A reinvestigation of the Gn-RH (Gonadotrophin-releasing hormone) systems in the goldfish brain using antibodies to salmon Gn-RH

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Summary. The organization of Gn-RH systems in the brain of teleosts has been investigated previously by immunohistochemistry using antibodies against the mammalian decapeptide which differs from the teleostean factor. Here, we report the distribution of immunoreactive Gn-RH in the brain of goldfish using antibodies against synthetic teleost peptide.

Immunoreactive structures are found along a column extending from the rostral olfactory bulbs to the pituitary stalk. Cell bodies are observed within the olfactory nerves and bulbs, along the ventromedial telencephalon, the ventrolateral preoptic area and the latero-basal hypothalamus. Large perikarya are detected in the dorsal midbrain tegmentum, immediately caudal to the posterior commissure. A prominent pathway was traced from the cells located in the olfactory nerves through the medial olfactory tract and along all the perikarya described above to the pituitary stalk. In the pituitary, projections are restricted to the proximal pars distalis. A second immunoreactive pathway ascends more dorsally in the telencephalon and arches to the periventricular regions of the diencephalon. Part of this pathway forms a periventricular network in the dorsal and posterior hypothalamus, whereas other projections continue caudally to the medulla oblongata and the spinal cord. Lesions of the ventral preoptic area demonstrate that most of the fibers detected in the pituitary originate from the preoptic region.

Key words: Gonadotrophin-releasing hormone – Teleosts – Immunohistochemistry – Neuroendocrine control – Reproduction – Goldfish (*Carassius auratus*)

The primary structure of a teleostean Gn-RH isolated from extracts of chum salmon brain has been recently reported as (Trp^7-Leu^8)-luteinizing hormone-releasing hormone (Sherwood et al. 1983). This confirms previous results by King and Millar (1980) and Barnett et al. (1982) demonstrating that the teleostean Gn-RH differs from the mammalian Gn-RH (mGn-RH) by amino-acid substitution in position 7 and 8 of the ten amino-acid peptide. Recent data indicate that this structure is also found in other teleost species, including the goldfish (Breton et al. 1984; Sherwood et al. 1984). Differences in the structure of Gn-RH in vertebrates have also been documented in birds where the sequence of one form of Gn-RH has been found to be (Gln^8)-mGn-RH (King and Millar 1982; Miyamoto et al. 1982) and a second form to be (His^5, Trp^7, Leu^8)-mGn-RH (Miyamoto et al. 1984). However, there is no information concerning the distribution of these native peptides in fish and birds. To date, with the exception of a short report on the sole (Nunez-Rodriguez et al. 1985), antibodies to only mGn-RH have been used in order to investigate the distribution of immunoreactive (ir) material in the central nervous system of non-mammalian vertebrates. In teleosts, mGn-RH-ir structures have been described in the brain and pituitary gland of many species, in particular the rainbow trout (Goos and Murathanoglu 1977; Dubois et al. 1979), platyfish (Schreibman et al. 1979, 1982, 1983; Münz et al. 1981, 1982; Halpern-Sebold and Schreibman 1983), common carp (Nozaki and Kobayashi 1979), goldfish (Kah et al. 1982, 1984a; Münz et al. 1982; Stell et al. 1984), three-spined stickleback (Borg et al. 1982) and African catfish (Goos et al. 1985). Although these results have been obtained by means of a heterologous system, their value is reinforced by the fact that ir perikarya and fibers can be demonstrated, in most cases, in areas known to be involved in the central regulation of reproduction (see Peter 1983), to concentrate labeled steroids (Kim et al. 1978) and to exhibit high levels of aromatase activity (Callard 1983).

The availability of antibodies to salmon Gn-RH makes it possible to reinvestigate the distribution of ir structures using an homologous system and thus to check the results obtained with antisera to mGn-RH. Because of the low cross-reactivity of antibodies to mGn-RH with salmon Gn-RH in vitro (B. Breton, unpublished observations), one could expect a better recognition of the native peptide using antisera to salmon Gn-RH; this, in turn, should lead to more detailed information. Here, we report our observations concerning the organization of Gn-RH systems in the central nervous system of male goldfish.

In order to investigate the origin of the Gn-RH fibers in the pituitary gland, electrolytic lesions were placed in a number of forebrain nuclei. The distribution of ir material

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was also examined in the brain and olfactory bulbs of animals after section of the olfactory tracts. Another aim of this study was to provide the morphological data needed for the interpretation of further Gn-RH investigations based on radioimmunoassays.

Materials and methods

Materials. 50 male goldfish (*Carassius auratus*) weighing 15–35 g were either obtained from a natural pond located near Bordeaux (France) or purchased from Ozark Fisheries, Stoutland, Missouri, USA. Most males were in mating condition at the time experiments were conducted.

Antibodies. For immunocytochemistry, three different antisera directed towards salmon Gn-RH were employed. Two antisera were raised against salmon Gn-RH synthesized at the University of Bordeaux (Laboratoire de Cristallographie et de Physique Cristalline) by Drs. G. Précigoux and S. Geoffre (see Breton et al. 1984, 1986), and the third was against salmon Gn-RH prepared at the Salk Institute by Drs. J.E. Rivier and W.W. Vale. Each antiserum was raised in a rabbit by injecting the peptide coupled to bovine serum albumin with glutaraldehyde, mixed with Freund's complete adjuvant.

Immunocytochemical procedure. The brains (including the olfactory bulbs) and pituitaries were fixed in 4% paraformaldehyde in phosphate buffer (0.1 M; pH 7.4) either by immersion for 12 h or by perfusion via the aortic bulb followed by a 12 h immersion. In some animals, the retinae and a piece of the rostral spinal cord were also dissected out. The tissues were rinsed and immersed overnight in 12% sucrose in phosphate buffer. After freezing on dry ice, the brains were placed in a cryostat for temperature equilibration at $-15°$ C and cut at 10–15 μm. The sections were collected on gelatin-coated slides, allowed to dry and rehydrated in phosphate buffer (0.1 M; pH 7.4) which was used for all further dilutions and rinses. The slides were incubated overnight in the presence of antisera to salmon Gn-RH diluted 1:3000 to 1:8000, rinsed three times (10 min each) and exposed for 2 h to swine immunoglobulins against rabbit immunoglobulins (1:200; Dako, Denmark). After rinsing the sections were incubated for 1 h with peroxidase-antiperoxidase complexes (1:1000; Dako, Denmark). Peroxidase activity was revealed using 0.025% 3-3′-diaminobenzidine (Sigma, Grade II) to which 0.006% hydrogen peroxide was added. The sections were then dehydrated and mounted in Permount.

Brain lesioning studies. Electrode placements were carried out in different areas of the forebrain according to the procedure developed by Peter and Gill (1975). In the preoptic region, large medial lesions were performed at the level of the anteroventral nucleus preoticus periventricularis (NPP; A = 1.2; M; V = 2.2; $n = 6$) or bilaterally at the same level (A = 1.2–1.4; L = 0.2; V = 2.0 then R = 0.2; V = 2.0; $n = 8$). Lesions were also placed at different levels in the ventral telencephalon along the midline (A = 1.6–1.9; M; V = 1.3–1.4; $n = 5$). The electrolytic lesions were induced using a radiofrequency current generator set at 25–30 V for 45–60 s. To ensure the total disappearance of immunoreactive material in the degenerating structures, the operated fish were allowed to survive for at least 11 days. The anterior brain, i.e., the area encompassing the lesions, was fixed in formalin or Bouin's solution for routine histology whereas the remaining part of the brain and the pituitary were processed for immunocytochemistry. The extent of the lesions was evaluated by drawing transverse sections every 50 μm. In one case, the entire brain of a fish with a large lesion of the preotic area was studied by immunocytochemistry.

Olfactory tract sections. 5 animals with bilateral sections of the olfactory tracts were studied. After checking the sections at the time of dissection (under the dissecting microscope), both brain and pituitary gland were processed for immunohistochemistry.

Control procedures. The specificity of the antisera was checked by radioimmunoassay (Breton et al. 1984; RE Peter and CS Nahorniak, unpublished results). In addition, routine tests for the specificity of the immunocytochemical reaction were performed. Replacement of the primary antiserum by normal rabbit serum, omission of one step of

Fig. 1. Gn-RH cell body (*arrow*) in the antero-ventral telencephalon (*Tel*). Longitudinal section, anterior on the left. × 285

Fig. 2. Gn-RH perikarya (*arrows*) located at the ventral surface of the telencephalon (*Tel*). Longitudinal section, anterior on the left. × 285

Fig. 3. Gn-RH perikarya (*arrows*) in the ventro-lateral preoptic area (*POA*). Longitudinal section, anterior on the right. × 280

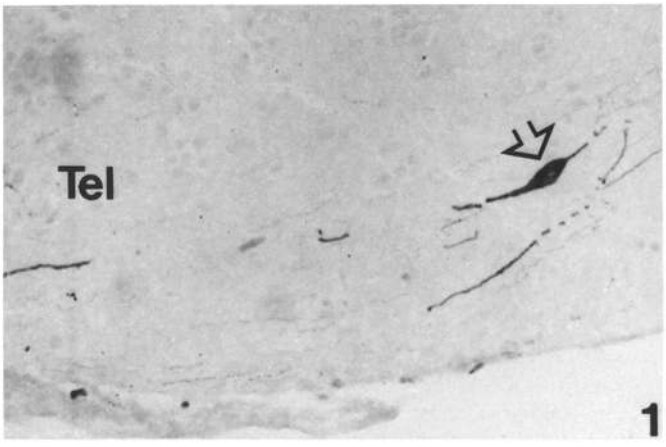
Fig. 4. Gn-RH cell body (*large arrow*) and fiber tract (*small arrow*) running caudally in the ventro-lateral preoptic area (*POA*). Longitudinal section, anterior on the left. × 180

Fig. 5. Gn-RH cell body (*large arrow*) located in the anterior basal hypothalamus (*Hyp*) along the fiber tract (*small arrow*) running from the preoptic region to the pituitary gland. Longitudinal section, anterior on the left; *ct* connective tissue. × 375

Fig. 6. Gn-RH cell bodies (*arrows*) located close to the midline in the anterior dorsal midbrain tegmentum (*MT*). Transverse section. × 240

Fig. 7. The pathway located in the medial olfactory tract (*mot*) and the ventral telencephalon (*Tel*). Dark field, longitudinal section, anterior on the right. × 115

Fig. 8. More caudally, this pathway appears to divide into two parts, one ascending dorsally (*large arrow*), the other running ventrally (*small arrow*). Dark field, longitudinal section, anterior on the right. × 115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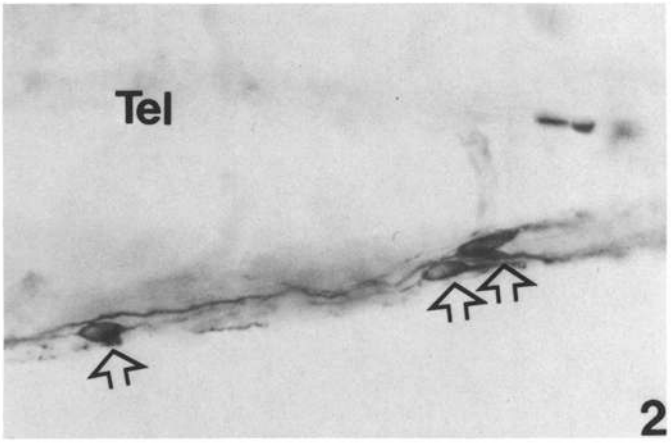
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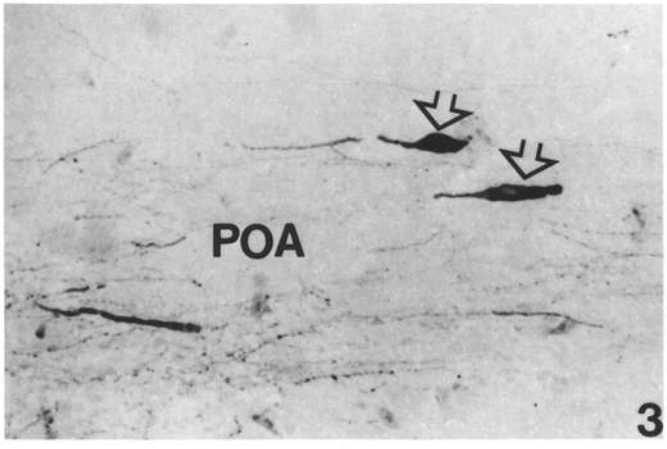
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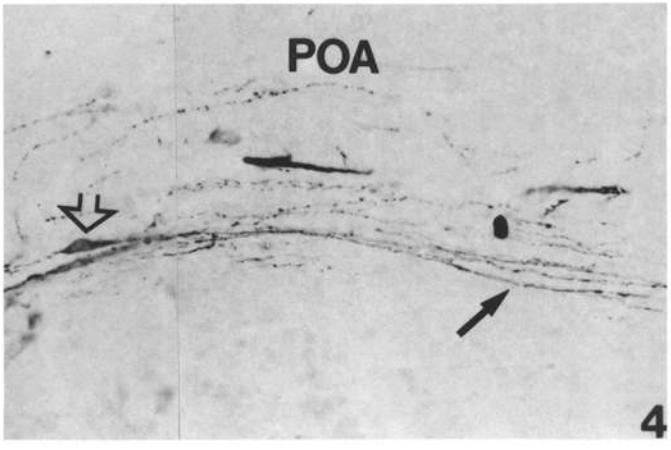
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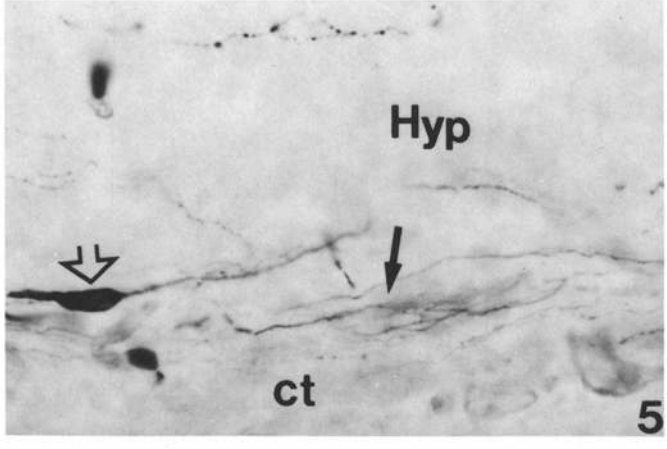
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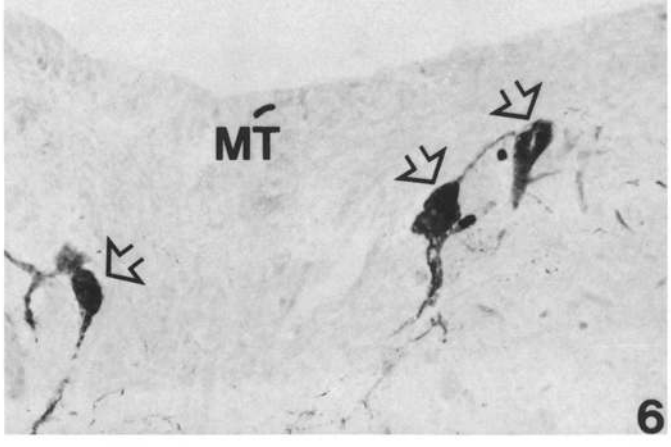
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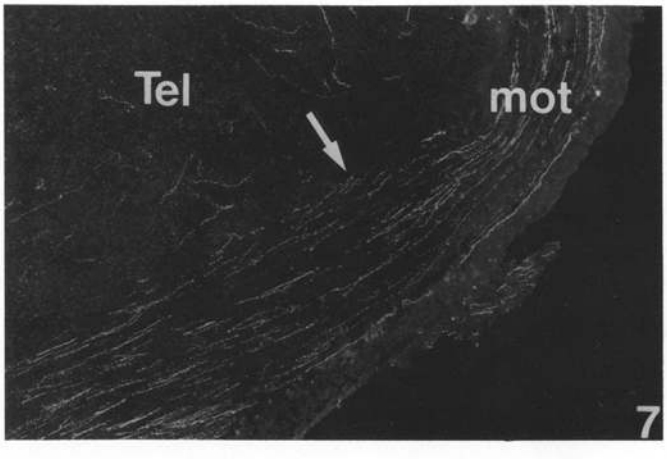
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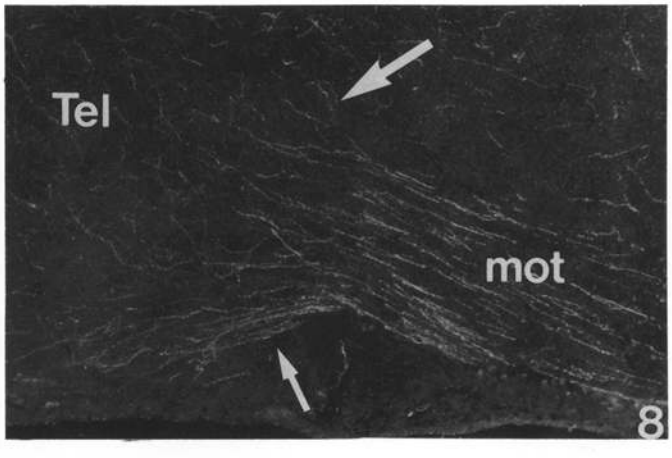
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the reaction or liquid phase absorption of the specific immuneserum with salmon Gn-RH alone or coupled with bovine serum albumine resulted in the suppression of the immune reaction.

Results

Control animals

Each of the three antisera used in this work gave similar results in terms of the general distribution of the peptide. There were differences, however, in the intensity of the reaction and the density of the ir structures. No significant differences were noted between the different groups of fish studied in France and Canada.

Most ir cell bodies and fibers are found along or close to the midline. The perikarya are not confined to any classical brain nucleus, but tend to be located along a nearly continuous column extending from the rostral olfactory bulb to the ventral telencephalon and hypothalamus. However, it is possible to distinguish several groups based on their shape, size, localization and frequency.

The most rostral cell bodies were observed in the olfactory nerves and lobes. In the nerves, large multipolar somata were found immediately rostral to the olfactory bulbs. They possessed numerous dendritic processes, none of which appeared to extend up to the rostral aspect of the nerve or to the nasal epithelium. From these perikarya, very thick smooth axons ran caudally around the periphery of the olfactory bulbs and into the olfactory tracts. Along their course, a few isolated cell bodies were detected. In addition to these large axons seen at the periphery, thin varicose fibers were found in the inner layers of the bulbs.

A small group of ir perikarya was consistently observed immediately caudal to the junction between the medial olfactory tract (mot) and the ventral telencephalon. More posteriorly, isolated cell bodies were found in the vicinity of the midline close to the ventral surface of the telencephalon, and in some cases more dorsally (Figs. 1, 2). These perikarya were observed along the rostro-caudal extent of the telencephalon up to the preoptic region. They were small and characterized by an elongated shape because of their bipolar aspect; most were oriented rostro-caudally.

The preoptic area contained the highest density of ir structures in terms of both fibers and cell bodies. From the rostral part of the preoptic recess to the posterior extent of the optic chiasma, ir cell bodies were seen lateral to the periventricular nuclei (Figs. 3, 4; nucleus preopticus periventricularis: NPPv; nucleus preopticus: NPO; nucleus anterior periventricularis: NAPv; Peter and Gill 1975). These bipolar rostro-caudally oriented perikarya were located near the ventral surface of the brain in the area anterior to the optic chiasma and more posteriorly immediately dorsal to the optic tracts. They tended to be situated more laterally in the caudal preoptic area. A few isolated cells were also found close to the optic tracts at the level where they ascended laterally towards the tectum. Additional isolated perikarya were also detected in the basal hypothalamus at the level of the nucleus lateralis tuberis, pars lateralis (NLTl; Peter and Gill 1975; Fig. 5).

The last group of ir cell bodies was found in the dorsal tegmentum immediately caudal to the posterior commissure. These large multipolar perikarya were located along or close to the midline (Fig. 6).

Compared with the relatively small number of ir cell bodies, the density of Gn-RH fibers was extremely high. Gn-RH fibers branched profusely and appeared as well-defined oriented pathways of parallel running fibers, or in other areas as loose networks without any particular orientation. The majority of the fibers were located in close vicinity to the midline, although some were also present in more lateral regions.

A distinct pathway of smooth axons originating from the large cell bodies located in the olfactory nerves and bulbs runs caudally at the periphery of the bulbs and enters the ventral portion of the mot; thin varicose fibers were detected more dorsally in the mot (Figs. 7, 8). Numerous thin varicose fibers were observed in the deeper layers of the olfactory bulbs. From the junction of the mot with the telencephalon, two main pathways were observed to run caudally (Fig. 8).

A continuous bundle of fibers could be traced from

Fig. 9. Dark field micrograph showing the numerous fibers located in the preoptic area (*POA, large arrow*) and in the optic nerve (*OpN, small arrows*). Longitudinal section, ventral on the right. × 200

Fig. 10. The preoptico-hypophyseal pathway (*arrow*) in the anterior basal hypothalamus (*Hyp*). Dark field, longitudinal section, anterior on the left; *ct* connective tissue. × 120

Fig. 11. Immunoreactive fibers in the neurohypophysis (*nh*) and entering (*arrows*) the adenohypophysis (*ah*) at the level of the proximal pars distalis. × 170

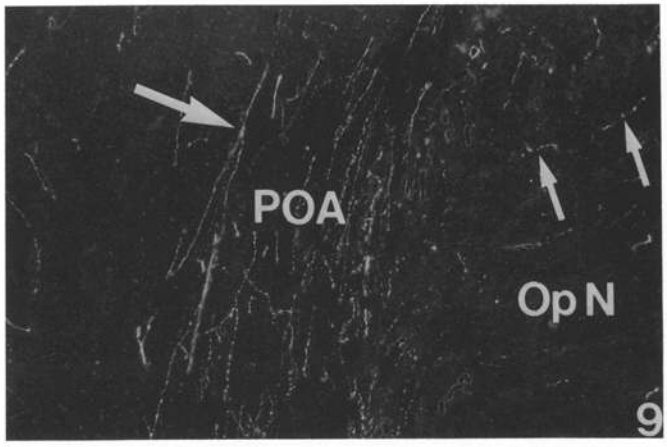
Fig. 12. Dark field micrograph showing the numerous fibers running in the lateral regions of the preoptic area (*POA*) adjacent to the ascending optic tracts. Longitudinal section, anterior on the left. × 200

Fig. 13. Transverse section showing the periventricular pathway at the level of the nucleus anterior periventricularis (*NAPv*). Dark field. *III* third ventricle. × 180

Fig. 14. Longitudinal section showing the projections ascending in the periventricular regions from the ventral to the dorsal telencephalon (*Tel*). Dark field. × 200

Fig. 15. Gn-RH fibers (*arrow*) between the inner plexiform layer (*ipl*) and the inner nuclear layer (*inl*) of the retina; *dr* distal (outer) retina. × 250

Fig. 16. Transverse section at the level of the preoptic recess (*por*). Numerous fibers are located in the ventral wall (*arrow*). *NPP* Nucleus preopticus periventricularis. × 235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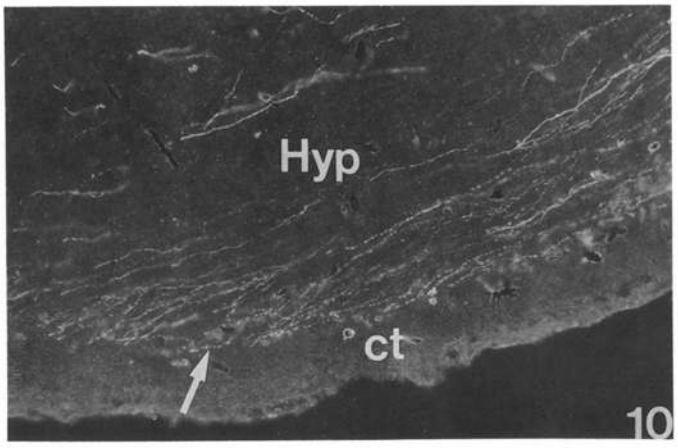
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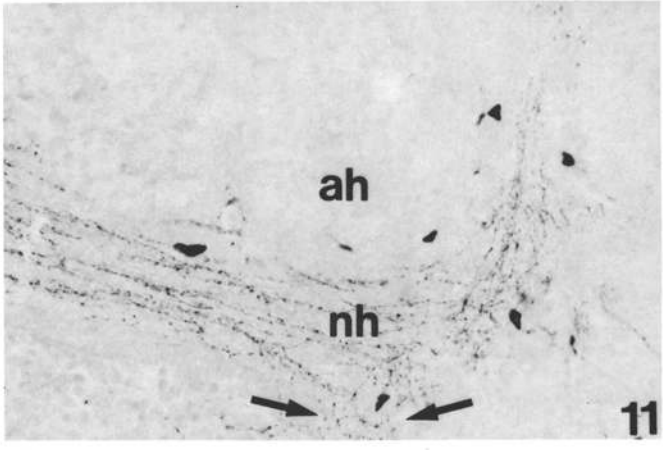
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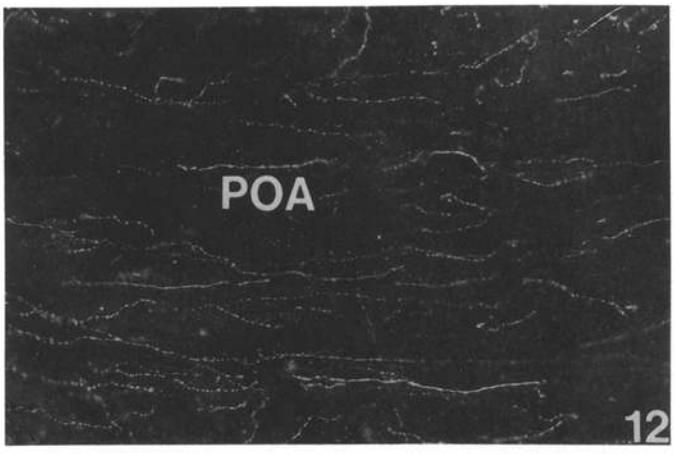
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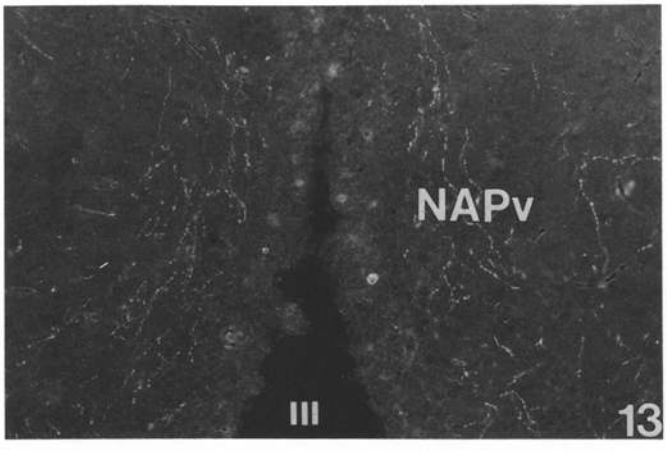
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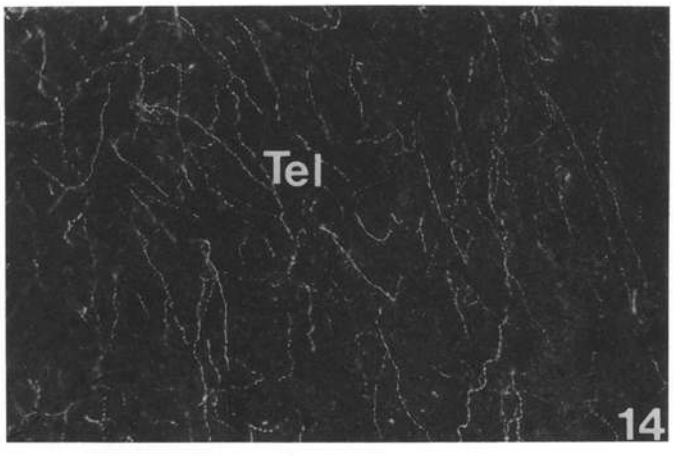
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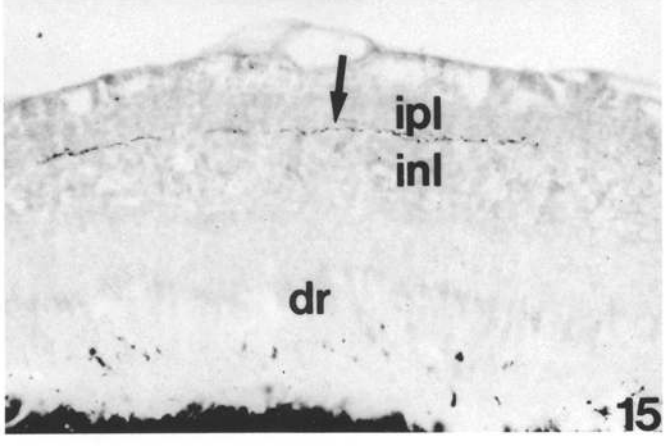
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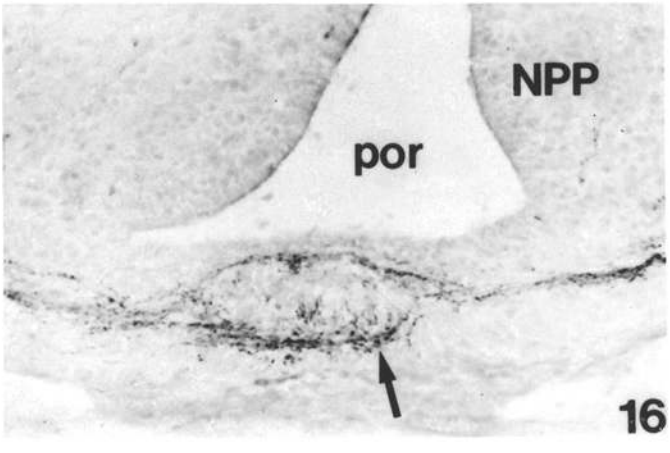
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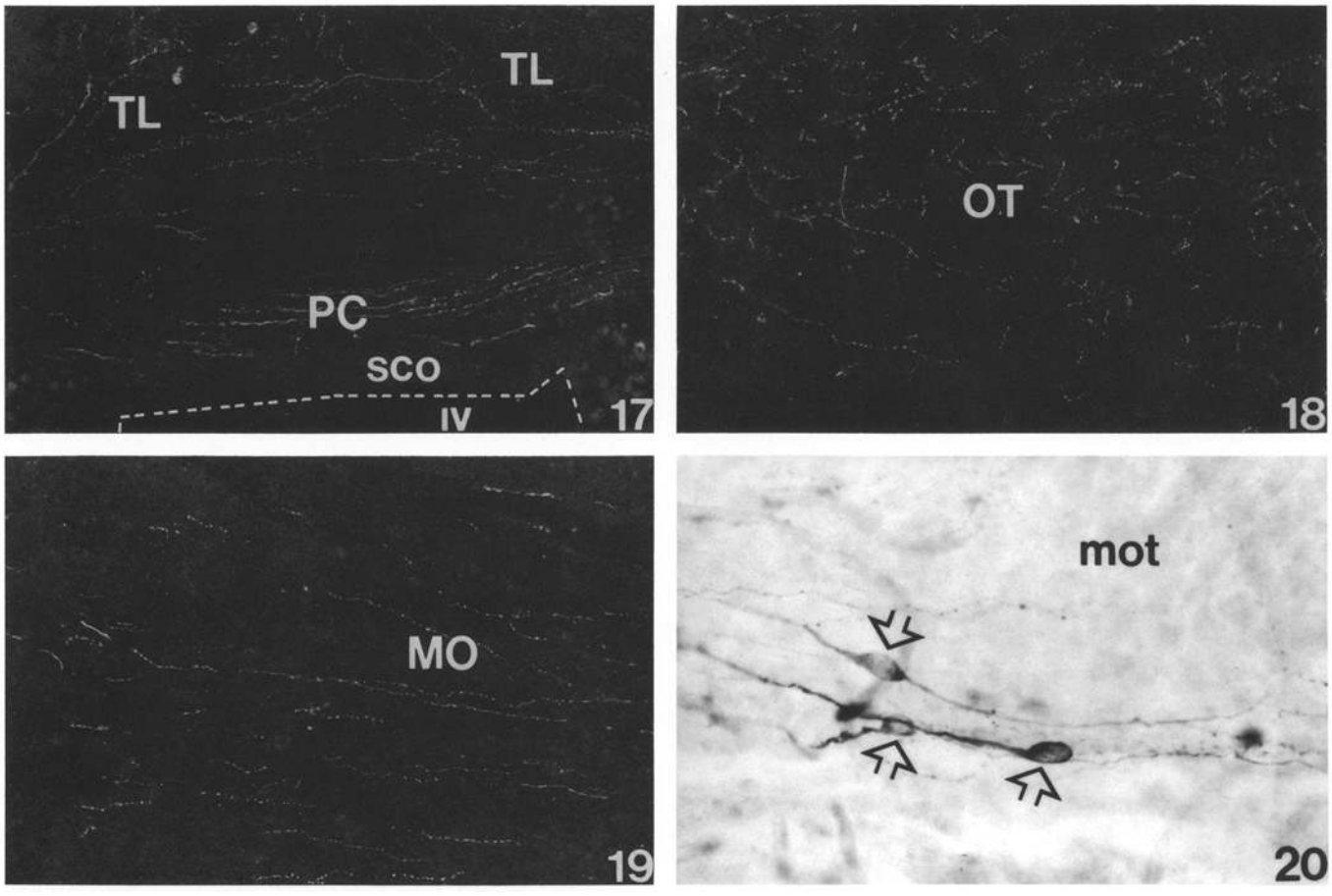

Fig. 17. Transverse section at the level of the posterior commissure (*PC*). Numerous fibers decussate in the commissure whereas none are observed in the subcommissural organ (*sco*). Dark field. *TL* torus longitudinalis, *IV* IV ventricle. × 190

Fig. 18. Immunoreactive fibers in the optic tectum (*OT*). Dark field, longitudinal section. × 190

Fig. 19. Immunoreactive fibers in the medulla oblongata (*MO*). Dark field, longitudinal section. × 190

Fig. 20. Gn-RH bipolar perikarya (*arrows*) observed in the caudal parts of the medial olfactory tracts (*mot*) after their bilateral section. Longitudinal section, anterior on the right. × 525

the rostral telencephalon to the ventral preoptic area and medio-basal hypothalamus (Figs. 9, 10). This tract ran in the vicinity of the perikarya described above, making the precise contribution of the different cell bodies difficult to discern. The density of ir fibers at the level of the rostral preoptic area was very high (Fig. 9). From the perikarya in the preoptic region, fibers ran laterally in a caudal direction above the optic tracts (Figs. 4, 9), they arched ventrally along the nucleus anterior hypothalami (NAH; Peter and Gill 1975), reached the ventral surface of the hypothalamus (Fig. 10), and converged towards the pituitary stalk. In the pituitary gland itself, fibers were mainly located in the proximal pars distalis (Fig. 11), although a few were also detected in the pars intermedia. These fibers were located in the neurohypophysis and in the adenohypophysis, in close contact with the secretory cells. No fibers were observed in the rostral pars distalis.

The second pathway observed in the anterior telencephalon ascended dorsally and arched through the telencephalon and the medial and lateral forebrain bundles (Fig. 12) to reach the periventricular regions of the dorsal and posterior hypothalamus. These fibers did not form a well-defined pathway, but a periventricular network was observed at the level of the nucleus anterior periventricularis (NAPv; Fig. 13), nucleus posterior periventricularis (NPPv), nucleus recessus lateralis (NRL) and nucleus recessus posterioris (NRP). These projections were difficult to trace and may also have contributed to the innervation of the pituitary.

In addition to the preoptico-hypophyseal and periventricular pathway, numerous other projections were observed in the forebrain. Fibers were seen in the dorsal and lateral parts of the telencephalon, especially at the level of the anterior commissure, supracommissural regions and pars medialis of the dorsal telencephalon (Dm; Peter and Gill 1975). The ependyma also received numerous ir fibers (Fig. 14). Their origin is not known, but all cell bodies of the telencephalon (including those from the olfactory bulbs) and rostral preoptic region may contribute to these projections.

Gn-RH fibers were observed in many parts of the diencephalon. In addition to the dense network of ir fibers in the preoptic area, a number of ir fibers were seen to enter the optic tracts (Fig. 9) and may have contributed to ir

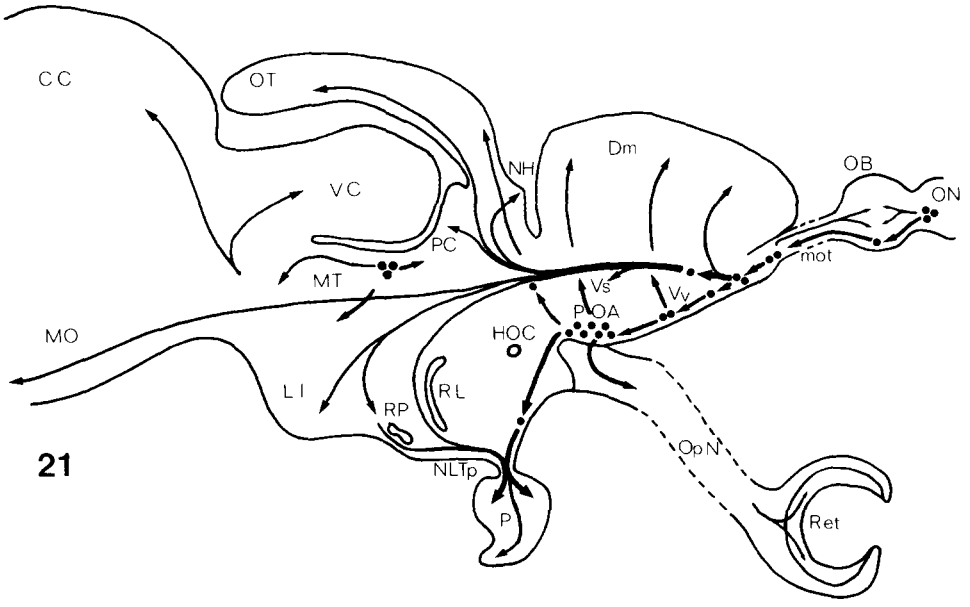

Fig. 21. Diagram summarizing the proposed organization of the Gn-RH systems in the goldfish brain as seen in a longitudinal section. *Black circles* represent the cell bodies and *arrows* indicate their main projections. *CC* corpus of the cerebellum; *Dm* area dorsalis telencephali, pars medialis; *HOC* horizontal commissure; *LI* inferior lobe; *MO* medulla oblongata; *mot* median olfactory tract; *MT* tegmentum of the midbrain; *NH* habenular nucleus; *NLTp* nucleus lateralis tuberis, pars posterior; *OB* olfactory bulb; *ON* olfactory nerve; *OpN* optic nerve; *OT* optic tectum; *P* pituitary; *PC* posterior commissure; *POA* preoptic area; *Ret* retina; *RL* lateral recess; *RP* posterior recess; *VC* valvula of the cerebellum; *Vs* area ventralis telencephali, pars supracommissuralis; *Vv* area ventralis telencephali, pars ventralis

fibers in the inner plexiform layer of the retina (Fig. 15). From the perikarya located in the ventrolateral parts of the rostral preoptic area, numerous fibers were seen to reach the ventral wall of the preoptic recess (Fig. 16). Fibers were observed in the epithalamus: habenular nucleus, habenular commissure and saccus dorsalis. No observations were made in the pineal organ which normally remains attached to the cranium following dissection. Numerous projections were also found in the dorsal and ventral thalamus, mostly along the periventricular regions.

A large number of fibers was detected within the torus longitudinalis (Fig. 17) and optic tectum, particularly in the stratum album centrale (Fig. 18). Projections were observed in the pretectal area, posterior commissure (Fig. 17) and in all parts of the tegmentum (in particular the torus semicircularis). From the periventricular network described above, fibers were seen to continue caudally and to reach the ventral medulla oblongata (Fig. 19). These rostro-caudally oriented fibers continued into the rostral spinal cord. A few ir axons were also observed in the cerebellum and vagal lobe.

Experimental animals

Large electrolytic lesions were placed in a number of forebrain nuclei in order to gain more information about the origin of the Gn-RH fibers observed in the pituitary gland. No attempt was made to quantify the density of the ir material in the gland following the lesions: our results were therefore divided into two classes.

Lesions producing significant changes in the density and the distribution of ir fibers as compared with controls: In a number of fish (N = 6), we observed a dramatic reduction in the number of ir fibers in the proximal pars distalis. In all cases the lesions encompassed the ventral preoptic area (ventral NPP and surrounding area), whereas the mediobasal hypothalamus was intact. It was difficult to appreciate whether such lesions influenced the Gn-RH fibers of the neurointermediate lobe.

Lesions producing no significant changes in the density and distribution of ir fibers as compared with controls: such lesions were found in a number of forebrain nuclei (dorsal NPO, anterior or posterior Vv, AC, optic chiasma) without affecting the ventral preoptic area.

Since the forebrain was processed for routine histology, only the posterior part of the brain could be investigated by means of immunohistochemistry. None of the lesions performed produced dramatic changes in the distribution of ir fibers in the different areas investigated: optic tectum, torus longitudinalis, midbrain tegmentum and posterior part of the inferior lobe. However, in some cases, the density of immunoreactive material appared to be reduced. In particular, a slight reduction in the amount of ir material was noted in the optic tectum and habenular nucleus of animals with lesions of the anterior ventral telencephalon.

In one case we processed the entire brain of a lesioned animal for immunohistochemistry. The lesion was found to be located in the ventral preoptic area. Accordingly, the density of ir fibers in the proximal pars distalis was very low. In this fish, it was found that the entire telencephalon rostral to the lesion exhibited a very high density of ir fibers. In particular, the fiber tract originating from the mot was prominent. Compared with the high density of ir fibers observed rostral to the lesion, very little could be observed more posteriorly in the ventral diencephalon. Immunoreactive cell bodies were observed in close vicinity to the optic tracts where these ascend towards the tectum, and in the lateral parts of the basal hypothalamus.

Olfactory tract-sectioned animals. The brains of 5 olfactory tract-sectioned animals were processed for immunohistochemistry. In two of them, we detected numerous ir cell

bodies in the caudal portions of the mot, immediately rostral to their junction with the ventral telencephalon. These perikarya formed small clusters interconnected by ir fibers (Fig. 20). In one case, up to fourteen ir cells were detected on a single 10-μm-thick longitudinal section at the level of the rostral telencephalon, where the mot is still separated from the telencephalic hemisphere by a ventricular recess. These cells send processes rostrally and ir fibers were observed just caudal to the section of the mot. Caudally fibers were seen to ascend within the telencephalon as in control animals. Along these fibers, a few isolated cell bodies were detected. The distribution of ir profiles did not appear to be dramatically affected following the section of the mot, although the density of ir material was markedly reduced in some areas such as the dorsal telencephalon, the habenular nucleus and the optic tectum.

Discussion

The present investigation confirms the validity of the previous studies, using antibodies to mGnH-RH, in the goldfish brain and in other teleost species (see Introduction). In addition, it provides new information concerning the organization of Gn-RH systems in the goldfish, e.g. the presence of ir perikarya in the olfactory tracts, the ventral telencephalon, the latero-basal hypothalamus and the rostral midbrain tegmentum. New data have also been obtained concerning some major projections in particular the preoptico-hypophyseal and the periventricular pathways. Finally, this work confirms the widespread distribution of Gn-RH in the brain and outside of the central nervous system, as already demonstrated for the retina (Münz et al. 1981; Stell et al. 1984) and suggested for the spinal chord (Münz et al. 1981). Our results are summarized in Fig. 21, which provides a tentative representation for the organization of Gn-RH systems in goldfish brain.

The presence of a Gn-RH-containing group of cells related to both olfactory and visual systems was first reported in the platyfish by Münz et al. (1981, 1982). Using horseradish peroxidase transport, these authors were able to demonstrate that cell bodies located in the nucleus olfactoretinalis are connected to both olfactory bulbs and retina. According to these authors, such a system is present only in teleost species having olfactory bulbs closely attached to the telencephalon (Münz et al. 1982). However, Stell et al. (1984) have recently demonstrated in the goldfish that LH-RH immunoreactive perikarya, located in the rostro-medial aspect of the bulbs are connected to the pars supracommissuralis (Vs) and to the retina via fibers running in the ventral telencephalon and the optic nerves. Using horseradish peroxidase and cobaltous lysine, Springer (1983) has also clearly demonstrated that cell bodies located in the olfactory nerves send projections to Vs and the retina. Our results confirm the presence of ir structures in the bulbs, the Vs area and the retina. According to Stell et al. (1984), such a Gn-RH system is a component of the nervus terminalis, a cranial nerve having connections with the olfactory organs, the Vs and the retina (Demski and Northcutt 1983). The presence of cell bodies reacting to anti-mGn-RH has also been demonstrated in the terminal nerve of the guinea pig (Schwanzel-Fukuda and Silverman 1980) and the rat (Witkin and Silverman 1983).

In the present study, we have found numerous ir fibers in the deep layers of the olfactory bulbs. Most of these fibers originate from the cell bodies located within the olfactory nerve, since they are still present after section of the mot. However, some fibers may also originate from the cells detected within the caudal portion of the medial olfactory tract; these appear to have processes directed both rostrally and caudally. In the hamster, the terminal nerve sends projections to the deeper layer of the olfactory bulbs and to the amygdala (Phillips et al. 1980), a situation which also seems to occur in teleosts, since it has been proposed that the Vs area of fish is homologous to the amygdala of mammals (Northcutt 1981; Kyle et al. 1982). Thus, the organization of the terminal nerve with respect to olfactory and limbic projections (but not retinal) appears to be similar in fish and mammals.

It is not clear whether the Gn-RH cell bodies located within the medial olfactory tract at its junction with the telencephalon represent a ganglion of the terminal nerve or part of the Gn-RH cell population of the ventral telencephalon. Multiple interconnecting ganglia have been reported for the terminal nerve of mammals such as the hamster (Phillips et al. 1980) and guinea pig (Schwanzel-Fukuda and Silverman 1980) and dogfish (Stell, unpublished results). In the goldfish, Stell et al. (1984) have reported that the Gn-RH-cell bodies of the terminal nerve also react with antibodies to the molluscan cardio-excitatory peptide FMRFamide. According to a recent study, other Gn-RH perikarya of the goldfish brain do not stain for FMRFamide and based on this difference, the authors suggest that the Gn-RH neurons of the terminal nerve constitute a different population (Kyle et al. 1985). It would be interesting to investigate whether the Gn-RH neurons observed in the caudal medial olfactory tract also react to FMRFamide antibodies.

Neurobehavioral and neurophysiological investigations have suggested that the terminal nerve, rather than other classical olfactory centers, mediates responses to sex pheromones (Demski and Northcutt 1983). The bilateral section of the olfactory tracts reduces the response of males to pheromones of sexually-active female goldfish (Stacey and Kyle 1983), whereas electrical stimulation of these tracts in codfish induces courtship behavior (Doving and Selset 1980) or sperm release in male goldfish (Demski and Hornby 1982; Demski and Dulka 1984). The Vs area is of primary importance for sexual behavior in male goldfish (Kyle and Peter 1982; Koyama et al. 1984) and is a steroid-concentrating area in teleosts (Kim et al. 1978). The amygdala has been implicated in sexual behavior in mammals (Lehman et al. 1980) and is also a steroid-concentrating area (Sheridan 1979).

With the exception of the platyfish (Münz et al. 1981, 1982; Schreibman et al. 1982), no ir cell bodies have been reported in the ventral telencephalon of teleosts. In the platyfish, the localization and connections of the Gn-RH cells of the nucleus olfactoretinalis with both olfactory bulbs and retina suggest that they are equivalent to the Gn-RH cells of the goldfish terminal nerve and not to the perikarya of the ventral telencephalon described in the present study. This finding is interesting since the medio-ventral telencephalon is presumably homologous to the mammalian medial septum (Northcutt 1981) which also contains numerous Gn-RH cell bodies (see Merchenthaler et al. 1984) and is a steroid-concentrating area (Pfaff and Keiner 1973). The ventral telencephalon, immediately anterior to the anterior

commissure, has been reported to concentrate steroids in *Macropodus* (Davis et al. 1978).

In all previous studies based on antibodies to mGn-RH, the ventral preoptic region has been shown to contain ir cell bodies (see Introduction). In the African catfish, ir cell bodies have also been observed in the nucleus preopticus (Goos et al. 1985). The present work confirms that the highest density of perikarya is found in the ventrolateral parts of the preoptic area. These cells are not located in any classical brain nucleus; we consider the term nucleus preopticus basalis lateralis (NPBL), introduced by Münz et al. (1981) in the platyfish, to be the most appropriate of those previously used. The preoptic region is a steroid-concentrating area in the goldfish, however the distribution of Gn-RH cell bodies does not overlap that of steroid-concentrating neurons described by Kim et al. (1978). According to these authors, such cells are located along the preoptic recess within the nucleus preopticus periventricularis (NPP; Peter and Gill 1975), which is devoid of Gn-RH cell bodies. The precise functions of the NPBL are not clear, but it has been implicated in the control of sperm release (Demski and Hornby 1982) and sexual behavior (Koyama et al. 1984) in male goldfish. Lesioning the anterior preoptic area results in a dramatic increase in serum gonadotrophin level in the goldfish, as a result of the presence in the ventral preoptic region of a gonadotrophin release-inhibiting factor, which is suspected to be dopamine (Peter and Paulencu 1980; Chang and Peter 1983). Accordingly, dopaminergic cell bodies have recently been described in the ventral wall of the preoptic recess of the goldfish (Kah et al. 1984b). Because of the near proximity of these structures, it would be very difficult to destroy selectively the Gn-RH cell bodies in the NPBL without affecting the dopaminergic neurons or their projections to the pituitary. The present work demonstrates that the majority of the Gn-RH fibers observed in the pituitary gland originate from the NPBL, since large lesions of the preoptic area severely reduce the amount of ir Gn-RH fibers in the pituitary, whereas lesions of the ventral telencephalon do not significantly modify their distribution. The remaining fibers observed in the pituitary after the lesion of the preoptic area probably originate from the cell bodies observed along the preoptico-hypophyseal tract and/or from the periventricular network. The projections to the neurointermediate lobe do not appear to be affected by any of the lesions and their origin is unknown. Gn-RH cell bodies occur in the preoptic region in all classes of vertebrates, in particular in mammals (see Merchenthaler et al. 1984), in which this region is also a steroid-concentrating area (Pfaff and Keiner 1973).

We report the presence of numerous Gn-RH fibers in the ventral wall of the preoptic recess. Their topographical localization and the fact that this area is located outside the blood brain barrier (it is sensitive to monosodium glutamate; Peter et al. 1981) suggest that this area is homologous to the mammalian organum vasculosum laminae terminalis (OVLT) as already proposed by Wenger and Törk (1968). In mammals, the OVLT also receives numerous Gn-RH projections from cell bodies located in the preoptic area (Jennes and Stumpf 1980; Bennett-Clarke and Joseph 1982). These similarities suggest that, in fish, Gn-RH is released into the blood at the level of the ventral wall of the preoptic recess as described in the OVLT of mammals (Weindl and Sofroniew 1978). Nevertheless, ultrastructural immunocytochemical studies are necessary to ascertain this point. Gn-RH has been reported in other circumventricular organs such as the subcommissural or the subfornical organs (Weindl and Sofroniew 1978). Although the posterior commissure contains numerous Gn-RH fibers, none has been observed in the subcommissural organ of the goldfish. No ir cells contacting the cerebrospinal fluid, similar to those described in the stickleback (Borg et al. 1982), have been detected in the present investigation.

The presence of cell bodies reacting to mGn-RH antibodies in the dorsal midbrain tegmentum was first reported by Münz et al. (1981) in the platyfish. Our observations confirm these data and suggest that these perikarya send numerous projections to the entire tegmentum of the mesencephalon and possibly to other brain areas. Such neurons have never been reported in the dorsal tegmentum of mammals which, however, is a steroid-concentrating area (Morrell et al. 1975). To date, no precise function can be attributed to this area in teleosts. In mammals, it is known that mGn-RH injections in the dorsal tegmentum can elicit a lordosis reflex in the female rat (Sakuma and Pfaff 1980). Furthermore, neurons responsive to mGn-RH have been reported in the midbrain central gray (Samson et al. 1980). Therefore, we can speculate that, as in mammals the dorsal tegmentum of fish is involved in reproductive events, especially sexual behavior.

In the present study, we have not observed any Gn-RH cell bodies in the posterior NLT similar to those already reported (Schreibman et al. 1979; Kah et al. 1984a). The posterior NLT has been implicated previously in the neuroendocrine control of reproduction (see Peter 1983). Lesioning the posterior NLT results in a decrease in the gonadosomatic index without affecting the basal levels of gonadotrophin (Hontela and Peter 1980). However, the daily fluctuations of gonadotrophin are abolished in sexually mature animals, suggesting that the NLT is implicated in the control of gonadal recrudescence. There are also indications for direct relationships between the NLT and the pars distalis (Zambrano 1971; Kah et al. 1983). In addition, the posterior NLT is a steroid-concentrating area (Kim et al. 1978). We cannot exclude the possibility that Gn-RH cell bodies are present in the posterior NLT and remain undetected by the immunocytochemical procedure used in this study. It is well known that Gn-RH cell bodies are difficult to visualize (Barry 1979) because of the low concentration of the peptide in the perikarya or the occurrence of a prohormone masking antigenic determinants. However, the almost total absence of Gn-RH fibers in the pituitary after lesions sparing the entire basal hypothalamus rather suggests that the NLT supplies very few Gn-RH fibers to the pituitary compared with the preoptic region. The presence of two forms of Gn-RH in teleosts has been demonstrated by Sherwood et al. (1983). Perhaps the second form of Gn-RH is more prevalent in the NLT. It is interesting to note that a similar controversy occurs in mammals concerning the presence of Gn-RH perikarya in the arcuate nucleus (see Merchenthaler et al. 1984) which is also a steroid-concentrating area (Pfaff and Keiner 1973).

It is clear that further investigations are necessary to elucidate the organization of Gn-RH systems in the brain of teleosts. However, we have obtained new information concerning the main pathways, in particular those sending projections to the pituitary gland. Most of these originate from the cell bodies of the NPBL and travel to the pituitary following a route similar to that of the preoptico-infundibu-

lar pathway extensively described in mammals (see Merchenthaler et al. 1984). The periventricular network originates from more anterior cell bodies located in the ventral telencephalon and/or the olfactory bulbs and sends projections to the posterior hypothalamus (possibly the pituitary), the inferior lobe and the posterior brain (medulla oblongata and spinal chord). Such a periventricular pathway originating from the septal area and sending projections to the dorsomedial hypothalamus, median eminence and mesencephalon has been reported in rat brain (Merchenthaler et al. 1984). This pathway also supplies the mammillary complex which in the present study has been found to be devoid of any ir material.

Fibers originating from the olfactory bulbs and associated with the terminal nerve seem to project not only to the Vs and retina (Demski and Northcutt 1983; Stell et al. 1984), but also more caudally as already suggested (Springer 1983). In particular, they may contribute to the innervation of the habenular complex and the optic tectum. However, the optic tectum also receives projections from the preoptic area. In the platyfish, Schreibman et al. (1984) describe the existence of a tract originating from the nucleus olfactoretinalis and running to the habenular nucleus.

In conclusion, there is a high degree of similarity between the Gn-RH systems in teleost fish, as exemplified by the goldfish, and mammals, not only with regard to the distribution of the perikarya in homologous areas of the "primitive brain", but also in the organization of the main pathways. In both groups, with the exception of the midbrain tegmentum, the distribution of the steroid-concentrating areas overlaps that of the regions containing Gn-RH cell bodies (olfactory bulbs, septal and preoptic areas, latero-basal hypothalamus) or a high density of fibers (Vs-amygdala). The functions of these different areas also seem to be similar suggesting that such a system has been highly preserved along the course of evolution.

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