Glycoproteins of the extracellular brain fluid mediate plasticity in the central nervous system

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The anatomical specificity of synaptic connections within neuronal microcircuits is assumed to code for the ("semantic") contents of acquired information. Biochemical reactions, on the other hand, provide ("syntactic") mechanisms modifying the efficacy of individual synapses. An appealing hypothesis suggests that mechanisms triggered by synchronous activity of converging neurons during associative learning may be related to those promoting epigenetic differentiation and regeneration of neurectodermal cells. Glycoproteins in particular, have been proposed to guide the migration of neurites, to regulate intercellular adhesion, and to modulate synaptic transmission.

Goldfish brain ependymins (37, 32 and 26 kDa; 15) are acidic, secretory, calcium binding (8) glycoproteins. During memory consolidation of a vestibulomotoric training task (9, 15) and active shock avoidance conditioning (10), during regeneration of the retinotectal projection (7), and after long-term potentiation of the rat hippocampus (2), ependymins become preferentially synthesized and secreted into the brain extracellular fluid. They share immunoreactivity against the HNK-1 antibody and 3-sulfatated glucuronic acid in their carbohydrate moieties (18) with cell adhesion molecules of the N-CAM family. The primary structures of ependymin precursors deduced from cDNA cloning (3, and unpublished results) comprise a hydrophobic leading sequence typical of secretory proteins, two N-glycosylation sites, and two clusters of negatively charged residues suitable for calcium binding. Ependymins form a family of closely related sequences, differing in point mutations and the degree of glycosylation (14, 16). Physiologically, the mature proteins occur predominantly as dimers and they respond to decreasing calcium concentrations by polymerization to long fibers (16). Immunohistologically, they were first encountered in the periventricular grey of the ependymal zone in cells tentatively identified as glial (1). In fish, the ependymal zone is rich in neurosecretory cells intermingled with glial elements and must not be confused with the monocellular ependymal layer typical of mammals. Because ependymins have also been demonstrated in synaptosomal preparations (12) and in pyramidal neurons of the rat hippocampus (13), we reinvestigated their distribution by immunohistofluorescence and electron microscopy, using rabbit antisera raised against the deglycosylated immunogen.

Immunohistofluorescence staining was strictly confined to the nervous tissue, in accordance with earlier radioimmunoassay measurements (11). Ependymins were observed in the subependymal layer of the optic tectum, cell clusters of the torus longitudinalis, the hypothalamic nucleus recessus lateralis, the eminentia mediana, the vagal lobes, areas surrounding the aqueduct and central channel and in the leptomeninx. Furthermore, pyramidal neurons were labelled in the stratum griseum periventriculare sending apical dendrites to the stratum fibrosum et griseum superficiale and the stratum opticum. Electron microsopical localization of ependymins was achieved by the avidin-biotin-peroxidase technique in adult goldfish, perfused with ethanolic Bouin's fixative: Many ependymin-positive pyramidal neurons were seen in the stratum periventriculare. The immunoreaction product was deposited in narrow cytoplasmic envelopes surrounding the large cell nuclei (unstained) and in dendrites displaying spines typical of pyramidal neurons. Ependymins were identified in new-born zebra fish *(Brachydanio rerio, Cyprinidae)*, at a time when much remodeling takes place in the CNS. In cryostat sections strong immunoreactivity was also observed in the extracellular space and

brain ventricles when juvenile zebra fish or adult goldfish brains were frozen without fixation in melting 2-methylbutane, supporting earlier evidence on secretion of ependymins *in vivo* and *in vitro* (4, 11, 12).

Secretion of ependymins is a step towards their interaction with remote structures, because anti-ependymin antibodies interfere with memory consolidation after vestibulomotoric training (9, 17), after shock avoidance conditioning (6), and with the sharpening of multiunit receptive fields during retinotectal regeneration (7). Furthermore, ependymins become incorporated into particles of the synaptosomal fraction (12), and they provide a suitable substrate for outgrowing axons of goldfish retinal ganglion cells *in vitro* (collaborative results with C. STÜRMER and J. SCHMIDT). It is suggested that the decrease in extracellular calcium concentrations (5) induced by synchronous activity of converging neuronal pathways triggers post-translational modifications in ependymin molecules which ultimately consolidate the activated synaptic connections. (Supported by the Deutsche Forschungsgemeinschaft)

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