TITLE:

Expression of ZFOR1, a δ opioid receptor, in the central nervous system of the zebrafish *(Danio rerio)*

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ABSTRACT

Opioid receptors, besides mediating the effects of analgesic compounds, are involved in drug addiction. Although a large amount of work has been done studying these receptors in mammals, little information has been obtained from non-mammalian vertebrates. We have studied the regional distribution in the central nervous system (CNS) of the zebrafish of the recently cloned δ opioid receptor homologue ZFOR1 using non-radioactive in situ hybridization. Our findings show that different nuclei within the main subdivisions of the brain displayed specific mRNA signal. The expression is widespread throughout the brain, but only specific cells within each nucleus displayed ZFOR1. Stained cells were abundant in the telencephalon, both in the olfactory bulb and telencephalic hemispheres; and in the diencephalon, where expression was observed in all the different subdivisions. In the mesencephalon, expression of ZFOR1 was abundant in the periventricular layer of the optic tectum. In the cerebellum, expression of ZFOR1 was detected in *valvula cerebelli*, *corpus cerebelli* and *lobus vestibulolateralis* in both granule and Purkinje cells. In the myelencephalon, cells expressing ZFOR1 were also distributed in the *octavolateralis* area, the reticular formation and the raphe nuclei, among others. Also, ZFOR1 was detected in cells of the dorsal and ventral horn of the spinal cord. This work presents the first detailed distribution of a δ opioid receptor in the CNS of zebrafish. Distribution of ZFOR1 expression is compared with that of the δ opioid receptor described in mammals.

INTRODUCTION

The existence of at least three main types of opioid receptors, namely μ, δ and k, has been pharmacologically characterized depending on their affinity for different agonists (Lutz and Pfister, 1992; Martin et al., 1976). They mediate the effects of the different families of endogenous opioid peptides derived from pro-opiomelanocortin, proenkephalin and prodynorphin, and that of alkaloid natural drugs such as morphine and its synthetic derivatives. Opioid receptors have been implicated in a broad range of physiological functions, including nociception and cognition, as well as affective, endocrine, cardiovascular, gastrointestinal, immune, respiratory, and autonomic regulation (Cox, 1988). They also mediate the adverse side effects of opiate analgesics, such as tolerance and addiction (Kreek, 1996).

From radioactive ligand binding and in situ hybridization studies, a broad vision of the opioid receptor anatomy in mammals has been reported (George et al., 1994; Mansour et al., 1994; 1995). δ opioid receptor is detected in olfactory bulb, neocortex, striatum, hippocampus, amygdala, pontine nuclei and dorsal horn of the spinal cord. This receptor has also been related to analgesia, gastrointestinal motility and hypothalamic regulation. μ opioid receptor is expressed in striatum, thalamus, hippocampus, nucleus of the solitary tract and spinal cord, and has been related to analgesia, and respiratory and cardiovascular functions. κ opioid receptor is detected in nucleus accumbens, hypothalamus, amygdala, brainstem and spinal cord in a pattern similar to that of μ opioid receptors. This receptor has been associated with analgesia, with the maintaining of water balance and with different neuroendocrine functions.

Since the discovery of opioid receptors in 1973 (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973), the genes encoding the different members of the opioid receptor

family: δ (Evans et al., 1992; Kieffer et al., 1992; Simonin et al., 1994), μ (Wang et al., 1993; 1994), and κ (Simonin et al., 1994; Yasuda et al., 1993), have been cloned in rodents and humans. Recently, a new opioid receptor-like gene (ORL1), which has been shown to code for a receptor that binds a new endogenous peptide known as nociceptin or orphanin FQ (Meunier et al., 1995; Reinscheid et al., 1995), has also been characterized (Mollereau et al., 1994). These proteins share a high degree of homology with the G-protein coupled superfamily of receptors, and the corresponding genes conserve the intron-exon boundaries, which suggests a probable origin from a common ancestor gene (Li et al., 1996). The presence of opioid receptors in organisms other than mammals was demonstrated through binding studies soon after their discovery (Buatti et al., 1981), and their endogenous peptidic ligands were identified even in invertebrates (Leung and Stefano, 1984). The molecular evidence for the existence of these receptors has been recently achieved by the cloning of a μ opioid receptor in a teleost (Darlison et al.,1997). Also, we have recently cloned a δ opioid receptor in the zebrafish (Barrallo et al., 1998), that appears to be fully functional (binding experiments in progress). In this work, using non-radioactive in situ hybridization, we provide the first detailed description of the distribution of the mRNA of a δ opioid receptor in the CNS of a non-mammalian vertebrate. Also, we compare our results with those described for the mammalian δ opioid receptor.

MATERIAL AND METHODS

Tissue preparation

Adult zebrafish of both sexes obtained from a local supplier were deeply anesthetized with tricaine methanesulfonate (MS-222, Sigma, St. Louis, MO), and fixed overnight by immersion in freshly prepared 4% paraformaldehyde, soaked in 30% sucrose, serially

sectioned on a cryostat (Leica) in 25 µm thickness and finally thaw-mounted onto gelatin-coated microscope slides. Parasagittal and coronal sections were fixed in 4% paraformaldehyde, followed by one wash in 0.1M phosphate-buffered saline pH 7.4 (PBS), and dehydration in graded ethanol series (65%, 80%, 90% and 100%). All procedures were in accordance with the guidelines of the European Communities Directive (86/609/EEC), the current Spanish legislation for the use and care of animals (BOE 67/8509-12, 1998), and conform to NIH guidelines.

Probe design

Two different oligodeoxynucleotides were designed from the cDNA sequence of ZFOR1: 5' ACC AGT GCG ATG CAA GTG CCA GCT A 3' and 5' GCA GAC TGT TGT ATT CTG ATT TGT CAC TCT AGT GA 3' (available in EMBL DataBase under accession number AJ001596), and purchased from a synthesis laboratory (MWG- Biotech; Boehringer Mannheim, Darmstadt, Germany). Due to the high homology among the opioid receptor genes, oligodeoxynucleotides were designed from the more variable regions of these receptors, mainly the second and third extracellular loops, which are involved in ligand selectivity (Meng et al., 1996). These oligodeoxynucleotides were labeled at the 5' end with digoxigenin for immunohistochemical detection.

In situ hybridization

Tissue was prehybridized in hybridization buffer (Omnibuff; Wak Chemie, Medical GMBH; Bad Homburg, Germany) for 30 minutes at room temperature, and hybridized overnight at 37°C with two different oligonucleotides probes. Sections were then washed in PBS for 10 minutes, once at 37°C and twice at room temperature. Mouse antidigoxigenin antibody diluted in TBS (50mM Tris, 800mM NaCl, pH 7.4) was used at 4°C overnight for immunological amplification. The slides containing the sample tissue were washed for 5

minutes at room temperature in TBS, incubated in a humid chamber with biotinylated goat anti-mouse antiserum in TBS for 40 minutes, and then washed in TBS. Streptavidin-peroxidase conjugate and 3-3'-diaminobenzidine were used for immunological detection. To ensure the specificity of the method, adjacent sections were used as controls. The following approaches were performed: pre-treatment with RNase, incubation without probe and incubation prior to hybridization with an excess of non-labeled oligodeoxynucleotide and then hybridization with a labeled one. Absence of signal in adjacent sections hybridized as negative controls confirmed the specificity of the hybridization. To ensure the presence of mRNA signal in the different cells that comprise each nucleus, some sections were counterstained with hematoxylin.

RESULTS

ZFOR1 expression has been specifically localized in the CNS of the zebrafish *Danio rerio* by in situ hybridization. Labeled elements were observed in all subdivisions of the brain (telencephalon, diencephalon, mesencephalon, metencephalon, myelencephalon and spinal cord; Fig. 1; Table I). Our findings are described from the rostralmost regions of the brain, beginning with the telencephalon and ending with the spinal cord.

Telencephalon

The telencephalon of the zebrafish is constituted by the olfactory bulbs and the telencephalic hemispheres. In the olfactory bulb, ZFOR1 mRNA was detected in small, rounded cells located within the internal cellular layer (Fig. 1a). Labeled elements were abundant in the telencephalic hemispheres where we have observed specific signal in both ventral and dorsal areas (Figs. 1b, 2a, 4a). In the ventral area, dense clusters of stained cells were observed in the periventricular region of the dorsal, ventral and supracommissural

nuclei (Figs. 1b, 2a). Only occasional labeled cells were observed in central and lateral nuclei of area ventralis. In the dorsal area (Figs. 1b, 2a, 3a, 4a), we detected numerous labeled cells in the medial, dorsal, lateral and posterior zones, and a smaller number of labeled cells in the central zone. Cells were especially abundant in the boundary between lateral and posterior zone of area dorsalis (Fig. 4a).

Diencephalon

The diencephalon is the most complex region in the teleostean brain. Seven main subdivisions have been established in the zebrafish brain (Wullimann et al., 1996) that in dorsoventral arrangement are: preoptic area, epithalamus, dorsal thalamus, ventral thalamus, hypothalamus, posterior tubercle, and synencephalon (pretectum). The distribution of ZFOR1 is widespread in the zebrafish diencephalon. Specific hybridization signal was detected in all the major subdivisions (Fig. 1c-f; Table I).

In the preoptic area, we observed ZFOR1 mRNA in cells located close to the ventricle, with more stained elements in caudal regions, especially in the posterior parvocellular preoptic nucleus and in the magnocellular preoptic nucleus. Abundant labeled cells were observed in the habenula. In the thalamus, we have observed a wide distribution of labeled elements. Aggregates of labeled cells were observed in the dorsal thalamus, especially abundant in the anterior thalamic nuclei, but also in the dorsal posterior and central posterior nuclei. The ZFOR1 signal was also abundant in the ventral thalamus. Small, round cells displayed ZFOR1 mRNA signal, and they were located close to the diencephalic ventricle in the ventrolateral and ventromedial thalamic nuclei.

Labeled cells were observed both in the periventricular part and migrated nuclei of the posterior tuberculum. In the periventricular part, we observed ZFOR mRNA in the posterior tuberal nucleus, in the paraventricular organ and in the periventricular nucleus of the

posterior tuberculum. We also observed staining in migrated preglomerular nuclei, in the corpus mamillare and in the torus lateralis.

In the hypothalamus, abundant labeling was found from rostral to caudal levels of the periventricular hypothalamus and scarce ZFOR1 stained cells were observed in the anterior tuberal nucleus (Figs. 2b, 4b). High density of labeled cells was found in the dorsal hypothalamus close to the ependyma, and in the nuclei of the inferior lobe, with larger stained cells in the central than in the diffuse nucleus (Fig. 3c).

The synencephalon includes structures intermediate between the diencephalon and the mesencephalon (Braford and Northcutt, 1983). We have found cells of medium size in the nucleus of the medial longitudinal fascicle (Fig. 3b) and small stained cells in the periventricular pretectum. According to the neuromeric model of Puelles and Rubenstein (1993) the synencephalon originates all pretectal nuclei, including those of the central and superficial pretectum, in which we have found a dense ZFOR mRNA signal in the nuclei immersed within the optic tract (Fig. 1c).

The retina develops from the optic vesicle of the diencephalon during the embryonic development, being considered as a diencephalic derivative. Within this region we have observed individual labeled cells located in the outermost region of the ganglion cell layer. In addition, we have observed a very intense labeling in the inner nuclear layer.

Mesencephalon

The zebrafish mesencephalon is comprised by the optic tectum, the tegmentum and the semicircular torus. The optic tectum is constituted by two cupuliform structures that are organized in a layered fashion. This region can be differentiated in six strata from the most internal to the most external: periventricular, central white, central gray, superficial fibrosus and gray layer, optic layer and marginal layer. Many of the round cells of the periventricular

layer of the optic tectum displayed ZFOR1 mRNA. In addition, scarce and disperse cells, with different shape and size in the other strata were also observed (Fig. 3d).

The longitudinal torus connects both hemitecta in the whole rostrocaudal extension of the mesencephalon. In this region we observed labeled cells in the most ventral portion. Also, disperse cells were observed in both the central and ventrolateral regions of the semicircular torus. Within the mesencephalic tegmentum, we have detected ZFOR1 mRNA signal in the rostral and dorsal tegmental nuclei (Fig. 3b) close to the torus semicircularis and in the mesencephalic superior reticular formation (Fig. 3f).

Metencephalon

The teleostean metencephalon comprises three areas: *valvula cerebelli*, *corpus cerebelli* and *lobus vestibulolateralis*. *Valvula and corpus cerebelli* are laminated structures with an internal granule cell layer, a monocellular layer formed by the somata of Purkinje cell layer, and an external molecular layer. The *lobus vestibulolateralis* comprises the *eminentia granularis* and the caudal lobe. ZFOR1 gene expression was very abundant in the granule cell layer of *valvula* and *corpus cerebelli*, and in the *eminentia granularis*. Together with the periventricular strata of the optic tectum, these are the regions with highest density of cells expressing ZFOR1-mRNA in the zebrafish brain (Figs. 1c-g, 3d,e). We distinguished numerous small cells with only a thin rim of stained cytoplasm around the negative nucleus, and we have identified them as granule cells. Other cells were bigger and more intensely stained than these presumptive granule cells. They were arranged in a monocellular layer, and may correspond to Purkinje cells (Fig. 3e).

Myelencephalon

We have observed a widespread expression of ZFOR1 in several myelencephalic nuclei of the zebrafish brain. Small labeled cells were densely packed in the central gray and

nucleus lateralis valvulae. Scarce stained cells were detected in the isthmic nucleus, the medial funicular nucleus and in the cerebellar crest. We have detected mRNA signal in the different nuclei of *octavolateralis* area: anterior, magnocellular, tangential, descendens and posterior octaval nuclei and in the medial octavolateral nucleus, and also in the secondary octaval population (Fig. 1 g,h). Scarce labeled cells were observed in the whole rostrocaudal extension of the reticular formation and in the dorsal and inferior raphe nuclei. In the caudal myelencephalon, we observed abundant stained cells in the facial, glossopharyngeal and vagal lobe, and in the glossopharyngeal and motor vagal nuclei (Figs. 1h-j, 2c).

Spinal cord

The structural arrangement of the zebrafish spinal cord resembles the organization in gray and white matter described for mammals. Specific labeling has been detected in the gray matter (Figs. 1j, 2d). In the dorsal horn, we have observed stained cells mainly close to the midline. In the ventral horn, the labeled cells are distributed in a more spread pattern.

DISCUSSION

Abundant data is available on the distribution of the different opioid receptors in mammalian CNS (George et al., 1994; Mansour et al., 1988, 1994, 1995). Nevertheless, to date little information has been published on this issue in relation to non-mammals. The recent cloning of μ and δ opioid receptors in teleosts (Darlison et al., 1997; Barrallo et al., 1998) has made possible the use of in situ hybridization techniques to analyze the expression of opioid receptors in different tissues. This technique provides better resolution than that obtained in ligand binding autoradiography studies. We report here the first detailed map of the distribution of a δ opioid receptor mRNA in the CNS of the zebrafish, an anamniote vertebrate. Furthermore, comparison of our results with the expression pattern

of δ opioid receptor mRNA in rodents suggests a high conservation throughout vertebrate evolution, further supported by the high degree of molecular homology of ZFOR1 with the mammalian opioid receptors (Barrallo et al., 1998).

Although interspecies differences in the cytoarchitecture, neuronal types and connectivity of the olfactory bulb have been described, there is a remarkably constant structure in vertebrates (Allison, 1953). This region shows high ZFOR1 and δ opioid receptor mRNA expression in mammals (Mansour et al., 1993) and in zebrafish. The different areas of the teleost telencephalic hemispheres are not easily comparable with the cortical and subcortical structures of the mammalian telencephalon, but some homologies have been proposed (Butler and Hodos, 1996), and some of these areas are homologous to the limbic structures of the mammalian forebrain that have been shown to be influenced by opiates. We have detected abundant ZFOR1 expression in the supracomissural part of the ventral area of the telencephalon , which is considered homologous to the amygdaloid complex of mammals (Butler and Hodos, 1996).

Regarding the diencephalon, the preoptic area and the hypothalamus are well developed areas in teleosts. By contrast, the dorsal and ventral thalamus are more complex in amniotes than in teleosts (Butler and Hodos, 1996). The widespread distribution of ZFOR1 mRNA in the zebrafish diencephalon contrasts with the scarce expression of the δ opioid receptor in mammals. In zebrafish, specific labeled cells are detected clearly in almost all nuclei of the periventricular hypothalamus, whereas in rodents only the ventromedial nucleus of the hypothalamus displays some signal (Mansour et al., 1993). Nevertheless, the fact that previous studies (Bird et al., 1988) showed a high opiate binding in this area of the teleostean brain confirm our findings. Analysis of opiate binding in the mammalian thalamus demonstrated only low levels (Mansour et al., 1993), whereas the expression was abundant in the zebrafish thalamus. Due to the high complexity of the diencephalon in the teleosts and to its capital role in the integration of sensory, motor and neuroendocrine functions, the

abundance of δ opioid receptor in this region in zebrafish suggests a wide and diversificate role of opioid signal transduction. Although there is evidence that morphine has a modulatory effect in the corticotropin-releasing activity in fish (Bird et al., 1987; Mukherjee et al., 1987) and that the opioid peptides interact with the neuroendocrine system of mammals (Grossman and Rees, 1983), the involvement of δ opioid receptor in the modulation of hormone release from the hypothalamic-hypophysary axis seems to be of minor importance in mammals (Mansour et al., 1993).

The optic tectum is a conservative region in the brain of all vertebrates, homologous to the superior colliculus of mammals (Jungherr, 1945), in which δ opioid receptor mRNA labeling has been detected (Mansour et al., 1993). Most of the cells of the periventricular layer of the optic tectum displayed ZFOR1 mRNA and disperse cells in the other layers were observed. The inferior colliculus of mammals presents cells with δ opioid receptor mRNA expression (Mansour et al., 1993). This is the homologous region to the semicircular torus of teleosts (Butler and Hodos, 1996) where disperse cells were observed in both the magnocellular and parvocellular regions. ZFOR1 mRNA is also observed in the longitudinal torus, an integrative region of vision and balance (Vanegas et al., 1984), reinforcing the hypothesis of a role for the opiate receptors in the sensory pathways (Atweh and Kuhar, 1983).

The basic organization of the cerebellum is conserved throughout vertebrate evolution. Nevertheless, there are some cytoarchitectonical and functional peculiarities exclusive to teleosts such as that the *valvula cerebelli* is not present in other groups of vertebrates (Meek and Nieuwenhuys, 1991). In addition, Purkinje cells are not the efferent elements of the cerebellar cortex. They send their axons to connect with the eurydendroid cells, located just beneath the Purkinje cell layer. These cells have been compared to deep cerebellar nuclei neurons of other vertebrates (Nieuwenhuys and Nicholson, 1969; Finger, 1983) and

represent a cell type exclusive of teleosts that leave the cerebellum to innervate diencephalic mesencephalic, and myelencephalic areas (Finger, 1983). Our results show a broad range in the levels of ZFOR1 mRNA expression within the cerebellum. We have found a high number of stained granule and Purkinje cells, which is in contrast with the scarce cells dispersed within the deep cerebellar nuclei and in the cerebellar cortex observed in mammals (Mansour et al., 1993). This result agrees with the results of Bird et al. (1988), that found the highest level of opiate binding, although not in an homogeneous pattern, in fish cerebellum. Hence, opiate receptors seem to play an important role in zebrafish cerebellum that has not been detected in mammals. In this sense, cerebellum ablation in goldfish induces an increase in tactile and pain sensitivity (Karamyan, 1962) suggesting its involvement in nociception.

The expression of ZFOR1 in myelencephalon and spinal cord is more restricted than in the other regions. In zebrafish, numerous cells expressing this gene are present in the *octavolateralis* area (both in the auditory and vestibular nuclei), a region with high divergence with the analogous nuclei of the auditory and vestibular processing in other vertebrates (Butler and Hodos, 1996). The abundance of ZFOR1 mRNA expression in this area, that plays a key role in the integration of stimulus in the fish brain, supports the hypothesis of a sensory role for the opioid receptors (Atewh and Kuhar, 1983). In the reticular formation of mammals, δ opioid receptors are involved in analgesic mechanisms (Mansour et al., 1993), as might be in teleosts, due to the presence of ZFOR1 mRNA in the whole rostrocaudal extention of the reticular formation.. ZFOR1 mRNA expression has been detected in dorsal and ventral horns of the spinal cord of zebrafish similar to that previously reported in mammals where δ opioid receptor binding and mRNA expression has been observed in scattered cells on several laminae of ventral and dorsal horns (Mansour et al., 1993).

The distribution of opioid receptors in fish has only been described from binding data (Bird et al., 1988). There is certain mismatching in the opioid binding distribution with

respect to our results on the localization of ZFOR1 opioid receptor expression. Thus, binding was highest in the molecular layer of cerebellum, while mRNA expression is localized in the Purkinje cell and granule cell layer. Nevertheless, it should be noted that this binding study was done with non-specific ligands as etorphine and in a different species of teleostean fish (the rainbow trout *Oncorhynchus mykiss*), and that this divergence between binding sites and receptor mRNA localization has been also described in mammals (Mansour et al., 1988) and may be due to an intracellular transport from the somata to the neuropil or be a consequence of the different sensitivity between in situ hybridization and binding studies. Specific antibodies raised against the protein sequence of ZFOR1 will help in localizing this receptor immunohistochemically. The comparison between immunohistochemical detection and the distribution of ZFOR1 mRNA, may solve this apparent mismatch.

In conclusion, although expression of ZFOR1 mRNA is widespread throughout zebrafish CNS, striking variatons have been detected between different areas, in a similar pattern to that previously described in mammals for the δ opioid receptor, although the expression is higher in the caudalmost areas in zebrafish as compared to the hindbrain of mammals. Our results suggest a high conservation throughout evolution of the δ opioid receptor, not only under the molecular point of view as reported previously (Barrallo et al., 1998), but also in its distribution within the central nervous system.

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TABLE I

CNS Region	ZFOR1
I Telencephalon	
Olfactory bulb	++
Dorsal telencephalic area	+++
Ventral telencephalic area	+++
II Diencephalon	
Preoptic area	+++
Epithalamus	++++
Dorsal thalamus	+++
Ventral thalamus	+++
Posterior tubercle	++
Hypothalamus	+++
Synencephalon (NMLF)	+
Pretectum	++
III Mesencephalon	
Optic tectum	++++
Torus semicircularis	+++
Tegmentum	++
IV Metencephalon	
Valvula cerebelli	++++
Corpus cerebelli	++++
Lobus vestibulolateralis	++++
V Myelencephalon	
Octavolateralis area	++
Reticular formation	++
Raphe nuclei	++
Vagal lobe	++
Glossopharyngeal lobe	++
Facial lobe	++
VI Spinal cord	
Ventral horn	+
Dorsal horn	+

TABLE I: Relative density of ZFOR1 labeled cells in the main subdivions of the zebrafish CNS. Density has arbitrarily been assigned to values as follow: + = low abundance; ++ = moderate abundance; +++ = high abundance; ++++ = very high abundance

FIGURE LEGENDS

FIGURE 1

Schematic representation of ZFOR1 mRNA expression in the zebrafish CNS. Each map (a-j) represents coronal levels through the zebrafish CNS and was reconstructed using the atlas of Wullimann et al. (1996).
Scale bar = 1 mm

FIGURE 2. a: Coronal section of zebrafish brain showing the localization of ZFOR1 mRNA by in situ hybridization at level of the precommissural telencephalon (a), rostral diencephalon (b), caudal myelencephalon (c) and spinal cord (d). Scale bar = 500μm

FIGURE 3. a: Dorsal telencephalic area. Small, round cells displayed ZFOR1 mRNA in a thin cytoplasmic rim around the negative nucleus. **b**: ZFOR1 labeling in cells of the nucleus of the medial longitudinal fascicle (arrows). Note the smaller size of positive neurons in the adjacent dorsal tegmental nucleus (open arrows). **c**: Small stained cells in the diffuse nucleus of the inferior lobe. **d**: ZFOR1 mRNA labeling in the optic tectum. High density of cells was observed in the periventricular layer (open arrow), and scarce cells with different morphologies were observed in other strata (arrows). **e**: ZFOR1 expression in the caudal lobe of the cerebellum. Abundant granule cells and some Purkinje cells (arrows) were labeled. **f**: High magnification of ZFOR1 labeled cells in the superior reticular formation, showing the variety of sizes and shapes of positive cells.
Scale bar in a-e = 100 μm; in f = 25 μm

FIGURE 4. In situ hybridization for ZFOR1 mRNA counterstained with hematoxylin, showing the presence of non-hybridizing cells in the positive areas. **a**: Dorsolateral and dorsoposterior telencephalic areas **b**: Ventral periventricular hypothalamus.
Scale bar = 100 μm

ABBREVIATIONS

A: anterior thalamic nucleus

CC: cerebellar crest

CCe: corpus cerebelli

CIL: central nucleus of the inferior lobe

CM: mammilary body

CON: caudal octavolateralis nucleus

CPN: central pretectal nucleus

Cpost: posterior commisure

Ctect: tectal comissure

DAO: dorsal accessory optic nucleus

Dc: central zone of dorsal telencephalic area

Dd: dorsal zone of dorsal telencephalic area

DH: dorsal horn

DIL: diffuse nucleus of the inferior lobe

Dl: lateral zone of dorsal telencephalic area

Dm: medial zone of dorsal telencephalic area

DON: descending octaval nucleus

DOT: dorsomedial optic tract

Dp: posterior zone of dorsal telencephalic area

DTN: dorsal tegmental nucleus

ECL: external cellular layer of olfactory bulb

EG: eminentia granularis

Fd: dorsal funiculus

Fld: dorsal part of lateral funiculus

Flv: ventral part of lateral funiculus

Fv: ventral funiculus

GC: central gray

GL: glomerular layer of olfactory bulb

H: habenula

Hc: caudal zone of periventricular hypothalamus

Hd: dorsal zone of periventricular hypothalamus

Hv: ventral zone or periventricular hypothalamus

ICL: internal cellular layer of olfactory bulb

IMRF: intermediate reticular formation

IO: inferior olive

IR: inferior raphe

IRF: inferior reticular formation

LC: locus coeruleus

LCa: caudal lobe of cerebellum

LIX: glossopharyngeal lobe

LVII: facial lobe

LX: vagal lobe

MaON: magnocellular octaval nucleus

MFN: medial funicular nucleus

MLF: medial longitudinal fascicle

MNV: mesencephalic nucleus of the trigeminal nerve

MON: medial octaval nucleus

NI: isthmic nucleus

NLV: nucleus lateralis valvulae

NMLF: nucleus of the medial longitudinal fascicle

NX: vagal motor nucleus

P: posterior thalamic nucleus

PGc: caudal preglomerular nucleus

PGl: lateral preglomerular nucleus

PGm: medial preglomerular nucleus

PPp: parvocellular preoptic nucleus, posterior part

PPv: periventricular pretectal nucleus, ventral part

PSm: magnocellular superficial pretectal nucleus

PSp: parvocellular superficial pretectal nucleus

PTN: posterior tuberal nucleus

PVO: paraventricular organ

SGT: secondary gustatory tract

SO: secundary octaval population

SR: superior raphe

SRF: superior reticular formation

T: tangential nucleus

TeO: tectum opticum

TL: torus longitudinalis

TLa: torus lateralis

TPp: periventricular nucleus of posterior tuberculum

TSc: central nucleus of torus semicircularis

TSvl: ventrolateral nucleus of torus semicircularis

Val: lateral division of valvula cerebelli

Vam: medial division of valvula cerebelli

Vd: dorsal nucleus of ventral telencephalic area

VH: ventral horn

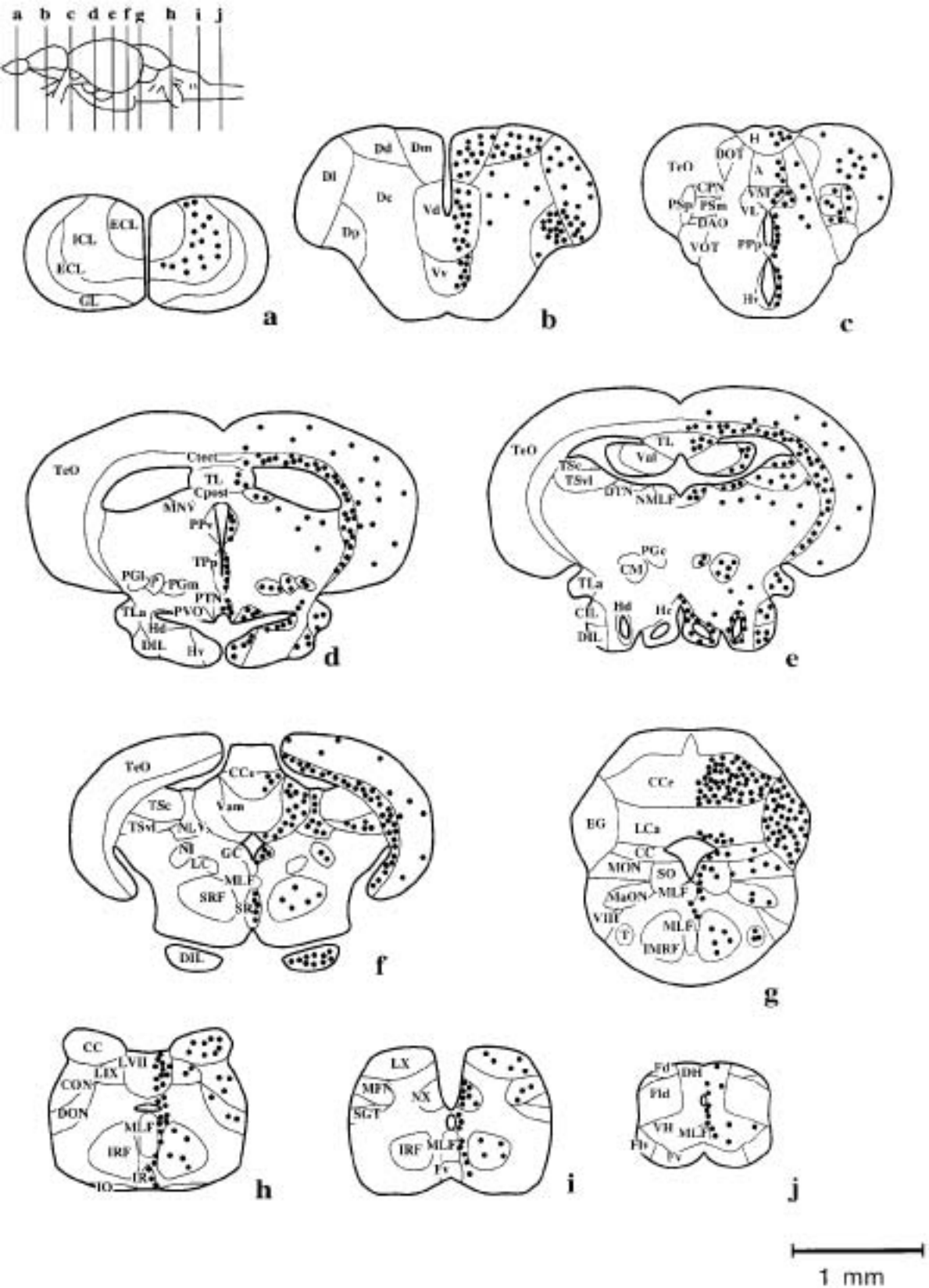
VIII: octaval nerve

VL: ventrolateral thalamic nucleus

VM: ventromedial thalamic nucleus

VOT: ventrolateral optic tract

Vv: ventral nucleus of ventral telencephalic area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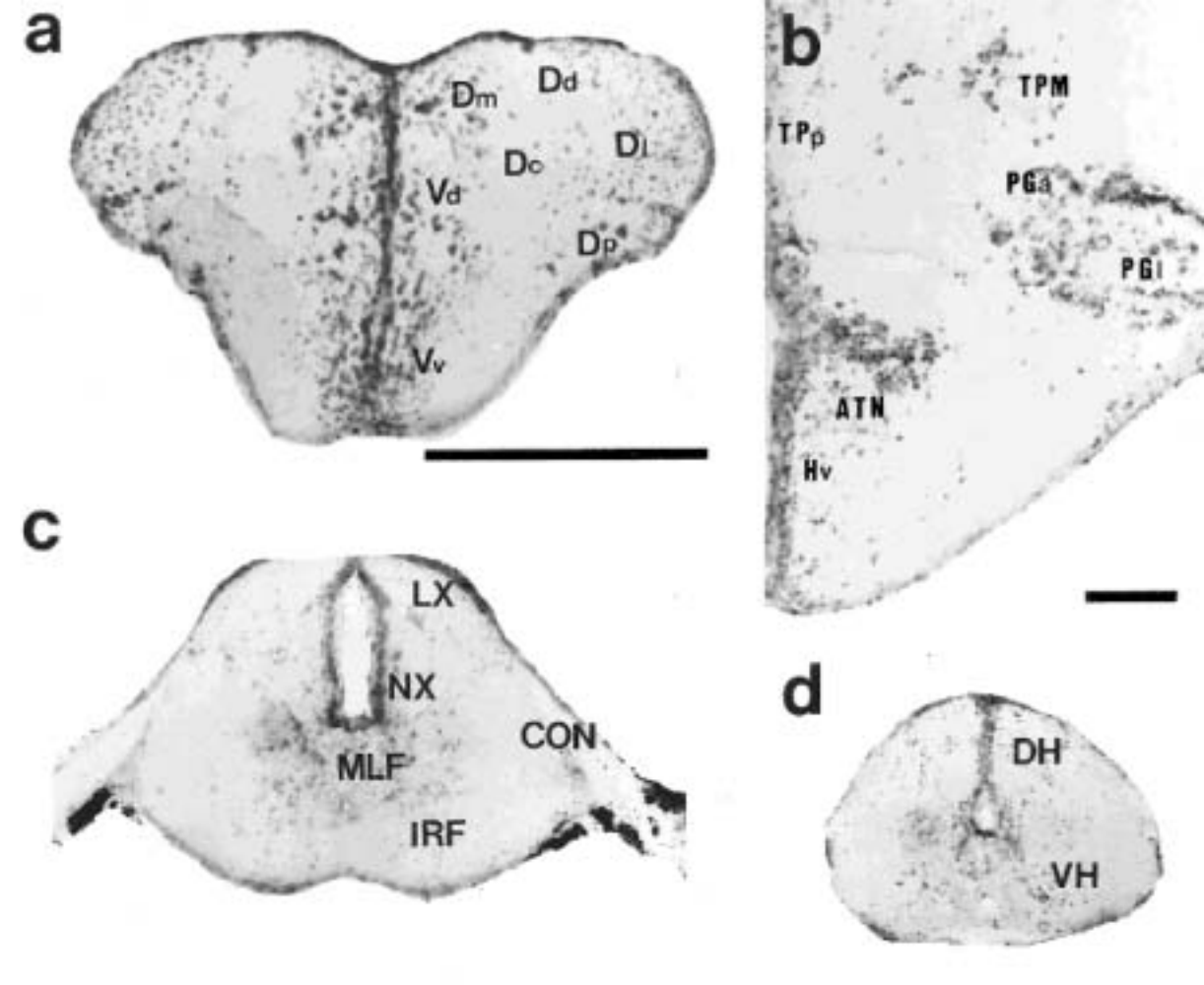
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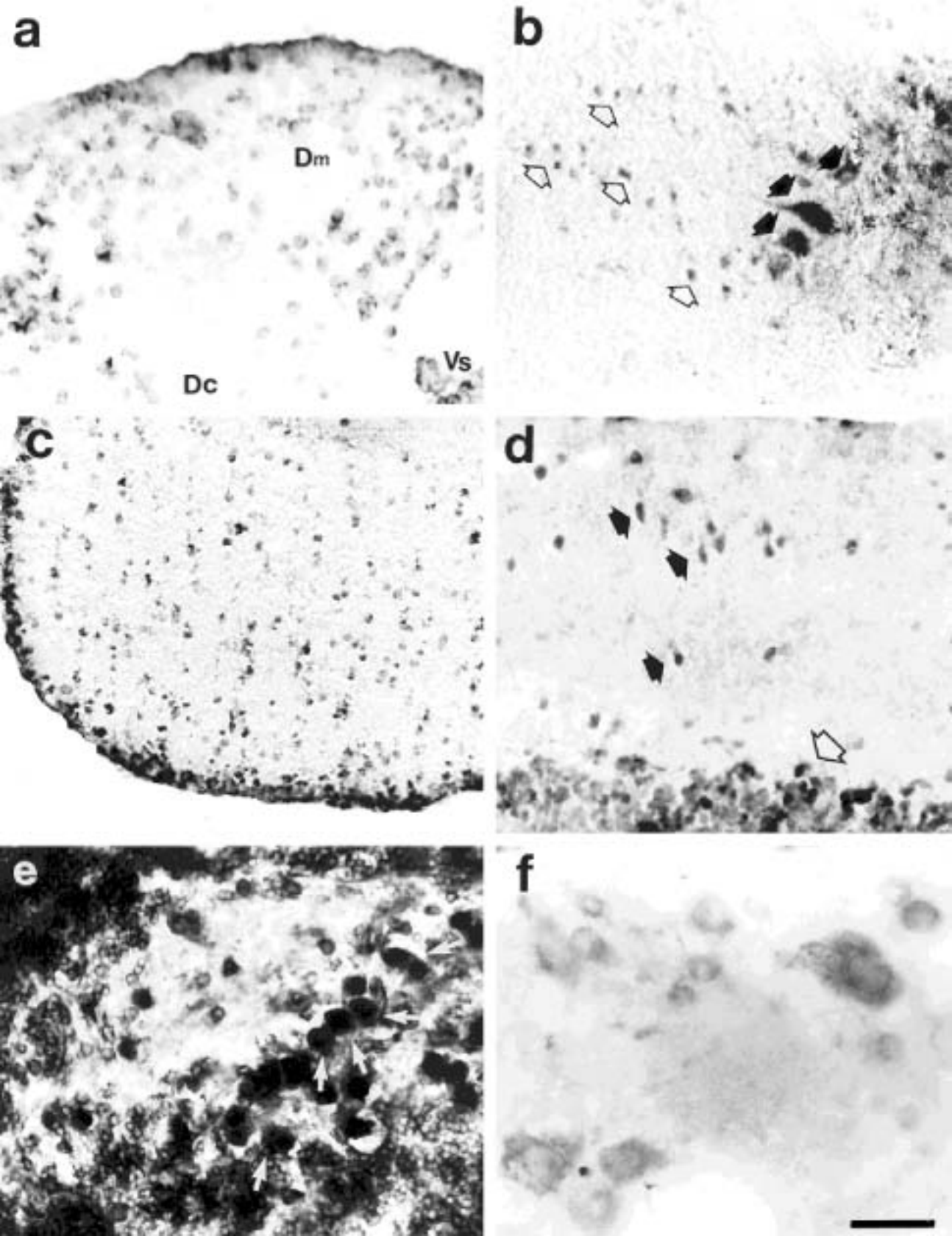
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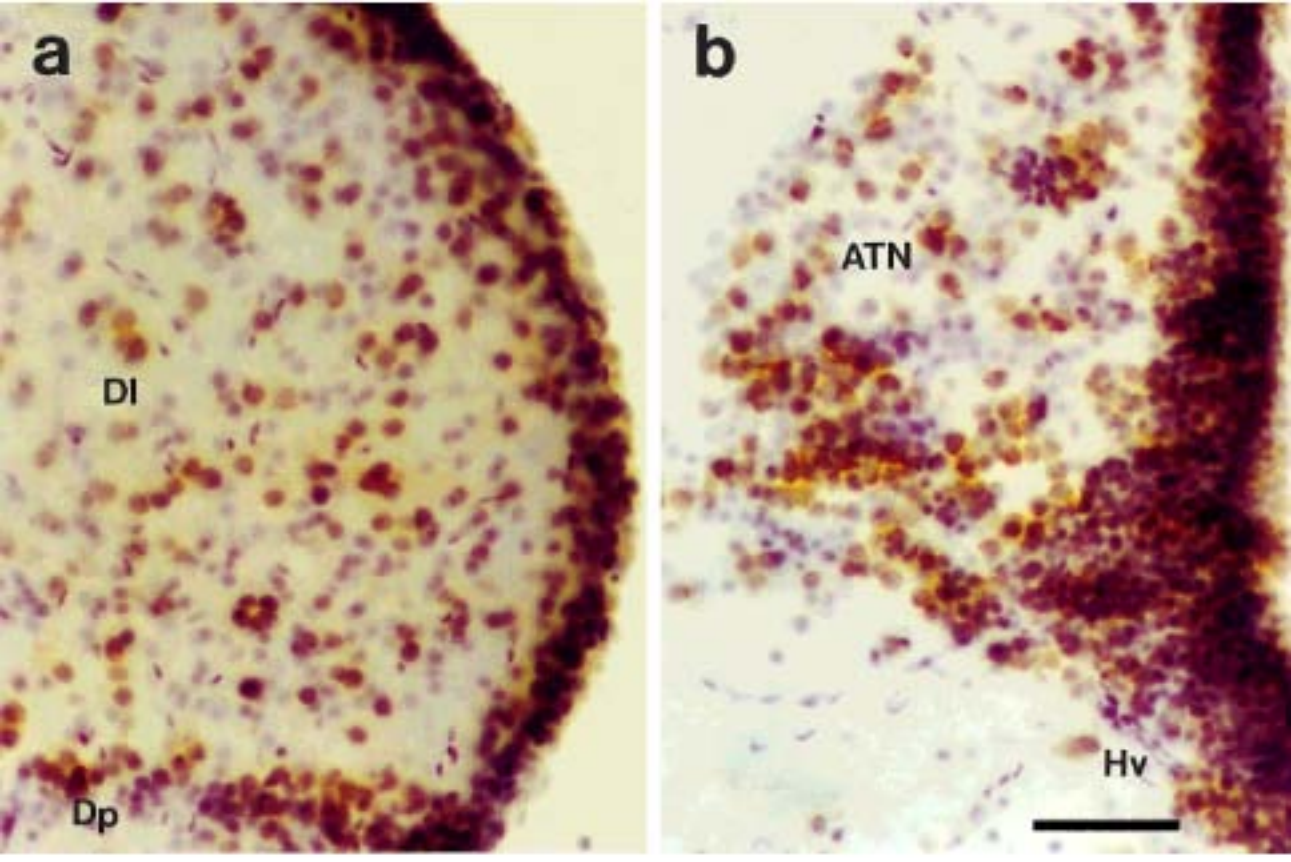
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