Distribution of enkephalin-like immunoreactivity in the central nervous system of the rainbow trout: an immunocytochemical study

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ABSTRACT

The distribution of enkephalin-like immunoreactive (ELI) cell bodies and fibres in the brain of the teleost Salmo gairdneri L. was demonstrated with the indirect peroxidase–antiperoxidase immunocytochemical technique using a highly specific antiserum.

In the telencephalon, ELI cell bodies were located in the area ventralis. In the diencephalon, they were found in the nucleus ventromedialis of the thalamus, nucleus lateralis tuberis, nucleus recessus lateralis, and nucleus recessus posterioris. In the mesencephalic tegmentum, ELI cell bodies were found in the nucleus of the rostral mesencephalic tegmentum, and in a group of neurons which was located dorsal to the nucleus of the rostral mesencephalic tegmentum. In the medial torus semicircularis, small numbers of immunoreactive cell bodies were found. In the cerebellum, numerous cell bodies were observed in the granule cell layer and at the border between the granular and molecular layer. ELI cell bodies were also seen in the nucleus tegmenti dorsalis lateralis and nucleus fasciculi solitarii.

ELI fibres were widely distributed in the rainbow trout brain. The highest density of immunoreactive fibres was found in the area ventralis telencephali, the mesencephalic tegmentum, the stratum opticum of the optic tectum, the central gray of the brainstem, the caudal part of the fasciculi solitarii and the dorsal horn of the spinal cord. In the stratum fibrosomum et griseum superficiale, stratum griseum centrale and stratum album centrale of the optic tectum, a moderate number of immunoreactive fibres was observed. In the olfactory bulb only a few immunoreactive fibres were present. No effect in the labelling was found after colchicine injections.

These results provide the first complete mapping of the ELI in a fish brain. It is clear that enkephalins show a similar distribution pattern in Salmo gairdneri to that in other vertebrates; however, the number of ELI cell bodies in the fish brain is smaller than in land vertebrates. The distribution of enkephalins in specific hypothalamic nuclei, visual areas, and in the brainstem of the rainbow trout brain, suggests that these peptides are involved in the modulation of neuroendocrine and as well in visual and somatosensory functions.

INTRODUCTION

Since the discovery of opiate receptors (Pert & Snyder, 1973; Pert et al, 1974) and subsequently of enkephalins (Hughes et al, 1975), many biochemical and immunocytochemical studies have described the localisation of enkephalins in the mammalian CNS: for humans (Bouras et al, 1984; Rougeot et al, 1988), monkeys (Haber & Elde, 1982a, b; Di Figlia & Aronin, 1984), cats (Coveñas et al, 1986, 1988; Léger et al, 1986), guinea pigs (Tramu et al, 1981) and rats (Elde et al, 1976; Simantov et al, 1977; Sar et al, 1978; Finley et al, 1981). The enkephalins are located particularly in regions involved in the control and transmission of pain messages. Such regions include the spinal cord, the raphe nucleus, the reticular formation, the periaqueductal gray and the thalamus (Hökfelt et al, 1977; Simantov et al, 1977; Sar et al, 1978; Finley et al, 1981). In addition, previous studies have suggested that enkephalins play an important

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role in neuroendocrine functions (Micevych & Elde, 1980), control of the reproductive axis (Yui, 1983), visual processes (Pickard, 1985), and inhibition of release of substance P (SP) from sensory fibres (Way, 1981).


In fishes, the presence of enkephalins has been demonstrated by using radioimmunoassay (King & Millar, 1980). Opiate receptors have also been found in fish (Pert et al. 1974; Buatti & Pasternak, 1981). ELI has been described in the neuroendocrine system of the carp (Folladius & Dubois, 1979a, b; Yamada et al. 1988), the preoptic nucleus, cerebellum, neuroendocrine cells, gustatory lobes, visceral nuclei and hypothalamus of the goldfish (Reaves & Hayward, 1979 a,b; Finger, 1981; Schulman et al. 1981), in the nucleus of the rostral mesencephalic tegmentum of rainbow trout and Atlantic salmon (Vecino et al. 1991); in the gustatory lobes, visceral nuclei and cerebellum of catfish (Finger, 1981), and in the telencephalon of the African lungfish (Reiner & Northcutt, 1987). The comparative distribution of neuropeptide-immunoreactive systems (including enkephalins) in the brain of the green molly has also been studied (Batten et al. 1990).

To understand the possible functional role of enkephalins in the CNS of fish, it is essential to know the anatomical distribution of these substances, but no detailed studies on the fish brain have been published. In the present work, our aim was to study the distribution of fibres and cell bodies containing enkephalins in the brain of the rainbow trout, using immunocytochemical techniques combined with intraventricular injections of colchicine. The colchicine injections were undertaken to enhance the immunoreactivity of the ELI-perikarya, since colchicine is known to block the axonal transport system (Bayon et al. 1980). The results have been compared with the distribution of enkephalins in the CNS of amphibians, reptiles, birds and mammals.

**MATERIALS AND METHODS**

Eighteen adult rainbow trout (Salmo gairdneri) 18–25 cm in length, obtained from commercial sources, were used in this study. After anaesthesia with 0.03% tricaine methanesulphonate (MS-222, Sandoz), 13 animals were placed in a stereotaxic apparatus and a part of the cranium was removed to expose the brain and colchicine was injected intraventricularly (25–75 μg in 0.5–1.5 μl of distilled water). The removed piece of cranium was replaced after the injection and sealed with cyanocrylate. Two animals (without colchicine injection) were used as controls for the immunocytochemical studies.

The injected fish were kept in aquaria at either 4°C or 16°C for 1–2 d. The fish were then reanaesthetised and perfused transcardially with physiological saline solution for several minutes, followed by perfusion with a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB) pH 7.2. The brains were postfixed in the same fresh fixative for 12 h. Transverse (coronal), horizontal and sagittal sections (50–70 μm) were cut serially on a vibratome and collected in PB.

The sections were processed for the indirect immunocytochemical staining method. Tissue sections were incubated in PB containing 1% normal sheep serum and 0.3% Triton X-100 for 30 min. The sections were then incubated overnight in the same solution containing the antienkephalin antibody at a dilution of 1/1000 (this antibody was kindly provided by Professor M. Hamon, INSERM U-288, Paris). After a 30 min wash with PB, the sections were incubated for 2 h with a second antibody, sheep antirabbit IgG coupled to horseradish peroxidase (Pasteur, Paris), diluted 1/250 in PB. Finally, the sections were washed in PB and the peroxidase activity was revealed by the 3,3′-diaminobenzidine method. The sections were mounted on glycerol-PB 1/1.

It is important to note that the antibody used in this study recognises both methionine- and leucine-enkephalin. When preabsorption of the enkephalin antibody was carried out with synthetic leucine-enkephalin or methionine-enkephalin (100 μg per ml diluted antibody), no immunoreactivity was observed in either instance. However, no reduction of the immunolabelling was found when the enkephalin antibody was preabsorbed with synthetic dynorphin A1-B2, dynorphin B, Met3-Enk-Arg4-Gly5-Leu6, B-endorphin, B-endorphin or peptide E (100 μg per ml diluted antibody). In addition, the specificity of the immunostaining was controlled by omission of the antienkephalin serum from the first incubation bath. In this latter situation, no residual immunoreactivity was observed. The term ‘enkephalin-like immunoreactivity’ (ELI) will be used in descriptions of labelling in our material.

Three trout brains were embedded in paraffin and
sectioned serially in the sagittal, transverse and horizontal planes. They were subjected to Nissl and haematoxylin-eosin staining and used as an anatomical reference. Neuronal size was determined by measuring the major axis with a Zeiss ocular micrometer. The nomenclature used in this work is that previously proposed for the telencephalon (Northcutt & Davis, 1983), the hypothalamic nuclei (Billard & Peter, 1982) and for the brainstem (Nieuwenhuys & Pouwels, 1983).

RESULTS

The overall distribution of ELI cell bodies and fibres in the brain of Salmo gairdneri is illustrated in a series of transverse section drawings (Fig. 1A–P). We describe the ELI profiles as they appear in a rostrocaudal sequence.

Colchicine pretreatment improved the staining of cell bodies and dendrites, but did not reveal any new populations of immunoreactive cells. The different water temperatures had no effect on the labelling.

**Olfactory bulbs and telencephalon**

No ELI cell bodies were present in the olfactory bulbs. However, weakly labelled fibres were detected in the internal and external cell layers of the olfactory bulbs (Figs 1A, B, 2).

In the rostral telencephalic area (Fig. 1B) an abundance of thin ELI fibres was observed in the medial zone of the area dorsalis telencephali, and a moderate number in the dorsal and lateral zones of the area dorsalis telencephali. More caudally in the telencephalon, in the ventral nucleus of the area ventralis telencephali (Fig. 1C, D), we found the highest number of ELI fibres in the telencephalon (Fig. 3). In the medial zone of area dorsalis telencephali a moderate number of ELI fibres were seen (Fig. 4). At the same transverse section level, few ELI cell bodies were observed in the ventral nucleus of the area ventralis telencephali. These neurons were small (6 μm in diameter) with round perikarya (Fig. 5). The anterior commissure showed no immunoreactivity (Fig. 1D).

**Diencephalon and midbrain**

In the diencephalon the highest numbers of ELI cell bodies were found in the nucleus ventromedialis thalami, nucleus lateralis tuberis, nucleus recessus lateralis and nucleus recessus posterioris (Fig. 1E–I).

In the nucleus ventromedialis thalami, the ELI cell bodies had processes that penetrated to the ependymal wall of the ventricle and appeared to be in contact with the ventricular cavity (Fig. 1F). These neurons were fusiform and of medium size (10–15 μm).

In the nucleus lateralis tuberis and nucleus recessus lateralis, 2 kinds of ELI neurons (8 ± 2 μm) with scanty cytoplasm were found: (1) monopolar neurons with their cell bodies located close to the ventricle and their processes directed away from the ventricle (Figs 6–8); (2) neurons with a variable morphology (monopolar and round cell body, or bipolar and fusiform cell body). These ELI neurons were located far from the ependymal layer of the ventricle (Figs 1F, G, 9). A high number of immunoreactive fibres were observed within the nucleus habenularis, dorsomedialis thalami, preopticus, lateralis tuberis, recessus lateralis and recessus posterioris (Fig. 1E–I).

Large ELI neurons, 20–30 μm in diameter with variable morphology (fusiform or pyramidal, bipolar or multipolar) were observed in the nucleus of the rostral mesencephalic tegmentum as described by Vecino et al. (1991) (Figs 1H, 10, 11). Dorsal to the ELI neurons of the nucleus of the rostral mesencephalic tegmentum, another group of 20 to 25 small bipolar ELI neurons (8 ± 2 μm) were observed (Figs 1H, 12, 13).

The torus semicircularis showed a moderate number of ELI cell bodies and a high density of ELI fibres (Figs 1G–I, 14, 15). In the ventral tegmentum, numerous ELI fibres were seen close to the nucleus nervi oculomotorii. However, no ELI cell bodies were seen in this nucleus.

A high number of ELI fibres was observed in the tractus opticus marginalis lateralis (Figs 1E–F, 16). In the optic tectum no ELI cell bodies were seen, but ELI fibres were observed in the stratum opticum, fibrosum et griseum superficiale, griseum centrale and album centrale (Fig. 17). The highest number of ELI was found in the stratum opticum where 3 sublayers were conspicuous. These corresponded to the pars superficialis, pars intermedia and pars profunda (Vanegas et al. 1974). The fibres in the pars superficialis were clearly immunoreactive. In the pars media no immunoreactivity was found and in the pars profunda a large number of immunoreactive fibres was intertwined in fascicles that formed a netlike sublayer (Fig. 17). This organisation of the ELI fibres was most clear in horizontal sections at the most rostral levels of the optic tectum. In addition, radially oriented ELI fibres extended from the deeper stratum album centrale to the most-superficial stratum fibrosum et griseum superficiale. A band of immunoreactive fibres was observed between the stratum
Fig. 1. A–E. For legend see page 442.
Enkephalin immunoreactivity in teleosts

Fig. 1. F-G. For legend see page 442.
Fig. 1. H–I. For legend see page 442.
Fig. 1. J–L. For legend see page 442.
Fig. 1. Camera lucida drawings of serial transverse sections of the trout brain, showing the distribution of enkephalin-like immunoreactive (ELI) cell bodies and fibres. The levels of the sections were taken from the rostralmost (A) to the caudalmost (P) part of the brain. Bar, 400 μm.

Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AC</td>
<td>anterior commissure</td>
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<tr>
<td>AnxC</td>
<td>commissura ansulata</td>
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<tr>
<td>Cb</td>
<td>cerebellum</td>
</tr>
<tr>
<td>CCb</td>
<td>corpus cerebelli</td>
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<tr>
<td>cgr</td>
<td>granule cell layer of cerebellum</td>
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<td>cm</td>
<td>molecular layer of cerebellum</td>
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<td>cnd</td>
<td>dorsal horn</td>
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<td>cnv</td>
<td>ventral horn</td>
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<tr>
<td>crcb</td>
<td>crista cerebellaris</td>
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<tr>
<td>Dc</td>
<td>central zone of area dorsalis telencephali</td>
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<tr>
<td>Dd</td>
<td>dorsal zone of area dorsalis telencephali</td>
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<tr>
<td>Dl</td>
<td>lateral zone of area dorsalis telencephali</td>
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<tr>
<td>Dm</td>
<td>medial zone of area dorsalis telencephali</td>
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<tr>
<td>ECL</td>
<td>external cellular layer of the olfactory bulb</td>
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<td>flm</td>
<td>lateral funiculus</td>
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<td>FM</td>
<td>Maathner's fibre</td>
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<td>gc</td>
<td>griseum centrale</td>
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<td>GL</td>
<td>glomerular layer of the olfactory bulb</td>
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<td>gs</td>
<td>tractus gustatorius secundarius</td>
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<tr>
<td>H</td>
<td>hypothalamus</td>
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<tr>
<td>ICL</td>
<td>internal cellular layer of the olfactory bulb</td>
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<tr>
<td>iv</td>
<td>nucleus lateralis valvulae</td>
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<tr>
<td>NAT</td>
<td>nucleus anterior tuberis</td>
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<tr>
<td>NAPv</td>
<td>nucleus anteriors periventricularis</td>
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<tr>
<td>NDL</td>
<td>nucleus dorsolateralis thalami</td>
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<tr>
<td>NDLI</td>
<td>nucleus diffusus lobi inferioris</td>
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<tr>
<td>NDM</td>
<td>nucleus dorsomedialis thalami</td>
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<tr>
<td>NDTL</td>
<td>nucleus diffusus tori lateralis</td>
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<tr>
<td>NH</td>
<td>nucleus habenularis</td>
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<tr>
<td>NI</td>
<td>nucleus isthmi</td>
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<tr>
<td>NLT</td>
<td>nucleus lateralis tuberis</td>
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<tr>
<td>NPO</td>
<td>nucleus preopticus</td>
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<td>NPPv</td>
<td>nucleus posterior periventricularis</td>
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griseum centrale and stratum fibrosum et griseum superficiale.

Cerebellum and brainstem

In the valvula and corpus cerebelli a moderate number of ELI cell bodies and ELI fibres were seen (Fig. 1G–N). In the valvula cerebelli the ELI cell bodies were mostly located between the granular and the molecular layers (Fig. 18), whereas in the corpus cerebelli the ELI cell bodies were mainly distributed in the granular layer. These ELI cell bodies were round and of medium size, and their processes were often seen along the limit of the granular and molecular layer of the valvula cerebelli. The shapes and sizes of the ELI cell bodies that we have described in the present study, and the orientation of these ELI cells within the cerebellar layers, match Pouwels’ (1978) descriptions of the Golgi cells. ELI fibres in the trout cerebellum were found in the granular layer. These fibres were branched with swellings or varicosities and according to the description of Pouwels (1978) these ELI fibres are probably mossy fibres.

ELI cell bodies were seen in the area situated dorsolaterally to the fasciculus longitudinalis medialis, at the level of the raphe nucleus (Fig. 1J). This area has been previously described as the nucleus tegmenti dorsalis lateralis (Ekström & van Veen, 1984; Ekström & Ebbesson, 1989) or area 6 (Frankenhuis-van den Heuvel & Nieuwenhuys, 1984). It was possible to follow ELI fibres from this area to the corpus cerebelli (Fig. 19). We also found ELI fibres crossing the nucleus isthmi (Fig. 20) and few small ELI cell bodies in the area B9 described by Ekström & Ebbesson (1989) (Fig. 1K). At more caudal levels of the brain, a large number of ELI fibres was found in the griseum centrale, whereas moderate numbers were seen in the nucleus raphe dorsalis, raphe medius and nucleus reticularis superior (Figs 1J–L, 21).

Medulla oblongata and spinal cord

In the nucleus fasciculi solitarii a large number of ELI fibres surrounding the ELI cell bodies were also seen (Figs 1M, N, 22). Moderate numbers of ELI fibres were present close to the nucleus descendens nervi trigemini, tractus gustatorius secundarius and tractus tectobulbaris cruciatus (Figs 1N–O, 22).

No ELI cell bodies were found in the spinal cord. However, the dorsal horn showed a large number of ELI fibres. Moderate immunoreactivity was seen in the ventral horn (Figs 1P, 23).

DISCUSSION

We did not find any difference in the number of immunoreactive cell bodies between the fish treated with colchicine and the controls. Similar results have been reported by Schulman et al. (1981) when they studied ELI in the cerebellum of mammals, birds, amphibians and teleosts. This may depend on a limited diffusion of colchicine when injected intraventricularly, or on a different action of colchicine on microtubules in fishes than in mammals (Strömberg et al. 1989). In our experiments, the different temperatures in which the fish were kept did not noticeably influence the distribution of ELI.

The distribution of ELI in the olfactory bulbs of the

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NPT  nucleus posterior tuberis
NRL nucleus recessus lateralis
nRMT nucleus of the rostral mesencephalic tegmentum
NRP nucleus recessus posteriortis
ndt nucleus tegmenti dorsalis lateralis
msf sol nucleus fasciculi solitarii
msg nucleus gustatorius secundarius
NYM nucleus ventromedialis thalami
nV nucleus trigeminus
OB olfactory bulb
optl tractus opticus marginalis lateralis
OT optic tectum
OTr tractus opticus
Pit pituitary gland
rd nucleus raphe dorsalis
rme nucleus raphe medius
rmV motor root of the trigeminal nerve
Rs nucleus reticularis superior
SAC stratum album centrale of the optic tectum
SC spinal cord

SFGS stratum fibrosum et griseum superficiale of the optic tectum
SGC stratum griseum centrale of the optic tectum
SM stratum marginale of the optic tectum
SO stratum opticum of the optic tectum
SPV stratum periventriculare of the optic tectum
ibc tractus tectobulbaris cruciatus
ibr tractus tectobulbaris rectus
TEG tegmentum
TEL telencephalon
TL torus longitudinalis
TS torus semicircularis
V ventriculus mesencephali
Vc commissural nucleus of area ventralis telencephali
VCh valvula cerebelli
Vd dorsal nucleus of area ventralis telencephali
Vi lateral nucleus of the area ventralis telencephali
Vs supramissural nucleus of area ventralis telencephali
Vv ventral nucleus of area ventralis telencephali
HV nucleus of the oculomotor nerve
Xm motor nucleus of the vagus nerve
rainbow trout is similar to that observed in the African lungfish *Protopterus annectens* showing only ELI-fibres (Reiner & Northcutt, 1987), but differs from that described for the dogfish *Squalus acanthias*, where ELI positive neurons have been observed (Northcutt et al. 1988). In the reptile *Caiman crocodilus*, small enkephalin containing cell bodies were observed within the granule cell layer of the olfactory bulb (Brauth, 1984). Numerous leu-enkephalin (LENK) positive neurons have been observed in the olfactory tubercle of turtles (Reiner, 1987) and met-enkephalin (MENK) immunoreactive cell bodies and fibres have
Fig. 6. ELi cell bodies (arrows) and fibres in the nucleus lateralis tuberis (NLT). Bar, 50 μm.
Fig. 7. ELi cell bodies (arrows) and fibres in the nucleus recessus lateralis (NRL). Bar, 25 μm.
Fig. 8. ELi cell body (arrow) in the nucleus recessus lateralis (NRL). Notice the distance to the ventricle (V). Bar, 10 μm.
Fig. 9. ELi cell bodies in the nucleus recessus lateralis located far from the ependymal cell layer of the ventricle. Bar, 10 μm.
Figs 10 and 11. ELI cell bodies in the nucleus of the rostral mesencephalic tegmentum. Bars, 100 μm.
Figs 12 and 13. Tegmental ELI cell bodies dorsally to the nRMT. Bars, 100 μm.
Fig. 14. ELI fibres in the rostradorsal part of the torus semicircularis (TS). Transverse section, medial is to the right. Bar, 100 μm.
Fig. 15. ELI cell body in the torus semicircularis (TS). Higher magnification of Fig. 14. Bar, 100 μm.
Fig. 16. ELI fibres in the tractus opticus marginalis lateralis (optl). Bar, 100 μm.
Fig. 17. ELI fibres in a dorsolateral area of the optic tectum. In the stratum opticum (SO), 3 sublayers are clearly distinguished (arrows) in the more superficial layer and in the deep layer are ELI fibres. In the stratum fibrosum et griseum superficiale (SFGs), stratum griseum centrale (SGC) and stratum album centrale (SAC). ELI fibres are less numerous. Bar, 100 μm.
Fig. 18. ELI cell bodies in the valvula cerebelli (vCb) placed in the border between the granular (cgr) and the molecular (cm) layer. Bar, 100 µm.

Fig. 19. ELI fibres (arrows) connecting the nucleus tegmenti dorsalis lateralis (ntdl) and the cerebellum (Cb). Transverse section, medial is to the left. V, ventricle. Bar, 100 µm.

Fig. 20. ELI fibres crossing the nucleus isthmi (NI). Bar, 100 µm.
In the medial pallium of the lungfish, more LENK-positive fibres were found in the pars dorsalis than in the pars intermedia. LENK-positive neurons and many labelled fibres were also seen in the lateral and medial subpallium (Reiner & Northcutt, 1987). In the trout, ELI cell bodies and fibres were seen in the ventral nucleus of the area ventralis telencephali and ELI fibres in the medial, dorsal and lateral zones of the area dorsalis telencephali.

It is interesting to note that a homology between the lateral zone of the area dorsalis telencephali of teleosts and the medial pallidum of land vertebrates has been postulated. The ventral nucleus of the area ventralis telencephali of teleosts seems to be equivalent to the medial septal nucleus of land vertebrates (Northcutt & Braford, 1980). On the other hand, LENK-positive fibres and perikarya have been found to be widely distributed in the telencephalon of the turtle (Reiner, 1987). A higher number of ELI cell bodies have also been described in the ventrolateral area of a reptile telencephalon (Braith, 1984). This brain region has been suggested to be equivalent to the mammalian corpus striatum, where MENK-containing cell bodies have been described (Beckstead & Kersey, 1985). In conclusion, the distribution of enkephalins in the telencephalon of teleosts is similar to that described in amphibians (Reiner, 1987), reptiles (Braith, 1984) and mammals (Beckstead & Kersey, 1985). These data are in agreement with the proposal that enkephalins have been present since primitive vertebrate lineages (Reiner & Northcutt, 1987).

In the rainbow trout the richest network of ELI fibres and cell bodies was found in the hypothalamus. A similar distribution of enkephalins was found in the teleost green molly (Batten et al. 1990). The location of ELI elements in the nucleus lateralis tuberis and nucleus recessus lateralis suggests that enkephalins may be involved in reproductive processes, since it has been pointed out that both nuclei innervate the hypophysis, (Simon & Reinboth, 1974; Ekengren & Terlou, 1978), probably regulating its function (Holmes & Ball, 1974; Terlou & Ekengren, 1979). A similar distribution of enkephalins in nuclei involved in the regulation of reproductive functions has been found in other vertebrates. Proenkephalin immunoreactive fibres and cell bodies also have been observed in the amphibian hypothalamus (Merchenthaler et al. 1987). A prominent accumulation of LENK-positive neurons has also been reported in the preoptic region and hypothalamus of turtles (Reiner, 1987).

No ELI cell bodies were seen in the optic tectum of the rainbow trout. This is in agreement with the results found in the Atlantic salmon (Vecino & Ekström, 1982). These data point to an abundance of ELI neurons in the olfactory bulbs of amphibians, reptiles and mammals, but few or none in the olfactory bulb of fishes.

Fig. 21. Transverse section of the medial brainstem showing a large number of ELI fibres in the griseum centrale (gc), and a moderate number in the nucleus raphe medius (rme) and nucleus reticularis superior (Rs). No ELI fibres are present in the nucleus of the medial longitudinal fasciculus (fml). Bar, 100 μm.

also been seen in the main olfactory bulb of the hamster (Davis et al. 1982). These data point to an abundance of ELI neurons in the olfactory bulbs of amphibians, reptiles and mammals, but few or none in the olfactory bulb of fishes.
Fig. 22. Transverse section of the medulla. The nucleus of the fasciculus solitarius (nufsol) possesses a large number of ELI fibres and cell bodies. Moderate numbers of ELI fibres are present in the surroundings of the tractus gustatorius secundarius (gs) and in the tractus tectobulbaris crucatus (tbc). No immunoreactive fibres are present in the medial longitudinal fasciculus (flm). creb, crista cerebellaris. Bar, 100 μm.

Fig. 23. Transverse section of a rostral segment of the spinal cord showing large numbers of ELI fibres in the dorsal horn (cnd), moderate numbers in the proximity of the lateral funiculus (fl) and absence of immunoreactivity in the ventral horn (cnv). Bar, 100 μm.

1990) and green molly (Batten et al. 1990). On the other hand, ELI cell bodies in the stratum periventriculare of the optic tectum were found in anurans (Kuljis & Karten, 1982), reptiles (Reiner, 1987) and birds (Reiner et al. 1982). In all cases the ELI cell bodies sent apical processes towards the superficial layers of the optic tectum.

The stratum opticum displayed the highest amounts of ELI fibres. This stratum receives direct inputs from the retina in fish (Réperant & Lemire, 1976). However, we did not find ELI fibres in the optic nerve, and eye enucleation experiments have not modified the pattern of immunoreactivity in the optic tectum of Salmo salar (Vecino & Ekström, 1990). So far, strong evidence for ELI retinotectal axons has been presented only in anurans (Kuljis & Karten, 1982; Kuljis et al. 1984), whereas in fish, reptiles, birds and mammals ELI axons in the optic tectum appear to have other origins.

Some of the ELI fibres found in the optic tectum of rainbow trout have their origin in the ELI neurons of the nucleus of the rostral mesencephalic tegmentum (Vecino et al., 1991). This group of ELI neurons may be the equivalent to those of the nucleus dorsalis commisuralis posterioris of turtles (Reiner, 1987) and the lateral spiriform nucleus of birds (Reiner et al. 1982) that also project to the optic tectum. It is also possible that some of the ELI fibres observed in the optic tectum might originate, for example, in the telencephalon or torus semicircularis where we have found ELI cell bodies. Tectal afferents arising from these regions have been described previously in teleosts (Vanegas & Ito, 1983).

In the cerebellum we have found numerous ELI mossy fibres, and ELI Golgi cells in the corpus and valvula cerebelli but we have not found ELI Golgi cells in the eminencia granularis. Similar results have been obtained in goldfish, albino rat and rabbit cerebellum (Schulman et al. 1981). In these animals ELI cells were more numerous in those parts of the corpus cerebelli which are related to the vestibular and spinal systems but were uncommon in the eminencia granularis. In catfish, cats and pigmented rats (Schulman et al. 1981), on the other hand, the distribution of ELI-Golgi cells appeared to be evenly distributed through the corpus cerebelli, whereas in
the teleost *Poecilia latipinna* (Batten et al. 1990) no ELI cell bodies or fibres were seen in the corpus or valvula cerebelli. The abundance of ELI Golgi cells in the cerebellum varies with species and is unrelated to phylogenetic position.

The mossy and climbing fibres are the afferent fibres in the teleost cerebellum. Golgi cells and mossy fibres both synapse on granule cells, but while Golgi cells are inhibitory, mossy fibres have been shown to be excitatory (Eccles et al. 1967). The role of enkephalins in cerebellar function is unknown, but electrophysiological studies have revealed that mossy fibres receive visual input (Reid & Westerman, 1975; Kotochabakdi, 1976a, b). Thus the presence of enkephalins in the optic tectum, nucleus rostral is of the mesencephalic tegmentum and mossy fibres points to the importance of enkephalin in the visual system of fishes.

We have found ELI cell bodies in the area 6 described by Frankenhaus-van den Heuvel & Nieuwenhuys (1984) in rainbow trout. This nucleus is equivalent to the tegmentum dorsalis lateralis of the three-spined stickleback (Ekström & van Veen, 1984) and sockeye salmon (Ekström & Ebbesson, 1989). In this nucleus, serotonergic cell bodies and fibres have been described (Ekström & van Veen, 1984; Frankenhaus-van den Heuvel & Nieuwenhuys, 1984; Ekström & Ebbesson, 1989; Movéreus et al. 1989). We were able to follow some ELI fibres which originated from this nucleus and entered the cerebellum (Fig. 19). The same pathway has been found in the serotonergic cells of area 6 (Frankenhaus-van den Heuvel & Nieuwenhuys, 1984). Since serotonin and enkephalins have been found to be colocalised (Glazer et al. 1981) it is possible that serotonin and enkephalins are colocalised in the above mentioned ELI cell bodies.

ELI cell bodies in the dorsal horn of the spinal cord have been demonstrated in mammals (Sar et al. 1978; Glazer & Basbaum, 1981), and in nonmammalian vertebrates such as the frog *Rana esculenta* (Lorez & Kemali, 1981), the lizard *Anolis carolinensis* (Naik et al. 1981) and the turtles *Chrysemys picta* and *Pseudemys scripta* (Reiner, 1987). The presence of ELI cell bodies in the spinal cord of the above mentioned groups contrasts with their absence in *Varanus exanthematicus* (Wolters et al. 1986), the lamprey (Buchanan et al. 1987) and the rainbow trout.

The present study provides a detailed account of the distribution of ELI cell bodies and fibres in the rainbow trout brain. The distribution of ELI found in different regions suggests that these peptides may be involved in the modulation of neuroendocrine, visual and somatosensory mechanisms. Electrophysiological and tracing studies combined with immunocytochemical studies are necessary to elucidate the origin of immunoreactive fibres and the function of enkephalins in the fish nervous system.

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**REFERENCES**


enkephalin distribution in turtle nervous system. Society of Neuroscience Abstracts 9, 439.


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