal extracellular environment. The nervous system of fish, ectothermic anamniotes, develops and functions with less sophisticated protection from such directly acting (i.e., direct thermal and chemical

effects on cellular function; Grafstein, Forman and McEwen, 1972; Springer and Agranoff, 1977) and sensory (i.e., transduced by sensory receptors and transmitted synaptically) effects of variations in the organism's environment. Neural maturation in fish might thus be normally more subject to environmental influences than the normal maturation of homologous mammalian systems.

Furthermore, variation within the normal environment of fishes is responsible for a considerable degree of intraspecies variation in gross anatomical features demonstrated even during post-larval (life-long) maturation (Brett, 1979). This developmental plasticity may have considerable survival value as growing animals adapt to a changeable and changing environment in the wild. The non-invasive ecological approach to the study of morphological plasticity is potentially more revealing of plasticity's consequences for the normal developing animal than are observations of the effects of experimental lesions.

The "adult" fish brain also demonstrates substantial regenerative abilities, such as the nearly precise restoration of retinofugal connectivity following the crushing of the optic nerve (Springer, 1981; Springer and Gaffney, 1982). A remarkable, if less perfect, regeneration of caudalmost spinal cord segments following their removal has been shown (Anderson and Waxman, 1981). The mechanisms underlying these regenerative phenomena and their relationships to normal maturational events are uncertain.

Since the earliest investigations of neurosecretion, studies of the preoptic area (PA) of fish have provided important clues to the general principles of neuroendocrine structure and function (Scharrer,

1941; Palay, 1960; Kandel, 1964). While morphological studies of neurosecretion are now more commonly performed on mammals, the current interpretation of neurosecretory electrophysiological function was until recently largely dependent on intracellular recordings almost exclusively obtained from fish (Mason and Bern, 1977).

Previous investigators using traditional stains (e.g., Palay, 1945; Leatherland and Dodd, 1969), or electrophysiological antidromic identification (Kandel, 1964; Hayward, 1974) have reported a preoptico-hypophysial projection in various species of fish. Recent immunohistochemical localization of neurophysin, the associated neurosecretory hormones arginine vasotocin and isotocin, and/or the hormone enkephalin (met- or leu- undetermined) in a continuum of small to very large (5-70μm) neurons of the PA (Watkins, 1975; Goossens, Dierickx and Vandesande, 1977a, 1977b, 1978; Hoheisel, Ruhle and Sterba, 1978; Follenius and Dubois, 1979; Schreibman and Halpern, 1980; Reaves and Hayward, 1980) and in fibers of the pituitary (Goossens et al., 1977b, 1978; Hoheisel et al., 1978; Gill, Burford, Lederis and Zimmerman, 1977; Schreibman and Halpern, 1980) of fish[1] further supports its general (discussed further below) homology with some or all parts of the paraventricular, supraoptic and so-called accessory magnocellular neurosecretory systems of tetrapods.

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[1]Neurons of the PA of teleosts contain the hormones arginine vasotocin, isotocin and/or enkephalin. Cyclostomes have vasotocin but apparently no isotocin or related hormone. The lungfish, like amphibians, have mesotocin and vasotocin. Cartilaginous fish have vasotocin and one or more variants other than isotocin. For a review of the evolution and function of the neurophysin/vasotocin family of hormones, see Sawyer and Pang, 1979.

The preopticohypophysial system, found in some form in all vertebrates, can also serve as a useful landmark for the topological (homological) analysis of the vertebrate forebrain. The origin of the PA (telencephalic or diencephalic) and the placement of developmentally significant preoptic/hypothalamic and related telecephalic/diencephalic borders is controversial (Keyser, 1979). The paraventricular and supraoptic nuclei of mammals, which are clearly preoptic based on comparative data (despite their developmental and phylogenetic translocation), are nonetheless considered to be hypothalamic by most investigators. Fish are convenient for the investigation of this question since their PA is morphologically more distinct from the hypothalamus proper, being more clearly contiguous with the basal telencephalon and ventral thalamus. Whether neurosecretory and other nuclei of the PA are developmentally associated with either telecephalon, ventral thalamus or hypothalamus proper as various workers have proposed (see Keyser, 1979) is a question of some importance to hodologists interested in the interpretation of any of the nuclei of this important region of the vertebrate brain. This developmental and hodological approach is in contradistinction to the prevailing definition of hypothalamus as an entity including hypothalamus proper plus the PA (Crosby and Showers, 1969).

The hypophysial and spinal efferent projections of the PA were examined in goldfish of various sizes using the retrograde transport of horseradish peroxidase (HRP). HRP was introduced via intraperitoneal (IP) injection (intramuscular, intravenous, intracardiac and subcutaneous injections being difficult or impossible in small fish), in order to reach the hypophysial parenchyma via its fenestrated capillaries

(Abraham, Kieselstein and Lisson-Begon, 1976). A functional blood-brain barrier exists in fish (as in mammals) except at the pituitary, and potentially at certain other circumventricular organs (Bernstein and Streicher, 1965; Brightman, Reese, Olsson and Klatzo, 1971; Bundgaard, Cserr and Murray, 1979; and unpublished observations). Intravascular injection of mammals with HRP yields labeling of neurons in neurosecretory, autonomic preganglionic and cranial and spinal motor nuclei, that is, neurons with axons projecting outside of the blood-brain barrier (Broadwell and Brightman, 1976). The pattern of neuronal labeling following systemic HRP administration resembles that seen after intrahypophysial injection in rats (Armstrong and Hatton, 1980). Therefore, it was hypothesized that IP HRP injections would produce, after a sufficient survival time, labeling of at least a major portion of the neurons in the preoptic nuclei of goldfish.

Ample evidence has recently emerged for a substantial system of descending projections from the mammalian paraventricular nucleus to numerous brainstem and spinal loci (Armstrong, Warach, Hatton and McNeill, 1980 and others). As similar projections, some immunohistochemically demonstrated to contain appropriate hormones have also been reported from cells of diverse nonmammalian vertebrates (Schober, Trautmann, Naumann and Sterba, 1977; Goossens et al., 1977b; Hoheisel et al., 1978; Ten Donkelaar, Kusuma and Boer-van Huizen, 1980; see also Hoheisel et al., 1978 and Smeets and Timerick, 1981 for further references), HRP was applied to the transected spinal cord of goldfish. Comparisons were made between the populations of cells labeled by the two procedures. Since this study of preoptic projections was followed by an ultrastructural

examination of neuronal maturation and positional variation, these experiments were performed on goldfish ranging widely in body and brain size.

Neurosecretory cells of diverse vertebrate and invertebrate forms have the capacity to periodically synthesize large amounts of hormone(s) for release at neurohemal junctions and elsewhere (Mason and Bern, 1977). When dictated by stimuli within the animal or its external environment to adjust the rate of hormone synthesis and release, numerous and dramatic morphological and functional changes can be seen in both homologous and nonhomologous neurosecretory systems (Knowles and Vollrath, 1966; Hatton and Walters, 1973; Mason and Bern, 1977; Morris and Steel, 1977; Armstrong, Gregory and Hatton, 1977; Morris, Nordmann and Dyball, 1978; Tweedle and Hatton, 1976, 1977, 1980, 1982; Gregory, Tweedle and Hatton, 1980; Theodosis, Poulain and Vincent, 1981; Hatton and Tweedle, 1982 and others).

The maturation of the goldfish PA was examined since, like any part of the fish brain, it is potentially undergoing continual structural change. Furthermore, like any neurosecretory system, morphological changes might occur in response to external variables and these morphological changes are potentially interpretable in terms of the physiology of the animal and its neurosecretory system (Tweedle and Hatton, 1976, 1977, 1980, 1982; Gregory et al., 1980; Theodosis et al., 1981; Hatton and Tweedle, 1982). In the present studies, it was assumed that capacity for neurosecretory hormone synthesis and release increases as body weight increases many fold during "adult" (i.e., sexually mature) life. If so, then ultrastructural features of preoptic neurosecretory cells of

larger fish might more closely resemble the physiologically stimulated and morphologically altered homologous neurons of thirsty, pregnant or lactating mammals (Hatton and Walters, 1973; Tweedle and Hatton, 1976, 1977; Armstrong et al., 1977; Morris et al., 1978; Gregory et al., 1980; Hatton and Tweedle, 1982 and others).

METHODS

Light Microscopic Experiments

Goldfish (Carassius auratus) of the common variety were obtained commercially. Some of the larger fish which were used in subsequent ultrastructural studies, with which comparisons have been made, were obtained from a pond in Lansing, Michigan. Fish receiving IP injections of HRP for light microscopic analysis were 1.2-75.0 g (3.2-11.7 cm standard length[1]). Similar fish (4.3-44.5 g, 4.8-10.5 cm SL) had HRP applied to the transected spinal cord for light microscopic analysis. Fish were kept in 10 or 20 gallon (37.8, 75.7 l) aquaria maintained at 20-24°C, subject to laboratory illumination with aeration and filtration. The sex of fish was not considered, since it was not always assessable. To investigate the preopticohypophysial projection of small goldfish, thirty-six (36) fish (1.7-8.1 g, 3.2-6.0 cm SL) were given IP injections of 0.01-7.14 mg HRP per gram body weight (gbw) in freshwater fish Ringer's solution (Young, 1933). Sigma Type VI horseradish peroxidase was used in all experiments. Three (3) additional fish of similar size were repeatedly injected, on alternate days, with 10 mg HRP, for a total of 30 or 40 mg (8.8-11.4 mg/gbw). Two similar fish were given IP

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[1] Standard length (SL) is the length of a fish measured from the rostral end of the head to the caudal end of the vertebral column, i.e., excluding the tail.

injections of HRP (2.44, 4.35 mg/gbw) and sacrificed six (6) or two (2) hours later, respectively. The brains of these two fish were processed for the light microscopic demonstration of HRP in order to assess the competence of the blood-brain barrier. Pituitaries of some HRP IP fish were processed for the ultrastructural demonstration of HRP. Three (3) larger fish (38.1-75.0 g, 10.5-11.7 cm SL) received 75-100 mg HRP IP (1.0-2.62 mg/gbw) and were sacrificed after three (3) or five (5) days and processed for light microscopy. Intraperitoneal injections, as well as surgery and perfusions, were performed on fish which had been anaesthetized by immersion in 0.1% tricaine methanesulfonate (Sigma).

Due to the size of the spinal cord of the small goldfish, it was not possible to discretely inject one side of the cord, or to hemisect it, in order to determine the laterality of descending projections. Therefore, the cord was transected, and HRP applied to the cut end. Utilizing a dissecting microscope and iridectomy scissors, a 2-3 mm incision was made followed by dissection to vertebrae from a position lateral to the vertebral column at the level of the anterior margin of the dorsal fin. This level was reliably located in fish of all sizes, was convenient for surgery and was sufficiently caudal to spare opercular (respiratory) and pectoral fin innervation which greatly increased chances for survival. The lamina of a vertebra was then removed, exposing one segment of cord which was excised. Approximately 1-3 µl of 30% HRP in water or 0.75% NaCl containing 0.5% dimethyl sulfoxide (Mallinckrodt) was placed via micropipet into the space which had been occupied by the removed segment of cord. After five (5) minutes, powdered absorbable gelatin sponge (Gelfoam, Upjohn) was placed into

this space within the vertebral column. Alternatively, similar amounts of HRP solution and Gelfoam were mixed and allowed to dry to a crust which was packed into the open vertebral column. The remaining cavity in the axial musculature was filled with Gelfoam moistened with Ringer's solution, and was covered with cyanoacrylate (Elmer's Wonder Bond). Fish were revived by flushing the gills with fresh aerated water, and maintained in 0.05% sulfadimethoxine (Roche) or amoxicillin (Utimox, Upjohn) which was changed daily. HRP was applied to transected cords of nine (9) small (4.3-6.6 g, 4.8-5.7 cm SL) and five (5) larger (16.0-44.5 g, 7.2-10.5 cm SL) fish, which were sacrificed after a 2-14 day survival period. As a partial control for the labeling of neurons via other than uptake from transected preoptic axons (e.g., by the diffusion of HRP through the cerebrospinal fluid surrounding the spinal cord to more rostral terminals of preoptic projections, or by diffusion of HRP into the blood which would carry it to the hypophysis, and thus possibly label preoptic neurons), the same amount of HRP was applied to the exposed but uncut spinal cords of two (2) small fish. The threshold for preoptic retrograde HRP labeling by systemic HRP was also established for this HRP reaction procedure (see below) by a qualitative dose-response analysis of the HRP IP data. The IP injections of small doses of HRP served as controls for neuronal labeling of spinal transected fish via a vascular-hypophysial route. For comparison with the IP dosages administered to small fish (0.01-11.4 mg/gbw), the HRP applied to the spinal cord represented 0.004-0.02 mg HRP per gbw. Brainstem sections of some spinal and IP cases were processed and

examined in order to establish the labeling of other systems by vascular or spinal means.

All fish were perfused through the bulbous arteriosus under microscopic control with heparinized freshwater teleost Ringer's solution followed by a buffered aldehyde fixative. The cranial cavity was then opened in order to expose the brain, and the head was immersed in the same fixative, or the brain was extracted immediately following the perfusion and placed in the same fixative, in either case yielding a total period of 2-24 hours in fixative. Fixatives consisted of various combinations of formaldehyde, paraformaldehyde and/or glutaraldehyde (2-4% total aldehydes) in 0.1 M sodium phosphate or cacodylate buffer, pH 7.0-7.2 containing 0-1.0% dimethyl sulfoxide. Following fixation, brains were placed in a 10% sucrose solution in the same buffer for 12-24 hours. The brain was then frozen by immersion in 2-methylbutane which had been chilled in liquid nitrogen. Coronal sections (30-60 μm) were taken on a cryostat. Sectioning began at the telencephalon just caudal to the olfactory bulbs, and extended at least throughout the diencephalon, and often through the caudal medulla. Sections of spinal cord were sometimes taken. Due to the small size of most sections, section loss was sometimes a problem, and sections were not maintained in exact serial order. Sections were collected in 0.1 M phosphate buffer (pH 7.0-7.2), usually intensified with cobalt chloride (Adams, 1977) and were then reacted for the demonstration of peroxidase using diaminobenzidine tetrahydrochloride (Sigma or Aldrich), generally according to the method of Nauta, Pritz and Lasek (1974). Reacted sections were then arranged into approximately serial order and mounted on subbed slides.

Sections were lightly counterstained with thionine or cresyl violet, and were examined using brightfield and darkfield microscopy and Nomarski optics using a Zeiss Universal microscope fitted with a 35mm camera.

Ultrastructural Experiments

Common goldfish used in ultrastructural experiments were obtained commercially or netted during the summer or fall from the east pond of Fenner Arboretum in Lansing, Michigan. The pond had been surreptitiously stocked several years previously with similar common goldfish. The PA, and in some cases ventral thalamus and other regions were examined from twenty four (24) fish (1.7-283.1 g; 3.8-18.0 cm SL). Seventeen (17) fish (1.7-51.6 g) were obtained commercially and seven (7; 70.1-283.1 g) were caught from the pond. Two (2) of these fish (105.6, 283.1 g) were caught in late August, while the other five (5) fish (70.1-164.4 g) were caught in mid-November. Fish were maintained in indoor aquaria as described above. Pond fish were perfused within one day except one fish subjected to experimentation (132.1 g, HRP IP).

In order to selectively label the population of preoptic cells possessing an axon which projects beyond the blood-brain barrier (potentially functional as neurosecretory cells), some fish received IP injections of HRP. Successful HRP IP experiments were performed with 3.2 g (2.3 mg HRP/g, 3 day survival), 4.2 g (3.6 mg/g, 4 day) and 6.6 g (2.7 mg/g, 10 day) fish. Additional HRP and normal ultrastructural data were obtained from 1.7, 13.7, 16.6, 51.6 and 132.1 g fish in which experimental parameters did not produce labeling as extensive as seen in corresponding light microscopic experiments. Several other fish

(included in the 24 of this study) had HRP or cytochrome c (Polysciences 5042; Malmgren, Olsson, Olsson and Kristensson, 1978; Nässel, Berriman and Seyan, 1981) applied to the transected cord. The present ultrastructural report is primarily limited to cells in areas which have been shown in light microscopic and ultrastructural HRP and cytochrome c experiments to project largely to the pituitary but not the spinal cord (Figures 1, 2, 5, 6A-C, 7A-G, 8B; Gregory and Tweedle, 1981).

After survival times of 3-10 days, EM experimental fish were anesthetized and perfused with heparinized Ringer's solution followed by aldehyde fixative. For the demonstration of HRP or cytochrome c, the fixative consisted of 1.25% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium phosphate or cacodylate buffer, pH 7.0. Following perfusion, 1 mm thick coronal slices were taken through the PA and entire diencephalon. Surrounding structures were left attached for orientation purposes and the tissue was left in chilled fix for an additional eight (8) hours. An en bloc reaction procedure was utilized to demonstrate the presence of HRP or cytochrome c, despite a probable loss of sensitivity, since positional information was important and the neurons of interest spanned an extensive region of periventricular brain. Following several 10 minute buffer rinses, the tissue was preincubated in a solution consisting of 40-50 mg diaminobenzidine in 100 ml of 0.1 M sodium phosphate buffer, for 45-180 minutes. Following preincubation, 4 ml of 0.6% hydrogen peroxide was added. After 30-60 minutes, the tissue was rinsed in buffer or an additional 1 ml of hydrogen peroxide solution was added for another 30 minutes. After several buffer rinses, tissue was placed in 1.0% osmium tetroxide in 0.1 M phosphate buffer,

pH 7.0 for 2-3 hours at room temperature. In some cases the brainstems of these fish were cut on a cryostat, reacted and examined with light microscopy in order to assess fixation and the quality and position of neuronal labeling related to cranial nerves or brainstem descending systems.

Fish (2.3-283.1 g) which were not experimentally treated were perfused with a mixture of 2.0% paraformaldehyde and 2.0% glutaraldehyde or 3.5-4.0% glutaraldehyde in 0.09-0.1 M sodium phosphate or cacodylate butter, pH 7.0-7.2. Fixatives were varied because no procedure is known to yield consistently good preservation of the fish brain (R. E. Coggeshall, personal communication). Following perfusion, tissues were placed in refrigerated fixative for an additional 18-24 hours. Immediately after perfusion or 4-6 hours subsequently, 1-2 mm thick coronal slices were taken through the PA and diencephalon with surrounding structures left attached for orientation purposes. Following aldehyde fixation, tissue was briefly rinsed in buffer and placed in buffered osmium for 2-3 hours at room temperature. In several cases a 1.5% potassium ferrocyanide - 1.0% buffered osmium solution (Dvorak, Hammond, Dvorak and Karnovsky, 1972) was used. Osmicated tissues were briefly rinsed and stained <u>en bloc</u> with refrigerated 4.0% aqueous uranyl acetate overnight. Tissues were then rinsed, dehydrated and embedded in Spurr's or Epon resins. Tissues were embedded so that thin sections were taken in the coronal plane. Block faces were left as large as necessary (extremely large in some areas of large fish, e.g., Figures 12A, 12B, 13, 14A, 14B, 15B, 17A, 18, 19) in order to preserve orientation and positional information on the thin section and to avoid

trimming away structures of interest (see also Figures 1-8). Thin sections (gray to light gold) were taken with diamond or glass knives on a Sorvall MT-2B ultramicrotome and poststained with lead citrate. In some cases thin sections were taken and examined every 30-50 μm from rostral to caudal through the entire extent of the PA. Rostral and caudal parts of the periventricular ventral thalamus (nucleus ventromedialis of Schnitzlein, 1962) received cursory examination in some fish. Most sections were examined with the Philips 201 electron microscope of the Center for Electron Optics. Initially, the Philips 201 microscope of the College of Osteopathic Medicine's Electron Microscope Facility was utilized. Due to environmental and probable genetic variations among fish, variations in fixatives, buffers, stains and plastics, and the extremely limited information relevant to sampling within the PA of fish, observations reported herein are qualitative rather than quantitative.

RESULTS

Light Microscopic Retrograde HRP Experiments

Results from recent ultrastructural, maturational, connectional and immunohistochemical studies (Gregory and Tweedle, 1981, 1982; Pasquier, Cannata and Tramezzani, 1980; and Reaves and Hayward, 1980) discussed below raise questions regarding the utility and validity of the periventricular/parvocellular/magnocellular preoptic terminology which has been commonly applied to fish and amphibians (e.g., Crosby and Showers, 1969; Peter and Gill, 1975; Terlou and Ekengren, 1979). Although attempts will be made to relate the patterns of neuronal labeling to the previous terminology as variously conceived, especially the atlas of the goldfish forebrain by Peter and Gill (1975), this is not meant to imply that regional preoptic variation is necessarily consistent with the named subdivisions as distinct morphological, spatial, developmental or functional entities based on present evidence.

Systemic Peroxidase Labeling in Small Fish (1.7-8.1 g)

Labeling of endothelial cells was noted whenever HRP was introduced into fish (Figures 5, 6, 7, 8, 9B). Cerebral capillary labeling was faint but detectable with the smallest IP injection (0.01 mg/gbw). However, IP injections of up to 0.5 mg/gbw gave no detectable preoptic

neuronal labeling. Extracellular space and glia were free of reaction product even with short survival times (2-6 hours) or after massive repeat injections.

With injections of HRP approaching 1.0 mg/gbw, a few granules of reaction product were found in tightly packed neurons (5-15 μm diameter) located beneath the ependyma along the preoptic recess (third ventricle in the PA). These cells appear to be in both the rostroventral parvocellular and immediately adjacent caudodorsal magnocellular portions of the preoptic nucleus of Peter and Gill (1975), possibly extending rostrally into the rostral part of their periventricular preoptic nucleus, although these "nuclei" are difficult to separate rostrally. Peter and Gill (1975) describe their periventricular preoptic nucleus as also extending caudally in a position dorsal to their preoptic (neurosecretory) nucleus. Labeled cells were not found in this position, or in their anterior periventricular nucleus which is ventral to their preoptic nucleus (however, see results below with larger doses). Labeling of neurons in these fish was never seen in the nucleus tuberalis (but see further results) which is also neurosecretory (Fryer and Maler, 1981). Very light nongranular reaction product was occasionally seen in the neurons of the oculomotor nucleus.

Labeling of neurons in all of the above labeled areas was much enhanced and reliable with dosages of HRP exceeding 2 mg/gbw, and neurons of certain other areas were also labeled. Granules of reaction product were found in the majority of neurons in an extensive complex extending through the PA and beyond (Figure 1A). A more diffuse labeling of neuronal somata and dendrites was seen in certain brainstem motor groups

(e.g., oculomotor). A large proportion of the neurons of the tuberal nucleus were labeled in these fish.

The majority of neurons along the rostroventral end of the third ventricle, rostral to the optic chiasm and ventral to the anterior commissure, were found to contain a small number of granules (per cell) of reaction product following large HRP IP injections (Figures 5A-F). Such cells were tightly packed in a group extending laterally about 100 μm from the ependyma. These cells were about 5 μm in diameter. Whether these cells are in the periventricular preoptic or anterior periventricular nuclei of Peter and Gill (1975) or in the adjacent parvocellular preoptic subnucleus is not clear since there is no obvious boundary between these groups rostrally. Directly dorsal and caudal, contiguous with the most rostral labeled cells and extending dorsal to the optic chiasm and between the optic tracts, there is a mass of closely packed neurons having more intense labeling with HRP than cells in the more rostral group (Figures 5I, 5G, 6A, 6B). As these neurons were closely packed, heavily labeled and were perfused by a dense capillary bed which was also labeled, little detail can be noted of the morphology of these cells with light microscopy. However, it appears that the great majority of these cells were labeled, and cellular diameters were 7-15 μm with most cells about 10 μm (see also Figure 9B, 9C). These more easily labeled preoptic cells appear to correspond to the magnocellular preoptic nucleus of Charlton (1932) and Crosby and Showers (1969) and to the magnocellular and parvocellular subnuclei of the preoptic nucleus of Peter and Gill (1975), although distinctions between the periventricular and other preoptic nuclei, and between the

Figures 1-2. Tracings of representative coronal sections, from rostral to caudal through forebrain of 4.3 g, 38.1 g and 75.0 g (Figures 1A, 1B and 2, respectively) adult goldfish following intraperitoneal injection of HRP. Labeling is pictures in Figures 5-7. Compare Figures 1A, 1B and 2 (75.0 g fish brain cut at a slightly different angle), observing increase in brain size with body growth. Blackened area (arrows) indicates position of neuronal (neurosecretory cell) labeling resulting presumably from HRP which was carried to the fenestrated capillaries of the pituitary via the blood, and taken up by neurosecretory terminals. Reaction product was found in neurons extending through an extensive portion of the PA (see text for discussion of preoptic subdivisions), continuing caudally into the caudal ventral thalamic area. Note similarity of labeling pattern despite maturational differences in brain and body size. Labeling in tuberal hypothalamic nuclei has not been indicated here. Section from 75.0 g fish of Figure 2 which contained the SCN was lost.

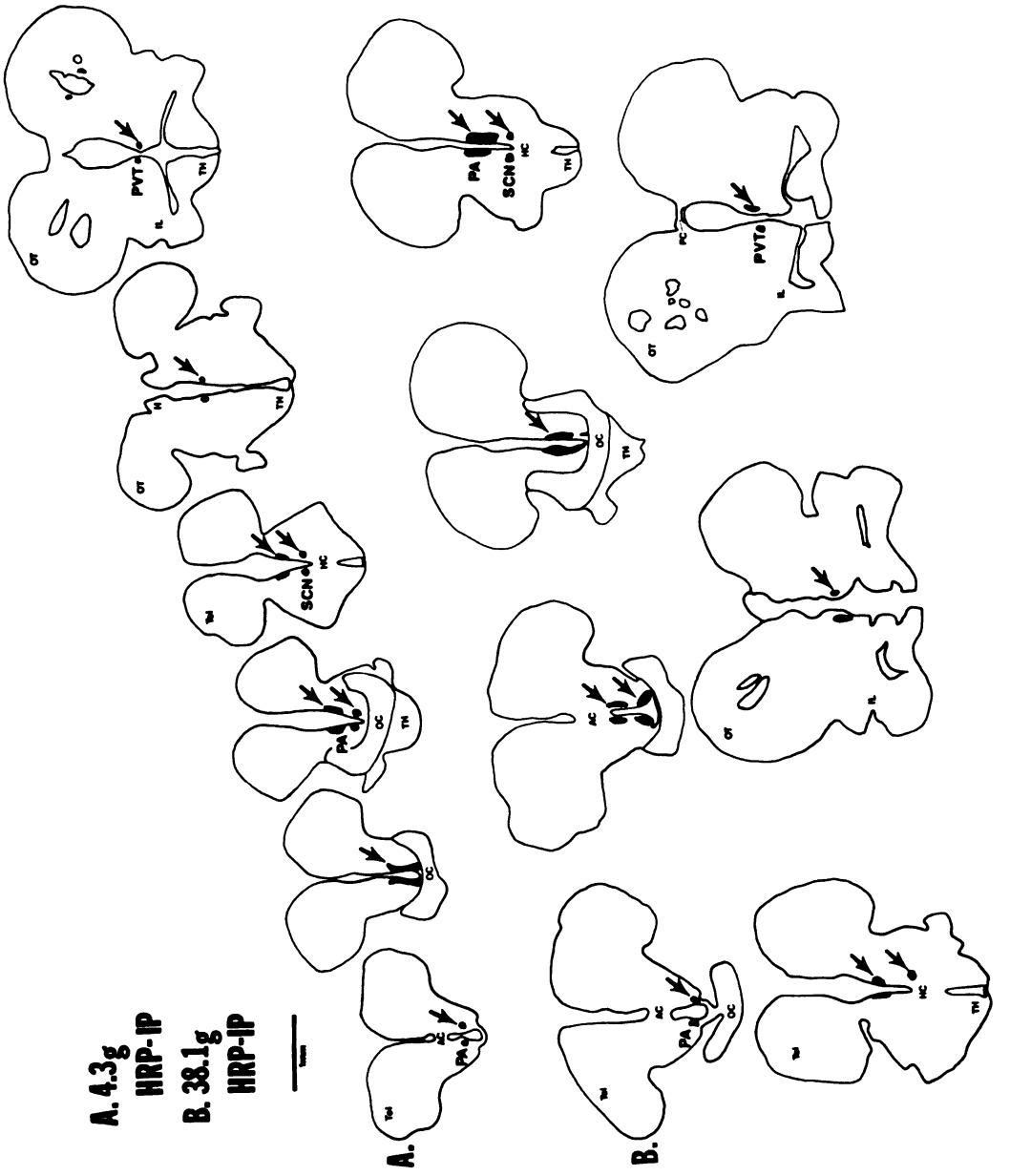

Figure 1

Figures 3-4. Position of forebrain neuronal labeling which was observed following the application of HRP to the transected spinal cords of 4.3 g (Figure 3) and 42.3 g (Figure 4) goldfish.

Blackened area (arrows) indicates position of forebrain neuronal labeling which was observed following the application of HRP to the transected spinal cords of 4.3 g (Figure 3) and 42.3 g (Figure 4) goldfish (also illustrated in Figure 8). A horizontal column of labeled cells was found through parts of the preoptic and ventral thalamic areas. Note similar patterns of labeling despite differences in brain and fish size (compare Figures 3 and 4). Compare also Figures 3 and 4 with 1A and 1B, respectively, for an indication of the considerable portions of the PA which apparently project to the hypophysis but not the spinal cord, and observe the potential overlap of the hypophysial and spinal efferent cell populations, some cells of which could conceivably project to both regions (but see text and Figure 8).

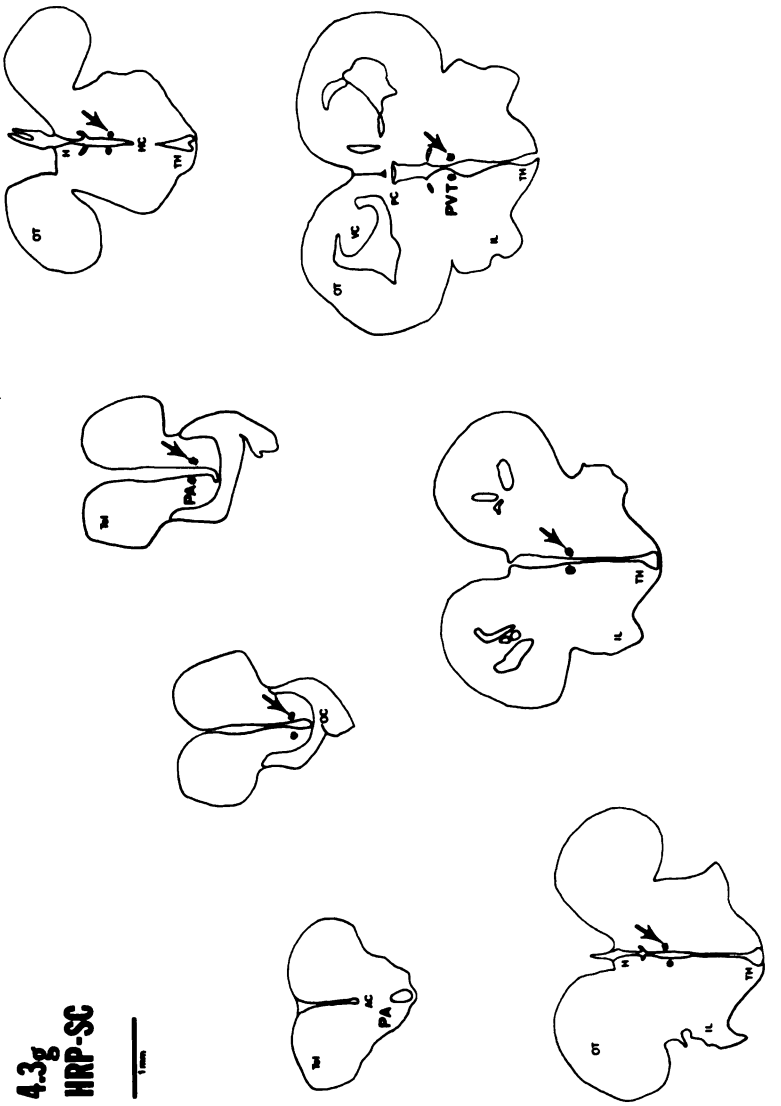

Figure 3

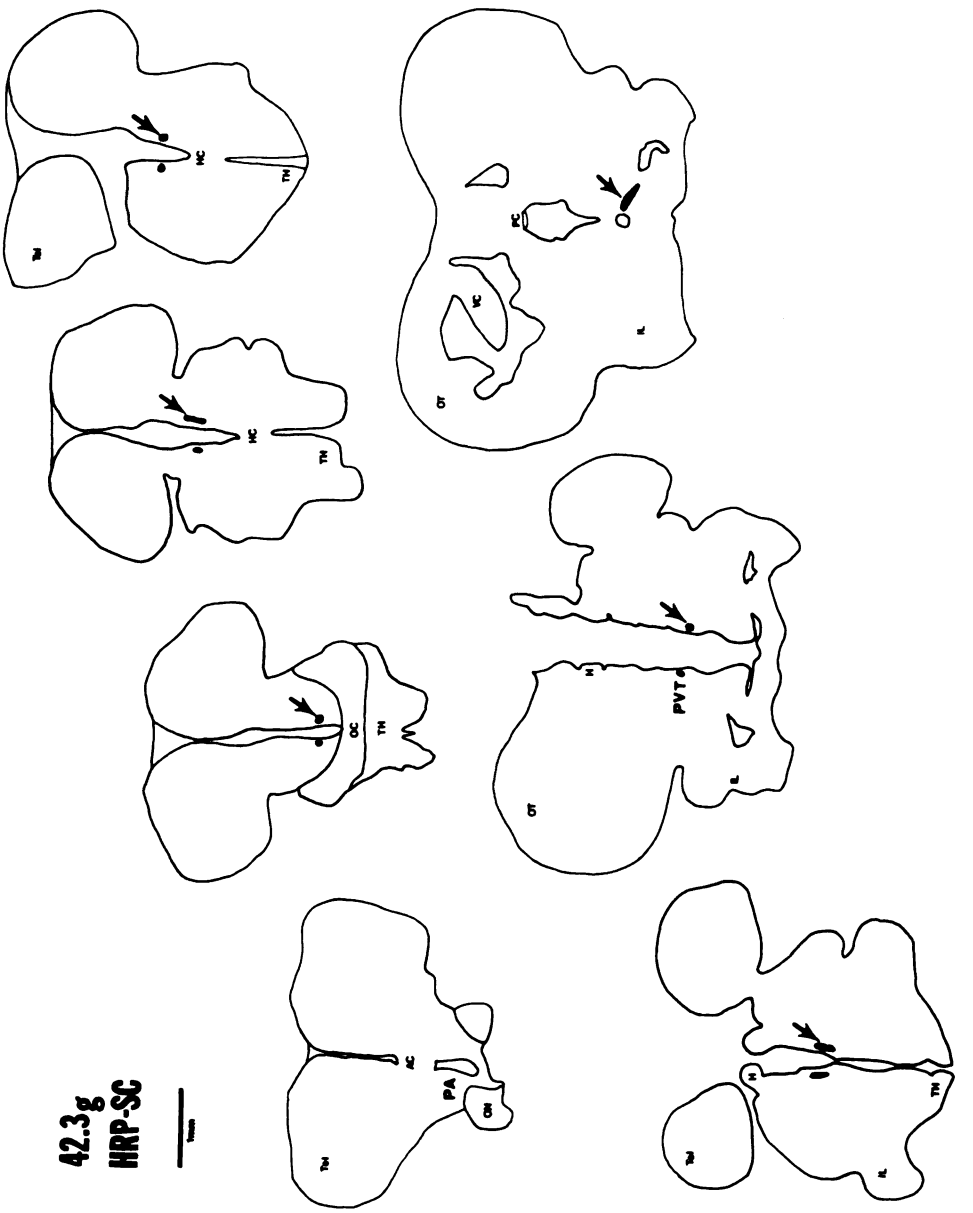

Figure 4

Figure 5. Labeling of presumed neurosecretory cells in coronal sections through rostral and intermediate portions of the PA of a 4.3 g (small adult) goldfish following IP injection of HRP. The extent of neuronal labeling is also plotted in Figure 1A. Note increase in cell size and intensity of neuronal labeling from rostral to caudal (Figures 5 and 6), and similarly, between small and large (Figure 7) fish. Thionine stain. See text for discussion of the issue of nuclear subdivision and boundaries.

A. Section through rostral preoptic recess. Capillary endothelial cells and meningeal macrophages are labeled with reaction product. Darkness of preoptic ependyma is due to basophilia of ependymal cells, endothelial cell labeling, curling of section edge and subependymal neuronal labeling but does not reflect label in ependymal cells, glia or extracellular space. Asterisk indicates area enlarged for Figure 5B. Scale = 1 mm for Figure 5A, 5C and 5G.

B. The great majority of these subependymal neurons (about 5 μm diameter) contain granules of reaction product in a small rim of cytoplasm around a spherical nucleus (open arrows). Solid arrow indicates ependyma out of plane of focus. Scales = 5, 50 μm.

C. More caudal section through the optic chiasm with similar subependymal neuronal labeling. Asterisk indicates area which was enlarged for Figure 5D, 5E and 5F.

D. Open arrows indicate lightly labeled small neurons. Solid arrow indicates ependyma/subependyma. Scale = 10 μm.

E,F. Similar area further enlarged with brightfield (E) and Nomarski optics (F). Focusing through section reveals that virtually all neurons here are similarly labeled. Scales = 10 μm.

G. Further caudal section through the PA. Letters H, I and J on Figure 5G indicate positions of contiguous neuronal labeling which correspond to the periventricular preoptic, parvocellular preoptic and anterior periventricular nuclei, respectively, of Peter and Gill (1975).

H. Several loosely packed small cells (open arrows) containing granules of reaction product were found dorsal to the highly vascular, closely packed and more readily labeled preoptic cells (Figure 5I). Scale = 10 μm.

I. Small tightly packed neurons in a highly vascular region which can be heavily labeled by systemic HRP, apparently corresponding to the parvocellular preoptic nucleus (Peter and Gill, 1975). Scale = 10 μm.

J. Minimally labeled mass of small (approximately 5 μm diameter) neurons (open arrows) ventral to the preoptic nucleus, in an area termed the anterior periventricular nucleus by Peter and Gill (1975). Scale = 10 μm.

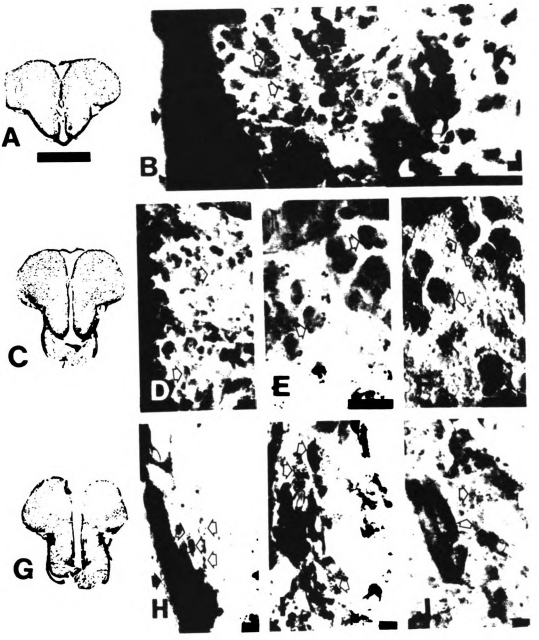

Figure 5

Figure 6. Labeling of presumed neurosecretory cells in caudal preoptic and adjacent ventral thalamic regions of a 4.3 g goldfish following IP injection of HRP.

A. Section through caudal optic chiasm and PA. Asterisk indicates area of neuronal labeling enlarged for Figure 6B. Scale = 1 mm for Figure 6A, 6C and 6E.

B. Small lightly labeled cells are found in a periventricular position (small open arrow). A few slightly larger and more heavily labeled neurons are found dorsally (large open arrows). Cells in this position have been termed magnocellular since in larger fish neuronal diameters can reach 70 μm. e = erythrocyte. Solid arrow denotes ependyma. Asterisks (also Figure 6F, 6G) indicate large blood vessels coursing through the large-celled region. Scales = 10, 100 μm.

C. Section through transition zone between caudal PA and ventral thalamus. An isolated neurosecretory cell was found at the arrow. Several small labeled cells were found in the SCN on each side. Asterisk indicates position of Figure 6D.

D. Labeled neurons (arrows) in SCN. Scale = 10 μm.

E. Section through caudal diencephalon (ventral) and valvula cerebelli and optic tectum (dorsal). Arrows indicate position of labeled neurons in caudal ventral thalamus (dorsal hypothalamus?), enlarged in Figure 6F and G.

F. Arrows indicate labeled neurosecretory cells in the ventral thalamus. Scale = 100 μm.

G. Enlargement of the left side of Figure 6F showing labeled ventral thalamic neurons (arrows). Scale = 25 μm.

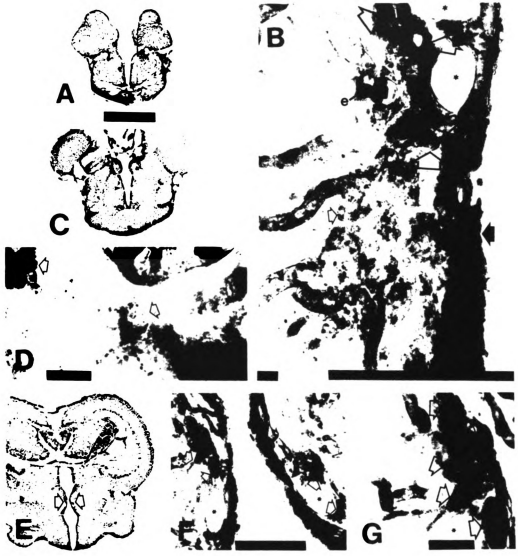

Figure 6

parvocellular and magnocellular subnuclei are not readily apparent, especially in these small fish.

In well-labeled preparations, small (5-10 μm, but usually 5 μm diameter) labeled neurons were found ventral to the heavily labeled preoptic cell group, between it and the optic chiasm and horizontal commissure (Figures 1A, 5G, 5J, 6A, 6B). This region has been termed the anterior periventricular nucleus (Peter and Gill, 1975). A few labeled cells were also found in a ventrolateral extension of this general area, in a portion of the preoptic area which receives a particularly heavy retinal input (Springer and Gaffney, 1981) and can be called suprachiasmatic nucleus (SCN; Figures 1A, 6C, 6D). Scattered small cells of the periventricular preoptic nucleus (Peter and Gill, 1975) contained a few granules of reaction product (Figures 5G, 5H). These cells were dorsal to the more heavily labeled and larger cells of the more vascular preoptic nucleus (Figures 5G, 5I).

Cells resembling the heavily labeled 10-15 μm neurons of the highly vascular preoptic (parvocellular/magnocellular) nucleus extended caudally far beyond the PA, however defined. The PA of fish is interposed between basal telencephalon rostrally and definitive diencephalon (especially putative ventral thalamus, in a periventricular location). About one to five heavily labeled multipolar cells were found per section per side in both rostral and caudal parts of the periventricular ventral thalamic area (PVT, Figures 1A and 6C, 6E-G), specifically in Schnitzlein's (1962) "nucleus ventromedialis (ventral thalamus) pars medialis". The largest neurosecretory cells observed in the small fish

were up to 25 μm diameter labeled neurons in the caudal ventral thalamus (Figures 6E-G).

The direct placement of HRP into the fish hypophysis without substantial diffusion or extraneous damage was not achieved. Limited success has, however, been attained with several HRP injections via orbital dissection (unpublished observations). Transorbital hypophysial injection virtually obliterated the pituitary and allowed HRP to spread to surrounding spaces, but also yielded heavy labeling of neurons in the PA corresponding to that described above. However, the brain was also damaged as light extracellular reaction product was found in areas of telencephalon, where some neurons were lightly labeled. Heavy labeling was observed in brainstem fibers and somata attributable to cranial nerves II-VI coursing in the vicinity of the pituitary and compromised orbit. Somata were labeled in the oculomotor, trochlear and abducens nuclei, and anterograde labeling was noted in the optic nerve and tract and in putative trigeminal fibers seen in the facial lobe. Laterality was clearly evident in this brainstem labeling (but knowledge of the laterality of sections was lost in the processing of this tissue), and no label was seen in more caudal nuclei which could have been labeled only by vascular spread. Thus, these few intrahypophysial HRP injections add support to the contention that at least a major portion of the IP HRP-labeled preoptic neurons do indeed have axons projecting to the pituitary.

Systemic Peroxidase Labeling in Larger Fish (38.1-75.0 g)

Brain size increases noticeably with body size as the fish grows (compare Figures 1-4). Nonetheless, the pattern of neuronal labeling

following the systemic administration of HRP remains similar (Figures 1A, 1B and 2). A path of labeled cells was again found stretching from the rostroventral preoptic recess in a periventricular location to the caudal parts of the ventral thalamic area. Neurons in most areas were larger in larger fish. The most rostroventral labeled cells, however, were still about 5 μm in diameter, resembling similarly placed small cells in small fish. Such cells had little cytoplasm surrounding a spherical nucleus, and contained only a few granules of reaction product (Figures 7A, 7B). Intermixed with these cells, and caudodorsal to them there were larger cells (about 10 μm diameter) containing relatively more cytoplasm and many more granules of reaction product (Figures 7C, 7D). This group (the preoptic nucleus of Peter and Gill, 1975, initially their rostral parvocellular subdivision) could be followed dorsocaudally as cell size gradually increased, with caudal preoptic cells reaching at least 30-40 μm diameter in the 38.1 g fish (Figures 7E-G, see also 50 μm somatic profile from 35.0 g fish in Figure 12A), and with some cells clearly approaching 50 μm in the 75.0 g light microscopic HRP IP fish. These large cells were filled with granules of HRP reaction product and were closely packed in a highly vascular region (Figure 7G). Electron microscopy has revealed that caudal cells are even larger in larger fish, with 70 μm diameter neuronal profiles not unusual in the caudal PA of 105.6-283.1 g fish (Figure 17B). Small lightly labeled cells were also found in the SCN, and larger more heavily labeled cells were found in the ventral thalamic area in positions corresponding to the sites of labeling in smaller fish (Figures 1B, 2 and 7H-J).

Figure 7. Neuronal labeling in preoptic and ventral thalamic areas of 38.1-75.0 g fish following HRP IP administration.

A. Section through rostral PA of 48.1 g fish. Asterisk indicates location of small labeled neurons enlarged in Figure 7B. Scale = 1 mm for Figure 7A, 7D, 7E, 7H and 7J.

B. Small cells with little cytoplasm (arrow)lightly labeled by systemic HRP. Asterisk denotes ependyma. Scale = 10 μm.

C. Slightly larger neurons (arrows) containing more granules of reaction product per cell. These cells are in closer proximity to capillaries than those of Figure 7B. Enlargement of area from Figure 7D. Scale = 10 μm. Nomarski optics.

D. Section through PA slightly caudal to level of Figure 7A.

E. Further caudal section through PA of 38.1 g fish. Neuronal labeling in area of asterisk enlarged in F.

F. Closely packed neurons (open arrows) in highly vascular caudal PA of 38.1 g fish. Tendency for location of larger cells dorsal and caudal in PA, more evident in even larger individuals, is morphological basis for variously described parvo-magnocellular subdivisions of the PA. e = erythrocyte. Solid arrow denotes ependyma. Scales = 100, 20 μm.

G. Large (giant in larger fish) labeled dorsal cells (open arrows) from an adjacent section viewed with Nomarski optics. These cells are characteristically located around a large blood vessel (asterisk) communicating with a rich capillary plexus. Solid arrow = ependyma. e = erythrocyte. Scale = 25 μm.

H. Portion of section through rostral ventral thalamus, habenula and tuberal hypothalamus of a 75.0 g fish which received IP HRP. Arrow indicates position of several large labeled neurosecretory cells (per side) located in the ventral thalamus, caudal to the PA.

I. Labeled cells (arrows) from position of arrow in Figure 7H. Medial is located to the right. Scale = 25 μm.

J. Section through posterior commissure, valvula cerebelli, tectum, ventral thalamus and inferior lobe of 75.0 g fish. Arrow indicates position of labeled neurons (enlarged in Figure 7K) in presumed ventral thalamic area.

K. Neurosecretory cells of the caudal ventral thalamus. Medial is located to the left. Scale = 25 μm.

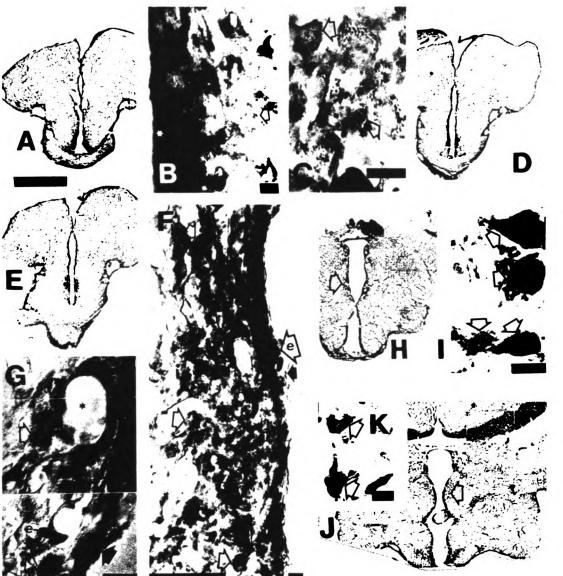

Figure 7

Forebrain Descending Projections in the Small Fish (4.3-6.6 g)

The application of HRP to the transected spinal cord of small fish produced light labeling of neurons in portions of the preoptic and ventral thalamic areas (Figures 3 and 8E-F). Capillary endothelial cells were lightly labeled throughout the brain of spinal fish (Figure 8), indicating the spread of HRP to the blood and thus the potential for labeling via hypophysial projections. Vascular leakage is not a likely explanation for the neuronal labeling which was obtained, however, since the total amount of HRP which was applied to the transected cord ($\leq$ 0.02 mg/gbw) is above the threshold for capillary labeling but far below the threshold for slight neuronal labeling ($\approx$ 0.5 mg/gbw) for small fish via HRP IP injection, and the application of the former amount directly to the uncut spinal cord gave slight capillary but no neuronal labeling. Likewise, following spinal transection in larger fish, moderately intense labeling was seen in a few cells per section, with much of the surrounding PA entirely unlabeled (Figures 8A-D, and compare Figures 1A and 3), whereas preoptic labeling of this intensity via vascular HRP would require much larger systemic administration, and would then label neurons in a much larger area. Furthermore, while the HRP-labeled preoptic and ventral thalamic spinal projecting cells have ultrastructural features resembling neurosecretory cells, they appear to be distinct from the latter, having relatively few DCVs (unpublished observations). The precise pattern of labeling resembled that obtained in larger fish, described below (compare Figures 3 and 4).

Figure 8. Neuronal labeling in caudal preoptic and ventral thalamic areas following the application of HRP to the transected spinal cord of 42.3 and 4.3 g fish.

A. Section through the caudal PA of 42.3 g fish. Arrow denotes position of neuronal labeling, enlarged in Figure 8B. Scale = 1 mm for Figure 8A, 8D, 8F and 8H.

B. Open arrows indicate labeled neurons, located on the ventrolateral edge of the caudal preoptic neurosecretory group. Cells to the left of the dotted line were predominantly unlabeled in spinal experiments but were heavily labeled when HRP was circulating in the blood after IP injection. Small solid arrows indicate lightly labeled border cells. Large solid arrow indicates ependyma. e = erythrocyte. Asterisks denote large blood vessels (also in Figure 8C). Scales = 25, 100 μm.

C. Enlargement from Figure 8D of rostral ventral thalamus. Small arrows indicate peroxidase-labeled neurons. Large arrows indicate large unlabeled cells, presumed (from other experiments) to be probably neurosecretory. Scales = 50, 200 μm.

D. Section through habenula and tuberal hypothalamus of 42.3 g fish. Arrows indicate position of labeled neurons (enlarged in Figure 8C) in rostral ventral thalamus.

E. Neuronal labeling (open arrows) from similar rostral ventral thalamic position of a 4.3 g spinal fish (enlarged from Figure 8F). Solid arrow indicates ependyma. Nomarski optics. Scales = 10, 50 μm.

F. Section through rostral ventral thalamus (arrow) of 4.3 g fish. Labeled neurons enlarged in Figure 8E.

G. Labeling of further caudal ventral thalamic neurons (small arrows) of 42.3 g fish (enlarged from Figure 8H). Large arrow indicates ependyma. Scales = 10, 50 μm.

H. Arrow indicates position of labeled ventral thalamic cells (enlarged in Figure 8G), from 42.3 g fish.

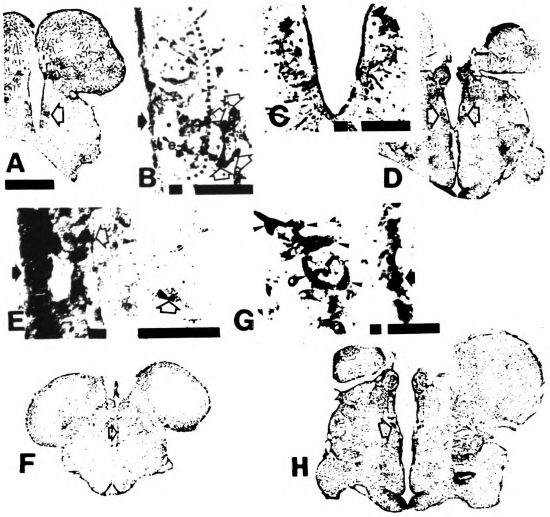

Figure 8

Forebrain Descending Projections in Larger Fish (16.0-44.5 g)

Labeled cells were not found in the portion of the PA which is actually located rostral to the optic chiasm (the preoptic nidulus of C. L. Herrick, 1892; Figures 3, 4) or in more rostral telencephalic regions. Labeled neurons were found in a horizontal column which begins dorsal to the optic chiasm (Figures 3, 4) located on the ventrolateral edge of the hypophysial projecting cells of the preoptic nucleus of Peter and Gill (1975; see Figures 8A, 8B and compare the positions of spinal and hypophysial labeling in Figures 4, 3, 1B, 1A, respectively). The spinal efferent cell population continued caudally from the PA into the ventral thalamus.

In the ventral thalamus, nucleus ventromedialis pars medialis of Schnitzlein (1962), there were typically 3-6 loosely packed pleomorphic labeled cells per section per side (Figures 8C-H). Occasionally, one or more large unlabeled cells were found amongst the smaller heavily labeled multipolar spinal efferents. These unlabeled cells resemble in size, shape, position and number the cells of the ventral thalamic area which were labeled following IP HRP injection (Figures 8C-D, compare with Figures 7H-J and 6E-G). Comparisons of the brainstems of fish receiving IP or spinal cord HRP revealed very different patterns of neuronal labeling, a further argument against labeling via vascular leakage. IP HRP produced light retrograde labeling clearly associated with cranial nerves. This was generally not the case in spinal experiments, where reticular-like cells were labeled, sometimes intensely. However, some neurons of the oculomotor nucleus were intensely labeled

(more so than in IP experiments) in spinal fish. Such cells may be homologs of cells in the nucleus of the medial longitudinal fasciculus of larval zebrafish which project to the spinal cord (Kimmel, Powell and Metcalf, 1982).

Ultrastructure

The ultrastructural appearance of goldfish preoptic neurosecretory cells is generally consistent with previous reports on various teleosts (Palay, 1960; Lederis, 1962, 1964; Follenius, 1963; Ötzen, 1966; Leatherland and Dodd, 1969; Ekengren, 1973; Peter and Nagahama, 1976; Vigh-Teichmann, Vigh and Aros, 1976; Fryer and Boudreault-Chateauvert, 1981; Cumming, Reaves and Hayward, 1981; Reaves, Cumming and Hayward, 1982) as well as descriptions of homologous regions of other vertebrates (Zambrano and deRobertis, 1967; Tweedle and Hatton, 1976, 1977; Morris et al., 1978; Gonzáles and Rodríguez, 1980; Gregory et al., 1980; Theodosis et al., 1981; Hatton and Tweedle, 1982 and others). To facilitate comparisons between electron micrographs, most (except Figures 17B and 19) are reproduced at the same magnification (4250x).[1]

1.7-6.6 g Fish

Neurons of the caudal PA of small fish have rudimentary morphological features typical of neurosecretory cells. Somatic profiles in this region of a 1.7 g fish were roughly 10 μm in diameter, with small

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[1] In order to comply with margin requirements of the graduate school, most figures have been photographically reduced from plates prepared for publication.

stacks of granular reticulum, nearly spherical nuclei, a small Golgi and with prominent lysosomes and DCVs (Figure 9A). The size of similarly positioned cells in 4.6-6.6 g fish was typically 10-15 μm (Figures 9B, 9C, see also Figures 5A, 5B). Clumps of neurons with areas of direct soma-somatic apposition were separated by neuropil. Virtually all of the neuronal somatic profiles were labeled in HRP IP experiments. Located within the neuropil between neurons were numerous electron dense glial cells which may be oligodendrocytes. These cells were either isolated or in chains which tended to very loosely and incompletely parallel the surface of a few neurosecretory somata (Figure 9B). The shape of neuronal nuclei was clearly irregular in a few of the largest cells in the larger fish in the group (Figure 9C). More rostral and ventral, in areas which were labeled in light microscopic HRP IP experiments (Figure 5A-F, 5J), neuronal somatic profiles had spherical nuclei with sparse strands of granular reticulum and few or no DCVs. However, occasional somata and more commonly their processes did contain DCVs (Figure 10A). Similar and further rostral smaller cells lacking DCVs contained reaction product in EM IP HRP experiments (Figure 10B) as did similarly placed cells in analogous light microscopic experiments.

12.9-16.6 g Fish

Neurosecretory cells of the caudal PA of these fish clearly differed from correspondingly placed cells of smaller fish. Neuronal cell bodies of this area were larger, often extensively apposed, containing larger and more irregularly shaped nuclei (Figure 11A). Granular reticulum was more extensive, clearly organized into stacks, and DCVs

Figure 9. Electron micrographs of neurosecretory cells of the caudal PA of small (1.7-6.6 g) fish. Position corresponds to the traditional "magnocellular" preoptic nucleus of larger individuals. Scale = 10 μm.

A. Largest neuronal somata found in the PA of a 1.7 g fish are small, but otherwise typical of vertebrate neurosecretory cells.

B. Similar area from 4.2 g fish sacrificed four days after HRP IP injection. Clusters of soma-somatically apposed neurons are labeled with reaction product in lysosomes (smaller arrows). Neuropil separates groups of apposed neurons. Strings of oligodendrocytes (larger arrows) in neuropil loosely surround some neurosecretory somata (compare with Figures 13, 14A, 14B).

C. Occasional labeled somata of the caudal PA of 4.2 g fish are slightly larger, with nuclei more convoluted than those of smaller labeled cells (compare with Figures 9A, 9B). Larger arrows indicate reaction product in lysosomes, and smaller arrows denote endothelial labeling.

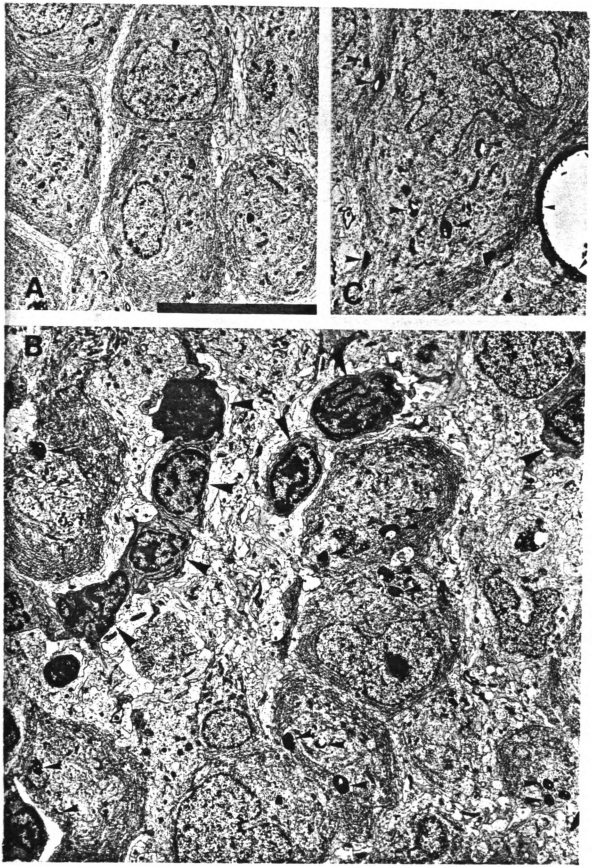

Figure 9

Figure 10. Electron micrographs of neurons of the rostral preoptic area of small (6.0-6.6 g) fish. Neurons rostral to those of Figure 9 in small fish have few or no DCVs and are smaller than more caudal cells. Scale = 10 μm.

A. Cells with rudimentary stacks of granular reticulum, a few DCVs in soma (smaller arrows) but containing more DCVs in a process. Large arrow indicates a lysosome. 6.0 g non-HRP fish.

B. Rostral PA of a 6.6 g HRP IP fish. Somata rarely contain DCVs (smaller arrows). Many cells contain electron dense reaction product in lysosomes (larger arrows). Labeled lysosomes were far more electron dense than nonlabeled ones. Similar appearance of lysosomes in Figures 10A and 10B results from matching contrasts of prints for plate.

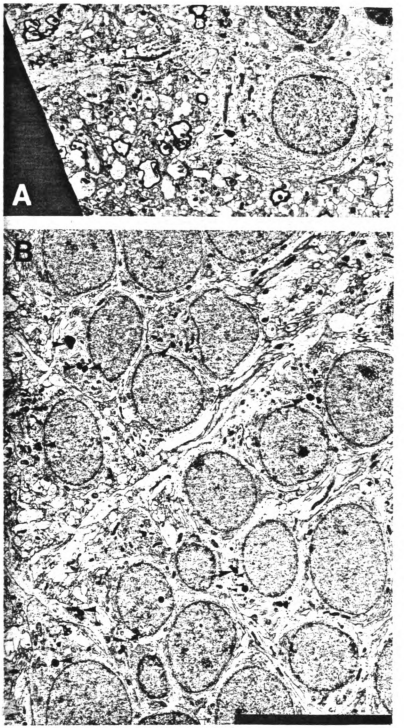

Figure 10

were generally prevalent. More rostral cells were progressively smaller, with more nearly spherical nuclei and less extensive granular reticulum (Figure 11B). The most rostral and smallest cells of this preoptic neuronal population were not heavily populated with DCVs. The few profiles of granular reticulum present were generally distributed through the cytoplasm of these cells, although rudimentary stacks consisting of several parallel profiles of cisternae were sometimes seen. Further rostral cells bore no obvious resemblance to neurosecretory cells, lacking DCVs and possessing a small complement of organelles in their limited amount of cytoplasm.

26.8-70.1 g Fish

A few neurons in the caudal PA of these fish were extremely large (Figure 12A). Profiles with diameters of 50 μm had large irregular nuclei which sometimes appeared to be multiple. Enormous masses of dispersed and stacked granular reticulum were present, intermixed with numerous DCVs, lysosomes and mitochondria. The cytoplasm of similar large somata of this area occasionally contained vacuoles filled with a flocculent substance (Figure 12B). Some 5-10 μm diameter profiles in the neuropil of this region (possibly proximal dendrites) were filled with a similar appearing substance, surrounded by only 0.1-1.0 μm of cytoplasm which in some instances contained DCVs (Figure 12C).

Slightly rostral to the largest, and apparently multinuclear caudal preoptic neurosecretory cells of large fish, were several moderately large (20-40 μm) neurons either tightly or loosely encased by electron dense glia tentatively termed oligodendrocytes

Figure 11. Electron micrographs of the preoptic area of larger (12.9-16.6 g) fish. Correspondingly placed cells are larger than in smaller fish. Scale = 10 μm.

A. Closely packed neurosecretory cells of the caudal PA of a 13.7 g fish. Soma-somatic appositions between adjacent cells are common. Stacks of granular reticulum (asterisks) are larger than in smaller cells of smaller fish. This fish received an IP injection of HRP, but few of the lysosomes were clearly labeled (arrows).

B. More rostral cells of a 15.3 g fish are smaller with small stacks of granular reticulum, a few DCVs (arrows) and nearly spherical nuclei.

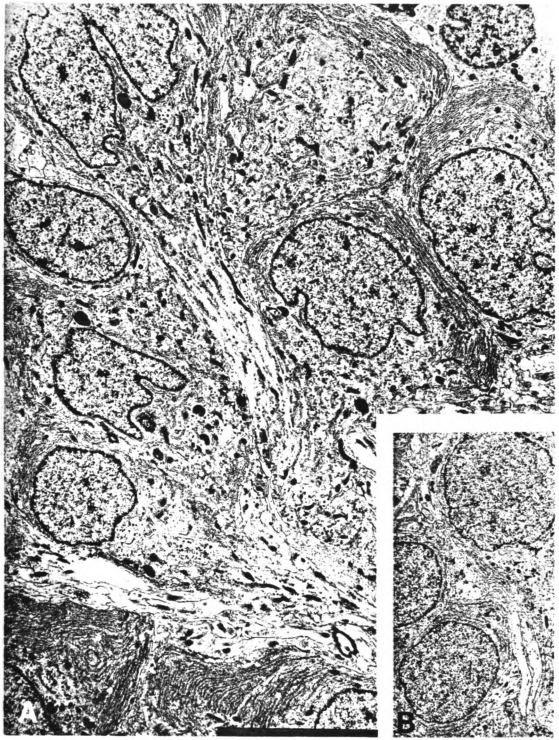

Figure 11

Figure 12A. Electron micrograph of apparently multinuclear 50 μm diameter neuron in the caudal PA of a 35.0 g fish. Similar cells in larger ($\geq$ 105.6 g) fish reach 70 μm. Note extensive complement of granular reticulum, free ribosomes, lysosomes and mitochondria, and zones of soma-somatic membrane apposition (arrows). Scale = 10 μm.

B. Somatic vacuole (arrow) containing a flocculent substance from a similar cell in the caudal PA of the same fish.

C. Vacuoles extend into processes (larger arrows) located in adjacent preoptic neuropil. Taken from a 138.5 g fish obtained from a pond in late fall. Vacuole is surrounded by a thin ring of cytoplasm containing a DCV (smaller arrow).

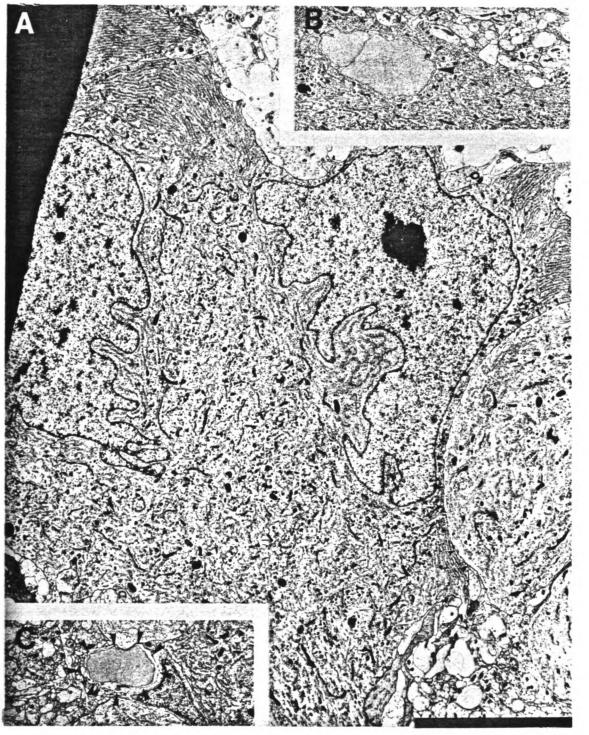

Figure 12

(Figures 13, 14). Neuronal soma-somatic appositions were not possible where cells were encased, but occurred when glial encasement was incomplete (Figure 13). Electron lucent glial processes (probably radial glial and/or astrocytic) frequently separated the oligodendrocytic sheath from the neuronal somata (Figures 13, 14B). Synapses were occasionally seen on these neuronal somata, beneath the oligodendrocytic sheath (Figure 14B). Processes extended from the oligodendrocytes deep (5-10 μm) into the neuronal somata (Figure 14A) or formed end feet on capillaries (Figure 13). Unidentified electron lucent processes, probably astrocytic or radial glial, also penetrated these neuronal somata (Figure 13). Some of these neuron-glial complexes were located immediately beneath an attenuated ependyma (Figure 14A). The nuclei of glial-encased neurons were either extremely convoluted (Figures 13, 14B) or likely multiple (Figure 14A). Small (5-7 μm) nearly spherical cells with electron lucent cytoplasm were frequently invaginated in the somata of these glial-encased neurons and occasionally in other large neurosecretory cells of the area (Figure 14B). Membrane specializations such as synapses, gap junctions and desmosomes were <u>not</u> observed between these small cells and the much larger neurosecretory cells in which they were embedded. Whether these small cells were neurons is not entirely clear, but they do not appear to be glia. Processes have not been traced from these cells, and they have not been observed as either presynaptic or postsynaptic elements. They were not, however, studied with extensive serial sectioning. In a larger (105.6 g) fish, a 4 μm spherical DCV-containing cell was deeply embedded into a 20-25 μm diameter glial-encased neuron. In one 6.0 g fish, a 30 μm

Figure 13. Electron micrograph of glial-encased neurosecretory cell with lobulated nucleus from 35.0 g fish.

Some neurosecretory cells intermediately positioned between large caudal and small rostral DCV-containing preoptic cells of large ($\geq$ 35.0 g) fish are surrounded by oligodendrocytes (intermediate sized arrows) which can form foot processes in capillaries. Astrocytic processes (large solid arrows) frequently separate oligodendrocytes from neuronal soma. Small solid arrows indicate neuronal soma-somatic membrane apposition where glial encasement is incomplete. Open arrows indicate processes of undetermined type which extend deeply into the neuronal soma. Note lobulated appearance of large nucleus. 35.0 g fish, scale = 10 μm.

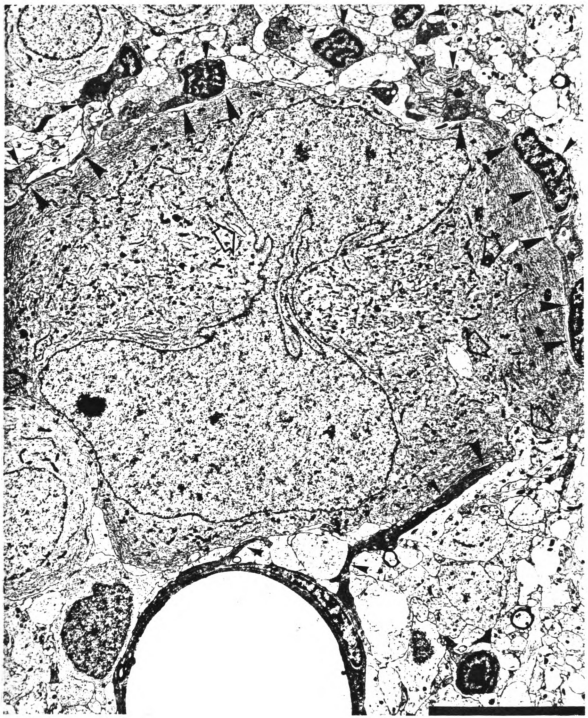

Figure 13

Figure 14. Electron micrographs of neurosecretory cells surrounded by oligodendrocytes (compare with Figures 9B and 13). PA of 35.0 g fish, scale = 10 μm.

A. Oligodendrocytes (solid arrows) closely surround and penetrate (open arrows) the multinuclear soma of a neurosecretory cell located immediately beneath an attenuated ependyma (Ep). Large vessel is visible at lower right.

B. Larger neurosecretory cell surrounded by oligodendrocytes (large solid arrows). Astrocytes and neuronal processes (intermediate sized solid arrows) and a synapse (smaller open arrow) fit between the oligodendrocytes and this neuronal soma. The nucleus of this large neuron is probably lobulated. A tenuous connection between nuclear profiles is nearly complete within this plane of section (small solid arrows). Note small electron lucent cell embedded within the large glial-encased neuron (larger open arrow).

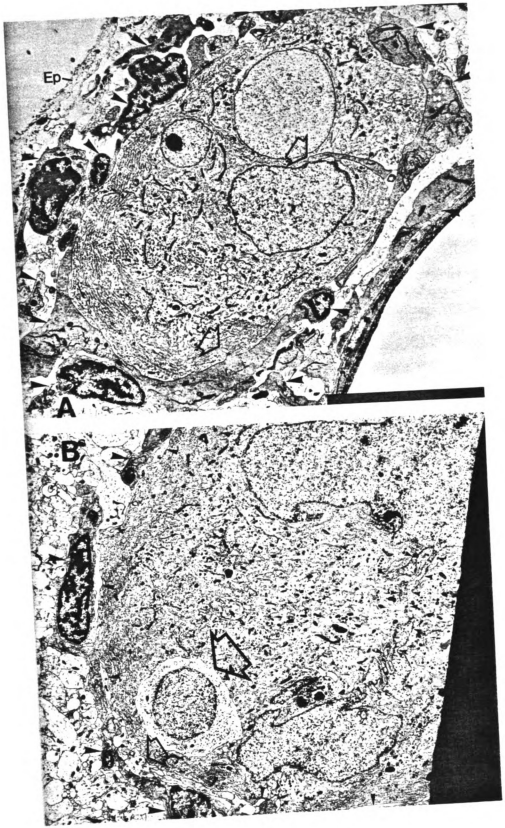

Figure 14

neurosecretory cell with a roughly spherical single nucleus was completely and tightly ensheathed by oligodendrocytes. This cell was also deeply penetrated by an oligodendrocytic process. More typically, neurons in the preoptic nucleus of small fish are smaller and occasionally surrounded by oligodendrocytes, which are not as flat as those which surround larger neurons (Figures 9A, 13, 14A, 14B).

More rostral preoptic neurons were mononuclear, with a gradation of cell size (larger caudal to smaller rostral) and ultrastructural features typical of neurosecretory cells (i.e., granular reticulum, DCVs, Golgi, neuronal soma-somatic membrane appositions) varied in relation to cell size (Figure 15A).

105.6-283.1 g Fish

Preoptic neurosecretory cells of these large fish assumed a wide range of forms. Cells of 10-70 μm diameter had similar morphological features typical of neurosecretory cells. As electron microscopic HRP IP experiments were not entirely successful in these large fish, it is not clear whether smaller further rostral cells lacking DCVs should be included in the same preoptic neurosecretory cell population. Rostral 10-15 μm diameter cells had spherical nuclei and an assortment of organelles including DCVs (Figures 16A-C). Such cells were usually in extensive soma-somatic apposition with surrounding cells (Figure 16B), but were occasionally separated from nearly adjacent similar cells by velate astrocytic processes (Figures 16B, 16C). More caudally placed neurosecretory cells were progressively larger, with larger and more irregularly shaped nuclei. Neuronal soma-somatic membrane appositions

Figure 15. Electron micrograph of neurosecretory cells of the rostral PA of a 35.0 g fish, and microglia and degeneration in a non-experimental 105.6 g fish.

A. Neurosecretory cells of the rostral PA of 35.0 g fish. Note nearly spherical single nuclei of these neurons, compared with the large lobulated and multiple nuclei found in glial-enwrapped and further caudal and larger cells. Small cell at right contains a few DCVs (small arrows), while a similar cell near the top (intermediate-sized arrows) lacks DCVs in this section. Observe variation in nuclear and somatic size, extent of granular reticulum and number of DCVs which could be indicative of differences in functional (maturational) state of otherwise similar neurons. Large arrow indicates a probable microglial cell, resembling those of Figure 15B. Scale = 10 μm

B. Electron dense cells (larger arrows) containing numerous dense includions were infrequently associated with degenerating material (smaller arrows) in large non-experimentally treated fish. Caudal PA of 105.6 g fish.

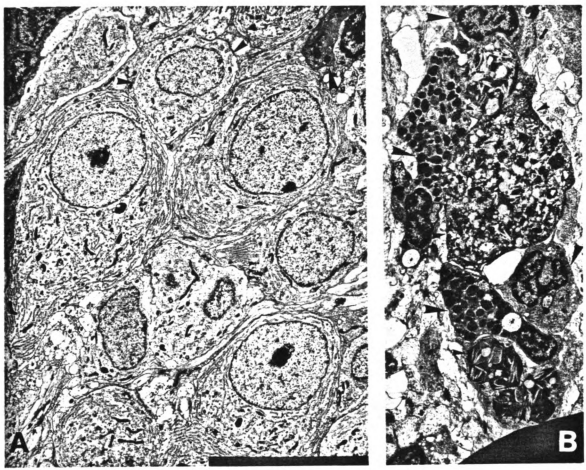

Figure 15

Figure 16. Electron micrographs of neurosecretory cells of the rostral preoptic area of a 105.6 g fish. Sequence of DCV-containing small rostral to large caudal (Figure 17) neurosecretory somatic profiles. 105.6 g fish, scale = 10 μm.

A. Small rostral neurons containing DCVs (arrows), small spherical nuclei and a limited complement of organelles.

B. Larger rostral cells with more organelles. Note large amount of soma-somatic neuronal membrane apposition (small arrows). Neuronal somata are occasionally separated by fine astrocytic processes (larger arrows).

C. Neurons separated by a double layer of fine astrocytic processes (arrows) from an area close to cells of Figure 16B in the rostral PA.

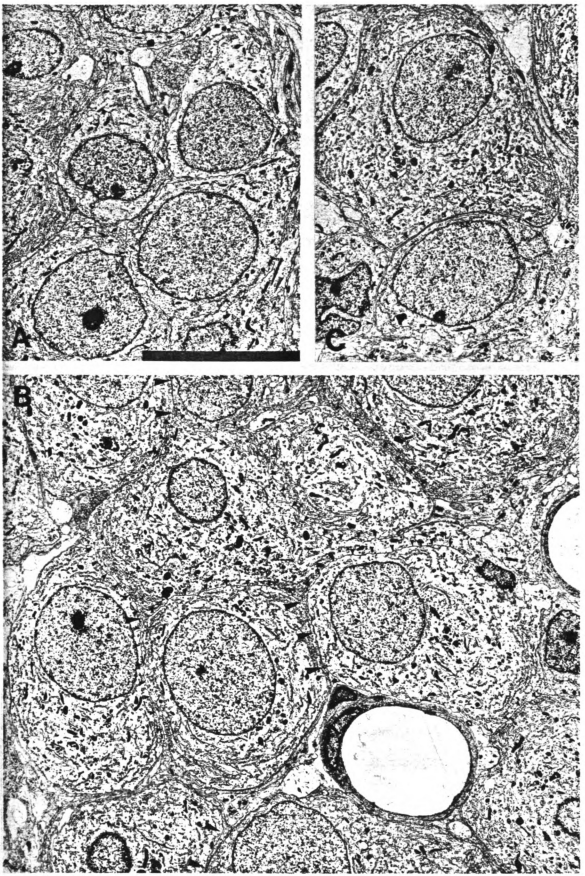

Figure 16

remained common (Figure 17A). Caudal preoptic somatic profiles were as large as 70 μm in diameter, and apparently multinuclear. Vacuoles containing a flocculent material of variable appearance reached 50 μm in diameter in some large caudal cells (Figure 17B). Extravacuolar cytoplasm was tightly packed with organelles typical of neurosecretory cells, including granular reticulum, Golgi, lysosomes, mitochondria and DCVs. Extensive soma-somatic neuronal membrane appositions were common in all parts of the preoptic nucleus, except for the oligodendrocyte-encased cells. In the caudal PA, profiles of only a few very large cells were found per section but these were extensively apposed, with appositions of 20 μm in length not unusual (Figure 12A) and occasional appositions reaching 50 μm (Figure 18). Profiles with several 20 μm appositions were common. In more rostral sections where cells were more frequent and smaller, appositions of 10-20 μm were still common, and these smaller cells were also frequently apposed to several surrounding cells within the same thin section (Figures 17A, 16B).

Microglia

One of more large vessels (40-60 μm diameter in large fish) course longitudinally through the dorsocaudal PA and PVT (Figures 6, 7, 8, 14A). These vessels are often surrounded by 1-3 layers of smooth muscle. Perivascular cells with electron dense cytoplasm and numerous dense inclusions frequently were clustered around these large vessels. These pericytes were sometimes clearly surrounded by the basal lamina of the smooth muscle, but were also found in the preoptic neuropil.

Figure 17. Electron micrographs of neurosecretory cells of intermediate size and position, with extensive soma-somatic apposition, and of large vacuolated caudal cell, from a 105.6 g fish.

A. Intermediate-sized cells located intermediate between small rostral (Figure 16) and large caudal cells (Figure 17B, see also Figure 12A). Note large convoluted nuclei, masses of organelles and extensive soma-somatic apposition (smaller arrows) which is occasionally interupted by astrocytic processes (larger arrows). 105.6 g fish, scale = 10 μm.

B. Large multinuclear vacuolated neuron of the caudal PA of 105.6 g fish. Note magnification reduced to 1700X. Scale = 10 μm. Enormous vacuole is surrounded in extensive areas by only a thin layer of cytoplasm (small arrows). Inset, at same magnification, depicts a portion of the cell which was obscured by a grid bar, taken from an adjacent section. Contrast of inset altered to demonstrate intravacuolar contents. Large arrows denote extensions of cytoplasm into vacuole. Arrows of intermediate size indicate membranous structures of various forms located within the vacuole. Small arrow in inset same as in remainder of cell. The entire vacuole was filled with an electron lucent fluocculent substance, which was more dense within some of the membranous structures.

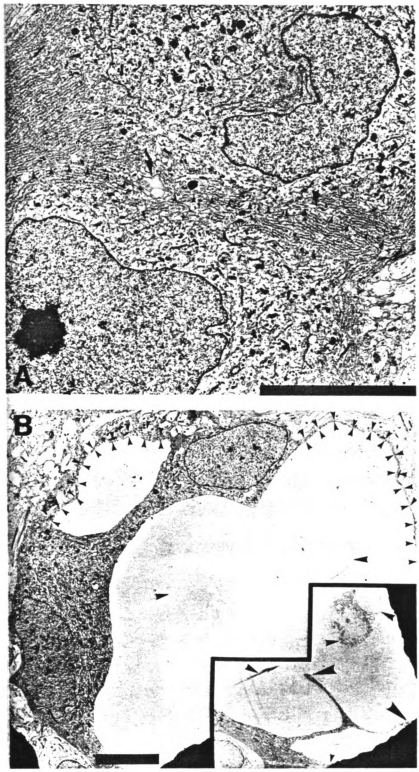

Figure 17

These cells are tentatively classified as microglia since they were occasionally located around degenerating material in the PA of apparently normal large fish (Figure 15B).

Seasonal Variations in Structure and the Issue of Gap Junctions

Extensive examination throughout the preoptic neurosecretory area of a considerable number of small and large fish revealed no definitive indication of neuronal gap junctions involving neurosecretory somata, although small or infrequent junctions could easily have been missed (see below). Desmosomes were present between apposed membranes. Subsurface cisternae were not infrequently seen closely associated with apposed neuronal membranes.

However, most of the fish of this report were maintained indoors at room temperature or were obtained from a Michigan pond during the summer, the mating season for goldfish. Several similar large fish were captured from the same pond in mid-November. These fish were extremely sluggish as they were removed from cold water on a relatively dark day. Gross examination after perfusion revealed that the gonads of these fish were much reduced in size and often indistinct compared with even the smallest of fish adapted to warm water. Thus physiological and endocrine differences between seasons are likely. In these late fall individuals neuronal soma-somatic membrane appositions were extremely common among preoptic neurosecretory cells and appear to be more extensive than during the summer, although regional variations in cytoarchitecture and the small number of fish precluded adequate

sampling for quantitative investigations. Soma-somatically apposed neuronal membranes were frequently jagged in late fall fish, producing a degree of interdigitation between apposed neuronal somata rarely seen in summer individuals (Figure 18). Zones of soma-somatic membrane apposition 50 μm in length were not uncommon in fall fish. Appositions of this length and interdigitating apposed membranes were infrequently observed in 35.0-283.1 g warm water fish. In one fall fish processed with potassium ferrocyanide-osmium several distinct gap junctions were seen between neurosecretory somata and neurites (Figure 19). In goldfish neurosecretory cells this fixative advantageously preserved cellular membranes and lysosomes, making them extremely electron dense, but rendered the appearance of cells atypical. Ribosomes, chromatin and DCVs which are striking features of typically prepared neurosecretory cells were only lightly stained after the ferrocyanide procedure. Very extensive and interdigitating appositions were also seen in fall fish processed without ferrocyanide treatment, but no gap junctions were observed. This difference may result from sampling or real differences between animals, or may be the result of the improved preservation of membranes with ferrocyanide treatment. The nuclei of large cells in large fall fish were not highly convoluted as were those of correspondingly positioned cells of summer fish (compare Figures 17A and 18). Nuclear profiles were nearly spherical except for a scalloped conformation in the larger cells (Figure 18). Large cells contained multiple nuclear profiles of this shape and thus were probably multinuclear rather than lobulated. Single, multiple and enlarged nucleoli were frequent in thin sections of fall preoptic neurosecretory cells

Figure 18. Electron micrograph of semi-caudal neurons of the preoptic area of a 164.6 g fish obtained in late fall, with long and interdigitating soma-somatic membrane appositions. Scale = 10 μm. Tissue was processed with ferrocyanide-osmium which rendered Golgi and lysosomes extremely electron dense. Note large amounts of soma-somatic membrane apposition (arrows). Observe extreme length of apposition of membranes (sometimes cut tangentially) between upper two cells. Apposition between cells at upper and lower left extended further to the lower left a similar distance. Curvature of apposed membranes permits pronounced interdigitation rarely seen in summer fish (compare with curvilinear appositions of Figures 11, 12A, 13, 16A, 16B, 17A). Granular reticulum was commonly dispersed and only rarely organized into large stacks. Extremely convoluted nuclei were rare, even in large cells. Nuclear profiles often exhibited a scalloped appearance as in cell at lower left. This cell contained within this section another nuclear profile of similar size and shape. These profiles were separated by about 10 μm of cytoplasm. Nucleoli are prominent during late fall.

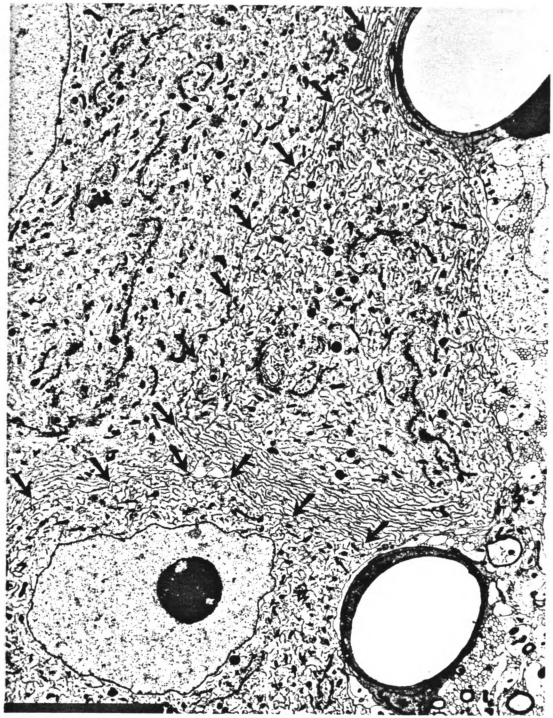

Figure 18

Figure 19. Electron micrograph of gap junction between neurosecretory cell and neurite.

A. Appositions between neurosecretory somata above (smaller solid arrows) and between somata and unidentified neurite below, from semi-caudal PA of 164.4 g late fall ferrocyanide-osmium fish. 25,000 x, scale = 1 μm. Larger solid arrows indicate DCVs. Open arrow indicates position of gap junction between neurosecretory somata and neurite, enlarged in Figure 19B.

B. Gap junction between preoptic neurosecretory cell (upper left) and neurite (lower right). 100,000x, scale = 100 μm.

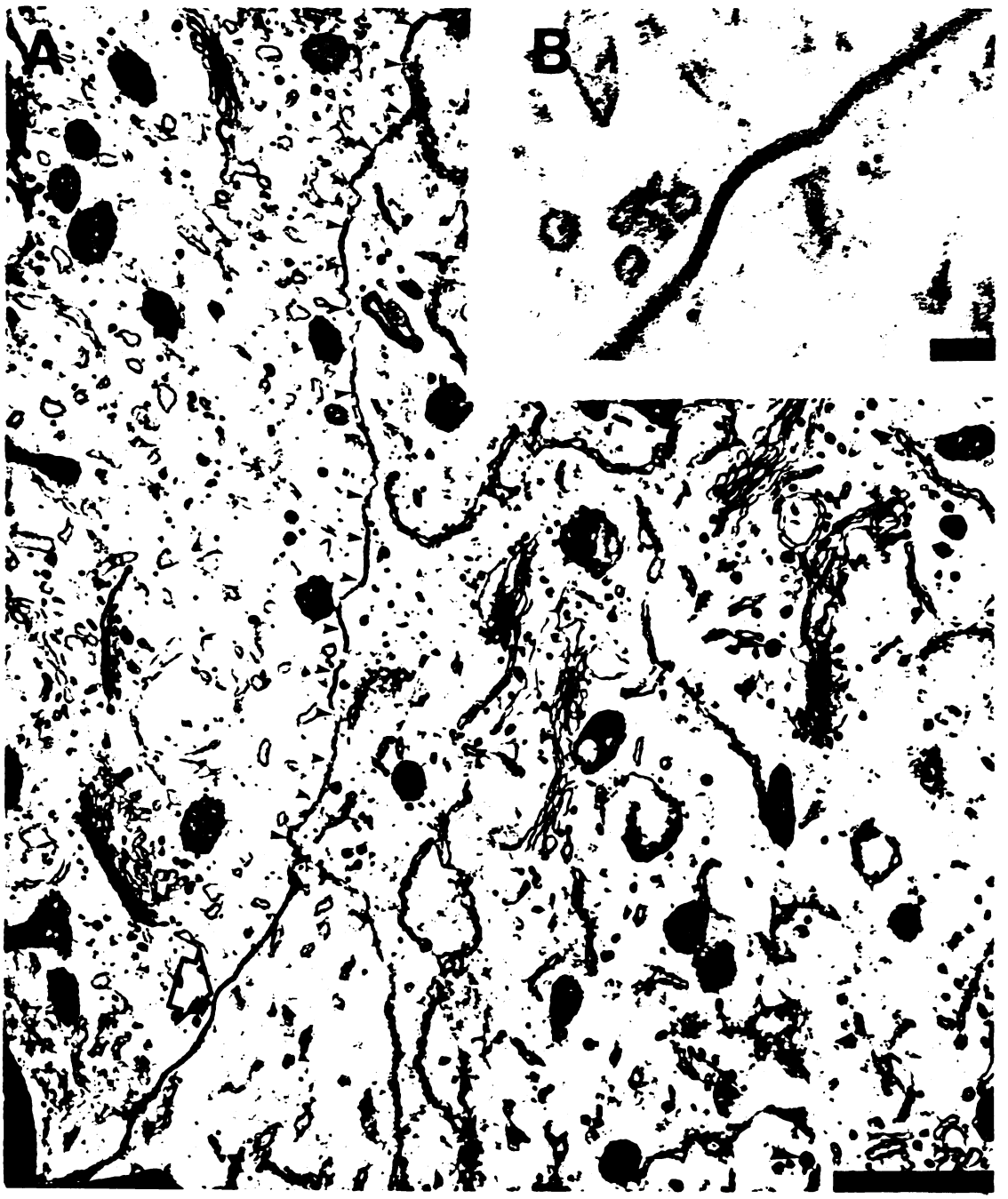

Figure 19

(Figure 18, compare size of nucleolus with nucleoli in Figures 9B, 10B, 12A, 13, 14A, 15A, 16A-C, 17A). Granular reticulum was less frequently organized in large stacks in these fall fish (Figure 18). Cytoplasmic vacuoles were found in the somata and in neurites (Figure 12C) of the caudal PA of these fish. The somatic vacuoles and vacuolated somatic profiles seemed smaller than the correspondingly placed structures of large summer fish, although morphometry was not carried out.

Cells Projecting to the Spinal Cord

Some neurons of the PA and PVT contained reaction product following the application of HRP or cytochrome c to the transected spinal cord (unpublished observations), in confirmation of analogous light microscopic results (Figures 3, 4, 8). These cells are ultrastructurally and spatially distinct from the cells discussed in the EM portion of the present report. Cells which were labeled in spinal experiments had very few or no DCVs but large numbers of ribosomes, both in rosettes and associated with dispersed (non-stacked) reticulum and contained a surprisingly large number of mitochondria (at least after spinal transection).

DISCUSSION

Light Microscopic Experiments and Preoptic Hodology

Despite previous reports of immunohistochemically and/or physiologically/morphologically identified neurosecretory cells in the teleost PA (Hayward, 1974; Watkins, 1975; Goossens et al., 1977a; Reaves and Hayward, 1980), the precise location of these identified cells has not been described in detail. It is apparent, however, that this immunohistochemically defined population of 5-70 μm diameter (in large goldfish) neurosecretory cells stretches across the putative parvocellular/magnocellular nuclear boundaries which have been variously described. The extension of this cell population across traditional boundaries has been confirmed in this report of neuronal labeling following the systemic administration of HRP, which demonstrated labeling in Peter and Gill's (1975) preoptic nucleus and beyond. These workers considered all of their preoptic nucleus, including rostroventral parvocellular and dorsocaudal magnocellular portions, to be neurosecretory since they found its neurons stained by paraldehyde fuchsin. Adjacent periventricular preoptic and anterior periventricular nuclei were not considered to be necessarily neurosecretory since these nuclei were not positively stained by the same procedure. Other investigators have subdivided the rostral periventricular/parvocellular/magnocellular preoptic cell mass into only two major divisions, a rostroventral

periventricular and/or parvocellular and an adjacent magnocellular portion (Charlton, 1932; Crosby and Showers, 1969; Terlou and Ekengren, 1979), with some magnocellular preoptic nucleus considered to be neurosecretory since the time of Scharrer (1941). A similar rostrocaudal distinction was made by C. L. Herrick (1892) who subdivided the area, relative to the optic chiasm, into adjacent preoptic and postoptic niduli which were considered to probably comprise a single functional entity. The postoptic position of a substantial portion of this entity in vertebrates (including mammals, Keyser, 1979) is largely ignored and obscured by literal interpretations of the term preoptic. Although a tendency for a rostrocaudal gradient of cell size is clearly found, subdivisions based on cell size or position are, according to present knowledge, arbitrary, since graded cellular variation occurs without apparent discrete boundaries (Charlton, 1932; Palay, 1960) and regional differences in function are obscure. Ultrastructural examinations of cellular variation as functions of position indicate a gradual transition of morphological features from small rostral to larger caudal cells (Palay, 1960; Gregory and Tweedle, 1981, 1982) with similar changes in cell size and ultrastructural features occurring in all regions as neurons enlarge (possibly by hypertrophy, discussed below) with increases in body size (Gregory and Tweedle, 1981, 1982). Therefore, the relationships between the periventricular, parvocellular and magnocellular nuclei of different workers are unclear, and reported "nuclear" boundaries are probably subject to maturational change through "adult" life. In addition, the terms parvocellular and magnocellular are used both in the fish PA and in the generally homologous paraventricular and related

"hypothalamic" nuclei of mammals. Further work is needed before any parvo-magnocellular distinction, even if proved valid for any species or age, can be homologously applied to other vertebrates. Reaves and Hayward (1980; T. A. Reaves, personal communication) consider the entire population of goldfish neurophysin- and/or enkephalin-containing hypophysial-projecting cells (5-70 μm diameter) to be "magnocellular" (despite size), as they apparently consider them all homologous with "magnocellular" mammalian neurosecretory cells. Possible roles of goldfish and mammalian "parvocellular" neurosecretory cells are discussed below. These issues should be considered if the fish PA is to be used as a prototype for intracellular neurophysiological investigations of neurosecretion (Mason and Bern, 1977).

Labeling of neurons was recently reported in parvocellular and magnocellular preoptic nuclei of large (80-85 g) goldfish following intravenous HRP injection (Fryer and Maler, 1981). The present findings are similar, but also include labeling in other preoptic regions and in the ventral thalamus (Gregory and Tweedle, 1980, 1981).

Systemic (IP) HRP also lightly labeled small cells in the anterior periventricular, periventricular preoptic and suprachiasmatic nuclei. Little is known of these "nuclei" in fish, but they have not been considered to be neurosecretory. The anterior periventricular cells possess a few DCVs resembling those of the adjacent neurosecretory preoptic nucleus (unpublished observation). The labeling of neurons in the goldfish SCN is interesting since its strong retinal input makes it probably homologous with the similarly placed mammalian SCN (Springer and Gaffney, 1981). The mammalian SCN is not known to project to the

pituitary or median eminence, but does contain an enigmatic population of so-called parvocellular neurophysin and vasopressin containing neurons (Sofroniew and Weindl, 1981; deVries, Bujis and Swaab, 1981 and others) which project to the organum vasculosum of the lamina terminalis (OVLT) and to limbic areas (deVries et al., 1981). The developmental and functional relationships between these cells and the larger population of mammalian neurophysin containing cells is unclear, and nothing has been reported (in the few limited immunohistochemical papers) regarding the presence or absence of neurophysin and vasotocin (or vasopressin) in nonmammalian suprachiasmatic nuclei. Information regarding the potential occurrence of vasotocin or vasopressin in nonmammalian SCN of vertebrates which secrete vasotocin with neurophysin from the preopticohypophysial system would lend insight into the evolution of the SCN, in particular whether mammalian neurosecretory and neurophysin containing SCN cells evolved (or developed) as subdivisions of a single neurophysin cell population or whether they evolved separately in close spatial but not temporal proximity within the PA. The labeling of goldfish SCN neurons following the larger systemic doses of HRP implies that their axons project beyond the blood-brain barrier, possibly to either the pituitary or the OVLT. The capillaries of parts of the goldfish OVLT are fenestrated (unpublished observation).

The preoptic hypophysial and spinal projecting cell group was found to extend far into the nucleus ventromedialis of Schnitzlein (1962), an area of the ventral thalamus which was described as extending rostrally into the "dorsal hypothalamus (preoptic)" and fusing caudally with the hypothalamus proper. Developmentally and functionally

appropriate boundaries of the hypothalamus are unclear (Keyser, 1979), particularly in fish. Schnitzlein (1962) considered scattered large cells within the confines of this ventral thalamic area to be dorsal hypothalamic. Cells with ultrastructural features suggestive of neurosecretory function, such as large numbers of ribosomes and a variable number of DCVs are found in this region (unpublished observation). Similarly placed cells resemble preoptic neurosecretory cells in staining properties (Charlton, 1932; Leatherland, Butz and Dodd, 1966; Leatherland and Dodd, 1969; and see Hoheisel et al., 1978 for further references). Likewise, vasotocin immunoreactivity has been reported in neurons of the PA and ventral thalamus of lamprey, the later region giving rise to most of the observed immunoreactive descending projections (Hoheisel et al., 1978). A similar pattern of labeling of neurons through the PA and ventral thalamus was seen in the toad following intrahypophysial HRP injection (Pasquier et al., 1980). A topologically similar distribution of vasotocin-neurophysin cells was recently reported in preoptic, hypothalamic and periventricular thalamic areas of the pigeon (Berk, Reaves, Hayward and Finkelstein, 1982).

The continuation of this identified system of neurons caudally into the ventral thalamus of fish and amphibians provides morphological support for the contention that the vertebrate PA (and the laterally adjacent entopeduncular nucleus), dorsal hypothalamus and parts of the ventral thalamus share a common diencephalic development in close association with the hypothalamus proper (Kuhlenbeck, 1977). Although possibly derived from the hypothalamus proper (Kuhlenbeck, 1977), some or all of the PA could instead develop as a rostral extension of the ventral

thalamus, and in mammals become juxtaposed with the hypothalamus proper (and borders become obscured) as more caudal parts of the ventral thalamus migrate away from the third ventricle. The preoptic neurosecretory region of fish has been termed the "preoptic nucleus of the thalamus" (Bernstein, 1970).

Retinal inputs to the preoptic/rostral hypothalamic area are probably common to all vertebrates possessing eyes (Ebbesson, 1970). Goldfish and diverse other fishes have an extensive retinal projection to the PA, especially to the suprachiasmatic and so-called magnocellular preoptic nuclei (e.g., Springer and Gaffney, 1981; Ebbesson and Ito, 1980; Ebbesson and Meyer, 1980; Ebbesson and O'Donnel, 1980; Northcutt, 1980; Smeets, 1981). In various species the forebrain retinal terminal fields extend caudally through nuclei of the PA and ventral and dorsal thalamus. Nucleus ventromedialis (Schnitzlein, 1962) is one of several thalamic areas receiving direct retinal input in goldfish (Springer and Gaffney, 1981), and corresponding areas are similarly innervated in the longnose gar (Northcutt and Butler, 1976), the Australian lungfish (Northcutt, 1980), the elasmobranch guitarfish (Ebbesson and Meyer, 1980) and the teleost piranha (Ebbesson and Ito, 1980). In the gar, the SCN and ventromedial thalamic nuclei also receive input from the tectum (Northcutt and Butler, 1980). The ventromedial nucleus also receives cerebellar input in gar (Northcutt, 1982) and catfish (Finger, 1978). Neurons of the ventromedial nucleus project to the tectum in the gar (Northcutt, 1980) and in the dogfish shark and thornback ray (Smeets, 1982). Cells of this area also project to the cord in the same shark and ray (Smeets and Timerick, 1981). Despite the ventral thalamic

position of the cells, these workers interpreted the spinal projection as nonetheless hypothalamic. HRP placed in the carp tectum has labeled neurons in the preoptic and ventromedial thalamic nuclei (Luiten, 1981a). A similar projection from the ventromedial nucleus to the tectum has been reported in goldfish (Grover and Sharma, 1981; see Northcutt, 1982 for discussion of the dorsomedial, i.e., dorsal thalamic, terminology used by Luiten, 1981a and Grover and Sharma, 1981 for this region). Vasotocin-containing cells reportedly project to the tectum in amphibians (Sterba, Ermisch and Ruhle, 1980). An ascending projection from the PA to the visual telencephalic central nucleus was reported in the nurse shark (Luiten, 1981b). Vasotocin-immunoreactive fibers enter the telencephalon from the PA of the lamprey and others descend to the brainstem from preoptic and ventral thalamic areas (Goossens et al., 1977b; Hoheisel et al., 1978). A mesotocin-containing projection is reported from the PA to the telencephalon in the African lungfish (Goossens et al., 1978). Rostral visual parts of the fish ventral thalamus have been considered homologous with the mammalian ventral lateral geniculate nucleus (Northcutt, 1982). The similarity of afferent and efferent connections of this ventral thalamic area and the rostrally adjacent PA are consistent with the tentative consideration of the PA as a ventral thalamic structure. As such, both regions relay afferent information utilizing both direct innervation (and/or modulation) and neurovascular means.

This possibility is supported by recent embryological studies of the rat diencephalon showing that neurons of the "hypothalamic" paraventricular, circularis and supraoptic nuclei arise from the ependymal

region of C. J. Herrick's (1910) sulcus ventralis, which separates the embryologically periventricular ventral thalamus from hypothalamus and subsequently forms an ependymal specialization in adults (Altman and Bayer, 1978b, c). Neurons of these neurosecretory nuclei are thus derived from neuroepithalium which is in close proximity to that which generates more definitively ventral thalamic structures, such as zona incerta, which arise from the upper bank of the same sulcus (Altman and Bayer, 1979a, b). These workers subdivided the rat thalamus based upon isochronicity of neuronal formation, and found that the entire ventral thalamic area (i.e., zona incerta, the ventral lateral geniculate, the subthalamic nucleus and the thalamic reticular nucleus), unlike remaining epi- and dorsal thalamic regions, was formed early over a relatively protracted period (embryonic days 13-15, Altman and Bayer, 1979a, b). Neurons of the paraventricular "hypothalamic" nucleus, which is immediately medial and rostral to the zona incerta in the adult (Armstrong et al., 1980), and the closely associated supraoptic nucleus were also formed on days 13-15, as were neurons of the medial preoptic area (Altman and Bayer, 1978a). Neurons of the adjacent hypothalamic areas (dorsal and lateral preoptic and lateral hypothalamic areas) were similarly formed on days 12-15. Thus, the formation of neurons in these preoptic and rostral hypothalamic nuclei in mammals is isochronic with the formation of neurons in the adjacent ventral thalamus.

Preoptic-ventral thalamic neurosecretory cell contiguity is less consistent with the interpretation of the PA as a telencephalic derivation (see Keyser, 1979), since it subdivides the cell population between major forebrain divisions. However, a small part of the neurophysin

containing cell population is found in the potentially telencephalic nucleus triangularis septi of mammals (Sofroniew and Weindl, 1978; Sofroniew, Weindl, Schinko and Wetzstein, 1979). In mammalian species such as the guinea pig, a continuum of neurophysin-containing cells stretches caudally from nucleus triangularis septi through the PA well into the hypothalamus, to the contiguous paraventricular and supraoptic nuclei (Sofroniew et al., 1979). In rats, a similar path of cells is discontinuous, with cells grouped into several distinct nuclei (Armstrong et al., 1980; Sofroniew and Weindl, 1978). The posterior paraventricular subnucleus of rats, a part of the homologous system, is located immediately medial to the presumably ventral thalamic zona incerta (Armstrong et al., 1980). Thus, the contiguity of neurosecretory cells across the PA, through a preoptic/ventral thalamic transition zone which might be termed dorsal hypothalamus, and continuing into the ventral thalamus of fish and amphibians may be seen as resembling the mammalian condition. Further clarification of preoptic/hypothalamic/ventral thalamic relationships could add considerable theoretical perspective to the interpretation of widespread nonhypophysial (especially peptidergic) projections from these regions of vertebrates.

The findings of descending projections from the goldfish preoptic and ventral thalamic areas, and similar findings in other nonmammals (Schober et al., 1977; Goossens et al., 1977b; Hoheisel et al., 1978; Ten Donkelaar et al., 1980; see discussion by Smeets and Timerick, 1981) imply that the recently discovered projection from the mammalian paraventricular nucleus to the brainstem and spinal cord (Armstrong et al., 1980 and others) is probably phylogenetically old. In rats, this

projection arises largely from the posterior paraventricular subnucleus which borders the zona incerta (Armstrong et al., 1980). In fish, the PA is probably the most rostral site with direct projections to the spinal cord, as no definitively telencephalic region is known to do so. The position of preoptic and ventral thalamic labeling and ultrastructural evidence imply that, in general, different cells project to the hypophysis and spinal cord, although some cells could conceivably project to both regions. Apparently few cells project to both the hypophysis and spinal cord in rat (Swanson, Sawchenko, Wiegand and Price, 1980).

These light microscopic tracing studies have demonstrated that the pattern of neuronal labeling (Figures 1-4) in growing goldfish (1.2-75.0 g) remains similar, despite a considerable increase in the size of neurons and brains during this developmental period and beyond. Capacity for hormone synthesis and release apparently increases by neuronal hypertrophy as body size and need for hormone increase. This response may (see further discussion below) resemble the neuronal hypertrophy seen early in mammalian development, and, continuing during adult life when conditions promote increased hormone secretion (Hatton and Walters, 1973; Tweedle and Hatton, 1976; Armstrong et al., 1977; Theodosis et al., 1981 and others). In fish, this maturational hypertrophy may involve a common system of neurons more extensive than previously recognized. Included are the previously described periventricular, parvocellular and magnocellular preoptic "nuclei", the anterior periventricular and suprachiasmatic "nuclei" (also of the PA), and the ventromedial thalamic area, most of which have not been considered to be neurosecretory. Detailed topographical immunohistochemical studies are needed to determine

which of these areas contain neurophysin, as other peptides and amines may also be present in the PA, and these may also project to the pituitary. Information on the functional and developmental relationships among these areas of fish, and their vertebrate homologues, would be enlightening and could add to the understanding of mammalian function if an understanding of the hodology of peptidergic systems was attained. The advantages of the fish brain for other aspects of developmental investigation are discussed below.

Ultrastructure and Maturation

Preoptic neurosecretory cells ranging widely in size had similar rudimentary cytological features in graded form, as first noted by Palay (1960; Peter and Nagahama, 1976). These observations are consistent with the reported occurrence of vasotocin, isotocin, neurophysin and/or enkephalin immunoreactivity in cells ranging widely in size (5-70 μm) through the rostral-caudal extent of the PA (Goossens et al., 1977a, 1977b; Schreibman and Halpern, 1980; Reaves and Hayward, 1980). Although the distribution of immunoreactive labeling is not well known in fish, cells containing these hormones are reportedly extensively intermixed. Each hormone is reported in cells ranging widely in size, position and number of processes in what have been called magnocellular and parvocellular preoptic nuclei.

In fish of all sizes a gradient of small rostral to large caudal somatic profiles was observed in the PA. In small fish ($\leq$ 6.6 g) only neurons of the caudal PA possessed ultrastructural features typical of neurosecretory cells, and these cells were relatively small (10-15 μm; Figures 9A-C). DCVs and small stacks of granular reticulum were present.

Small (10 μm) profiles tended to have spherical nuclei, while nuclei of slightly larger cells (15 μm) were more irregular (Figure 9C). Caudal preoptic neurosecretory cells appeared to be invariably labeled in these electron microscopic IP HRP experiments. More rostral cells with sparse granular reticulum and few or no DCVs were also labeled with HRP (Figure 10B), indicating that their axons projected beyond the blood-brain barrier.

Cells in the caudal PA, traditionally part of the poorly defined magnocellular preoptic nucleus were progressively larger in larger fish. More rostral placed cells were also larger in larger fish. Stacks of granular reticulum and substantial numbers of DCVs which are characteristic of typical mature neurosecretory cells were found rostrally only in larger fish (compare Figures 10A, 10B, 11B, 15A, and 16A-C). In large fish (105.6-283.1 g) some caudal cells reached 70 μm in size (Figure 17B). Far rostrally, 10 μm preoptic neurons with modest complements of granular reticulum and DCVs were found (Figures 16A, 16C). Intermediately positioned between these extremes were cells of various intermediate sizes, also possessing features typical of neurosecretory cells (Figure 17A).

The ultrastructural features of neuronal somata could be predicted largely based on somal size, regardless of relative neuronal position. Thus, the variation in appearance of small rostral to large caudal neurosecretory somal profiles in large fish (Figures 16A, 16B, 17A, 17B) resembled the sequence of forms observed when cells of the caudal PA were compared from small through large fish (Figures 9A-C, 11A, 12A, 17B). Granular reticulum varied from an isolated strand to enormous

stacks. Larger cells also had regions of dispersed granular reticulum (Figures 11A, 12A, 13, 14A, 14B, 17A, 17B, 18). Nuclei varied from small (5-10 μm) and often spherical in small cells (Figures 9A, 9B, 10A, 10B, 15A, 16A-C) to greatly enlarged and convoluted (Figures 13, 14B). Some convoluted nuclear profiles extended as much as 50 μm across the somata. Potentially multinuclear neurons were also found (Figures 12A, 14A, 17B), although serial reconstruction might have shown these nuclear profiles to be connected (Figures 13, 14B). Small HRP-labeled cells lacking DCVs were found rostrally in small HRP IP fish (Figure 10B). Whether similar small cells in the corresponding position of large fish also project to the pituitary is less clear, since the HRP IP ultrastructural experiments were not entirely successful due to penetration and other technical difficulties. Similarly placed small rostral cells were lightly labeled in IP HRP material of 38.1-75.0 g fish processed for light nicroscopy (Figure 7A-D).

Studies with tritiated thymidine and cell counts show that neurons are clearly formed through life in the fish retina and tectum (Johns, 1977; Johns and Easter, 1977; Kock and Reuter, 1978; Meyer, 1978; Easter et al., 1981; Johns, 1981). Counts of neurons in various other regions and of axons in peripheral nerves imply that the formation of neurons is widespread through life in fish (Leonard et al., 1978; Birse et al., 1980). Although these later studies clearly demonstrated changes, they did not distinguish between the actual formation of neurons (terminal cell division) through life and the prolonged migration and hypertrophy of cells which were eventually counted and functional as neurons. Easter (1979) reported that the number of axons in the goldfish trochlear

nerve is fixed early in life (apparently the final number is found in fish resembling the smallest individuals of the present study). Thus, while the formation of neurons continues through life in parts of the visual system, it is unwise to assume that all parts of the nervous systems of all species of fish living under various and variable environmental conditions are similar in this respect.

Nonetheless, it is not unlikely, though undemonstrated, that small preoptic neurosecretory cells are added rostrally by the extended and perhaps intermittent formation of neurons from preoptic ependyma (Polenov et al., 1972), similar to the addition of neurons to the periphery of the enlarging retina. This could account for the labeling of small far rostral cells even in larger fish (Figure 7A-D) in IP HRP experiments. The smallest rostral cells seen in the large fish may, however, have been present even when the fish were as small as the smallest fish in this study. Thus, the small rostral cells labeled in HRP IP studies of small fish (Figure 5, 10B) may or may not be precisely comparable to the analagously placed similarly sized small HRP-labeled cells of larger individuals. Tritiated thymidine labeled cells, a proportion which decreases through adult life, in the rostroventral preoptic ependyma of frogs and various teleosts (Kirsche, 1967; Kranz and Richter, 1970; Polenov et al., 1972). These cells were not demonstrated to be neurons. No mitotic figures were seen there or elsewhere in the present study of goldfish but they were reported during certain seasons in the preoptic ependyma of "adult" teleosts, juvenile sturgeon and frogs (Polenov et al., 1972). The ultrastructural features and function of vertebrate neurosecretory cells are well known for responding

to stimuli in the internal and external environment of the animal (Mason and Bern, 1977; Tweedle and Hatton, 1976, 1977; Morris et al., 1978; Theodosis et al., 1981; Hatton and Tweedle, 1982 and others). In addition, chronic dehydration of young adult rats, which are no longer forming neurosecretory cells, leads to an increased formation of astrocytes and endothelial cells, demonstrated by thymidine studies of the supraoptic nucleus (Paterson and Leblond, 1977). The formation of glia and endothelial cells of the homologous fish PA is potentially subject to similar environmental control. Furthermore, since fish preoptic neurosecretory cells (neurons) are forming in the presence of substantial environmental variation regardless of when neurogenesis is completed in this system, experimental studies of environmental influences on neurogenesis and subsequent maturation of this system seem appropriate. For example, development could be observed of individuals raised in fresh, brackish or salt water, from a species which can live in either. The neuroendocrine function would almost certainly differ; would the morphology of neurons as well as glia be altered?

The apparent microglia which are found in the caudal PA could play a role in the ongoing maturation of the goldfish preoptic neurosecretory system beyond the normal defensive functions of this cell type. The congregation of microglia in this area might be related only to the presence of large blood vessels in the caudal region. Nonetheless, these cells apparently left the proximity of large vessels to contact large unidentified structures of debris (Figure 15B). This could be indicative of the developmental phenomena of neuronal cell death, which has not been investigated in neurosecretory systems.

In moderate to large fish, occasional 20-40 μm somal profiles were extensively surrounded by oligodendrocytes (Figures 13, 14A, 14B), which extended processes into the neuronal soma (Figure 14B). The cytoplasm of these glial-encased neurons was typical for neurosecretory cells of this size, and included numerous DCVs. Large more caudal cells were not surrounded by oligodendrocytes and contained convoluted or apparently multiple nuclei (Figures 12A, 17B, 18), while more rostral smaller cells were not wrapped in glia and contained relatively nonconvoluted or spherical single nuclei (Figures 15A, 16A-C). The cells surrounded by oligodendrocytes were the only preoptic neurosecretory cells not in extensive soma-somatic apposition, although they were apposed where the oligodendrocytic encasement was incomplete (Figure 13) and with the enigmatic small cells which were invaginated within their somata (Figure 14B). The nature, origin and function of the small embedded cells is unclear. Oligodendrocytic encasement is potentially a transient and dynamic process which occurs around neurosecretory cells as they reach a moderate size, nurturing them in preparation for further hypertrophy into unusually large (70 μm) neurons. During this time the nucleus of the engulfed large neuron seems to be transformed, as it becomes large, irregular and apparently multiple and/or lobulated. It would not be surprising if these neurons were in the act of becoming polyploid, analagous to the increasing polyploidy of promegakaryocytes as they grow extremely large for eventual subdivision of cytoplasm into platelets (Bloom and Fawcett, 1975, pp. 221-224). Claims for polyploidy among vertebrate neurons are disputed (Peters, Palay and Webster, 1976, pp. 48-50), but evidence is strong in giant invertebrate neurons

(Swift, 1962; Coggeshall, Yaksta and Swartz, 1970; Lasek and Dower, 1971). The diploid amount of DNA doubles as many as sixteen (16) times during the maturation of large Aplysia neurons (Coggeshall et al., 1970). A single diploid nucleus may be limited in the amount of cytoplasm with which it can relate, thus extraordinarily large neurons may be necessarily polyploid.

Oligodendrocytic ensheathment with glial processes extending into the neuronal soma is strongly suggestive of a symbiotic relationship between neurons and glia (Holmgren, 1900; Cajal, 1928). Neurons of the lateral vestibular nucleus of the rat are similarly ensheathed by oligodendrocytes, which are frequently separated from the neuronal soma by astrocytic processes and synapses (Sotelo and Palay, 1968). The functions of such arrangements and more generally of perineuronal oligodendrocytes are obscure (Peters et al., 1976, pp. 248-254). Each glial-encased neuron could be considered a somatic glomerulus, with glia functioning in some way to protect the neuron and/or its inputs from extraneous variables in the extracellular environment. The oligodendrocytic capillary end feet could function in the regulation of the neuronal environment, or possibly in relaying blood born humoral stimuli to the neurosecretory cell.

A few large caudal cells contained vascuoles which were themselves occasionally enormous (50 μm diameter, Figure 17B, see also 12B). Similar structures have been seen in other species of fish with light microscopy (Scharrer, 1941; Ötzan, 1963, 1966), in which the contents were stained with Masson trichrome and acid fuchsin. Caudal preoptic cells in one species, the platyfish, were extensively vacuolated even

though these "magnocellular" cells remained rather small (10-20 μm, Ötzen, 1963). These vacuoles have not previously been reported in goldfish (Palay, 1960; Peter and Nagahama, 1976). Vacuoles may be more common in the large caudal preoptic neurosecretory cells of salt water species (S. L. Palay, personal communication). Similar vacuoles have been reported in homologous neurosecretory cells of diverse vertebrates including pathologically dehydrated humans (Koep, Konigsmark and Sperber, 1970). Very large and vacuolated neurosecretory cells have been the subject of few investigations because they are rare in laboratory rats, the subjects of most investigations of neurosecretion. Such cells are however found in the caudal (post chiasmatic or tuberal) portion of the dog supraoptic nucleus, including adult male nondehydrated individuals, where cytoplasmic vacuoles may reach 100 μm (Verney, 1947; Jewell, 1953; Zambrano and deRobertis, 1967). It was also this tuberal portion of the supraoptic nucleus which was enlarged in desert species of rodents, although its neurons were apparently not unusually large in these much smaller animals (Hatton, Johnson and Malatesta, 1972). Rats deprived of water for even a short period develop a modest dilation of granular reticulum in supraoptic neurosecretory cells (Tweedle and Hatton, 1977). Rat neurosecretory cell and nuclear sizes increase with more sustained enhancement of secretion (Hatton and Walters, 1973; Armstrong et al., 1977; Morris et al., 1978; Theodosis et al., 1981). Krisch (1977) has reported that somata of vasopressin-containing neurons of nine (9) day water deprived rats are intensely immunoreactive for vasopressin due to the labeling of the contents of considerably dilated reticular cisternae. Cells in this functional state had few DCVs located in the

somata. This nonvesicular immunoreactive material may be labile, since it reportedly accounts for increased vasopressin immunoreactivity within minutes of brief immobilization stress of rats and the labeling of intracellular clefts (Krisch, 1978, 1979). A similar substance in dilated cisternae of neurons of the paraventricular and supraoptic nuclei of a lizard stained with pseudoisocyanine, aldehyde fuchsin, PAS and trichrome orange G but was not neurophysin immunoreactive unless sections were pretreated with trypsin, although the same cells were otherwise immunoreactive without enzyme pretreatment (Gonzáles and Rodríguez, 1980). It seems likely that the material seen in large vacuoles (sometimes termed vesicles) and more commonly in typical cisternae is a prohormone for neurophysin/hormone. In goldfish this substance was also observed in vacuolated neurites in the preoptic neuropil (Figure 12C). These observations may be relevant to the possible role of reticulum in the transport, storage and release of a nonvesicular hormone from neurosecretory axons, somata and/or dendrites (Silverman, 1976; Castel and Hockman, 1976; Rougon-Rapuzzi, Cau, Boudier and Cupo, 1978; Alonso and Assenmacher, 1979; Krisch, 1979) and the possible exocytotic release of hormones at other than obviously specialized presynaptic sites (Krisch, 1979; but see also Robinson and Jones, 1982). Experimental and immunohistochemical studies of neurosecretion in species other than laboratory rat (e.g., species possessing vacuolated neurosecretory cells such as dog, lizard, fish and potentially desert mammals) might be more revealing of these aspects of intracellular and intercellular neuronal function.

The extremely large vacuoles found in large neurons (Figure 17B) and in neurites (Figure 12C) may also play a role in the resculpturing of neuronal morphology. As these neurons have already become multinuclear, an increase in the size of the somatic vacuole could potentially lead to the formation of several separate neurons, by the process of amitotic cell division. It is also not inconceivable that large multinuclear neurons result from the fusion of smaller neurons.

Because of the possibilities of the extended formation of neurons, neuronal death, neuronal division, neuronal fusion and the hypertrophy and migration of neurons dorsal or ventral to the preoptic nucleus (i.e., the periventricular preoptic and anterior periventricular nuclei, which are also labeled by HRP IP; Figures 5G, 5H and 5J, and 6A, 6B), numerous models of development are consistent with the observations of the present studies. The simplest model explains regional and maturational differences by postulating that neurons in the fish PA remain in their same relative positions, without neuronal formation, death, or division but hypertrophy during growth in adult life. As there is, however, a strong possibility for continued formation of neurons and evidence for degeneration (Figure 15B), there may be a continued turnover of neurons, with larger cells replacing smaller ones. Since it is conceivable that large multinuclear neurons divide, this could be a source of additional cells caudally, which could perhaps hypertrophy into large cells.

In fish of all sizes, caudally placed DCV-containing neurons appear to be morphologically suited for greater synthetic capacity than smaller rostral cells. The gradation of size and form is apparent in part because the cell populaton remains contiguous, which is not true of most

mammals which possess several nonperiventricularly located neurophysin-containing cell groups. In large fish, the caudal cells are extremely large, perhaps by hypertrophy. Smaller rostral cells may also hypertrophy, gaining morphological features (e.g. DCVs) typical of neurosecretory cells. It is not necessary to assume that the very large caudal cells and the smaller and possibly more recently hypertrophied rostral cells function in parallel to release a larger quantity of neurosecretory hormones, even if cells of all sizes contain the same hormones and project to the pituitary. Regional differences in function are likely in the preoptic nucleus of teleosts, which appear to lack a hypothalamohypophysial portal system and instead utilize intrahypophysial diffusion and direct innervation for adenohypophysial control (Perks, 1969; Kaul and Vollrath, 1974; Abraham et al., 1976). Claims have been made for rostral ("parvocellular") preoptic control of adrenocorticotropin and growth hormone secretion in goldfish (Fryer and Boudreault-Chateauvert, 1981; Fryer, 1981). Potentially homologous "parvocellular" vasopressin and neurophysin-containing cells of the mammalian paraventricular nucleus influence adenohypophysial function via the release of hormones into the portal system of the medium eminence (Silverman, 1976; Castel and Hochman, 1976; Lobo-Antunes, Carmel and Zimmerman, 1977; Krisch, 1978 and others). Regardless of similarities or differences of function which are obscure in fish, and the exact nature of homologies among neurophysin-containing cell groups which is uncertain, the developmental and morphological similarity of the entire population of fish preoptic neurosecretory cells seems clear.

Although direct evidence is lacking, it is intriguing to speculate regarding the mechanisms which might direct neurosecretory cell hypertrophy in growing fish. Neuronal hypertrophy may be a response to increased demand for hormone secretion resulting from everincreasing body weight, analagous to the hypertrophy of the homologous neurosecretory cells in dehydrated adult rats (Hatton and Walters, 1973; Armstrong et al., 1977). Axons also presumably elongate as the fish brain grows larger (if connections remain otherwise unchanged, which is not always true, e.g., Johns and Easter, 1977), which might cause smaller increases in cell size even in non-neurosecretory systems. However, the fish brain clearly has features which resemble the embryonic and fetal mammalian brain (Johns, 1977; Johns and Easter, 1977; Kock and Reuter, 1978; Leonard et al., 1978; Meyer, 1978; Easter, 1979; Birse et al., 1980; Easter et al., 1981; Johns et al., 1981; Stevenson and Yoon, 1982). Maturational hypertrophy in fish may be analagous (or homologous) to hypertrophy occurring in mammalian neurosecretory cells before birth. The function of mammalian neurosecretory cells at this time and whether hypertrophy at this time is induced by demand or occurs otherwise in parallel or advance of demand is unclear. The source of new membrane in hypertrophied neurosecretory cells is also unclear, but could include the membranes surrounding DCVs, which fuse to the cell membrane during hormone release. It seems likely that dendritic morphology and physiological properties also change if neurons hypertrophy greatly during a protracted period of maturation.

The gradient of neuronal maturation, size and form which is seen in the fish PA encompasses the range of reported ultrastructural features

which the neuronal somata of homologous regions of assorted vertebrates adopt under diverse environmental conditions (Morris et al., 1978). Gradations are evident between forms, as are developmental transformations. This observation has clear implications regarding the categorization for convenience sake of neurons based on morphological features (e.g., parvocellular, magnocellular, etc.) since major transformations of structure and function occur within the lives of cells. There may be an even greater potential for variation as form is adapted depending on the extracellular and ultimately the extraorganismic environment. Thus, rather differently appearing cells could result from the position of a cell in different species or in different positions within a species, without genetically-coded information necessarily acting directly to dictate the specific detailed differences in form among related neurosecretory cells. Intraregional, intraspecies and interspecies differences in neurosecretory cell form could all be results largely of the adaptation of multipotential neurons to specific features in their extracellular environments, which show spatial, temporal and species differences.

Nonquantitative observations obtained from warm summer and cold late fall waters indicate that seasonal changes occur in the goldfish preoptic nucleus. Neuronal nuclei were not extremely convoluted as in large cells of summer fish, but nuclear profile(s) had a scalloped conformation. Nucleoli were more prominent in fall fish, where they were large and frequently found in thin sectioned nuclei, which often contained more than one nucleolus. Multiple nucleoli are produced in the homologous neurosecretory cells of rats under conditions of increased

hormone synthesis (Hatton and Walters, 1973; Armstrong et al., 1977). The more frequent observation of nucleoli and multiple nucleoli in thin sections implies that multiple nucleoli are formed during the fall in preoptic neurosecretory cells of goldfish subject to Michigan seasonal environmental changes. It may be that hormone synthesis is actually increased in the fall. However, reduced body temperature would tend to reduce synthetic capacity (Grafstein et al., 1972; Springer and Agranoff, 1977). Thus fall cells may require structural modification in order to synthesize adequate amounts of hormones at reduced temperatures.

Soma-somatic appositions were extremely common among neurosecretory cells of most regions of the fish PA, particularly in fish larger than about 10 g. Appositions were previously observed by Palay (1960), and are common but less frequent in the homologous neurosecretory cell groups of rats. Quantitative studies have shown that these appositions become more extensive in rats under conditions of increased hormone secretion such as mild dehydration and the prolonged stresses of longer term water deprivation, pregnancy and lactation (Tweedle and Hatton, 1976, 1977; Gregory et al., 1980; Theodosis et al., 1981; Hatton and Tweedle, 1982). These changes appear to occur via the active retraction and extension of astrocytic processes from between neuronal elements. Goldfish preoptic neurosecretory somata are occasionally separated by fine astrocytic (and/or radial glial) processes (Figure 16A-C). Reaves et al. (1982) reported that "the number of direct soma-somatic appositions is limited by the extent of glial intervention between adjacent neurons". This apparent contradiction between their observations and

ours may be indicative of an actual morphological difference between goldfish living in Michigan and those of Reaves et al. (1982) from Chapel Hill, North Carolina. Nonquantitative observations of our Michigan goldfish suggest a probable increase in apposition from summer to fall and changes in the shape of apposed neuronal membranes, producing slight but distinct interdigitations during the fall (Figure 18). In one fall fish, gap junctions appeared to be present between apposed somata and were definitely present between a soma and a neurite (Figure 19). Indications of gap junctions were only observed in fall fish, and these structures may be rare, small or only present under certain physiological conditions. Gap junctions have been reported, based primarily on dye coupling in hypothalamic slice experiments, among the homologous neurosecretory cells of rats (Andrew, MacVicar, Dudek and Hatton, 1981). If present on neuronal somata of rats, they also appear to be rare, small and/or variable (unpublished observations). Injections of Lucifer yellow-CH or Procion yellow into goldfish preoptic neurosecretory cells in vivo has yielded no indication of dye coupling (Hayward, 1974; Reaves and Hayward, 1980), although gap junctions do not permit dye coupling under all circumstances (Bennett, Spray and Harris, 1981). Intracellular injections of HRP often labeled multiple cells, a finding interpreted as supporting the presence of gap junctions (Reaves et al., 1982). As HRP does not cross gap junctions in other systems or laboratories (Bennett et al., 1981), multiple labeling is apt to be the result of multiple or extracellular injections. Recent physiological evidence shows that neurons may interact at neuron-neuron membrane appositions even in the absence of gap junctions, apparently by

the accumulation of potassium in restricted extracellular spaces (Alkon and Grossman, 1978; Yarom and Spira, 1982). The physiological significance of neurosecretory cell membrane appositions, which are extremely common, and of gap junctions which are of unknown frequency remains to be determined. The maturational and seasonal changes in morphology imply that major physiological features of this region also may be labile.

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APPENDIX

CLASSIFICATION OF FISH

APPENDIX

A CLASSIFICATION OF KNOWN CURRENTLY LIVING SPECIES OF FISH
(48% of Living Vertebrate Species)

I. Superclass Agnatha (jawless fish)

A. Class Cephalaspidomorphi

1. Subclass Cyclostomata

a. Order Petromizoniformes
(1). Petromizonidae (the lamprey family)
b. Order Myxiniformes
(1). Myxinidae (the hagfish family)

II. Superclass Gnathostomata (jawed fish)

A. Class Chondrichthyes (cartilaginous jawed fish)

*1. Subclass Holocephali (chimeras)
2. Subclass Elasmobranchii (sharks, skates and rays)

B. Class Osteichthyes ("bony" fish)

*1. Subclass Dipneusti (lung fish)
*2. Subclass Crossopterygii (primitive forms possibly ancestral to amphibians, one living species, the Coelacanth)
*3. Subclass Brachiopterygii (reed fish, e.g., Polypterus)
4. Subclass Actinopterygii (ray finned fish)

*a. Infraclass Chondrostii (sturgeons and paddlefish)
*b. Infraclass Holostoi (gars and bowfin)
c. Infraclass Teleostii (96% of living fish species)

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*Although these species are commonly classified as such, the relationships of species within and/or between these major groups are questioned.