Glycoproteins and memory formation: An introduction

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Morphological approaches

In ancient times, memory was conceived as a box filled by experience. Functional concepts proposed by various schools differed mainly with respect to the attention they paid to either acquisition ("learning" of new information in the sense of Aristotl) or retrieval ("remembering" of ideas in Plato's philosophy). The success of a materialistic approach to the analysis of living phenomena began in the 15^{th} century and was - against strong criticism (e.g., H. Bergson) - in this century extented to the so called higher brain functions. Many early studies concentrated on attempts to localize memory. Lashley's (1950) unsuccessful search for an anatomical, structural basis of memory within the brain tissue is typical of many studies before world war II.

Molecular biological approaches

The success of two disciplines fostered new theories on learning and memory in the fifties: Molecular biology revealed that genetically transmitted information is coded by a chemical alphabet of four nucleotides, and computer sciences demonstrated that simple structures, endowed with the ability to exist in only two different states, i.e., excitement and inexcitement, may be programmed to learn and to store tremendous amounts of information and to retrieve them in a relatively short time. Both ways to handle information provided attractive models for the mechanisms of learning and memory.

In analogy to studies on the genetic code much early biochemical work was dedicated to the identification of *the* particular class of chemical molecules that might encode learned information. Reminiscent of a Nuremberg Funnel, some of these ambitious experiments culminated in the attempt to transfer learned information molecules to the unexperienced subject, a chemical modification of the royal road of learning.

Short- and long-term memory

Learning is the process by which the brain converts new information into a form that can be stored as a retrievable memory trace ("engram"). Psychobiological and neurochemical investigations indicate that this process consists at least of two stages: (1) a short-term memory (STM) phase, which is susceptible to physical interference, such as application of electroconvulsive shock or cooling within seconds to minutes after acquisition of the information, and (2) a long-term memory (LTM) phase, which is not blocked by such events and may last for a lifetime (Agranoff, 1981; Menzel, 1983). The mechanism by which STM is transformed into LTM is called the memory consolidation process.

If we search for molecules in the central nervous system (CNS) that can fulfill this requirement for long-term stability, we find that everything except DNA is in a dynamic state (Shashoua, 1982). Regulation of the cell cycle and metabolic state may provide important mechanisms for plastic adaptations, however, there is no evidence, as yet, that DNA itself can be modified to encode experiential information. The average half-life of proteins ranges below 20 days; ribonucleic acid turnover can vary from half an hour to a day (Shashoua, 1974); lipids and carbohydrates are in a rapid state of flux. It appears unlikely, therefore, that long-lasting changes in the efficacy of synapses may be achieved via continuously elevated concentrations of particular substances, such as, e.g., enzymes or neurotransmitters.

The connectionist's view

Apparently, only the patterns of structure and connectivity in the CNS may be sufficiently stable for LTM storage. From such considerations, the neurochemical aspects of memory reduce to the identification of specific metabolic, physiological, and biochemical parameters, that provide the mechanisms to modify individual synapses and to alter neuronal circuits (Shashoua and Schmidt, 1987). The steady-state concentrations of those chemical factors, that induce permanent changes in the structural relationships during memory formation, may, then, soon return towards their resting levels.

Strategies for analysis

Three strategies have been used to study the biochemical changes that occur during acquisition and consolidation (Agranoff, 1981; Shashoua, 1982; Shashoua and Schmidt, 1987):

1. *The interventive approach* interferes with the formation of a long-term memory by blocking some discrete process in cell metabolism, e.g., nucleic acid transcription and translation by means of antibiotics, or presynaptic enzymatic reactions critical to transmitter synthesis and release by means of antibodies. Antagonists of postsynaptic neurotransmitter-receptors, of second transmitter systems, and ion channel blockers were used in other investigations. The disadvantage of this approach is, that a metabolic inhibitor may not block memory by its principal mode of biochemical action, but by some alternate and possibly unknown mechanism. The interfering agent might actually function as a general metabolic poison that is not even specific for the nervous system.

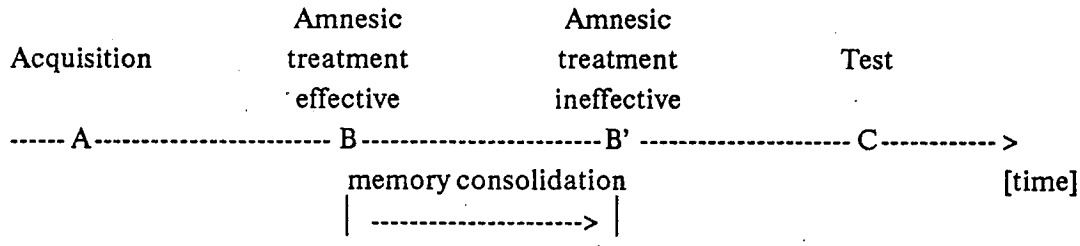
2. *The correlative approach* analyses biochemical changes that occur in the CNS during acquisition or memory consolidation. Radioactive precursor molecules are commonly used to detect newly synthesized nucleic acids and proteins with a possible role in memory formation. Advantageously, this procedure does not perturb the animals' behaviour, but the monitored metabolic alterations may also be related to secondary phenomena triggered by the experimental situation (e.g., stress, focused attention, or the performance of the behaviour as such).

3. *The modulative approach* makes use of hormones and agonists to promote acquisition and consolidation or to delay the forgetting. This approach has obvious pharmacologic implications. It is difficult, however, to distinguish side effects, such as increased blood flow in the brain, increased energy metabolism and sleep reduction, from a possible effect of a given substance on learning, memory or retrieval. The modulative approach, therefore, combines the disadvantages of the interventive and the correlative approaches.

It is questionable, whether molecular mechanisms, that are necessary and sufficient for memory consolidation, exclusively subserve this purpose, just as there is no particular gene or chemical set-up, e.g., for hunting, for singing or for aggressiveness.

Retrograde amnesia, a metabolic paradox?

Nevertheless, numerous experiments have demonstrated that inhibitors of the transcription or translation process in protein biosynthesis interfere with consolidation of declarative memory in vertebrates (Agranoff and Klinger, 1964; Davis and Squire, 1984; Flexner et al., 1963). Typically, such antibiotics cause retrograde amnesia only, if they are intracerebroventricularly administered before or shortly after acquisition of the new behaviour. When injected at a later time, they are without influence on recall in the test.



On the first sight, retrograde amnesia may appear as a metabolic paradox: Although nothing unphysiological happens at the time, when the animal learns (A), it cannot recall during the test session (C), if a protein synthesis blocker was applied at an intermediate time B. The same drug is, however, ineffective, when applied somewhat later, at B'. By some process, obviously, the new information becomes less susceptible to interference between B and B'; this process has been named the memory consolidation process and it has been compared to the chemical reactions that develop and fix a latent photographic image (Agranoff, 1981). On the other hand, protein synthesis is not required for acquisition and STM. Experiments did not reveal, however, whether STM and LTM are formed by independent mechanisms in parallel with each other, or wether STM is physiologically converted into LTM.

Specific proteins in memory formation

Changes in the synthesis or turnover of specific proteins were first reported for S-100 (Hydén and Lange, 1970) and 14-3-2 (see Zomzely-Neurath and Walker, 1977) after handedness-reversal and passive avoidance conditioning of rats, for tubulin (Stewart and Rose, 1978), acetylcholine esterase and muscarinic receptors (Rose and Stewart, 1978) after environmental stimulation in rats and chicks, and for ependymin after a vestibulomotor training in goldfish (Shashoua, 1976; Schmidt, 1987). Bock and Dissing (1975) identified 14-3-2 as a neuron specific form of the glycolytic enzyme enolase and thus were the first authors to explain a specific phenomenon in the sequel of learning by a well known biochemical "house-keeping" reaction in cellular metabolism.

Post-translational modifications in memory formation

Recently, postranslational modifications of protein molecules have received much attention in the context of plastic adaptations of the CNS:

Phosphorylation of synaptic proteins, in particular of ion channels in invertebrates, has been implicated to modulate synaptic transmission in simple forms of behavioural plasticity, such as

habituation and sensitization (Klein et al., 1980; Kupfermann et al., 1970). Later experiments referred to associative learning and revealed that phosphorylation induced changes in the ion conductivity are regulated by several second transmitter systems (Alkon, 1980; Alkon et al., 1988; Baudry et al., 1987; Kandel and Schwartz, 1982). These second messengers, in term, become activated via independent mechanisms operating onto the same neuron (synaptic inputs and depolarization). The synergistic effect of converging intracellular second transmitter signals on the same effector molecule may provide a basis for associative learning (Abrams and Kandel, 1988). As in mammals, however, long-term changes in the behaviour depend on protein biosynthesis (Nelson and Alkon, 1989).

Proteolysis accounts for other posttranslational modifications which have been discussed in relation to plasticity of the CNS (Lynch and Baudry, 1984; Shashoua and Schmidt, 1987).

Glycosylation: Most important for this workshop, the groups at Dublin and Magdeburg have documented increased glycosylation of proteins by measurements of ^{3}H-Man-N-Ac (a precursor for sialic acid in N-CAM; Doyle et al., 1991) and ^{3}H-fucose incorporation (Popov et al., 1980) in the sequel of learning experiments. Furthermore, a "false" sugar, 2-deoxy-D-galactose, acts as a specific inhibitor that prevents formation of Fuc(12)Gal linkages and induces amnesia in rats (Jork et al., 1986), chicks (Rose and Jork, 1987) and fish (own unpublished results together with R. Jork) when injected during a critical time period. Similar results have been obtained in long-term potentiation (LTP) experiments on the rat hippocampus (Krug et al., 1991), raising again the question, of whether LTP may be regarded as an electrophysiological analogue to - or even a partial mechanism in - LTM-formation (Teyler and DiScenna, 1984; Matthies, 1989a; 1989b).

Glycoproteins in differentiation and memory formation

Glycoproteins are well known to guide the migration of neurectodermal cells. As integral components in cell membranes and as constituents of the extracellular matrix glycoproteins regulate cellular adhesion and may modulate the efficacy of neuronal connections (Edelman, 1985; Künemund et al., 1988; Schmidt, 1989; Schachner, 1992). The critical time period, during which biochemical interference may prevent memory consolidation, lasts for 10^{4} sec or more. By 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, therefore, 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 (Breen and Regan, 1988; Doyle et al., 1991; Schmidt et al., 1991). May behavioural plasticity, then, involve micro-events in the morphological differentiation of CNS synapses? Such kind of mechanisms are slow and would determine only after learning, what is to be retained, rendering the paradoxical phenomenon of retrograde amnesia comprehensible.

There is an intriguing formal analogy in the recognition of self and non-self as a prerequisite for the molecular evolution of polynucleotides in phylogeny, for tissue differentiation via recognition molecules in ontogeny (e.g., of identical and different membrane properties on glial and on neuronal cells; compare Schachner, 1992), for the invasion of malignant cells (Linnemann and Bock, 1990), for development of children's ability to communicate (Spitz, 1957) and even for the dialectic structure of Hegelian philosophy.

Finally, I should like to pose a few rather general and provocative theses:

1. The excitation in a set of neuronal circuits may represent a sensory impression, a higher order association, or a set of commands in a motor programme. The word "represents", here, is meant in a monistic sense. Neuronal excitation is not a correlate of some information, but it is the information itself. We have to be aware of the fact, that methodologically different approaches use linguistically different descriptions of the same phenomenon (identity theory; compare Rose, 1981).

2. Whenever the same set of neuronal circuits is activated to the same extent, the excitation represents exactly the same information.

3. Learning is a change in the excitability of a set of neuronal circuits, that alters its probability of becoming activated.

4. The anatomical specificity of synaptic connections determines what is learned, so to speak, the semantic aspect of an "engram". The same set of neurons may, however, be involved in the storage of different informations. Furthermore, different aspects of the same behavioural situation may be coded in different anatomical structures within the brain.

5. Molecular events provide the mechanisms to create or modify individual synapses, i.e., the syntax of learning and retrieval. However, biochemical events are not the cause for learning, because they do not precede it. Nevertheless, they may induce a permanently altered cell biological and metabolic state in a neuron or a neuronal circuit.

6. In some instances, the repetition of sensory stimuli or the repeated activation of the same motor programme alone may be sufficient to increase the probability of their representation in the brain. However, at least the vertebrates distinguish between mere activation of a set of neuronal circuits and novel experience. In higher organisms there must exist mechanisms that weigh the biological importance of external and internal stimuli and evaluate the advantages and disadvantages of a newly acquired behaviour. Stress hormones, e.g., might provide such a kind of evaluating mechanism, because any important learning comes with a certain amount of stress, regardless of whether the animal searches food, looks for a mate, or even worse, it has to escape a predatory animal. Organisms, that consolidate exactly those neuronal circuits, which had been activated before the level of arousal and stress hormones decreased again, will certainly gain selective advantage in evolution.

7. It is not a priori clear, whether such alterations in the connectivity are achieved by an increased efficacy of existing synapses, or whether new synaptic connections are being formed. Activation of "silent", preformed synapses during behavioural acquition may provide a fast learning-mechanism. Stabilization of a few synapses via selection from excessive numbers of temporarily activated or even newly formed neuronal circuits during memory consolidation appeals by its similarity to neural regeneration and epigenetic development (compare J. Schmidt and Shashoua, 1988; J. Schmidt et al., 1991; R. Schmidt et al., 1991).

8. If glycoproteins are involved in memory formation (Rose, 1989), as we suppose, the required anatomical specificity may be achieved, because cell recognition molecules and neural growth- and elongation-factors may only be synthesized in the proximity of those neurons, which are involved in the processing of the newly acquired information. There is, however, a further possibility: Glycoproteins may be synthesized in cells away from their sites of action.

and be secreted and distributed as a message "To whom it may concern". Such glycoprotein factors may then interact with receptor molecules that have become primed at activated neuronal membranes. Alternatively, the secreted glycoprotein factor itself may become post-translationally modified by ectoenzymes and low molecular weight co-factors at the activated synapses (Schmidt and Makiola, 1991).

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