# Glycoproteins involved in long-lasting plasticity in the teleost brain

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The remarkable ability of the central nervous system (CNS) to cope with changing environmental conditions (acquisition of a new behaviour, or learning) results in functional alterations, which may succeedingly be utilized (retrieval or remembering) throughout the animals life (long-term memory, LTM). Using inhibitors of the transcription and translation process of the genetic information («antibiotics»), biochemical studies demonstrated the pivotal role of protein synthesis for LTM formation (Flexner et al., 1962; Agranoff and Klinger, 1964; Barondes and Cohen, 1966). The results of this interventive experimental approach have often been questioned, because the drugs produced side effects (Barondes and Cohen, 1967; Roberts et al., 1970). In carefully planned experiments (Squire et al., 1974), however, the side effects have been separated from the truly amnesic ones (reviewed by Shashoua, 1982; Davis and Squire, 1984; Shashoua and Schmidt, 1987). Biochemical studies on LTM received a new impetus by the application of specific antisera against defined proteins (Hydén and Lange, 1970) and recently by molecular biological techniques. The critical time period, during which experimental manipulation may prevent LTM formation, also called memory consolidation, lasts for $10^4$ sec or more. In its order of magnitude, so long a time comes closer to phenomena of growth and maturation ($10^7$ sec) than to the electrical events at the synapse ($10^{-2}$ sec). The stability of LTM, too, excludes that the neuronal excitation itself or individual chemical molecules might encode learned information. Regardless of their high metabolic turnover, proteins may, however, induce long-lasting finestructural alterations.

An appealing working-hypothesis suggests, that the mechanisms, which are triggered by synchronous activity of converging neurons during associative learning, may be similar to those promoting epigenetic differentiation or regeneration of neurectodermal cells. Glycoproteins have been proposed to guide the migration of neurites, to regulate intercellular adhesion and to modulate the efficacy of neuronal connections, either as integral components in cell membranes or as constituents of the extracellular matrix (Bogoch, 1967; Barondes, 1970; Edelman and Thiery, 1985). In particular, glycoproteins were shown to incorporate radiolabelled fucose during learning events (Popov et al., 1980; Rose and Jork, 1987).

In an attempt to identify proteins involved in memory consolidation, Shashoua (1976) trained goldfish to swim with an attached polystyrene foam float. After 4 hours the floats were removed and the fish were injected with radioactive valine into the brain ventricles. As compared with non-learning goldfish (active, passice and «stressed» controls), the precursor molecule was preferentially incorporated into a set of 3 related proteins migrat-

ing on electrophoretic gels with apparent molecular weights of 37, 32 and 26 kDa. The purified proteins were used to raise antisera. According to the immunohistochemical localization in the periventricular grey of the ependymal zone (Benowitz and Shashoua, 1977), the proteins were called ependymins.

Using a sensitive radioimmunoassay (RIA, Schmidt and Shashoua, 1981) ependymins were shown to be specific for the nervous system, encountered throughout the CNS (Schmidt and Lapp, 1987a). The highest concentrations were measured in the tectum, tegmentum, and the vagal lobes. Subcellular fractionation of goldfish brain revealed large amounts of ependymins in the cytoplasmic and some in the microsomal fraction, the highest specific concentration, however, was determined in the extracellular brain fluid (ECF; Schmidt, and Lapp, 1987b). Particles sedimenting in the synaptosomal fraction were shown to bind or incorporate radioactive ependymins.

The presence of ependymins in the periventricular region and in the ECF suggested that they may be secreted. Secretion of ependymins was confirmed in cell cultures derived from the optic tectum (Majocha et al., 1982) and was also shown in situ after the float-training (Shashoua, 1979), resulting in increased steady-state concentrations in the ECF (Schmidt, 1987). Antisera directed against ependymins prevented memory consolidation, when administered into the brain ventricles within 24 h after acquisition of the float swimming task (Shashoua and Moore, 1978; Schmidt, 1987).

The float-training activates many different brain functions and was favourably employed to increase biochemical changes for the identification of behaviourally relevant proteins. In this operant vestibulomotoric training, the experimenter does not have to interfere with the animals' behavior, but the learning-paradigm may not easily be compared to associative tasks. We have, therefore, applied an active shock-avoidance conditioning in the shuttle-box as a different (classical) learning paradigm. In this article, we discuss, whether similar alterations in the ependymin metabolism are to be observed after both learning paradigms. Furthermore, we compare the distribution of ependymins in goldfish and in zebra fish (*Brachydanio rerio*; Cyprinidae) and report some recent experiments on the molecular characterisation of ependymins.

## Methods

Goldfish were either trained in an operant vestibulomotoric learning-paradigm to swim with an attached polystyrene float (Shahoua, 1976) or in a classical active shock-avoidance conditioning in a shuttle-box (Horner et al., 1961). Trains of mild electroshock pulses (3 V, 1.25 mA) served as unconditioned stimuli; a conditioning light signal preceded the shock alternatively at either side of the shuttle-box. Within 25 one-minute cycles, the fish learnt to swim into the dark compartment of the tank whenever the light came on (Pironi and Schmidt, 1988). At various time points after acquisition, the fish were injected either with [$^3$H]-labelled valine (compare Fig. 2) or anti-ependymin antisera as described earlier (Schmidt, 1987).

By staining with the periodic acid Schiff's reaction, ependymins were identified as glycoproteins. By affinity chromatography on concanavalin A, ependymins may effectively be separated from the pool of soluble proteins (Schmidt and Shashoua, 1981). When the isolated proteins were further purified by isoelectric focusing, they were concentrated to three major bands with isoelectric points of 4.0, 4.1 and 4.2 (Fig. 1). Proteins were eluted with 0.1 M $\alpha$-methylmannoside in phosphate buffered saline (1.7 M, pH 8.4) and concentrated on Amicon PM10 filters. After reduction of disulfide bonds by $\beta$-mercaptoethanol all three major bands gave rise to the same two monomeric ependymin subunits migrating

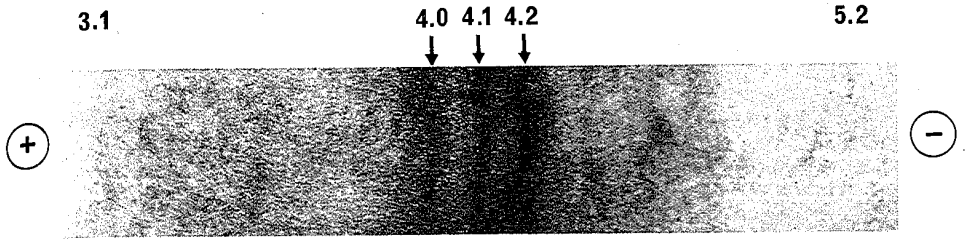



Fig. 1: Preparative isoelectric focusing of ependymins. Using Servalyt 3–5 (analytical grade; Serva, Heidelberg) on Sephadex G-75 (superfine; Pharmacia), three major bands were obtained at isoelectric points of 4.0, 4.1 and 4.2. In some preparations, the most acidic protein band was further separated into two constituents, and three additional minor bands were detected between pI 4.4 and 4.6; i. e., 7 bands at all.

on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at 37 and 32 kDa, respectively. Both monomers contain sugar moieties, since they both bind to concanavalin A (Schmidt and Shashoua, 1983). If ependymins were separated on SDS-PAGE without prior reduction, however, dimeric conformations of ependymins were obtained (Schmidt and Shasoua, 1981). Carbohydrate chains were removed from ependymins by incubation with glycopeptidase F from *Flavobacterium meningosepticum* (EC 3.2.2.18; Boehringer, Mannheim, F.R.G.) in 0.125 M Tris/HCl, pH 7.1, containing 0.5% SDS and 0.5% Nonidet-P40 at 37 °C for 24 h, or alternatively by treatment with endoglycosidase F (EC 3.2.1.96; Boehringer, Mannheim) at pH 5.0. Purified ependymins were used for biochemical analyses and to raise antisera in rabbits.

Methods used for immunofluorescence staining and for cDNA cloning of ependymin precursor molecules will be described elsewhere (Schmidt et al., 1990; Königstorfer et al., 1989a,b).

## Results

### Increased ependymin turnover after classical conditioning

Cytoplasmic fractions separated 4 h after acquisition of the active shock-avoidance conditioning exhibited a slight increase in the synthesis of proteins migrating at 32 and 37 kDa on electrophoretic gels (Fig. 2). By immunoprecipitation and RIA measurements the proteins were identified as ependymins. Increased valine incorporation was also observed at 75 kDa, probably into dimeric ependymins. At 8 h after injection six times as much radiolabel was incorporated into ependymins and other proteins of the cytoplasmic fraction, but significant changes induced by learning were no longer to be detected. The 37 and 32 kDa ependymins were also recovered in the ECF (Fig. 2). In this fraction, the change was small during the first 4 h, but 8 h after learning, secretion was enhanced by 21 and 23%, respectively (Fig. 3).

### Inhibition of memory consolidation by anti-ependymin antisera

Secretion of ependymins might either serve as a drainage device to remove excessive molecules, or rather be a step towards their interaction with remote target cells. Inactiva-

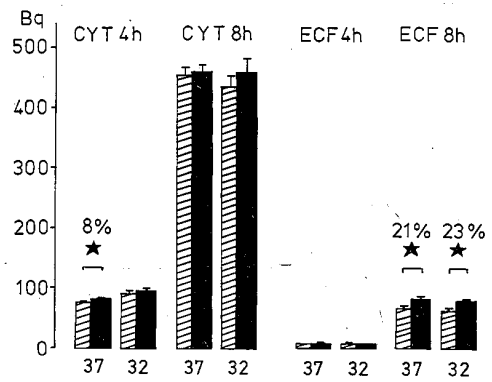



Fig. 2: Incorporation of [$^3$H]-valine into ependymins after active shock-avoidance conditioning. Fish were injected with 2 MBq [$^3$H]-valine (2 TBq/mmol) 15 min after acquisition (filled columns). Control fish (hatched columns) were injected simultaneously. To collect peptides of the ECF, brains were removed and incubated in buffer solution (Schmidt and Lapp, 1987a). They were then homogenized and separated by differential centrifugation to yield fractions enriched in various subcellular compartments (Schmidt and Lapp, 1987b). The fractions obtained were solubilized, further separated by SDS-PAGE and analysed for radioactivity incorporated into various proteins. Gels were cut to 40 slices of 3 mm each, digested with 1 ml of a tissue solubilizer (Beckman, BTS 450) for 24 h at 55 °C, mixed with scintillator and counted, using quench correction. The histogram blocks give the mean radioactivity (from n independent experiments) recovered with the total amount of ependymin from the respective fraction. Small bars indicate standard errors of the mean, asterisks indicate significant changes (Student's paired t-test). CYT, cytoplasmic fraction.

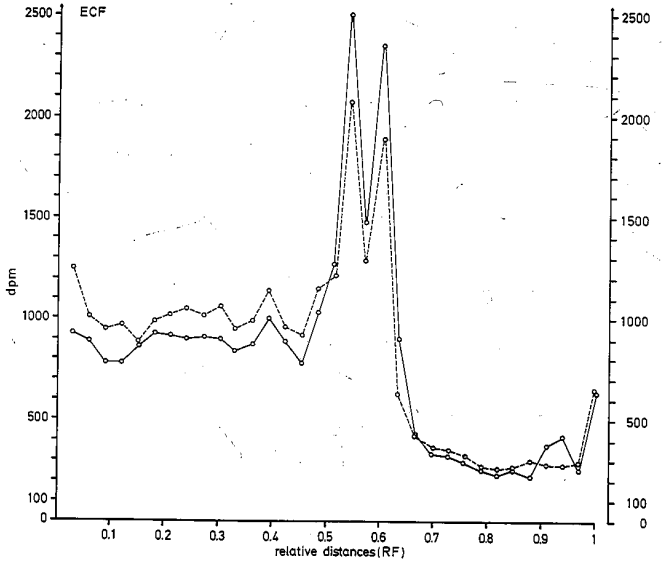



Fig. 3: Scintigram of proteins derived from goldfish extracellular brain fluid, separated by SDS-PAGE. Proteins migrating at 37 and 32 kDa (RF = 0.55 and 0.60, respectively) exhibit significantly enhanced secretion 8 h after acquisition of the shuttle-box task (solid line) as compared with controls (broken line).

Table 1: Dependence of the amnesic effect of anti-ependymin antisera on the time of injection.

| Shuttle-box training | | | Float-training | | |
|---|---|---|---|---|---|
| Time of injection [h] | | Retention score | Time of injection [h] | | Retention score |
| 1 | (n=66) | −0.03 | – | | – |
| 4 | (n=20) | 0.19 | 4 | (n= 28) | 0.36 |
| 24 | (n=10) | 0.05 | 4+16 | (n=165) | 0.34 |
| 48 | (n=16) | 0.53 | 48 | (n= 14) | 0.51 |
| 72 | (n=10) | 0.78 | 72 | (n= 13) | 0.77 |

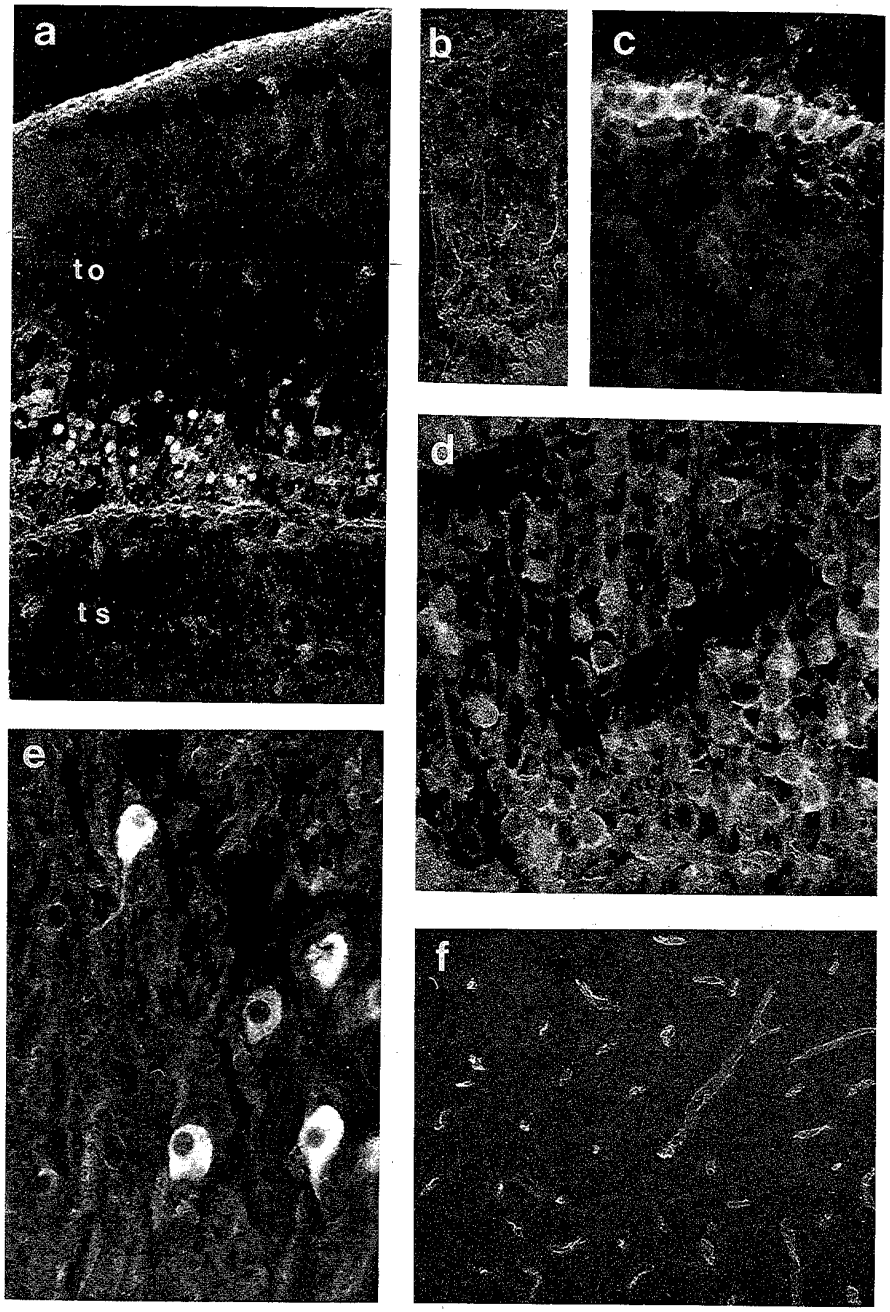
Goldfish were trained in the shuttle-box or float-swimming paradigm and injected with anti-ependymin antisera at various time points after the onset of training. Mean retention scores represent recall in the test session as a percentage of the performance acquired during training. Retention scores were pooled and recalculated using the data and algorithms from Shashoua and Moore (1978), Schmidt (1987), and Piront and Schmidt (1988). n, number of fish.

tion of extracellular ependymins by injection of antisera supports the second alternative: Following shock-avoidance conditioning, the antisera inhibited memory consolidation, when injected into the fourth brain ventricle 0.5, 4.5 or even 24 h after learning (Table 1). When tested on day 4, these fish did not recall the avoidance response, however, they learnt the task once again and then recalled it at a second test on day 6 (Piront and Schmidt, 1988). At 48 or 72 h after acquisition, memory consolidation was complete, and the antisera were ineffective. Antisera injected before training did neither interfere with learning nor with recall. A toxic effect of the antisera or an influence on the behaviour as such (performance) was ruled out by the time dependence of the amnesic action and by further control experiments making use of overtrained animals and of goldfish fleeing the conditioning light stimulus spontaneously.

### Immunofluorescence staining in goldfish and zebra fish

By immunohistofluorescence staining, ependymins are to be seen in the subependymal cell layer of the optic tectum (Fig. 4a), in the nucleus recessus lateralis in the hypothalamus, and they also occur in the vagal lobes. At higher magnification (Fig. 4d), piriform cells may be recognized in the stratum griseum periventriculare, that might represent pyramidal neurons sending weakly stained extensions (Fig. 4b) as far as to the stratum fibrosum et griseum superficiale and the stratum opticum. In addition, all anti-ependymin antisera stained the outer surface of goldfish brain (leptomeninx; Fig. 4a, c), and some of the antisera reacted with capillaries, especially within the optic tectum and the torus semicircularis (Fig. 4f). In agreement with the low ependymin concentrations measured by RIA (Schmidt and Lapp, 1987a), very little immunofluorescence was observed in the cerebellum. There was, however, a small population of intensely stained large cells, possibly equivalent to the Purkinje cells of mammalian brain (Fig. 4e).

Ependymins were also encountered in a similar distribution in other cyprinides, including the rudd *(Scardinius erythrophthalmus)* and zebra fish (*Brachydanio rerio*; Fig. 5). In cryostat sections of zebra fish, that were neither perfused nor fixed by immersion, strong immunoreactivity was observed in the extracellular matrix of the tectal and cerebellar




ventricular space (Fig. 5a,e,f), in addition to clusters of brightly staining cells situated in the subependymal layer, the torus longitudinalis, areas surrounding the tectal and third ventricle, and the central channel (Fig. 5b–g).

### Primary structure of prepro-ependymin I

Behavioural and morphological investigation both indicated, that ependymins might exert their physiological action via the extracellular matrix. To elucidate the molecular mode of action of these unique proteins, ependymins were studied biochemically. Analysis of the amino acid composition and peptide mapping after limited proteolysis with *Staphylococcus aureus* protease revealed that the 37 and 32 kDa ependymins are homologous (Schmidt and Shashoua, 1983). Furthermore, $^{125}I$ substituted into the 37 kDa or the dimeric ependymins was recovered with the 32 kDa protein after exposure to ECF. Gas-phase sequencing of both forms of ependymins now proved that they possess an identical N-terminal sequence, displaying microheterogeneities at positions 3, 8 and 13 (Königstorfer et al., 1989a). Radioactive oligonucleotides were synthesized as hybridization probes for screening of goldfish brain cDNA-libraries. The positive clones contained the regions encoding both, the N-terminal amino acid sequence and a fragment obtained from cyanogen bromide cleavage. The full length amino acid sequence of the ependymin precursor deduced from cDNA cloning (Hoffmann et al., 1988; Königstorfer et al. 1989a) comprises 216 amino acids and has a molecular weight of 24267 Da ($His^3$-$His^8$-$Thr^{13}$-variant, Fig. 6). It contains a hydrophobic, cleavable N-terminal signal sequence composed of 21 amino acid residues, characteristic of secretory proteins (von Heijne, 1983), which is followed directly by the ependymin sequence. After removal of the signal sequence, the molecular weight of the mature protein is 21960 (without sugar-moieties). A computer search did not reveal any homology to other sequenced proteins.

Two potential N-glycosylation sites were detected (Fig. 6), in accordance with the biantennary structure inferred from binding to concanavalin A (Shashoua et al., 1986). Following treatment with endoglycosidase F, ependymin mixtures were recovered as a single protein band after SDS-PAGE (Königstorfer et al., 1989b). Incubation of the 32 kDa ependymin molecule with glycopeptidase F revealed a fragment of 28 kDa and three bands migrating close together at an apparent molecular weight of approximately 22 kDa. Even the smallest fragment is recognized by the RIA, indicating that our antisera react with the protein backbone of the ependymin molecule. Incubation of the 37 kDa ependymin molecule, resulted in the same cleavage products, however, one additional fragment was obtained, comigrating exactly with the 32 kDa form of ependymins.

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Fig. 4: Immunofluorescence staining of goldfish brain. Goldfish were perfused through the heart with Bouin's fixative containing 53% ethanol and post-fixed by immersion. Coronal sections were cut in a cryostat and stained by anti-ependymin antisera followed by fluoresceinisothiocyanate-conjugated goat anti-rabbit IgG. a) Mesencephalon: brightly stained cells are observed in the subependymal cell layer of the optic tectum (to) dorsal to the torus semicircularis (ts). b) Weak staining of radially oriented fibers rising from cell bodies in the stratum periventriculare. c) Higher magnification of the outer dorsal surface (leptomeninx) in the optic tectum. d) High magnification of stained cells in the subependymal and periventricular cell layers. e) Large brightly staining neurons in the deep layers of the cerebellum. f) Capillaries reacted with antisera directed against the 37 and 32 kDa forms of ependymins (With antisera raised against sugarfree ependymins no immunoreactivity was observed in capillaries).

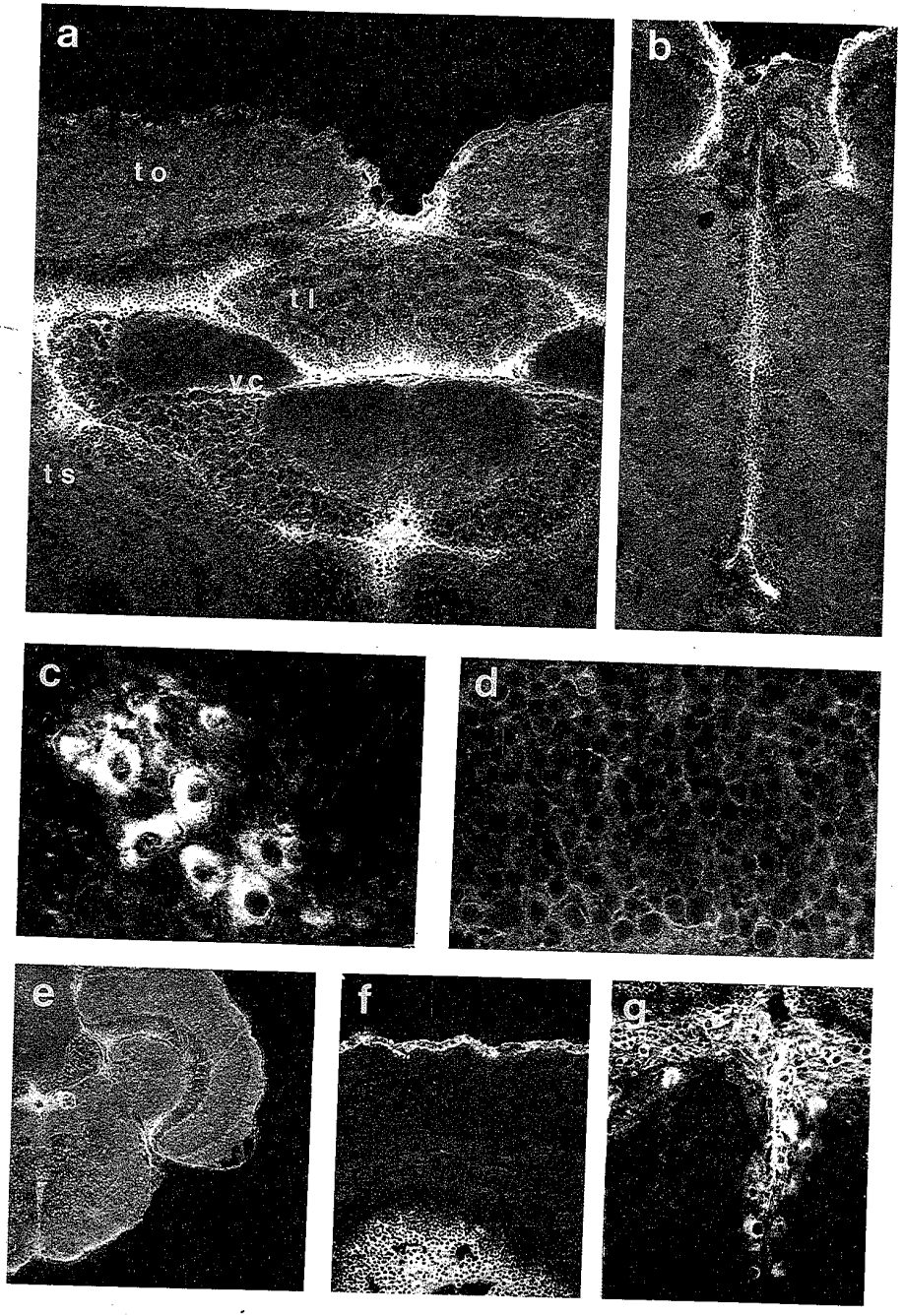


Fig. 5: Immunofluorescence staining of zebra fish brain. Prior to staining (compare Fig. 4), zebra fish brains were frozen in melting isopentane (2-methylbutane) at −159 °C and cut in a cryostat. a) Staining of the extracellular matrix in mesencephalic ventricles beneath the optic tectum (to) and torus longitudinalis (tl) and between the torus semicircularis (ts) and the valvula cerebelli (vc). Coronal sections through the «rostral» part of the third ventricle (b) and the caudal mesencephalon and diencephalon (e) displayed immunoreactivity in the extracellular matrix as well as in cell clusters depicted at higher magnification in c (tegmentum), d and f (subependymal layer and leptomeninx of the optic tectum) and g. Note fibers passing through the stratum periventriculare in e. Faint staining is observed in the valvula cerebelli and the torus longitudinalis (a, e).

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                MET-MET-HIS-THR-VAL-LYS-LEU-LEU-CYS-VAL-VAL-PHE-SER-CYS-LEU-
-21             CYS-ALA-VAL-ALA-TRY-ALA-SER-SER-HIS-ARG-GLN-PRO-CYS-HIS-ALA-
                                       -1 ↑ +1
-06             PRO-PRO-LEU-THR-SER-GLY-THR-MET-LYS-VAL-VAL-SER-THR-GLY-GLY-
010             HIS-ASP-LEU-GLU-SER-GLY-GLU-PHE-SER-TYR-ASP-SER-LYS-ALA-ASN-
025             LYS-PHE-ARG-PHE-VAL-GLU-ASP-THR-ALA-HIS-ALA-ASN-LYS-THR-SER-
                                                   ++++++++++
040             HIS-MET-ASP-VAL-LEU-ILE-HIS-PHE-GLU-GLU-GLY-VAL-LEU-TYR-GLU-
055             ILE-ASP-SER-LYS-ASN-GLU-SER-CYS-LYS-LYS-GLU-THR-LEU-GLN-PHE-
                                ++++++++++
070             ARG-LYS-HIS-LEU-MET-GLU-ILE-PRO-PRO-ASP-ALA-THR-HIS-GLU-SER-
085             GLU-ILE-TYR-MET-GLY-SER-PRO-SER-ILE-THR-GLU-GLN-GLY-LEU-ARG-
100             VAL-ARG-VAL-TRY-ASN-GLY-LYS-PHE-PRO-GLU-LEU-HIS-ALA-HIS-TYR-
115             SER-MET-SER-THR-THR-SER-CYS-GLY-CYS-LEU-PRO-VAL-SER-GLY-SER-
130             TYR-HIS-GLY-GLU-LYS-LYS-ASP-LEU-HIS-PHE-SER-PHE-PHE-GLY-VAL-
145             GLU-THR-GLU-VAL-ASP-ASP-LEU-GLN-VAL-PHE-VAL-PRO-PRO-ALA-TYR-
160             ======================
                CYS-GLU-GLY-VAL-ALA-PHE-GLU-GLU-ALA-PRO-ASP-ASP-HIS-SER-PHE-
175                                    ======================
                PHE-ASP-LEU-PHE-HIS-ASP
190
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Fig. 6: Amino acid sequence of an ependymin precursor (prepro-ependymin I). The amino acid sequence of the $His^{3}$-$His^{8}$-$Thr^{13}$-variant of prepro-ependymin I deduced from c-DNA cloning is consistent with the results obtained from gas-phase sequencing (positions 1–24 and 89–102; underlined) and the amino acid composition published by Schmidt and Shashoua (1983). The arrow indicates the cleavage site for the signal sequence. Positions 51–53 and 74–76 (marked by crosses) represent potential N-glycosylation sites. The highly negatively charged clusters at positions 160–165 and 181–186 are probably the two calcium binding sites.

Ependymins possess a high ratio of phenylalanine to tyrosine residues, typical of calcium binding proteins, and the primary structure of the ependymin molecule comprises two clusters of negatively charged amino acid residues (Fig. 6), as described for other calcium binding proteins like calsequestrin (Scott et al., 1988). Indeed, ependymins bind radioactive calcium in dialysis- and during electrophoresis-experiments (Schmidt, 1986). On an average 1.8 calcium ions were precipitated with each ependymin molecule by trichloroacetic acid.

## Discussion

Results obtained by amino acid analysis, cDNA sequencing and digestion by glycopeptidase F all revealed that the 37 and 32 kDa forms of ependymins differ mainly by the amount of glycosylation. Because two potential N-glycosylation sites were detected in the ependymin sequence, it is tempting to speculate that the 32 kDa ependymin represents a monoglycosylated, and the 37 kDa form a biglycosylated molecule.

In Western blots ependymins were recently shown to possess immunoreactivity against the monoclonal antibody HNK-1, which is lost after removal of N-linked carbohydrates (Shashoua et al., 1986). HNK-1 is known to cross-react with a set of cell adhesion glycoproteins, including the N-CAM family, the myelin associated glycoprotein MAG, and the epitopes L1 and J1 (Chou et al., 1985). Carbohydrates in ependymins comprise mannose, galactose, N-acetylglucosamine, N-acetylneuraminic acid, fucose, glucose and 3-sulfated glucuronic acid (Shashoua et al., 1986). The latter is typical of several surface active molecules reacting with the HNK-1 antibody (Chou et al., 1985).

The time limits during which retrograde amnesia was achieved by injection of anti-ependymin antisera after the classical conditioning (Table 1; Piront and Schmidt, 1988) were identical with those reported for the float-training (Shashoua and Moore, 1978). Furthermore, the time course of synthesis and secretion of ependymins was identical for both forms of learning. However, stimulation of synthesis and secretion was more pronounced after the float-training, as analysed both, by incorporation of radioactive valine (+ 60%; Shashoua, 1976) and by RIA (+ 39%; Schmidt, 1987).

We do not propose that ependymins are exclusively designed for memory consolidation. On the contrary, the crucial point is, to establish, in which basic reaction they participate from the extracellular fluid. Our working-hypothesis is, that synapses become temporarily activated by information processing during acquisition. If the new behaviour proves to be biologically advantageous, extracellular peptides might permanently modify all those synapses which had previously been activated. If such a mechanism is involved, ependymins might determine *after* learning what is to be retained and the phenomenon of retrograde amnesia would become comprehensible.

The results obtained with the HNK-1 antibody suggest, that ependymins might be involved in cell adhesion and recognition. May behavioural plasticity, then, be considered as a microevent in synaptic differentiation? The retinotectal projections favourably lend themselves towards an analysis of this question, because, in goldfish, they regenerate in a topologically correct pattern, reaching their targets in the tectum by guidance from chemical cues during migration. The sharpening of the multiunit receptive fields to approximately 12°, which is achieved at a later stage during regeneration, however, is promoted by synchronous activity of neighbouring afferent ganglion cells (Schmidt and Edwards, 1983). Infused anti-ependymin antibodies prevented this sharpening and froze the receptive fields at 30° (Schmidt and Shashoua, 1988).

It is suggested that ependymins are not only involved in memory formation, but whenever neurons are activated synchronously. How could this be achieved? The concentration of calcium ions in the extracellular matrix was shown to decrease as a consequence of long-term potentiation in the rat hippocampus (Krnjević et al., 1982), where ependymin-like immunoreactivity has been demonstrated in pyramidal cells (Schmidt et al., 1986) and in the extracellular fluid (Fazeli et al., 1988). Very recently, Morris et al. (1988) described a fall in extracellular calcium concentrations in the goldfish optic tectum after tetanization of the marginal fibers from the torus longitudinalis, in particular when combined with conditioning stimulation via the optic nerve. We found that ependymins bind radioactive calcium (Schmidt, 1986) and Shashoua (1988) has shown that they polymerize to long fibers in its absence. Furthermore, ependymins may be copurified with an EDTA-sensitive metallo-protease activity (Shashoua and Holmquist, 1986). Obviouly, polymerization, on the one hand, and monomerization and proteolysis, on the other, offer the means for a bimodal calcium-dependent regulation.

The synchronous activity of several synapses induces a temporary local decrease in extracellular calcium. In this time period, intracellular second messengers promote protein phosphorylations and change the conductivity of ion channels (Alkon, 1980; Kandel and Schwartz, 1982). At the same time extracellular molecules regulating cell adhesion may influence the ultrastructure of synapses by calcium-dependent changes in their conformation. As the calcium cannot permanently stay sequestered within the terminal, a later increase in its extracellular concentration will induce the decomposition of monomer ependymins.

## Abbreviations

CNS, central nervous system; ECF, extracellular fluid; LTM long-term memory; PAGE, polyacrylamide gel electrophoresis; RIA, radioimmunoassay; SDS, sodium dodecyl sulfate; Tris, tris (hydroxymethyl) aminomethane.


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## Summary

A glycoprotein specific of the nervous system, ependymin, is decomposed, resynthesized and secreted when goldfish learn an operant or a classical task. Immunofluorescence staining localized ependymin to the extracellular matrix and the subependymal cell layer in the goldfish and zebra fish brain. The primary structure of ependymin comprises two N-glycosylation sites, giving rise to glycoproteins of variable content in carbohydrates. Ependymin also contains clusters of negatively charged amino acids, suitable for binding of calcium ions. The configuration and conformation of this protein may be modified depending on the extracellular calcium concentration. Anti-ependymin antisera interfere from the extracellular matrix with plastic changes, that require synchronous neuronal activity.


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