



Network on Neural Mechanisms of Learning and Memory

ESF SCIENTIFIC NETWORKS

**BACKGROUND PAPERS
FOR THE WORKSHOP ON
NEURAL MECHANISMS OF
LEARNING AND MEMORY**

Palermo, 27 - 29 January 1989

March 1989

MOLECULAR APPROACH TO MECHANISMS OF INTERCELLULAR INTERACTION
DURING LONG-TERM MEMORY FORMATION

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Theoretical concept:

The stability of long-term memory excludes that the neuronal excitation itself might provide a storage device for learned information. The short half lives of the brain's chemical constituents (with the exception of DNA) also exclude that individual molecules might encode learned information. Regardless of their high metabolic turnover proteins may, however, induce long-lasting finestructural alterations. The specificity of synaptic connections is supposed to determine what is learned (the semantic aspect of an "engram"), but molecular events provide the mechanisms to modify individual synapses (the syntax of learning and retrieval). The respective synapses may perhaps be identified in lower invertebrates. Making use of correlative approaches (e.g., by incorporation-studies of radioactive precursor molecules) and interventive approaches (amnesic drugs and antisera against brain-specific constituents), however, the molecular mechanisms of neuronal plasticity may be analysed, even in vertebrates.

Posttranslational alterations in synaptic proteins, in particular phosphorylation of ion channels, have been demonstrated in invertebrates in the sequel of simple events of behavioural plasticity, like habituation and sensitization. However, no physiological mechanism has yet been elucidated that might provide a means to determine which of many possible associative pathways should be strengthened, or rather be ignored and demolished during long-term memory formation. Which criterion may provide a basis to differentiate between functional coincidences during activity of

neuronal circuits and acquisition of new information? Our working-hypothesis is that all synapses involved in information processing during acquisition of a new behaviour become activated temporarily. Only if the new behaviour proves to be biologically advantageous, some additional "factor" might permanently modify all those synapses that have previously been activated. Such a factor would have to be widely distributed, like a message "to whom it may concern", and should readily be available throughout the central nervous system. Like, e.g., the cell adhesion molecules, it should have the potential to modify finestructural and/or functional characteristics of synaptic connections. The factor should be responsive to the functional state of the neuronal micro-environment, e.g. to changes in ionic concentrations. It should be regulated by an evaluating function weighing the importance of the preceding behaviour for the biological fitness of the organism (e.g., dearousal). Such a kind of mechanism would determine only after learning what is to be retained, rendering the phenomenon of retrograde amnesia comprehensible.

The critical time period, during which biochemical manipulation may prevent long-term memory formation 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). An appealing working-hypothesis suggests, that some of the mechanisms, which are triggered by synchronous activity of converging neurons during associative learning, may be similar 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 the efficacy of neuronal connections, either as integral components in cell membranes or as constituents of the extracellular matrix. On the other hand, glycoproteins were shown to increase in turnover and to incorporate radiolabelled sugar moieties during learning events. May behavioural plasticity, then, become comprehensible as a microevent in synaptic differentiation?

Experimental support:

During the last decade, we studied ependymins, acidic, secretory calcium binding glycoproteins of the extracellular brain matrix, that mediate plasticity in the central nervous system. Ependymins share 3-sulfatated glucuronic acid in their biantennary carbohydrate moieties and immunoreactivity against the HNK-1 antibody with cell adhesion molecules of the N-CAM family. The primary structure of prepro-ependymin, deduced from cDNA cloning, comprises a leading sequence, characteristic of secretory proteins, two N-glycosylation sites, and two clusters of negatively charged amino acids, suitable for calcium binding. The mature protein is expressed in several closely related forms, which differ in the degree of glycosylation and respond to the ionic environment: ependymins polymerize when exposed to low calcium concentrations and may be copurified with an EDTA-sensitive metallo-protease activity. Obviously, polymerization, on the one hand, and monomerization and proteolysis on the other, offer the means for a bimodal regulation depending on the calcium concentration that is known to change in the extracellular compartment during synchronous neuronal activity.

Immunohistologically ependymins were observed in the periventricular grey of the ependymal zone. They are specific for the nervous system and enriched in neurons of the optic tectum, the hypothalamus and the vagal lobes, and are also encountered in Purkinje cells. They were found in several other species, including early stages of the zebra fish (*Brachydanio rerio*; Cyprinidae), but also in higher vertebrates, in particular in pyramidal neurons of the embryonic rat hippocampus. Secretion of ependymins was demonstrated both, in situ and in cell cultures derived from the optic tectum. The immunological cross-reactivity with carbohydrates involved in cell adhesion and recognition suggests that secreted ependymins interact with neurons from the extracellular fluid.

As shown by incorporation of radioactive amino acids, by radioimmunoassays and immunological techniques, ependymins are preferentially synthesized and secreted into the extracellular matrix, (1.) when goldfish learn an operant swimming-skill, or

(2.) a classical associative task, (3.) during regeneration of the retinotectal projections in goldfish, and (4.) following long-term potentiation of the rat hippocampus. By subcellular fractionation methods, particles sedimenting in the synaptosomal fraction were shown to bind or incorporate radioactive endymins.

Furthermore, anti-endymin antibodies injected into the brain ventricles prevent long-term memory formation up to 24 hours after both, acquisition of a float-swimming skill and an active shock avoidance conditioning in a shuttle-box. They also interfere with activity-dependent sharpening of the multiunit receptive fields during regeneration of retinotectal projections following optic nerve crush. The antisera are without influence on overtrained animals or on the performance of the behaviour as such.

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. At the same time extracellular endymins may influence the ultrastructure of synapses by calcium-dependent alterations in their conformation and binding characteristics. As the calcium cannot permanently stay sequestered within the terminal, a later increase in its extracellular concentration will induce the decomposition of monomer endymins.

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