A protein secreted from the ependyma contributes to the consolidation of experiential plasticity in the central nervous system

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Biochemical experiments have revealed that vertebrates can not establish long term memory whenever protein biosynthesis in the brain is impaired. A careful combination of biochemical and morphological analyses should permit the identification of the protein molecules involved and the anatomical sites of their action.

SHASHOUA (1) demonstrated that two brain glycoproteins (32,000 and 26,000 M.W.) exhibit an enhanced rate of synthesis when goldfish are forced to adopt a vestibulomotor training task. We have isolated and characterized them biochemically. The pure proteins, β and γ, were used to raise antisera. When such antisera were injected into the brain ventricles during a short defined period after the initial training, and the goldfish were tested some days later, they were unable to remember the task for which they had been trained (2). Benowitz used the antisera to localize β to cells of the periventricular grey in the ependymal zone, and named it "ependymin" (3).

I have developed a sensitive and specific radioimmunoassay (RIA) for quantitative determination of ependymin β (4). The RIA does cross-react, however, with the γ-protein. Biochemical analyses proved that β is the physiological precursor of the γ-molecule; the reaction is catalysed by a protease of the interstitial or the cerebrospinal fluid (CSF; 5). The RIA was used to investigate the proteins' distribution in untrained fish. They are specific for the nervous system and are highly enriched in the CSF and an isolated cytoplasmic fraction.

The presence of β in cells of the zona ependyma and the CSF suggested that it might be secreted. Therefore, we grew dissociated zona ependyma cells as primary monolayer cultures, which survived for at least 4 weeks. Certain cells stained with anti-ependymin antisera. Labeling studies and RIA data indicated that cells within the cultures can synthesize ependymin *de novo* and secrete it into their extracellular environment (6). *In vivo*, ependymin might be released for the purpose of its inactivation, or it might use the CSF as a transport vehicle and exert its physiological action at targets away from its site of synthesis, possibly after proteolytic modification to the γ-polypeptide. Such a mode of action could be similar to that of other extracellular peptide factors which play a crucial role in the maintenance and differentiation of neurons in culture. Indeed, anti-γ-antisera do not only stain the ependyma, but also neurons of the granular layers in the vagal lobes and the optic tectum. Although it is not yet known whether the stained cells represent physiological binding sites or centers for artificial redistribution, it is our working hypothesis that neuronal membranes become temporarily activated by the electrophysiological events during acquisition of new behavioural patterns, and permanently modified by extracellular peptide factors (e.g. γ), if the new behaviour proves to be advantageous. This, then, would be called long term memory.

We investigated whether the same or similar proteins can be detected in nervous tissue of higher vertebrates. The hypothesis was tested, in collaboration with Drs. MÜLLER and SEIFERT, on neuronal cells cultured from embryonic rat brain hippocampus. All pyramidal neurons stained strongly for ependymin γ. Several different

antisera directed against neuron-specific proteins were used to counterstain them. Contaminating astrocytes were never seen to react with anti-ependymins. In accordance with the biochemical data, the antigenic material was located within the cytoplasm, including neurites, which began to extend after a few hours in culture. The staining was most prominent in areas where synaptic connections are formed between neuronal extentions. The demonstration of this protein in the hippocampus is of particular interest, because neurological observations clearly indicate the importance of the hippocampus for long term memory formation.

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