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Introduction: molecular mechanisms of memory formation—from receptor activation to synaptic changes

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The neuronal syntax and semantics of memory

The brain is the central interface between incoming sensory information and the initiation of motor reactions. In humans, such reactions may depend on previous experiences, which have been encoded, memorised and are now retrieved. Mechanisms of encoding and retrieval of experiences in the brain is a key question of neuroscience. Owing to pragmatic constraints, however, neuroscientists prefer to examine the general cellular syntactic rules which are operative during learning over the neuronal semantics that correspond to the encoding of discrete items of information [1, 2]. The difficulty with neuronal semantics lies in the concept of neuronal representation, that is the idea of an internal code of the outside world. Indeed, the concept of representation is blurred by its overuse. The pioneering work of Tolman [3] on the cognitive map, that is cognitive representations of space, has opened a large debate on the nature of these representations. For some authors, representations operate in an analogue mode [4], whereas for others, they comprise associations between items in a propositional mode [5, 6]. Cognitive psychology, for which the concept of representation is central, has extended it to mental representations or mental images, widely neglecting the notion of a trace as an organic substrate. Since the formulation of Hebb's concept of synaptic plasticity and neuronal cell assembly in 1949 [7], neuroscientists have made many attempts to delineate the concept of internal representation by seeking correlations or equivalencies between neuronal activity and connectivity, and percepts and concepts [8, 9]. The Hebbian paradigm of a reverberating signal as the internal representation of experiences or stimuli has been refined to the theory of connectionism. Briefly, connectionist or parallel distributed processing models emphasise that the efficiency of a synapse (connection) is influenced by the frequency of its use and that neurones (units) are highly interconnected in interacting networks. Consequently, self-organisation of networks in the brain contributes to conceive information in a distributed manner within cell assemblies [5]. Although the model of Freeman and colleagues shares a number of basic characteristics with connectionist models, they argue that chaotic neural activity builds sense of the world, and they reject the notion of representations [10, 11]. Taken together, all these considerations have widened rather than narrowed the gulf between cellular and molecular mechanisms of neural elements and the emergent activity of the brain, that is cognitive processes.

While the knowledge about neural semantics is still limited due to the lack of appropriate concepts and technologies, the contrary applies for operations that govern the cellular and molecular response of the neuronal machinery to an incoming signal. It is remarkable, and this will be developed in the following articles, that neuronal syntax obeys universal rules that are valid for a variety of nervous systems ranging from invertebrates to mammals. One further unifying principle already raised by Bailey and colleagues [12] concerns the issue that neuronal syntax is conserved regardless of the memory system under investigation. Even though the different reviews of this issue have not explicitly formulated this idea, there is compelling support that both implicit and explicit forms of memory share common molecular mechanisms. This suggests then that neuronal systems use a similar syntax to encode information, and this may be independent of both the content

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of the information and mechanisms of its retrieval. It also indicates that to decipher the molecular syntax of memory will help little in understanding the complexity of the cognitive processes of mental representations. Nevertheless, progress in understanding cellular and molecular syntax will spur further experimental work on neuronal semantics. Only if both processes are understood in great detail will we be able to answer the question, What do we expect to find in the molecular biology of learning?

Stages of memory

Independent of the form of memory and its neuronal semantics, it is intuitively clear that memories may last for different lengths of time. The concept of there being at least two types of memory, termed short-term and long-term memory, dates back to William James [13], who distinguished a rapidly formed short-term memory lasting some hours from a long-term form lasting for hours, weeks or even years. This concept has been further corroborated during the last 40 years or so, and the arrival of molecular and cellular methods has generated a wealth of data especially for the cellular mechanisms of short-term memory. However, it is noteworthy that the use of different animal models for behavioural examination has led to several modifications of this basic theme [14, 15]. This multi-author review mainly follows the basic distinction between short-term memory that lasts for minutes and long-term memory that lasts hours or more.

The situation became more complex in 1900 when Mueller and Pilzecker [16] introduced the concept of memory consolidation. According to this idea, recent memories are subject to stabilisation over time, during which they are susceptible to interference and erasure. Over time, however, memories are no longer susceptible to interference—they are permanently stored. For Cajal [17] permanent memory storage was achieved by rewiring neuronal connections, and for Hebb [7] it was manifested by alterations in properties of the electrical network and the synaptic connections between neurones. We shall only marginally touch on this issue since our particular interest here centres on rather early processes of memory formation and the biochemical molecules that participate in short-term consolidation (cohesion [18]) and not long-term consolidation lasting for days and weeks.

Given this vulnerability of recent memory, early cellular and molecular processes of memory formation are by no means static. It is the dynamic interactions of some, many or all molecules both activated and inactivated after the learning event which bring about the formation of long-term memory. Some of these molecules and their changes due to learning have been investigated during the last 15 years. We have chosen a selection that follows a logic sequence. This sequence starts off with second messenger-dependent cascades and enzymes, continues with activation of transcription factors, transcription and translation to generate new proteins, and culminates in changes in synapse morphology and anatomy. Finally, a synthesis summarises dysfunctions of these molecules in Alzheimer's disease (AD).

Molecular mechanisms of memory formation

Following activation of both ionotropic and metabotropic receptors, the initial cascade of intracellular events induced by learning may start with alterations in the so-called second messengers. Probably the best, known second messenger is cyclic adenosine monophosphate (cAMP) which is generated by the hydrolysis of ATP by the enzyme adenylate cyclase (AC). AC comes in various isoforms with circumscribed cellular localisation. It is modulated via G-proteins or calcium, and its role in mechanisms underlying memory formation is reviewed by Nicole Mons, Jean-Louis Guillou and Robert Jaffard. Not much is known about all nine subtypes, but recent work has centred on type 1 and type 8 AC because both are located in hippocampus and both are stimulated by calcium and calmodulin. Calcium can be provided by activation of *N*-methyl-D-aspartate receptors, which occur in abundance in hippocampal CA1/CA2 and the dentate gyrus. Thus, both AC sybtypes are indicated in long-term potentiation and also learning and memory formation, but data are still sparse and controversial. The concrete function of hippocampal ACs in memory formation still remains elusive. What seems clear, however, is that both subtypes of AC may act as coincidence detectors when two or more separated inputs activate the cell in conjunction. Thus, AC stimulation may considerably magnify specific, sometimes weak, inputs and interact with a complex cellular network.

An alternative path for magnification consists of the activation of enzymes in order to catalyse a chemical reaction of the substrate. Enzymes that have become particularly important in memory formation are kinases, which typically facilitate phosphorylation via adenosine trisphosphate. A long tradition of research exists on the role of protein kinase C in memory formation, which itself has been subject of a complete issue recently. Thus, the review of Jacques Micheau and Gernot Riedel concentrates on other protein kinases such as cAMP-dependent kinase, calcium/calmodulin-dependent kinase, protein tyrosine kinase and mitogen-activated kinase. The data reviewed suggest a sequential pattern of activation of the different kinases during

early post-training stages of memory processing. Their differences in cellular compartmentalisation are indicative of a complex network of interactions that bring about long-term memory. Intervention with one step in this sequence may cause an imbalance in the activation profiles and the complex cross-talk between molecules, thus leading to subsequent block of memory formation. Kinases participate in all early stages of memory formation or consolidation but appear to be devoid of function during recall.

Given the importance of kinases outlined so far, it seems logical to predict a similar relevance for phosphatases in processes underlying memory formation. However, a search of the literature for relevant material does not live up to this expectation. Compared with the 'kinase world', reports on phosphatases are rather sparse and very recent. The review by Riedel thus summarises the few reports published on Aplysia and rodents and distinguishes between pharmacological and genetic interventions. Phosphatases are modulated by several kinases, and blockade of kinase activity impairs memory formation. One may thus argue that blockade of phosphatases may increase kinase activity and thus enhance memory. Contrary to this simple hypothesis, both blockade and overexpression of phosphatases inhibit memory formation. Because memory deficits are not apparent before about 60 min post-training, phosphatases may play an important part in the transformation of short-term into long-term memory. It therefore does not come as a surprise that some phosphatases have been implicated in memory decrements during ageing and dementia.

In a next step, information encoded in changes in the activity of enzymes may also affect transcription factors—better, transcription activators—as a further step downstream on the way to genomic activation. cAMP response element binding (CREB) is such a transcription factor, and it is of particular interest because it enables cAMP- and calcium-dependent gene expression and is phosphorylated by protein kinase A and calcium/calmodulin-dependent kinase IV. Thus, the review by Raphael Lamprecht begins with a biochemical introduction of CREB as activating genomic activity, thereby acting against CREM, the cAMP response element modulator or repressor. Through a complex circuit involving association of many proteins, CREB facilitates activation of immediate early genes. CREB thus has a pivotal role in various biochemical cascades and is essential for the generation of many, though possibly not all long-term memories. Inhibition or genetic alteration of CREB in both vertebrates and invertebrates provides compelling evidence for its potential role in long-term, but not short-term, memory. These data are reviewed, and a striking similarity appears for CREB function in molluscs, fruit flies and mammals.

An alternative way of activating transcription factors is described by Wolfgang Tischmeyer and Rita Grimm. As they show, activation of immediate early genes (IEGs) has been reported for many training paradigms in snails, flies and rodents alike. Activation of IEGs produces proteins among which are the inducible transcription factors. However, studies are complex, and interpretations are not always straightforward. Activation of IEGs is very sensitive to many sensory stimuli and may not always be related to learning and memory formation. It appears, however, that when side effects have been eliminated, IEGs play a pivotal role in the transformation of short-term to long-term memory formation.

As a consequence of learning-induced stimulation of genes and transcription factors, alterations in messenger RNA (mRNA) activity and protein composition within the respective cells should occur. Such changes have been monitored using various techniques. These techniques as well as their applications in behavioural investigations on the production of new proteins are reviewed by Oliver Stork and Hans Welzl. Although increased translation may be a product of the previous activation of specific enzyme cascades, one alternative hypothesis is put forward by Stork and Welzl. It is possible that transcription is stimulated during learning and thus occurs in parallel with second messengers activation. The consequent interpretation of the data thus might be twofold. First, it is feasible to assume that newly generated protein products are directly altered by some enzymes that are active because of the experience. Second messenger-induced protein activity would then have a role in short-term memory formation, and an additional, possibly separate function during manifestation of long-term memory. Second, there might be no modification of newly produced proteins by experience dependently activated enzymes. Under such circumstances, one might expect enzyme activation to be part of short-term memory formation, whereas transcription and translation exclusively subserve long-term memory. However, this latter view seems rather unlikely given the behavioural evidence reviewed in previous articles.

So far, we have considered intracellular cascades to be activated after the initial stimulation through the neurotransmitters. It follows that enhanced or reduced nuclear activity resulting in alterations of the proteins expressed should eventually result in anatomical or morphological alterations detectable especially in brain centres where long-term storage could take place. Although the hippocampus is not recognised as such a structure, May-Britt Moser shows in her review that anatomical changes can be detected, and that they do take place there. Learning-induced increases in synapses have also been reported for other brain structures, but changes are not as massive as in the hippocampi of

seasonal food-storing birds, for example. Thus it is striking not only that food-storing birds have bigger hippocampi than their nonstoring relatives, but also hippocampi may grow or shrink during the season. It is feasible that storing food in as many as several thousand hatches requires more hippocampal volume for spatial learning. Alternatively, it could be argued that storage of new spatial information requires more hippocampal volume, and shrinking takes place when spatial information about hidden food is no longer essential for survival, that is in spring. A distinction cannot be made at present, but data provided by Moser herself in rats clearly suggest that higher spine densities (and possibly more cells) enable better learning, especially when task demands are high. This could be a preparatory response of the neuronal network so that an ever-increasing number of experiences could be stored. Still, it remains to be seen whether formation of new synapses is the physical substrate of the memory trace.

With this sequence we have started at, or at least close to, the cellular membrane, and by virtue of describing many intracellular molecules participating in memory formation, we have also ended at the cellular membrane. A synthesis now follows which brings together some, though not all, of the above-mentioned processes and describes how dysfunction of such processes might interfere with normal memory formation in humans. As an example, Eva von Linstow Roloff and Bettina Platt have chosen the model of Alzheimer's disease (AD). While describing the pathological alterations established to date, it emerges that most of the memory mechanisms described in this multi-author review are disturbed in AD. Clearly, this may be one of the reasons why therapeutic approaches have failed so far. But Roloff and Platt then develop a hypothesis as to what common denominator might underlie the dysfunction. At the molecular level, they have identified oxidative stress as a link between pathological alterations. It appears, then, that mitochondrial malfunctioning is the cause of the disease, and this has multiple consequences on the functioning of cAMP, kinases, phosphatases, transcription factors and immediate early genes, transcription and translation, and morphological degeneration.

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