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Origin of the pituitary innervation in the goldfish

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Abstract. Despite the large number of studies devoted to the pituitary of teleosts, the origin of the direct pituitary innervation is still largely unknown. Although such a model is ideal for applying retrograde transport techniques, these methods involve the difficult in vivo injection of tracers into the pituitary and have never been applied. Recently, a lipophilic fluorescent dye (1-1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanin; DiI) has been introduced and shown to have the capacity of being transported by the membranes of paraformaldehyde-fixed tissues. Microcrystals of DiI were implanted via a ventral approach into the pituitary of goldfish previously fixed by intracardiac perfusion of paraformaldehyde. The goldfish heads were kept immersed in paraformaldehyde for various periods of time (2–6 weeks). The brains were then dissected and cut transversally using a Vibratome. The results demonstrate that hypophysiotrophic areas are essentially restricted to the preoptic region, the mediobasal hypothalamus and the nucleus dorsolateralis thalami. In addition, cell bodies probably containing gonadotrophin releasing-hormone were also retrogradely stained along a pathway that can be traced up to the olfactory bulbs. The results also confirm that cell bodies, located around the ventral aspect of the preoptic recess and probably corresponding to dopaminergic neurons, project to the pituitary. Large neurons have also been observed in the rostral dorsal midbrain tegmentum just caudal to the posterior commissure. Most neurons of the so-called paraventricular organ remain unstained. Finally, a fiber tract originating from an undetermined territory of the posterior brain has been observed. The results are discussed in relation to the possible chemical nature of the hypophysiotropic neurons.

Key words: Hypothalamus – Hypophysiotropic neurons – Pituitary innervation – Neuroendocrine control – DiI-Retrograde transport – Goldfish (Teleostei)

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Introduction

In all classes of vertebrates, the pituitary gland is divided into three main regions: the anterior lobe or pars distalis, which contains the classical secretory cell types [adrenocorticotropic (ACTH) cells, prolactin cells, thyrotrophs, somatotrophs and gonadotrophs], the intermediate lobe or pars intermedia and the neural lobe or pars nervosa. In tetrapods, the different functions of the anterior lobe are regulated by neurohormones (neuropeptides or neurotransmitters) released into the capillary plexus of the external zone of the median eminence; these neurohormones reach their target cells via the classical hypothalamo-pituitary portal system. On the other hand, in teleost fishes, the neurohypophysis penetrates deeply into the adenohypophysis and sends digitations into both the anterior lobe and the intermediate lobe. In addition, there is no convincing evidence favoring the existence of a functional portal system, and all parts of the adenohypophysis receive a direct innervation of central origin (Peter et al. 1990).

Depending on the species and cell type, nerve terminals may establish direct (sometimes synaptic-like contacts) on secretory cells or end on the basement membrane separating the neurohypophysis from the adenohypophysis. Furthermore, there is frequently an overlap between a particular cell type and the neurosecretory fibers involved in the regulation of this cell type. For instance, fibers containing gonadotrophin releasing-hormone (GnRH) are mostly restricted to the territory encompassing the gonadotrophs (Kah et al. 1986a). Fibers containing various neuropeptides (see Peter et al. 1990), monoamines (Kah et al. 1986b) or amino-acid neurotransmitters (Kah et al. 1987a, 1992a) have been characterized, but their origin is still largely unknown.

Efforts have been made to lesion different brain territories and to study the degeneration of terminals in the pituitary, but the interpretation of such studies is difficult because of the ever present possibility of lesioning the fibers of passage. A model, in which the pituitary receives afferents, without sending efferents or without

containing axons of passage, would appear to be ideal for using retrograde transport techniques in order to search for the origin of its innervation. However, these techniques have never been applied because of the difficulty of performing intrapituitary injections of tracer in vivo. The recent introduction of DiI, a carbocyanine lipophilic fluorescent tracer (1-1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanin), that can be transported by membranes of fixed tissue, overcomes the problem of in vivo injections of tracer. In this paper, we report the results obtained from a study on the origin of the pituitary innervation in the goldfish (*Carassius auratus*) following the implantation of microcrystals of DiI into the pituitary of animals previously fixed by intracardiac perfusion of paraformaldehyde.

Materials and methods

A total of 15 adult goldfish (30–50 g) of the common variety was used. Animals were purchased from a fish farm and kept in the laboratory at 18° C under a natural photoperiod (Bordeaux, France) until use. Following anesthesia with MS 222, animals were perfused via the aortic bulb with a saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Since the pituitary stalk of the goldfish is extremely fragile, the pituitary gland was exposed using a ventral approach by removing the ventral jaw and gently dissecting the tissues surrounding the pituitary. The rostral surface of the gland was dried out using filter paper to avoid precocious dissolution of the DiI. Using an insect pin, a small hole was made along the midline in the pars distalis, and a microcrystal of DiI was implanted using the insect pin. The skull was then removed to facilitate exposure of the brain to the fixative and the head was immersed in 4% paraformaldehyde in 0.1 M phosphate buffer. Samples were kept in an oven at 37° C for a duration of 2–6 weeks. The brain and olfactory bulbs were then carefully dissected out, embedded in Agar and cut transversally using a Vibratome. Sections of 50 µm thickness were obtained, mounted on slides and coverslipped in glycerol:phosphate buffer (1:1). The sections were observed using a Leitz fluorescence photomicroscope equipped with rhodamine filters. Unless otherwise stated, the nomenclature for brain nuclei and the level of the different transverse sections were taken from Peter and Gill (1975).

Results

Figure 1 provides a schematic sagittal representation of the hypophysiotropic areas and pathways in the brain of the goldfish. Because the main neurohypophyseal tract invading the neurointermediate lobe crosses the anterior lobe in the middle, and because of the extensive diffusion of the dye, it is not possible to identify with precision the location of the crystals. Therefore, the results have to be related to the whole pituitary and not to a particular region. With regard to time, it is clear that for the regions close to the pituitary stalk, such as the mediobasal hypothalamus, short times of exposure must be used to obtain a clear specific retrograde staining. Indeed, after long exposure, the tracer tends to diffuse from the point of insertion and to stain the whole mediobasal hypothalamus, making it difficult to observe any clear specific staining.

Despite these limitations, the results were very consistent from one fish to another. In two fish, the number of fluorescent structures was more important on one side of the brain than on the other, probably because the crystal was not inserted in the middle of the pituitary. This seemed to indicate that neurons of one side of the brain tended to innervate one side of the pituitary.

The different brain areas containing hypophysiotropic neurons are represented on a longitudinal diagram and a series of representative transverse sections taken from the atlas of the goldfish brain (Fig. 2a–i). The results are presented from rostral to caudal.

Olfactory bulbs and tracts

The more anterior fluorescent cells were small perikarya in the rostral and caudal part of the olfactory bulbs, and were observed 6 weeks after implantation of the DiI crystal (Fig. 1). The rostral cell grouping was found at the junction between the olfactory nerve and bulbs (Fig. 3), whereas the caudal cells were observed at the junction between the bulbs and the olfactory tracts, which connect the olfactory bulbs to the telencephalon in the goldfish. In whole-mount preparations of the olfactory tracts, a few isolated cell bodies (Fig. 4) and fine varicose fibers (Fig. 5) were consistently observed within the medial olfactory tract.

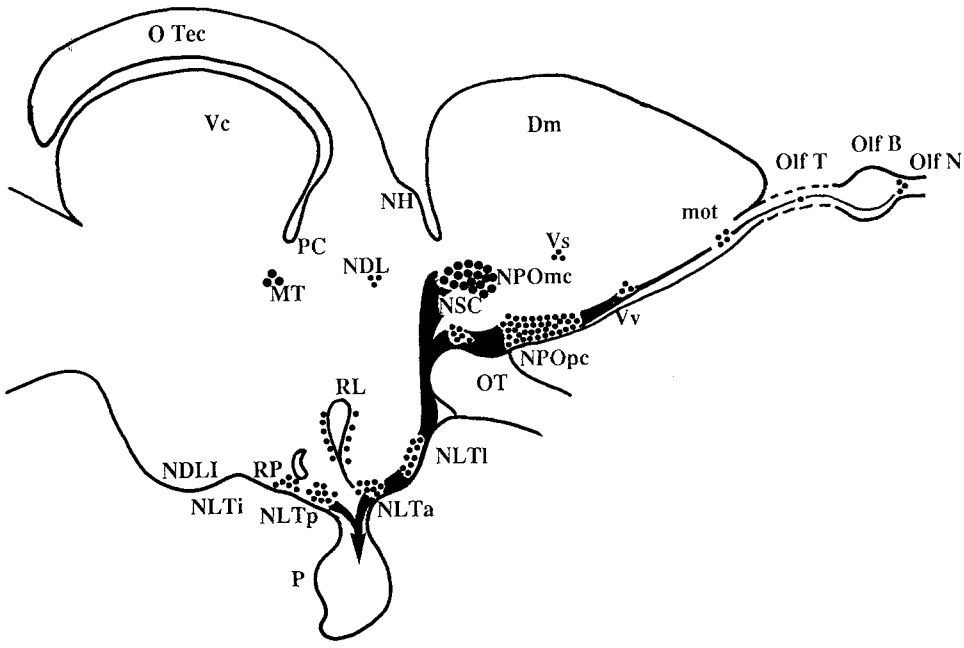
Telencephalon

Labeled cells and fibers were consistently found in the ventral part of the telencephalon (areas ventralis telencephali pars ventralis: Vv; Figs. 1, 2a). These cells were either small and located in the central and dorsal part of Vv (Fig. 6) or medium in size and located in a ventral position (Fig. 7). These fluorescent cell bodies were observed from the point of entrance of the medial olfactory tract to the anterior ventral anterior preoptic region. In the ventral telencephalon, longitudinally oriented labeled fibers were consistently observed close to the midline (Figs. 6, 7). The caudal portion of the nucleus (n.) entopeduncularis was also found to contain small retrogradely labeled cells (Figs. 2c, 9), whose axons traveled first towards the midline and then ventrally in the direction of the n. preopticus (NPO).

Diencephalon

In the diencephalon, two major areas were found to be hypophysiotropic: the preoptic region and the mediobasal hypothalamus.

Preoptic region. At this level, the terminology of Peter and Gill (1975) had to be slightly modified. The hypophysiotropic n. preopticus periventricularis (NPP) and the NPO of the atlas were simply divided into the n. preopticus, pars parvicellularis (NPOpc) and pars magnocellularis (NPOmc) according to the size of their perikarya. Labeled cell bodies were regularly observed around the preoptic recess (Fig. 2b, c), in an area that

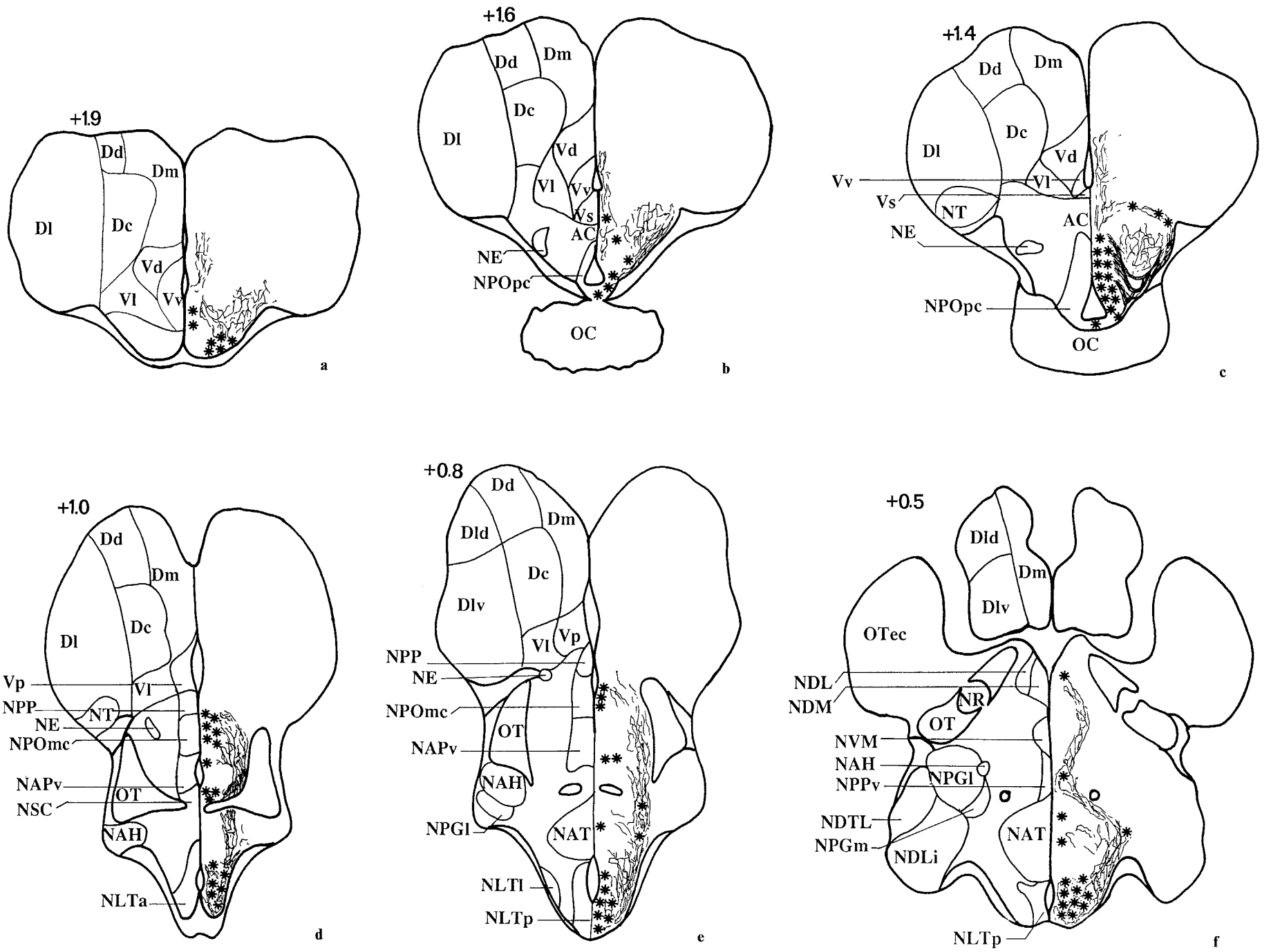


Abbreviations for **Figs. 1, 2:** *AC* anterior commissure; *AP* area pretectalis; *C* cerebellum; *CM* corpus mamillare; *Dc* area dorsalis telencephali, pars centralis; *Dd* area dorsalis telencephali, pars dorsalis; *Dl* area dorsalis telencephali, pars lateralis; *Dld* area dorsalis telencephali, pars lateralis; *Dlv* area dorsalis telencephali, pars lateralis ventralis; *Dm* area dorsalis telencephali, pars medialis; *HOC* horizontal commissure; *mot* medial olfactory tract; *MT* midbrain tegmentum; *NAH* n. anterior hypothalami; *NAPv* n. anterior periventricularis; *NAT* n. anterior tuberis; *NCH* n. cerebellosus hypothalami; *NDL* n. dorsolateralis thalami; *NDLI* n. diffusus lobi inferioris; *NDM* n. dorsomedialis thalami; *NDTL* n. diffusus tori lateralis; *NE* n. entopeduncularis; *NG* n. glomerulosus; *NH* n. habenularis; *NLTa* n. lateralis tuberis, pars anterior; *NLTl* n. lateralis tuberis, pars lateralis; *NLTp* n. lateralis tuberis, pars posterior; *NLTi* n. lateralis, tuberis pars inferior; *NP* n. pretectalis; *NPGc* n. preglomerulosus, pars medialis commissuralis; *NPGm* n. preglomerulosus, pars medialis; *NPGl* n. preglomerulosus, pars lateralis; *NPOmc* n. preopticus, pars magnocellularis; *NPOpc* n. preopticus, pars parvicellularis; *NPPv* n. posterior periventricularis; *NPT* n. posterior tuberis; *NR* n. rotundus; *NRL* n. recessus lateralis; *NRP* n. recessus posterioris; *NSC* n. suprachiasmaticus; *NSV* n. sacci vasculosi; *NT* n. taeniae; *NTP* n. posterior thalami; *NVL* n. ventrolateralis thalami; *NVM* n. ventromedialis thalami; *OC* optic chiasma; *Olf B* olfactory bulb; *Olf N* olfactory nerve; *Olf T* olfactory tract; *OT* optic tract; *OTec* optic tectum; *P* pituitary; *PC* posterior commissure; *RL* recessus lateralis; *RP* recessus posterior; *RPO* recessus preopticus; *SCO* subcommissural organ; *TC* tectal commissure; *Vc* valvula of the cerebellum; *Vd* area ventralis telencephali, pars dorsalis; *Vl* area ventralis telencephali, pars lateralis; *Vp* area ventralis telencephali, pars postcommissuralis; *Vs* area ventralis telencephali, pars supracommissuralis; *Vv* area ventralis telencephali, pars ventralis;

Fig. 1. Schematic representation of the major hypophysiotropic regions and tracts on a longitudinal section of the goldfish brain

corresponded to the NPOpc (level +1.7 to +1.2), anterior NPP of Peter and Gill (1975). In this nucleus, most perikarya appeared to be labeled (Fig. 10). In particular, the ventral wall of the preoptic recess (not represented in the atlas) contained two populations of hypophysiotropic cells: numerous small cell bodies and a few medium-sized perikarya (Fig. 8). In this area, a few cerebrospinal fluid (CSF)-contacting neurons were observed. In more lateral regions, close the optic chiasma, scattered fluorescent cells were also detected. In this region, sometimes called the n. preopticus basalis lateralis (Münz et al. 1981), the labeled fibers coming from the ventral telencephalon and the numerous axons that issued from the NPP (Fig. 10) joined together and formed a preoptico-hypophyseal tract that could be followed up to the pituitary stalk. The more rostral part of the NPO as given in the atlas of Peter and Gill (1975) at levels +1.3 and +1.2 was never seen to contain hypophysiotropic cells. More posteriorly and dorsally, from level +1.1 to 0.6, large cell bodies were labelled in an area that was named NPOmc (Figs. 2d, e, 11). The axons that issued from these cells joined the preoptico-hypophyseal tracts just at the level where the optic tracts arched dorsally (Figs. 11, 12). In the n. anterioris periventricularis (NAPv), only a few neurons, sometimes of the CSF-contacting type, were observed. A group of fluorescent perikarya was regularly observed just ventral to the NAPv and dorsal to the optic tract (Figs. 2d, 12, 13). This area is sometimes refered to by certain authors as the suprachiasmatic nucleus (NSC). At this level, the axons that issued from all these cell bodies formed large fluorescent tracts (Fig. 12).

Mediobasal hypothalamus. Just caudal to the end of the optic chiasma (level +1.0, +0.9), the preoptico-hypophyseal tract ran down to the mediobasal hypothalamus, bordering the n. anterior hypothalami (NAH) towards the n. lateralis, pars lateralis (NLTI). Scattered fluorescent cells were observed along the tracts, although they were difficult to observe because of the intense fluorescence of the tracts themselves. In the NLTI, large cell bodies were consistently labeled, as well as in the region


+1.9
Dd
Dm
Dl
Dc
Vd
Vl
Vv
a
+1.6
Dd
Dm
Dc
Dl
Vd
Vl
Vv
Vs
AC
NE
NPOpc
OC
b
+1.4
Dd
Dm
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Dl
Vd
Vv
Vl
Vs
NT
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NPOpc
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c
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NPP
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NPOmc
NAPv
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NSC
NAH
NLTa
d
+0.8
Dd
Dld
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Vl
Vp
NPP
NE
NPOmc
OT
NAPv
NAH
NPGl
NAT
NLTl
NLTp
e
+0.5
Dld
Dm
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NLTp
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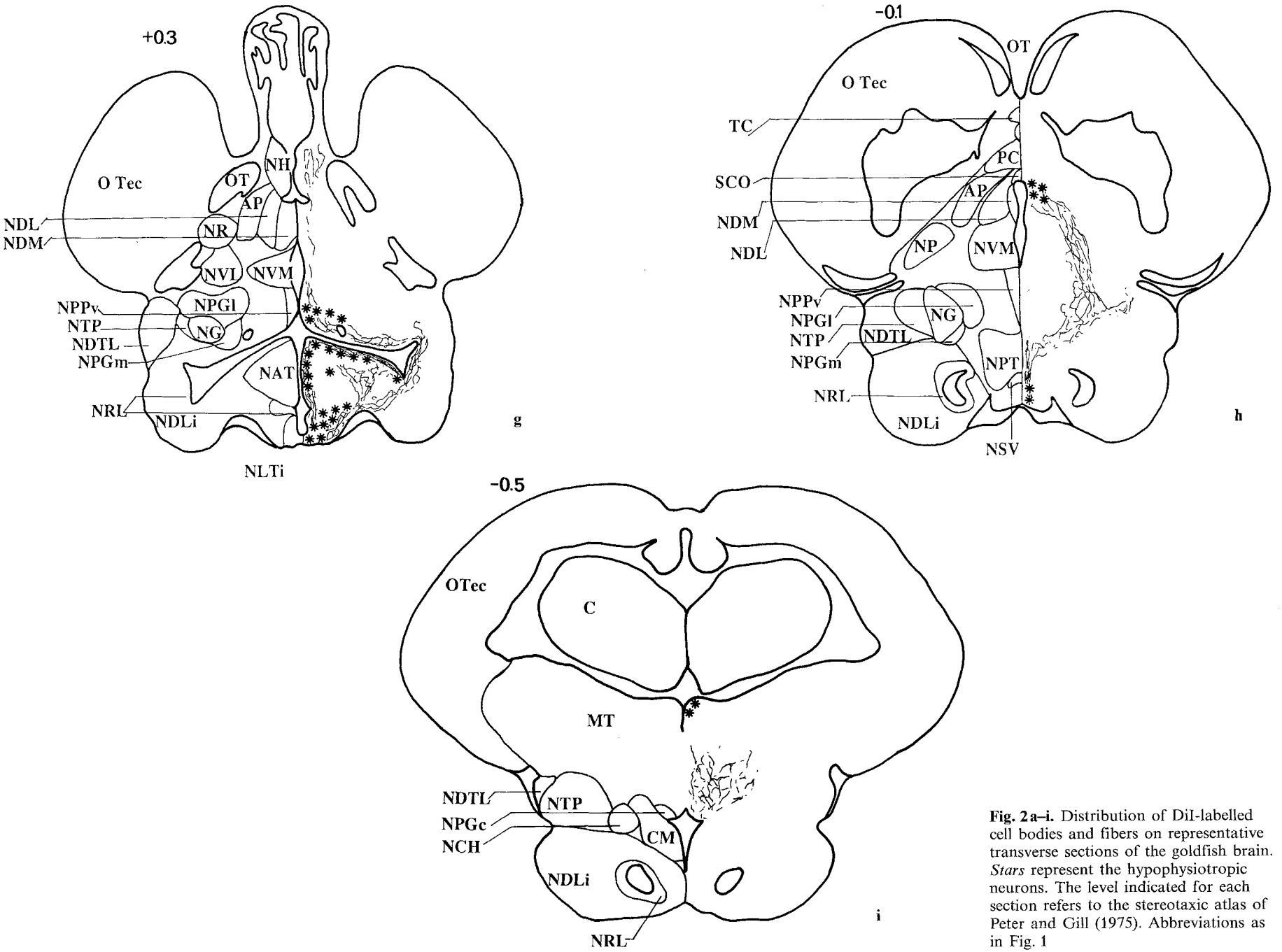
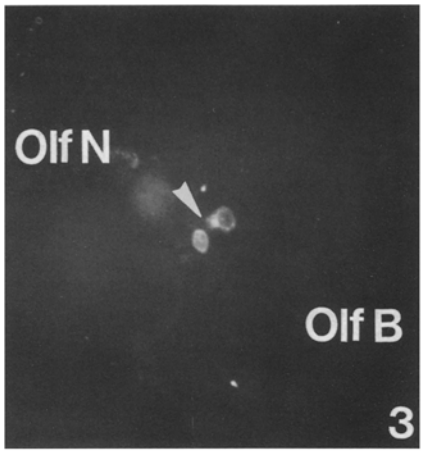
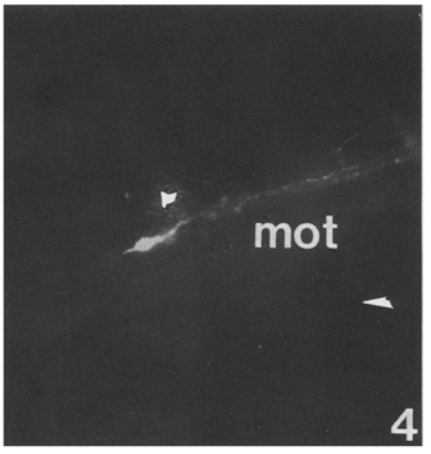


Fig. 2a–i. Distribution of DiI-labelled cell bodies and fibers on representative transverse sections of the goldfish brain. *Stars* represent the hypophysiotropic neurons. The level indicated for each section refers to the stereotaxic atlas of Peter and Gill (1975). Abbreviations as in Fig. 1


Olf N
Olf B
3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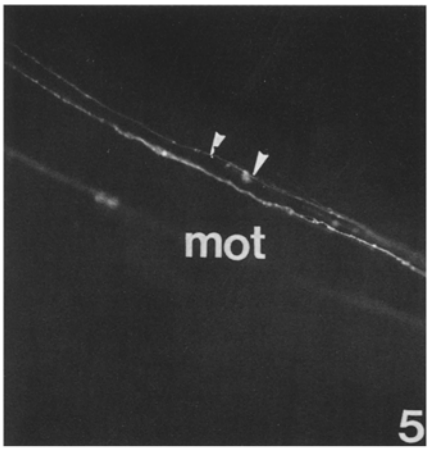
mot
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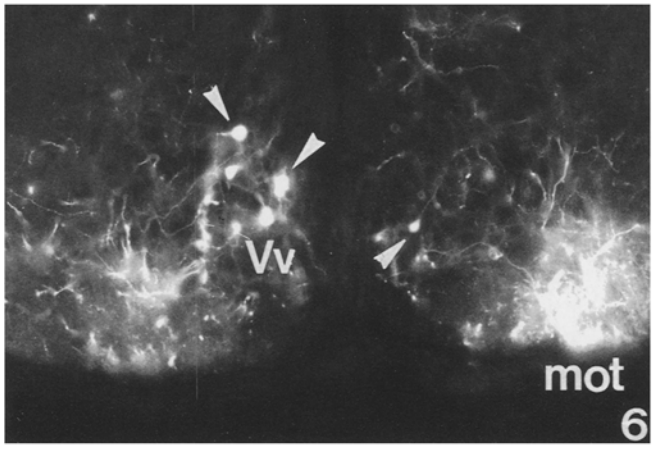
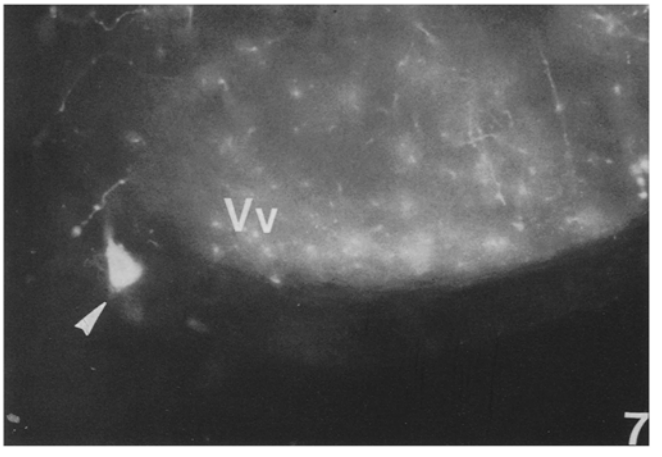

Vv
mot
6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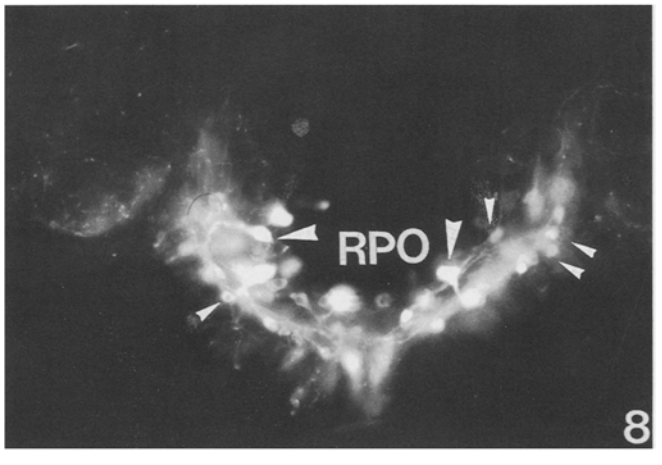
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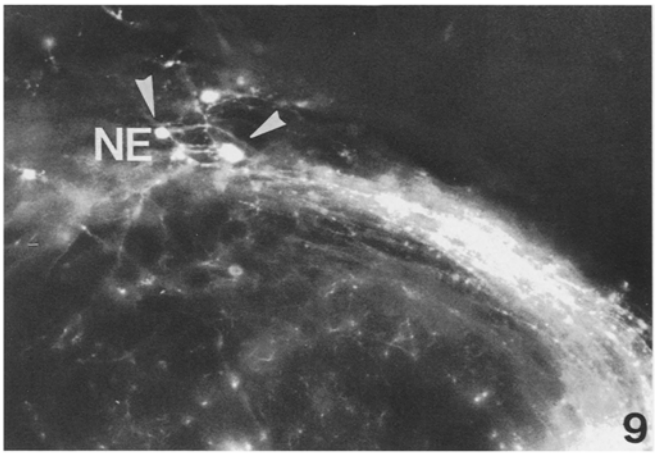
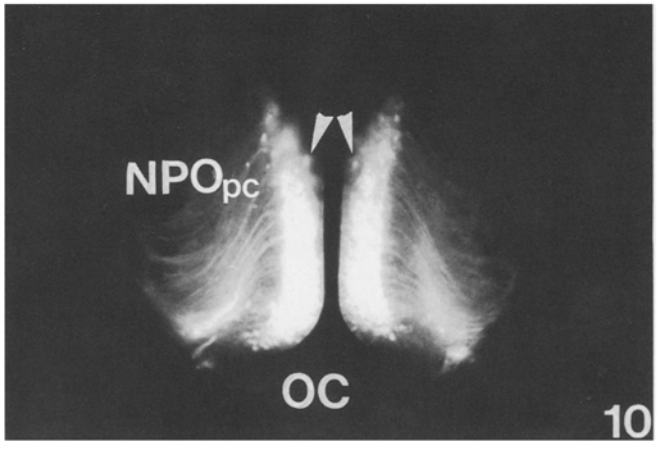
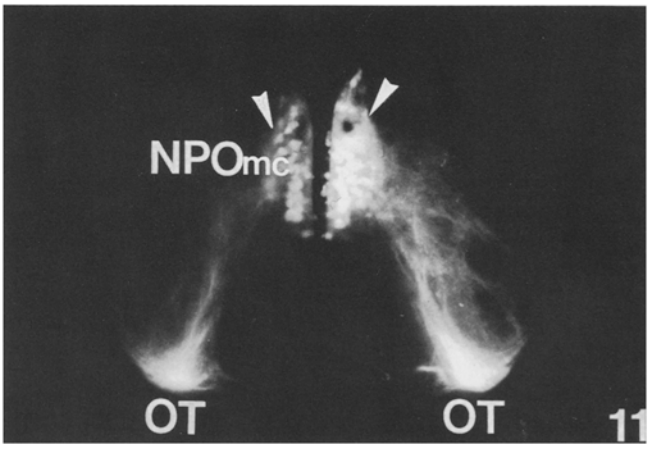

NE
9


NPOpc
OC
10


NPOmc
OT
OT
11

of the anterior n. lateralis tuberis (NLT), along the midline (Figs. 2d, 12). At the level of the pituitary, the tracts converged toward the stalk. More caudally, all parts of the NLT, posterior and inferior NLT (NLTp and NLTi), contained many fluorescent cells, some of them being of the CSF-contacting type (Figs. 2f, g, 14, 15). The n. anterior tuberis (NAT) contained a few scattered stained perikarya in its central or lateral regions and also along the ependymal layer (Fig. 2g). The n. posterior periventricularis (NPPv) was observed to contain a few retrogradely stained cells, but they were not within the ependyma. Finally, scattered labeled cells were observed around the lateral parts of the n. recessus lateralis (NRL). The CSF-contacting neurons of the NRL and n. recessus posterioris (NRP) were never observed to be fluorescent, although a few small round perikarya were found around the NRP (Fig. 17).

It is interesting to mention that, at the level of the pituitary stalk and only at this level, ependymal cells ressembling tanycytes were clearly retrogradely stained. Processes that issued from these ependymal cells could be followed up to the level of the stalk.

Thalamus

In all fish studied, a group of small fluorescent cell bodies was observed in the n. dorsolateralis thalami (NDL; Figs. 2h, 16). Axons that issued from these cells could be clearly followed from the thalamus to the pituitary stalk, travelling around the n. ventromedialis thalami and NAT.

Mesencephalon

Surprisingly, in all fish, fibers could be followed from the posterior hypothalamus to the tegmentum of the midbrain in which large fluorescent cell bodies could be observed along the midline just caudal to the posterior commissure (Figs. 2i, 18, 19), but also more laterally in the central nucleus of the torus semicircularis.

Discussion

From the results mentioned above, it is clear that the retrograde transport of DiI is a powerful tool for mapping the hypophysiotropic systems in a model such as the pituitary of teleosts. As these results were consistently observed from one fish to another, we feel confident about our observations. The same technique has previously been successfully applied in *Apteronotus leptorhynchus* (Johnston and Maler 1992). Although our results are similar to those observed in this latter study, we have found more hypophysiotropic cells, in particular at the level of the olfactory systems and mesencephalon, indicating that Johnston and Maler (1992) may not have allowed enough time for the tracer to reach these areas, which are far removed from the pituitary. This assumption is reinforced by the fact that we have observed retrogradely labeled perikarya in these regions only in brains incubated for the longest period (6 weeks). The short incubation times necessary here compared with the very long times (up to one year) employed by Johnston and Maler (1992) could be explained either by a difference in the size of the brain or by the fact that we incubated the samples at 37° C, whereas the brains studied by Johnston and Maler were incubated at room temperature.

Our study confirms the empirically established view according to which the hypophysiotropic areas in teleosts are essentially located in the classical neuroendocrine territories such as the preoptic region and the mediobasal hypothalamus, although this has never previously been formally demonstrated. Indeed, this assumption has been based mainly on classical histological techniques, and only a few attempts have been made to demonstrate conclusively that particular hypothalamic nuclei project to the pituitary. The specific staining of neurophysin-containing neurons of the magnocellular system by aldehyde fuchsin has clearly established that these neurons project to the neurointermediate lobe, but there has previously been little precise information about the so-called parvicellular systems. Fryer and Maler (1981) have reported the uptake and retrograde transport of peroxidase from the pituitary to the NPO and the NLT; however, the technique based on the uptake of intraperitoneally injected peroxidase has not proved to be very sensitive. Other attempts have been based on electrolytic lesions of particular brain nuclei followed by immunocytochemical or electron-microscopical examination of the pituitary innervation. In the goldfish, lesions have been placed at different levels of the paraventricular organ, and degenerating fibers in the pituitary have been demonstrated (Fryer et al. 1985); however this approach is dangerous because it is now clear from the present results and from those of Johnston and Maler (1992) that the paraventricular organ does

Fig. 3. A few fluorescent perikarya (*arrowhead*) can be observed at the junction between the olfactory nerve (*Olf N*) and bulb (*Olf B*) 6 weeks after implantation of the DiI crystal. ×320

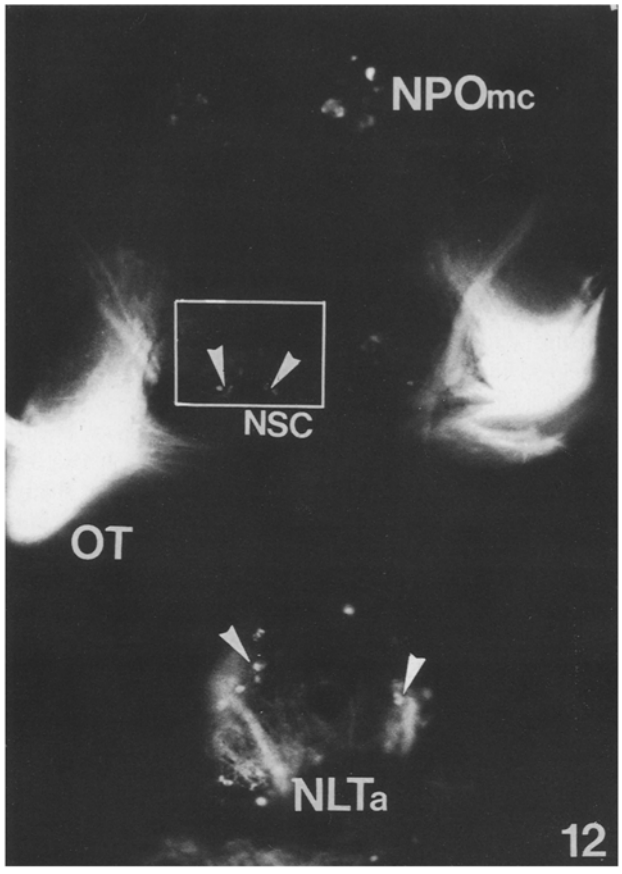
Figs. 4–5. Whole-mount preparations of the medial olfactory tract (*mot*) showing a fluorescent perikaryon (*arrowhead*; **Fig. 4**) and fibers (*arrowheads*; **Fig. 5**). ×110

Figs. 6, 7. Transverse sections at the level of the ventral telencephalon (*Vv*) illustrating the two populations of labeled neurons: small (*arrowheads*; Fig. 6) and large (*arrowhead*; Fig. 7). Note the numerous fluorescent fibers within the medial olfactory tract (Fig. 6, *mot*). Fig. 6: ×120, Fig. 7: ×290

Fig. 8. Transverse section at the level of the anterior ventral preoptic region showing small (*small arrowheads*) and larger (*large arrowheads*) labeled perikarya within the ventral wall of the preoptic recess (*RPO*). ×125

Fig. 9. Transverse section at the level of the caudal extent of the n. entopeduncularis (*NE*). *Arrowheads* Small retrogradely labelled cell bodies. Note the fluorescent pathway running first toward the midline and then ventrally. ×200

Figs. 10, 11. Low power view of transverse sections at the level of the pars parvicellularis (*arrowheads*; *NPOpc*) and magnocellularis (*arrowheads*; *NPOmc*) of the n. preopticus. Note the axons issuing from the cells and running ventrolaterally. *OC* Optic chiasma; *OT* Optic tract. ×50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NSC
OT
NLTa
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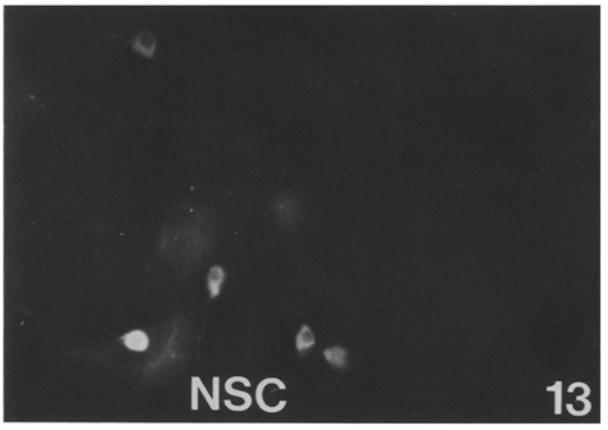
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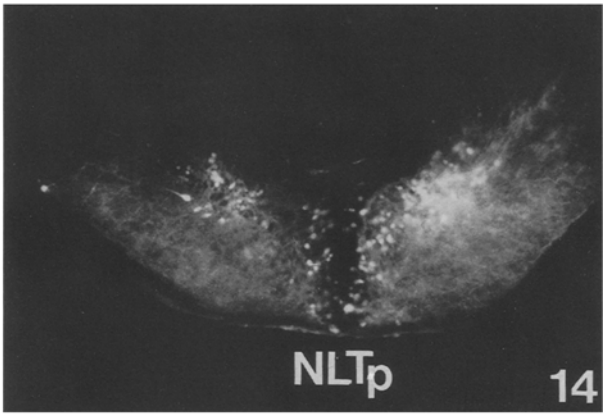
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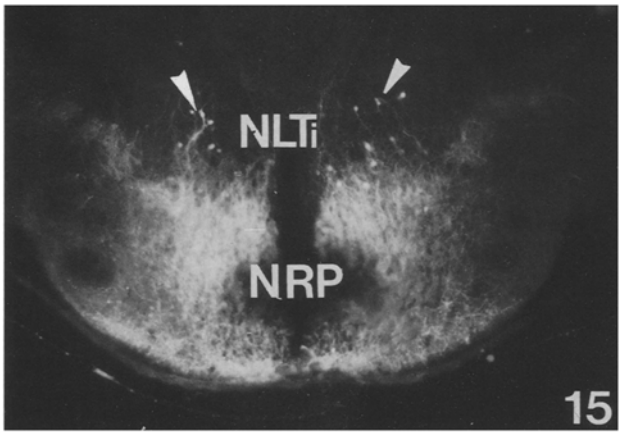
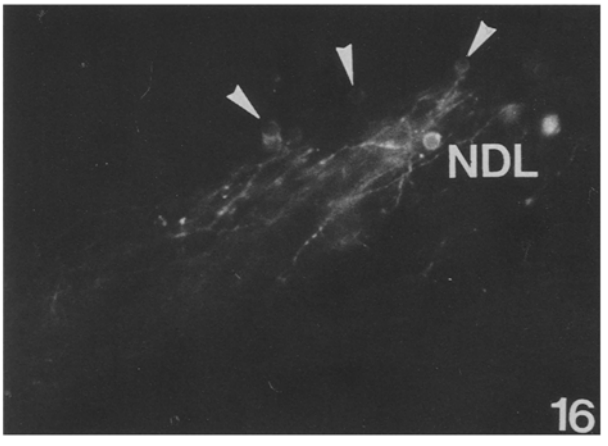
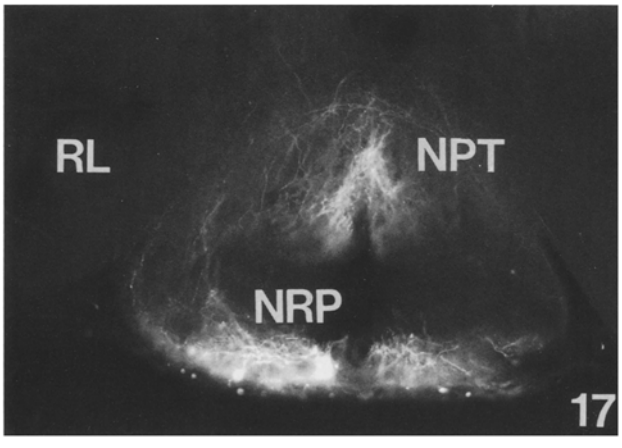
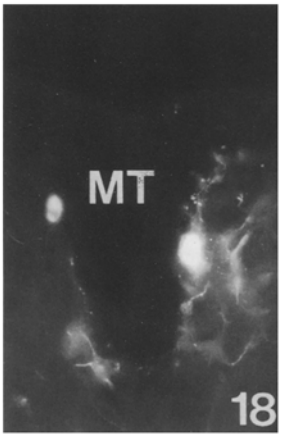
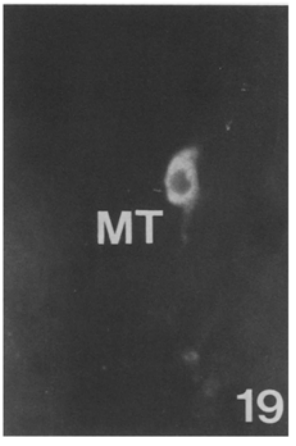

NLTi
NRP
15


NDL
16


RL
NPT
NRP
17


MT
18


MT
19

not project to the pituitary, demonstrating that the risk of destroying axons of passage is important. In addition to the classical neuroendocrine regions, this study demonstrates that extrahypothalamic neurons project to the pituitary. This is notably the case for the olfactory bulbs and tracts, the ventral telencephalon and the tegmentum of the midbrain.

Although we have attempted to process sections of DiI preparations for immunocytochemistry, we have not yet succeeded in staining retrogradely DiI-labeled neurons with any of the antibodies that we have tried, possibly because of the long incubation times at 37° C that may result in a loss of antigenicity. Therefore, the chemical nature of these neurons is not known, but by comparing the present study and others performed on the distribution of several peptides and neurotransmitters in the goldfish, it is possible to put forward some hypotheses.

The presence of hypophysiotrophic neurons in the ventral telencephalon, but not in the olfactory systems, has also been reported in *Apteronotus leptorhynchus* (Johnston and Maler 1992). It is probable that these neurons contain salmon-GnRH (sGnRH), since the labeled perikarya and fibers have exactly the location, size and shape of sGnRH-immunopositive neurons in the same areas (Kah et al. 1986b, 1993). In the goldfish, two forms of GnRH have been identified in the brain: sGnRH, which is more abundant in the anterior brain, and chicken-GnRH-II, which predominates in the posterior brain (Yu et al. 1988). The two forms of GnRH are present in the pituitary (Yu et al. 1988), but their origins are not known. The sGnRH fibers in the goldfish pituitary may originate from the olfactory bulbs and tracts, the telencephalon and the ventrolateral preoptic region, whereas the cGnRH-II might originate from the large neurons of the ventral tegmentum. Indeed, the present study indicates the existence of hypophysiotropic neurons along a fiber tract extending from the olfactory nerve to the pituitary and overlapping the distribution of sGnRH in the forebrain of the goldfish. However, the large sGnRH-containing neurons of the ganglion cells of the terminal nerve (Kah et al. 1986b) are not retrogradely labeled, in agreement with a recent study demonstrating that these neurons do not contribute to the pituitary innervation (Kobayashi et al. 1992). cGnRH-II fibers in the pituitary could originate from the large cell bodies that occur in the midbrain tegmentum and that have been found in the present study to be hypophysiotrophic. Although, it has not been demonstrated in goldfish, there is evidence in the Masu salmon (Amano et al. 1991) and the Siberian sturgeon (Kah et al. 1993) that cGnRH-II is only present in large cell bodies of the anterior dorsal midbrain tegmentum. GnRH neurons in the same location have been reported in the goldfish (Kah et al. 1986b), and will probably be found in the future to contain cGnRH-II.

The observation of retrogradely labeled neurons in the pars supracommissuralis of the telencephalon is interesting. Indeed, this region is well known for being strongly implicated in the control of spawning behavior in male goldfish (Kyle and Peter 1982). The chemical nature of the neurons in this area is poorly documented. So far, cell bodies containing gamma-aminobutyric acid (GABA; Martinoli et al. 1990), glutamate (D. de Monbrison, N. Mons and O. Kah, unpublished data) and neurotensin (I. Anglade, G. Tramu and O. Kah, unpublished observations) have been observed in this region, which also receives a heavy GnRH innervation (Kah et al. 1986b). Whether such neurons mediate part of the endocrine response associated with sexual behavior (Dulka et al. 1987) remains to be demonstrated. The finding of hypophysiotropic neurons in the n. entopeduncularis was not expected. Such neurons have only been observed in the caudal part of this nucleus, in which GABA-positive and neuropeptide Y-positive neurons have been reported (Martinoli et al. 1990; Pontet et al. 1989).

The most anterior neurons of the preoptic region, located around the ventral anterior part of the preoptic recess, probably correspond partly to the dopaminergic neurons responsible for inhibiting gonadotrophin-II release in goldfish (Kah et al. 1984). Indeed, electrolytic lesions of this are result in the disappearance of all tyrosine hydroxylase-positive fibers in the anterior lobe of the pituitary and in a spectacular increase in gonadotrophin levels (Kah et al. 1987b). However, we have also observed larger perikarya, whose chemical nature is unknown, in the same area. In the NPOpc, most cells are retrogradely labeled, as observed in *Apteronotus leptorynchus* (Johnston and Maler 1992). Many different neuropeptides and neurotransmitters have been identified in this nucleus sometimes refered to as the NPP (Peter and Gill 1975). As only part of the NPP was found to project to the pituitary, we choose to name this hypophysiotropic component the NPO, pars parvicellularis. Numerous galanin-positive cell bodies, probably contributing to the heavy galanin innervation of the pitui-

Fig. 12. Low-power view of a section at the level of the caudal preoptic region and the anterior n. lateralis tuberis (*NLTa*) illustrating the caudal nucleus preopticus, pars magnocellularis (*NPOmc*), and the suprachiasmatic nucleus (*NSC*). Note the large bilateral fluorescent preoptico-hypophyseal tracts above the optic tract (*OT*). ×75

Fig. 13. High-power view of the area framed in Fig. 12 illustrating the hypophysiotropic neurons of the suprachiasmatic nucleus (*NSC*). ×230

Figs. 14, 15. Two aspects of the n. lateralis tuberis, pars posterior (*NLTp*) and pars inferior (*NLTi*), illustrating the retrogradely labeled perikarya (*arrowheads*; transverse sections). Note on Fig. 15 that the anterior part of the n. recessus posterioris (*NRP*) does not contain any fluorescent structures. Fig. 14: ×60; Fig. 15: ×60

Fig. 16. Transverse section at the level of the n. dorsolateralis (NDL) of the thalamus showing a few retrogradely labeled neurons (*arrowheads*). ×170

Fig. 17. Transverse section at the level of the caudal hypothalamus, showing the absence of fluorescent structures within the n. recessus posterioris (*NRP*). Note the fluorescent axons ventral to the *NRP* and in the n. posterior tuberis (*NPT*). *RL* Recessus lateralis. ×45

Figs. 18, 19. Two aspects of the large perikarya observed in the anterior dorsal midbrain tegmentum (*MT*). Fig. 18: ×115; Fig. 19: ×190

tary, have been reported in this area (Olivereau and Olivereau 1991; I. Anglade and O. Kah, unpublished). Cell bodies immunoreactive for GABA (Martinoli et al. 1990), glutamate (D. de Monbrison, N. Mons and O. Kah, unpublished), somatostatin (Kah et al. 1982; Olivereau et al. 1984), CRF (corticotropin-releasing factor; Olivereau et al. 1984) and tyrosine hydroxylase (Hornby et al. 1987) have also been observed in this nucleus. The ventral preoptic region is the main steroid concentrating area in goldfish, contains high levels of aromatase or androgen receptor immunoreactivity (for a review, see Kah et al. 1993), and thus appears to be one of the major integrative neuroendocrine regions similar to the homologous region in mammals.

The large neurons of the NPOmc are known to contain the nonapeptides vasotocin, isotocin, and neurophysin (Cumming et al. 1982; Goossens et al. 1977) and to project to the neurointermediate lobe. In addition, neurons containing CRF, some of which also containing vasotocin, have been identified in this nucleus (Olivereau and Olivereau 1988). Recently, evidence that the magnocellular neurons are also immunoreactive for glutamate has been obtained in goldfish (D. de Monbrison, N. Mons and O. Kah, unpublished). Only a few neurons of the n. anterior periventricularis are hypophysiotropic. Neurons containing somatostatin (Kah et al. 1982; Olivereau et al. 1984) and GABA (Martinoli et al. 1990) have been located in this region. The finding of hypophysiotropic neurons in the suprachiasmatic nucleus, as also observed in *Apteronotus leptorhynchus* (Johnston and Maler 1992), is interesting because this nucleus is a component of the visual system (Springer and Mednick 1984). The homologous nucleus in mammals is supposed to be a central circadian oscillator (Moore 1983), but there is no such evidence in fish. Tyrosine hydroxylase-positive neurons are located in this nucleus (Hornby et al. 1987; I. Anglade and O. Kah, unpublished), but there is no convincing demonstration for dopamine immunoreactivity as claimed by Johnston and Maler (1992). Neurons positive for GABA (Martinoli et al. 1990) and glutamate (D. de Monbrison, N. Mons and O. Kah, unpublished) have also been reported in this particular region.

The NLT has been known for a long time to be a major hypophysiotropic area (Fryer and Maler 1981), although detailed evidence has been lacking. Many different neuropeptides and neurotransmitters have been reported in the NLT area in the goldfish: ACTH (Olivereau and Olivereau 1990), galanin (Olivereau and Olivereau 1991; I. Anglade and O. Kah, unpublished), somatostatin (Kah et al. 1982; Olivereau et al. 1984), neurotensin (I. Anglade and O. Kah, unpublished), GABA (Martinoli et al. 1990), glutamate (D. de Monbrison, N. Mons and O. Kah, unpublished), and GnRH (Kah et al. 1986b). This area is also steroid-concentrating territory in the goldfish (Kim et al. 1978) and represents another major integrative neuroendocrine region. The chemical nature of the hypophysiotropic neurons observed in the NAT is unknown, but the labeled neurons observed in the lateral parts of the NRL are located in the same position as neurons immunoreactive for TRH thyrotropin-releasing hormone) observed in the carp (Hamano et al. 1990).

The finding of hypophysiotropic neurons in the thalamus confirms the study by Johnston and Maler (1992) in *Apteronotus leptorhynchus*. No report of a similar projection has been presented in higher vertebrates. The nature of these neurons is not documented, although GABA-positive cell bodies have been reported in this nucleus (Martinoli et al. 1990). The thalamus is an important relay area in the processing of sensorial and motor information, and is known as a steroid concentrating area in the goldfish (Kim et al. 1978).

In contradiction to a well-established view (Fryer et al. 1985), the dopamine and serotonin CSF-contacting neurons of the three nuclei constituting the so-called paraventricular organ (NPPv, NRL, NRP; see Kah and Chambolle 1983) do not seem to project to the pituitary. This agrees with the results of Johnston and Maler (1992) in *Apteronotus leptorhynchus*, and with the fact that no serotonin fibers can be detected in the pituitary either by immunocytochemistry or radioautography (Kah et al. 1989), as one would expect if the paraventricular organ projects to the pituitary. The dopaminergic innervation of the pituitary (Kah et al. 1986a) originates from the anterior ventral preoptic region (Kah et al. 1987b) and other unidentified nuclei.

In conclusion, the present study provides new information about the hypophysiotropic systems of one of the most studied teleost models, the goldfish. In addition to confirming the hypophysiotropic nature of the preoptic region and the mediobasal hypothalamus, the finding of long projecting hypophysiotropic neurons in the olfactory system or the tegmentum of the midbrain is unexpected. It will be of high interest to elucidate the chemical nature of these neurons, although both are probably related to the GnRH systems. This work provides strong evidence that the hypophysiotropic brain far exceeds the limits of the classical neuroendocrine territories.

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