# Memory in Mammals:
# An overview of cellular mechanism and brainstructures involved in memory formation

*W.C.E.P. Verberk*

*Supervisor: Prof. dr. E.W. Roubos*

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 Department of Cellular Animal Physiology
University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

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## PREFACE

This paper is part of my research at the department of Cellular Animal Physiology (February 1997 – November 1997). My reasons for doing research at this department were my interests in brain functions with the emphasis on how memory works. While my research focussed on the functional role of sauvagine induced cAMP egress, this paper focuses anew on my interests concerning memory. In addition, there is a link between this paper and the before mentioned research. cAMP is an ubiquitous second messenger and also plays an important role in cellular signalling processes involved in memory.

## SUMMARY

This paper focuses on memory processes in mammals on both the cellular level and on the level of different brainstructures. The goal is to provide an overview of these different processes in order to gain a global understanding of how memory works. Memory is represented in the brain as neural activity patterns and learning is the changing of these activity patterns. Changing these activity patterns must occur in relation to the new information to be learned. Since this new information is also represented as (temporary) activity patterns, changing must occur in an activity-dependent way. At the cellular level this is implemented by the coincidence detection rule formulated by Hebb: the synapse linking two cells is strengthened if the cells are active at the same time.

There are several cellular mechanisms which can result in changes in synaptic transmission. Long-term potentiation is the most prevalent cellular model for memory formation. This mechanism enables a synapse to become potentiated (thus increasing synaptic transmission) in an activity-dependent way. Another cellular mechanism is the outgrowth and restructuring of dendrites and synapses. These processes are related to cell adhesion and cytoskeletal stability. When cell adhesion or cytoskeletal stability decrease, synaptic and dendritic outgrowth is promoted. In addition there is evidence for so-called silent synapses. These synapses are inactive, but can be recruited, thus leading to changes in activity patterns.

Besides these cellular processes there are modulating effects. These provide inhibitory constraints which must be removed in order for memory formation to occur. These constraints provide checkpoints at the cellular level in order to make sure that only relevant information is stored. Besides the inhibitory constraints, there is another modulating mechanism. Oscillations arise when ensembles of neurones fire in a repeated and synchronous way. Oscillations enhance the plasticity of cells and they play a role in selectively switching brainstructures into a plastic mode.

These cellular mechanisms are operative in larger ensembles of neurones, the different brainstructures. Different brainstructures are each specialised for a particular function and operate as a unit in the formation of memory. The brain is also organised in distinct modules which each are specialised to store a specific type of information. A memory consists of different aspects and each aspect is stored in separate modules. This means there is no single memory centre where an entire memory is stored.

Memory formation occurs in distinct stages. At first, during short-term memory, new inputs are represented as activity patterns in specific cortical regions (e.g. visual cortex, auditory cortex). This information then moves on to the association cortex and the prefrontal cortex. In the prefrontal cortex the information is organised and provided with structure, possibly with participation of attentional processes. From there, long-term memory takes over and the information is temporarily stored in the hippocampus. During a process of consolidation, information transferred from the hippocampus to the cortex for permanent storage. During this stage there is a possible reanalysis and selection of information.

In addition to the brainstructures involved in short-term and long-term memory processes, there is another brainstructure; the amygdala. This structure is selectively involved with modulating memory. Memory for emotional arousing events is selectively enhanced through the actions of stress hormones. This is yet another mechanism involved with selection of information.

It is concluded that selection and specialisation are important processes in the formation of memory. It is an important evolutionary advantage to store only relevant information. The communication between the different brainstructures is complex, depending on different transmitters, nerve cell types and brainstructures. Since the different structures function as a unit in the formation of memory, there should be more integrative studies. Specialised studies are crucial as well, but integrating studies can evaluate the importance played by each part in relation to the other parts, which will be necessary in order to unravel the precise workings of memory, a hard, but challenging task.

## SAMENVATTING

Deze literatuur scriptie richt zich op geheugen processen in zoogdieren zowel op cellulair niveau als op het niveau van verschillende hersenstructuren. Het doel is om een overzicht te geven van verschillende geheugen processen om zo een beeld te krijgen van hoe het geheugen werkt. Het geheugen ligt vast in de hersenen en bestaat uit activiteits patronen in de neuronen. Leren kan worden gezien als een verandering in deze activiteits patronen. Het veranderen van deze activiteits patronen, moet gebeuren in relatie tot de nieuw te leren informatie. Aangezien invloeden van buitenaf ook in de hersenen leiden tot activiteits patronen moet verandering plaatsvinden in relatie tot deze activiteit. Op cellulair niveau komt dit tot uitdrukking in de regel van Hebb die stelt dat een synaps die twee neuronen verbindt alleen versterkt wordt wanneer beide neuronen tegelijkertijd actief zijn.

Er zijn verschillende cellulaire mechanismen die kunnen leiden tot veranderingen in signaaloverdracht tussen neuronen. Eén daarvan is lange-termijn potentiatie (LTP). Dit is een proces waardoor een synaps wordt versterkt. Dit proces verloopt op een activiteits-afhankelijke manier en het wordt gezien als het belangrijkste cellulaire model voor geheugen processen. Daarnaast bestaat er ook herstructurering en het uitgroeien van synapsen en dendrieten. Deze processen zijn afhankelijk van de stabiliteit van het cytoskelet alsmede van celadhesie. Wanneer de stevigheid van het cytoskelet of de celadhesie afneemt worden herstructurering en uitgroei van synapsen en dendrieten bevorderd.

Naast deze cellulaire mechanismen zijn er een aantal mechanismen die invloed hebben op deze cellulaire processen. Zo bestaan er een aantal beperkingen die opgeheven dienen te worden voordat informatie voor lange duur vastgelegd kan worden. Deze beperkingen bieden controle-punten zodat alleen relevante informatie wordt opgeslagen. Naast deze beperkingen bestaat het fenomeen oscillaties. Hierbij vuren een aantal neuronen herhaaldelijk op hetzelfde moment. Door deze oscillaties wordt de plasticiteit van de neuronen verhoogd zodat lange-termijn potentiatie sneller optreedt. Van oscillaties wordt gedacht dat ze ook betrokken zijn bij het aanschakelen van specifieke hersenstructuren waardoor deze ontvankelijker worden voor nieuwe informatie.

Bovenstaande processen en mechanismen spelen ook een rol op een hoger niveau; dat van de verschillende hersenstructuren. Er bestaan een aantal verschillende hersenstructuren die betrokken zijn bij het vormen van geheugen. Deze structuren hebben ieder hun eigen, gespecialiseerde functie, maar functioneren als eenheid in het intacte brein. Daarnaast zijn de hersenen opgebouwd uit modules. Dit zijn kleine afzonderlijke groepen van zenuwcellen die ieder een specifiek type van informatie verwerken en/of opslaan. Een gebeurtenis bestaat uit verschillende aspecten (geluid, zicht, reuk etc) en elk van deze aspecten wordt opgeslagen in een aantal verschillende en afzonderlijke modules. Hoewel modules betrokken bij één enkel aspect (zoals bijvoorbeeld zicht) wel bij elkaar liggen, bestaat er dus niet één geheugen centrum waar alle aspecten van een gebeurtenis worden opgeslagen.

De formatie van geheugen vind plaats in een aantal verschillende stadia. Eerst worden nieuwe inputs in de hersenen vertaald naar activiteits patronen in de verschillende delen van de cortex (visuele cortex, auditoire cortex, etc). Daarna word deze informatie geïnterpreteerd in de associatie cortex en in the prefrontale cortex. In de prefrontale cortex wordt de informatie gestructureerd en mogelijk spelen aandachtsprocessen hierbij een rol. Dit zijn allemaal aspecten met een korte tijdsduur en behoren tot het korte-termijn geheugen. Wanneer de informatie lang genoeg wordt onthouden of als er genoeg aandacht aan wordt besteed wordt de informatie tijdelijk opgeslagen in de hippocampus. Dit gebeurt tijdens het ingespannen waken en hiermee begint het lange-termijn geheugen. Gedurende het ontspannen

waken en tijdens het slapen (met uitzondering van de REM slaap) wordt de informatie in een meer permanente vorm opgeslagen doordat er dan informatie overdracht van de hippocampus naar de cortex plaatsvindt. Dit is het vastleggen van informatie en mogelijk vindt hierbij wederom een selectie plaats op relevante informatie.
Naast de structuren betrokken bij de processen van korte-termijn en lange-termijn geheugen is er nog een andere hersenstructuur; de amygdala. Deze structuur is betrokken bij het moduleren van het geheugen bij stressvolle situaties. Het geheugen wordt bevorderd onder invloed van stres hormonen zodat de omstandigheden tijdens een stressvolle situatie beter worden onthouden.

Uit deze literatuur studie blijkt dat selectie van relevante informatie en specialisatie van hersenstructuren en zenuwcellen belangrijk zijn voor processen die betrokken zijn bij de formatie van geheugen. Selectie van relevante informatie biedt een organisme een duidelijk evolutionair voordeel. Communicatie tussen de verschillende hersenstructuren is complex; er zijn verschillende type neuronen en transmitters. Aangezien de verschillende structuren goed met elkaar moeten communiceren om als eenheid te kunnen functioneren zijn integrerende studies vereist. Gespecialiseerde studies die zich richten op één structuur of één transmitter zijn weliswaar noodzakelijk om inzicht te krijgen, maar, om na te kunnen gaan hoe belangrijk de rol is van elk deel in het geheel, zijn integrerende studies noodzakelijk. Zo zal uiteindelijk het precieze functioneren van het geheugen kunnen worden ontsluierd. Dit is een zeer lastige, maar uitdagende taak.

# 1. INTRODUCTION

## 1.1. Goal of this paper

A vast amount of articles is published on the subject of the working of the brain. Clearly a paper like this cannot summarise the diversity and detail of all these articles. Therefore choices have to be made as to limit down the enormous amount of information. This paper deals with both cellular processes involved in memory formation as well as processes involved with memory formation on a higher level; that of the functioning and co-operation of different brainstructures. In order to further limit down the amount of information, the goal of this paper is not to fit every detail about memory processes, but rather to present a number of mechanisms and structures involved in memory formation in order to gain an global understanding. The focus of this paper is on mammals including man.

My interest in this subject is broad concerning the workings of the entire brain in memory processes, and by limiting this study even further down by focussing on only part of the brain and its functions more details can be discussed, but I feel that important links, necessary for understanding the whole picture of how memory works, would be left undiscussed.

The following questions are discussed in this paper:

- What is memory?
- What cellular mechanisms are involved in memory?
- How can these cellular mechanisms be modulated?
- Where is memory stored?
- How is memory stored
- Which braincentres and pathways are important in short-term memory?
- Which braincentres and pathways are important in long-term memory?
- Which braincentres and pathways are important in modulating memory processes?

## 1.2. Nerve cells & synapses

The site of memory storage is the nervous system. The nervous system has two classes of cells: nerve cells and glia cells (Kandel, 1991). There are between 10 to 50 times as many glia cells as there are nerve cells. Glia cells are probably not essential for processing information, but they are thought to serve several other roles. They serve as supporting elements. They serve to separate and insulate groups of neurones from each other. Some glia cells form myelin, the insulating sheath which covers most large axons. Some remove debris after injury or neuronal death. They buffer the $K^+$ ion concentration in the extracellular space and, finally, some take up and remove chemical transmitters (Kandel, 1991).

In contrast, the nerve cells are essential for processing information. Although there are different types of nerve cells, each cell has a cell body which gives rise to two types of processes called the dendrites and the axons (figure 1). Dendrites serve as the main apparatus for receiving the input to the neuron from other nerve cells. The axon is the main conducting unit of the neuron and can extend up to a meter; it is capable of conveying information great distances by propagating in an all-or-none way a transient electrical signal called the action potential. Near its end, the axon divides into fine branches that have specialised swellings called presynaptic terminals. By means of its terminals, one neuron transmits information about its own activity to the receptive surfaces (the dendrites and cell bodies) of other neurones. The point of contact is called a synapse, and the pre- and postsynaptic cell are separated from each other by the synaptic cleft. Transmission of information can occur both electrically via gap junctions and chemically via neurotransmitters (Kandel, 1991).

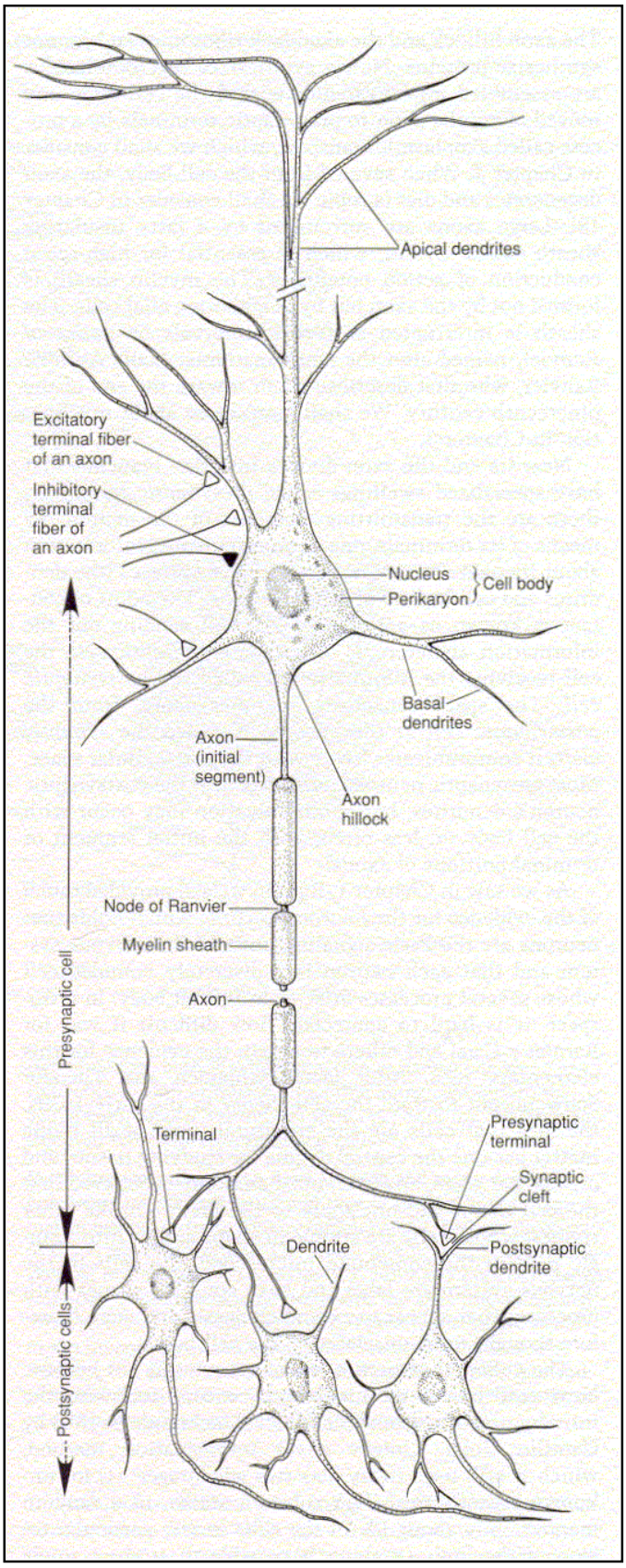


Figure 1. The anatomy of a neuron. Dendrites mediate input signals, while the axon mediates the output signal. Signal transfer occurs via synapses (from Kandel, 1991)

### 1.3. What is memory?

Learning is the process of acquiring knowledge. Memory is the retention of such knowledge. How does the brain store this knowledge? In short, the nerve cells are interconnected via synapses and thus form a neural network. In this network there are input neurones and output neurones and the input signal is processed in a specific way to produce an output signal. In this way, the network can encode information. Thus memory is represented as patterns in neural activity and learning is the modification of these activity patterns. Subsequently, forgetting can be represented as a loss of neural activity pattern.

Modification of the activity patterns occurs by changes in synaptic efficiency or by physical changes in the neuron such as the formation of new synapses and dendrites. These modifications cannot occur in random however, but must occur in relation to the new information to be stored. This new information (for example external events) are also represented in the brain as spatiotemporal patterns of neural activity, and these patterns themselves are the agents which induce the synaptic changes (figure 2). The location of information storage must therefore be found among those synapses which support activity-dependent changes in synaptic efficiency (Bliss & Collingridge, 1993). The idea of activity dependent change arose as

early as 1949 when Hebb proposed a coincidence-detection rule in which the synapse linking two cells is strengthened if the cells are active at the same time (Hebb, 1949). Thus when two cells are interconnected and active at the same time, their connection becomes strengthened. This enables specific ensembles of neurones, active at the time of the information representation to be co-activated again later on.

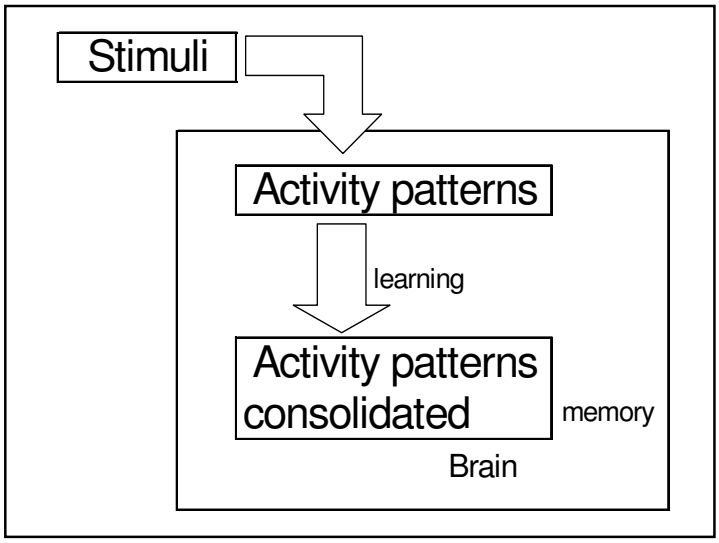


Figure 2. Stimuli are represented as activity patterns and these patterns cause an activity-dependent change in synaptic efficiency, called learning. Retention of these changes in activity pattern is called memory.

## 2. CELLULAR MECHANISMS INVOLVED IN MEMORY FORMATION

There are a number of mechanisms which can account for modification in neural patterns. These mechanisms are mostly important within their own specific time window.

### 2.1. Long-term potentiation

2.1.1. Properties

Long-term potentiation (LTP) is experimentally induced by brief trains of high-frequency stimulation which cause an abrupt and sustained increase in the efficiency of synaptic transmission (Bliss & Collingridge, 1993). LTP has three properties (Bliss & Collingridge, 1993): (1) co-operativity; the existence of an intensity threshold for induction of LTP. (2) associativity; for example, a weak input can be potentiated if it is active at the same time as a strong tetanus to a separate but convergent input. (3) input-specificity; other inputs inactive at the time of the tetanus do not share in the potentiation induced in the tetanised pathway.

The three properties can be explained on the assumption that a synapse will be potentiated only if the dendrite on which it terminates is sufficiently depolarised; i.e. activated. Evidence for this assumption was provided in experiments showing that low-frequency (1 Hz) low-intensity stimuli could produce robust LTP if repeatedly paired with depolarising pulses delivered through an intracellular recording electrode (Kelso *et al*., 1986).

LTP provides a cellular mechanism for synaptic change, which is important for learning and memory. Furthermore, LTP occurs in an activity dependent way and in accordance with the Hebbian coincidence-detection rule, since a synapse will be potentiated only if the activated presynaptic neuron terminates on an activated post-synaptic neuron. LTP has been reported in different brain structures (Hara & Kitajima, 1997), but is best studied in the hippocampus (e.g. Bliss & Collingridge, 1993; Frey & Morris, 1997; see section 4.1.3 & 4.3.). For these reasons it is understandable that LTP is the most prevalent cellular model for memory formation. The opposite of LTP, long-term depression (LTD) is thought to play a role in synaptic competition since it provides a mechanism for synaptic weakening (see Miller, 1996). This process also leads to changes in neural activity and both LTP and LTD may be important in learning.

2.1.2. N-methyl-D-aspartate receptor

LTP research has focussed on the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor (e.g. Tsien *et al*., 1996; Bliss & Collingridge, 1993). The reason for this focus on the NMDA receptor because this receptor behaves as a molecular coincidence detectors, which can implement the Hebb rule at the synaptic level. In order for the NMDA channel to open and trigger induction of LTP, both presynaptic activity (glutamate released by axonal terminals) and postsynaptic activity (depolarisation that releases the $Mg^{2+}$ block) are required (McBrain & Mayer, 1994). Depolarisation occurs through $Na^{+}$ influx via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors.

The properties of the NMDA channel can explain the properties of LTP: a sufficient strong stimulus has to be delivered in order to produce an adequate reduction of the $Mg^{2+}$ block, or the associative stimuli must be strong enough. In addition, input specificity is explained by the need for the presynaptic terminal to provide a sufficient concentration of L-glutamate to activate adequate numbers of NMDA receptor. Induction of LTP is prevented by a variety of NMDA antagonists (e.g. Coan *et al*., 1987; Bashir *et al*., 1990) showing NMDA receptor activation is an important trigger for the induction of LTP. However, NMDA

receptor activation is in itself usually not sufficient to induce LTP and other factors may be important.

2.1.3. Different forms of potentiation

There are many different forms of activity-dependent synaptic potentiation and different criteria exist to distinguish between the different forms, such as distinct temporal components, dependency on protein-kinases or potentiation can also be classified on the basis of whether or not its induction is blocked by antagonists of the NMDA receptor (Bliss & Collingridge, 1993). Short-term potentiation (STP) is protein kinase independent while LTP is dependent on protein kinase and can be dissociated in three phases (see Bliss & Collingridge, 1993). The first phase (LTP1 or early phase of LTP) lasts less than three hours and is independent of both transcription and translation. The second phase (LTP2) is dependent on translation, but independent on transcription. This involves translation of pre-existing mRNA. The third phase (LTP3) requires gene transcription. The second and third phase are also termed the late-phase of LTP (e.g. Nguyen *et al*., 1994; Frey *et al*., 1996).

In summary, a tetanus-induced potentiation proceeds in stages, beginning with a protein kinase-independent phase, STP, followed by 3 stages of LTP, requiring respectively, protein phosphorylation, protein synthesis from pre-existing mRNAs and gene transcription. The expression of synaptic potentiation probably involves both pre- and postsynaptic mechanisms, leading to an increase in transmitter release and increase in the number of receptor activated ion-channels or a change in property of receptor activated ion-channels.

2.1.4. Signal transduction pathway

The proposed signal transduction pathways are diverse and incomplete. However, some generalisations can be drawn. $Ca^{2+}$ influx is assumed to occur following the opening of NMDA channels and the rise in intracellular $Ca^{2+}$ is augmented by release from internal $Ca^{2+}$ stores (Bortolotto & Collingridge, 1993). A number of other receptors can play a role in the rise in intracellular $Ca^{2+}$. Activation of metabotropic glutamate receptors (mGluR) results in activation of 1,4,5-triphosphate (InsP3) which releases $Ca^{2+}$ from internal stores. In addition, voltage dependent $Ca^{2+}$ channels may also participate.

$Ca^{2+}$ can exert its effect on a number of proteins which are important during different stages in the formation of LTP (reviewed by Bliss & Collingridge, 1993). Phospholipid-dependent protein kinase (PKC) is an important kinase presumably involved in the transition from STP to LTP. Other kinases important for LTP are calmodulin and $Ca^{2+}$ / calmodulin-dependent protein kinase II (CaMKII). In addition, $Ca^{2+}$ can activate adenylyl cyclase, resulting in an increase in cAMP.

The rise in cAMP activates cAMP dependent protein kinase (PKA) which (possibly with CaMKII) activates cAMP-responsive element binding protein (CREB) and perhaps other constitutive transciption factors (Abraham *et al*., 1989). These transcription factors may initiate a gene transcription (Abraham *et al*., 1989; Qian *et al*., 1993). Because a similar molecular switch for late-LTP induction seems to be operative in invertebrates (Dash *et al*., 1990; Hawkins *et al*., 1993), this mechanism for gene induction may be quite general (Nguyen *et al*., 1994).

Thus the signal transduction pathway involves immediate effects of $Ca^{2+}$ leading to an increase in cAMP effects. cAMP in turn induces gene transcription. In addition, a retrograde messenger is suggested by a number of authors to ensure presynaptic changes leading to an increase in transmitter release (e.g. Bliss & Collingridge, 1993; Nguyen *et al*., 1994).

2.1.5. How is synapse specificity regulated?

An important aspect of long-term potentiation is synapse specificity. Frey and Morris (1997) show evidence for a synaptic tag which ensures input specificity. They stimulated two independent synaptic inputs (S1 and S2) to the same neuronal population in the CA1 region of hippocampal slices in vitro. S1 was tetanised and after 35 minutes the protein synthesis inhibitor anisomycin was added. 25 minutes later, S2 was tetanised which resulted in normal LTP (figure 3a). From these results they concluded that S2, when a tag was present, could use proteins synthesised as a result of LTP induction of S1. Also, when S1 was tetanised, a single tetanisation of S2 one hour later, normally inducing only early LTP, was shown to induce late LTP (figure 3b). Thus, only a single tetanisation is needed to induce the formation of the tag. They also showed that this tag decays in less than 3 hours. This tag has been speculated to be an phosphorylated kinase (Schuman, 1997).

The Hebbian hypothesis is thus extended: The time interval for pre- and postsynaptic co-activity to create the synaptic tag must be short (<300ms; Larson & Lynch, 1986). However, the persistence of LTP can be influenced hetero-synaptically by tetanisation of other synaptic affronts over a period of 2-3 hours. This shows that incoming signals at a given time may alter the effect of signals at a later time to different inputs of the same neuron, which would otherwise result in short-term changes in plasticity (early LTP). The prior and sequential activity state of hippocampal neurones is therefore also an important factor. This mechanism might explain why inconsequential events are typically remembered for much longer if they occur around the same time as well remembered events.

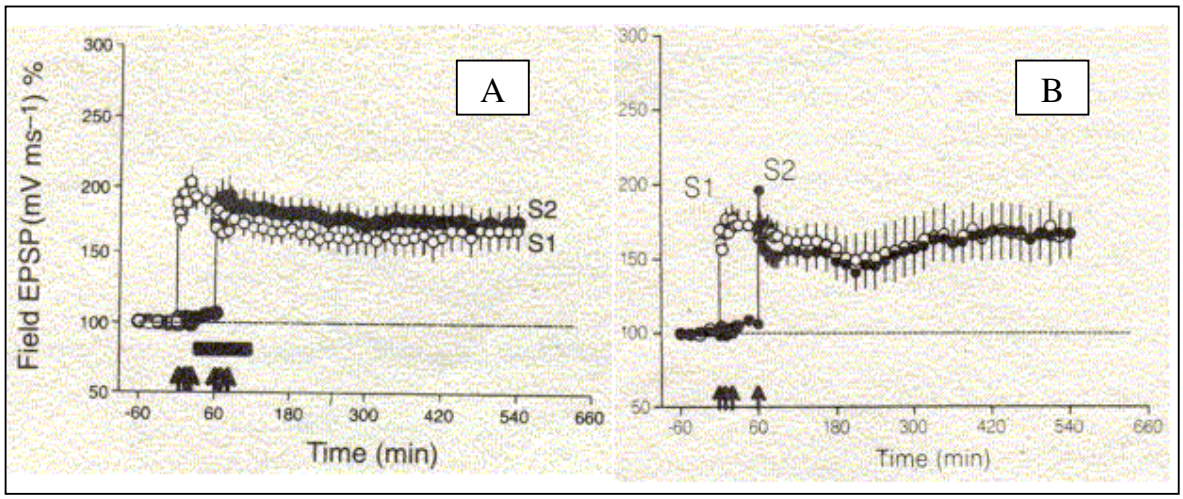


Figure 3. Induction of late LTP in the presence of a protein synthesis inhibitor (a) and induction of late LTP following a single tetanisation (b).

2.1.6. Critical notes concerning long-term potentiation

A role for LTP in memory is thoroughly studied and seems likely, but is there a causal relationship between LTP and memory? Evidence for a role of LTP in learning and memory has been reviewed by Shors and Matzel (1997). They point out that there is very little direct evidence coupling LTP to behavioural functions and wonder if LTP is both sufficient and necessary for memory formation and storage. They suggest that much of the present focus on LTP reflects a preconception that LTP is a learning mechanism, although empirical evidence does not support this. Furthermore, LTP cannot be a memory mechanisms since it is functional at the cellular level, while memory is functional at higher level, involving

numerous neurones. Also, they point to the eventual decay of LTP. They propose that LTP serves not as a memory storage device, but as a neural equivalent to an arousal attention device in the brain. LTP may facilitate the induction of memories in distant synapses by increasing discrete external stimuli.

In response, Abraham (1997) suggests that LTP might not always correlate with behaviour since, in addition to the learning as typically occurring in animal experiments, LTP could serve also for the storage during other kinds of cognitive activity and in multiple anatomical locations. Also learning itself is likely to involve distributed storage at many nodes in a network and therefore it should come as no surprise that LTP in a single synaptic pathway does not always correlate particularly well with learning or memory performance. Hawkins (1997) also points out that the lack of direct evidence between LTP and behaviour does not necessarily entail that the hypothesis is completely faulty. Rather, it should be made more sophisticated such as: There is a correspondence between a particular type and site of LTP and a particular type and aspect of learning. This can be explained for example by the fact that an animal might be able to learn the same task in different ways, so that one type of leering (and LTP) could compensate for the loss of another. Or conversely, two different types of learning might be required, so that behavioural performance could be poor even if one type of learning (and LTP) is normal.

Hara and Kitijama (1997) concede that LTP cannot be a memory mechanism, since it is a cellular and synaptic process. They suppose it should instead be viewed as a cellular storage mechanism, with many of such cellular storage mechanisms, producing the capability of memory formation. The integrative functions of the hippocampal systems include a capacity to store intermediate information. Active resetting of potentiated synapses may prevent saturation of LTP and makes the synapses more responsive than does passive decay (Linden, 1994). This could take place through LTD and the resetting of the synapse may be important for subsequent learning.

Although no direct causal link between LTP and memory and behaviour is present, compelling correlations have been found, including the results of Tsien and colleagues (1996). They created gene-knockout mice, with a deletion of the NMDA receptor, restricted to the CA1 region of the hippocampus. This method affected only one receptor and only in a restricted part of the brain to reduce non-specific effects. Still, both LTP and spatial memory are disrupted.

## 2.2. New outgrowth and restructuring of synapses and dendrites

In addition to changes in synaptic transmission, another mechanism can provide changes in neural activity patterns. This mechanism involves structural changes such as outgrowth and restructuring of synapses and dendrites and a large number of studies also indicate this mechanism is involved with learning (e.g. Abel *et al*., 1998; Woolf, 1998; Martin & Kandel, 1996). Through this outgrowth, additional connections can be made, resulting in additional information storage. Outgrowth and restructuring is thought to be related to cell adhesion and cytoskeletal stability.

### 2.2.1. Cell adhesion

Much evidence for a role of cell adhesion molecules comes from *Drosophila* and *Aplysia* studies. In the nerve muscle synapse of *Drosophila*, Fasciclin II (Fas II), a homologue of the vertebrate neural cell adhesion molecule (NCAM), is thought to be required for synapse remodelling and sprouting of additional synaptic contacts in activity-dependent processes, but

not in activity-independent processes (Schuster *et al.*, 1996a). In *Aplysia*, training decreases the expression of several isoforms of *Aplysia* cell adhesion molecules (apCAM) and internalisation of apCAM in sensory neurones. This causes defasciculation of the presynaptic preterminal processes and promotes the formation of new connections (Zhu *et al.*, 1994). The internalisation requires new protein syntheses and was mediated by elevations in intracellular cAMP (Bailey *et al.*, 1992). This may also be the case in *Drosophila*, explaining why only activity-dependent processes are affected by Fas II. In vertebrates, exogenously added fragments of NCAM were found to block LTP in rat hippocampal cultures (Lüthl *et al.*, 1994). In addition, both spatial learning and LTP were impaired in NCAM gene knockout mice (Fields & Itoh, 1996). These results suggest a regulatory function in synaptic remodelling for cell adhesion molecules in mammals as well (Fazeli *et al.*, 1990).

Fas II hypomorphs (expressing half the amount of Fas II) have a large increase in the number of synaptic boutons (Schuster *et al.*, 1996a). However, despite the increase in synaptic boutons in Fas II hypomorphs, synaptic efficiency is not increased (Schuster *et al.*, 1996b). In *Aplysia*, an increase in transmitter release from terminals of the sensory neurones is mediated in good part by cAMP and PKA (Abel *et al.*, 1998). Complementary genetic studies in *Drosophila* showed this signalling system was also present (Schuster *et al.*, 1996b). In odorant based learning tasks, expression of a CREB activator converts the need for spaced training to a single training trial (Yin *et al.*, 1995), indicating CREB activation is sufficient for conversion of short-term memory to long-term memory. These experiments show that cell adhesion molecules regulate the outgrowth of synapses but are in itself insufficient for an increase in synaptic efficiency. For an increase in synaptic efficiency, CREB activation is needed. CREB activation presumably leads to functional as well as structural changes (Abel *et al.*, 1998).

2.2.2. Cytoskeletal stability

Besides cell adhesion, cytoskeletal stability is also thought to be important in outgrowth and restructuring. Woolf (1998) focuses on the structural basis for memory storage in mammals in cholinoceptive cells. Activation of muscarinic receptors lead activation of phospholipases and protein-kinases. Protein kinases that are activated by the muscarinic G-protein complex include PKC, CaMKII and mitogen activated protein kinase (MAPK) and are known to phosphorylate microtubule associated protein 2 (MAP-2). Muscarinic receptor activation may also lead to MAP-2 dephosphorylation, since NMDA receptor activation is enhanced and MAP-2 is dephosphorylated through NMDA receptor activation. The role of phosphorylation and dephosphorylation in memory coding is not clear, but it is suggested that changes in the phosphorylation state of MAP-2 regulate dendritic plasticity. Phosphorylation of MAP-2 decreases its co-assembly with microtubules (e.g. Diez-Guerra & Avila, 1995; Ainsztein & Purich, 1994). Thus phosphorylation can reduce the cytoskeletal stability thereby favouring dendritic plasticity.

## 2.3. Silent synapses

Much evidence suggests the presence of silent synapses in many different systems and organisms during development and in the adult life (e.g. Jack *et al*., 1981; Charpier *et al*., 1995; Bolshakov *et al*., 1997). Postsynaptically silent glutamate synapses are found in the hippocampus (Liao *et al*., 1995; Tong *et al*., 1996). These are unable to detect the release of neurotransmitter. Presynaptically silent synapses were discovered in the spinal cord almost two decades ago (Jack *et al*., 1981) and some work suggests that they are present in the hippocampus (Tong *et al*., 1996).

The silent synapses are recruited by Sp-cAMP, a cell permeable analogs of cAMP (Ma *et al*., 1999). This recruitment of silent synapses is dependent on protein synthesis. Since the recruitment is completely prevented by the combination of NMDA and AMPA receptor antagonist this suggests that this recruitment also requires glutamate release from nerve terminals (though presumably not from the population of silent boutons) and calcium entry through NMDA receptors. It has been suggested that sp-cAMP could activate the silent synapse by activating the synaptic tag involved in maintaining input specific LTP (Bear, 1997). Awakening of silent synapses can be perceived as a decrease in the failure rate of transmission and in an increased number of quanta released per stimulus. Increased quanta release was indeed reported for hippocampal CA1 slices (Bolshakov *et al*., 1997) after recruitment of silent synapses.

There are several suggestions to explain the large numbers of silent synapses (Malgaroli, 1999). First, this might represent a safety factor; active connections might be too precious to afford losing them even for just the few minutes required to build new synapses. Second, synapses may alternate between silent and non-silent states without our noticing. Third, the brain synapses are not terminal compartments, but are very much interconnected. They are arranged ‘en passant’ at regular intervals (every 2-4 μm) along the same axon. *In vivo*, if we recruit more and more axons, we would trigger action potentials in all these postsynaptic neurones. Having silent synapses might be a way to tune down this flow of information with just a minority of all possible circuits left operative. Selectively recruiting silent synapses, could create a large number of possible alternative circuits in which to store information over longer times. Finally, in cases where pre- and postsynaptic activity are dissociated, it might be easier and less energetically costly to switch off the synapse rather than remove it altogether.

## 3. MODULATING EFFECTS

In addition to long-term potentiation and other cellular mechanisms, there are processes which regulate and modulate memory via the before mentioned cellular mechanisms. Of these processes, memory suppressor genes and oscillations will be discussed.

### 3.1. Memory suppressor genes

In addition to activation of positive regulatory mechanisms that favour memory storage, also the removal of inhibitory constrains preventing memory storage are required. These genes are termed suppressor genes in analogy with molecular studies of tumor formation (Abel *et al*., 1998). Most work comes from invertebrate studies with *Aplysia* and *Drosophila*, but similar mechanisms are thought to operate in mammals (Nguyen *et al*., 1994). If information is continually stored in existing synapses, retraining may be difficult. In this context, memory suppressor genes may decrease synaptic strength. Thus, limitations imposed by memory suppressor genes may provide an important condition for subsequent learning (Abel *et al*., 1998). Three inhibitory constraints on memory formation are discussed (figure 4).

*Aplysia* sensory neurones constitutively express ApCREB2, which is partially homologous to human CREB2 and can repress CREB1-mediated transcription. By injecting anti-ApCREB2, a single application of serotonin normally producing only short-term facilitation, was sufficient to produce long-term facilitation, lasting 24 hours. In Drosophila it was shown that expression of an inhibitory form of CREB blocks long-term memory (Yin *et al*., 1994) and over-expression of an activator of CREB increases long-term memory formation (Yin *et al.*, 1995).

A second class of memory suppressor genes are cell adhesion molecules. Down-regulation or endocytosis of these molecules decreases the interaction of neurites with each other and leads to the formation of new synaptic connections (Zhu *et al*., 1994). Phosphorylation of apCAM by mitogen-activated protein kinase (MAPK) promotes endocytosis (Bailey *et al*., 1997). Cell adhesion molecules have been discussed in section 2.2.

A third inhibitory constraint is relieved by the ubiquitin (Hegde *et al*., 1993), a proteasome induced rapidly after serotonin treatment (Hegde *et al*., 1997). Ubiquitin degrades the regulatory subunit of PKA (Greenberg *et al*., 1987), resulting in a decreased ratio of regulatory to catalytic subunits. Such a ratio decrease would result in a more responsive kinase and thus result in elevated kinase activity after cAMP has returned to basal concentrations. This process is suggested to bridge the transition from short- to long-term facilitation (Greenberg *et al*., 1987). It has also been suggested that increases in protease activity affect cleavage of proteins with extracellular domains, such as NCAMs (Fazeli *et al*., 1990) thus influencing memory on multiple levels.

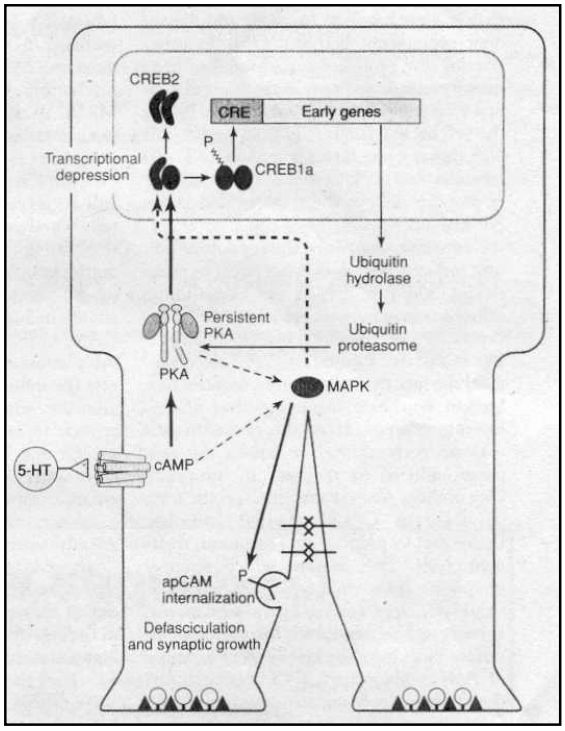


Figure 4. Overview of three inhibitory constrains on memory formation. Binding of serotonin (5-HT) leads to increase in cAMP levels. cAMP activates PKA which in turn activates CREB1. In addition to CREB-mediated transcription, turning on the long-term processes requires the removal of three inhibitory constrains. The first inhibitory constraint is provided by CREB2 which depresses transcription. Ubiquitin removes the second inhibitory constrain: the regulatory subunit of PKA. Ubiquitin is produced after gene transcription and RNA translation. The third inhibitory constraint is removed by MAPK, which is also activated by serotonin, then translocates to the nucleus, where it may phophorylate the transcriptional repressor CREB2. In addition, MAPK phosphorylates apCAM, which triggers the internalisation of apCAM. This allows fasciculation and synaptic growth to occur (from Abel *et al*., 1998).

### 3.2. Oscillations

Not only spatial activity patterns, but also temporal activity patterns, such as oscillations are important in modulating neural activity patterns. Coherent oscillations are ensembles of neurones firing in a repeated and synchronous manner. Synchronisation of neuronal activity is fundamental in the operation of cortical networks (Singer, 1993) and different frequencies of cortical oscillatory activity at the electroencephalogram (EEG) level have been correlated to distinct patterns of behaviour (Draguhn *et al*., 1998; Cobb *et al*., 1995). Of these different frequencies, there has been a focus on theta oscillations (4-7 Hz) since this frequency occurs in the hippocampal EEG during exploratory behaviour (Cobb *et al*., 1995; Vanderwolf, 1969) and during periods of learning (Huerta & Lisman, 1993; Winson, 1978). In addition, temporal patterns have been found to code olfactory information in the bee (Borst, 1999) suggesting a more universal role for temporal activity patterns.

How do these oscillations arise? There are several studies indicating a role for both acetylcholine and γ-aminobutyric acid (GABA) in the formation of oscillations. Brain cholinergic neurones are critical for memory function (Fibiger, 1991; Aigner & Mishkin, 1986) and one role of cholinergic neurones is to elicit an oscillatory activity called theta rhythm (Bland, 1986) in the hippocampus. Cholinergic neurones can possibly achieve this through the property of spontaneous firing (Khateb *et al*., 1992). In an experiment by Huerta and Lisman (1993) rat hippocampal CA1 (a specific area of the hippocampus) neurones were stimulated at 0.1 Hz with single shocks. When a cholinergic agonists carbachol (CCh) was added, theta oscillations were induced in most cases and CCh increased excitatory postsynaptic potential (e.p.s.p. – a measure of synaptic efficiency) substantially. This enhancement required presynaptic stimulation and was synapse specific. When CCh induced theta oscillations, the 0.1 Hz stimulation induced a long-term enhancement that would

normally cause no modification in synaptic efficacy. When CCh induced no oscillation or only small oscillations the enhancement was negligible. This suggests that the enhancement produced by CCh is not purely a biochemical process, but depends on the induction of an electrical oscillation.

GABAergic interneurones have been shown to effectively phase spontaneous firing and subthreshold oscillations in hippocampal pyramidal cells at theta-frequencies (Cobb *et al*., 1995). This GABAergic mechanism is sufficient to synchronise the firing of pyramidal cells by 'resetting' (figure 5) the regular firing in pyramidal cells. Thus cholinergic neurones induce oscillations and the phasing of firing may be achieved by activating GABAergic interneurones.

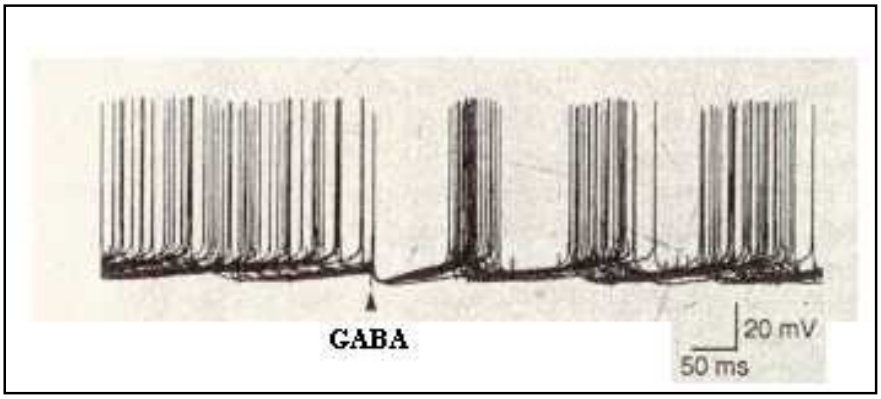


Figure 5. 'Resetting' of firing of hippocampal pyramidal cells by an application of GABA (from Cobbs et al., 1995).

There are several possibilities as to how these oscillations may be important in regulating functional processes in the brain. First, by synchronising presynaptic excitatory neurones, the co-operative action of these afferents might result in more efficient postsynaptic depolarisation, and thus contribute to changes in synaptic efficiency (Paulsen & Moser, 1998). Second, synchronised postsynaptic activity might serve as a temporal reference for the encoding of specific associations: for example the activity of a specific cell changes its phase in relation to the extracellular theta activity as a rat traverses the memory field of that cell (O'Keefe & Recce, 1993). Third, by phasing both presynaptic and postsynaptic principal cells, the temporal coincidence requirement for synaptic change may be set (Paulsen & Moser, 1998).

A connection between oscillations and the temporal coincidence requirement is also demonstrated by Huerta and Lisman (1993). When one input was stimulated by single pulses given at positive peaks of the wave ('in-phase'), while another pathway was stimulated at the negative valleys ('out of phase'), it was shown that in-phase stimulation greatly enhanced the e.s.p.s., even more than the enhancement produced by stimulation not synchronised with respect to the theta oscillations. In contrast, out-of-phase produced no enhancement. This strongly suggests that the oscillations are responsible for the heightened plasticity and an important role of cholinergic modulation may be to put the hippocampal networks into a highly plastic oscillatory state that is specialised for learning.

### 3.3. Overview of mechanisms in three components

The outlines of several cellular mechanisms involved in memory formation and agents modulating these mechanisms have been sketched. However, these are not the only mechanisms involved in memory formation. Receptor activation, neurotrophin effects, increase in transmitter release rearrangement of axonal release site are some mechanisms which are also believed to play a role in the transition from short-term memory processes to long-term processes (see Woolf, 1998). Here, the switch from short-to long-term plasticity is divided into three components (Abel *et al*., 1998) and each of these cellular mechanisms has a specific time window in which they are of importance.

(1) Initiation. Memory formation begins with in increase in cellular activity. In this specific time window, early LTP, immediate $Ca^{2+}$ actions, removal of CREB2 and activation of CREB1 are important. (2) Consolidation. Subsequently, there is a maintenance of this cellular activity. During this component late LTP, and the induction of immediate response genes such as ubiquitin hydrolase are important. (3) Stabilisation. During the final stage, cellular activity can decrease, because in this stage there is an outgrowth of new connections, dividing the activity over multiple connections.

Cascades of gene expression are turned on and off during memory consolidation and memory suppressor genes may provide a checkpoint for memory storage to ensure that only salient features are learned. This checkpoint is especially important because one of the hallmarks of human cognition is the ability to remember a signal from a noisy background by attending to and remembering only the most critical details. It is an evolutionary advantage for individuals to learn only facts that are important for survival, rather than storing in long-term memory everything that is encountered (Abel *et al*., 1998).

## 4. BRAIN STRUCTURE, CONNECTIONS AND FUNCTIONING

Of course, memory involves not only the important issue of how synaptic change occurs, but also questions the organisation and workings of the brain from the perspective of groups of neurones and brain structures. How is memory organised and where is it stored?

### 4.1. Structure of the mammalian brain and location of memory

4.1.1. Cortex is organisation in modules

Early studies have suggested that vertical columns of cells arranged perpendicularly to the cortical surface are the basic mode of organisation of the cortex (Mountcastle, 1979). This idea has been refined and has shown that the brain can be subdivided in distinct functionally homogenous assemblies of neurones, called modules, although in practice it is hard to determine where one module ends and another begins (for discussion, see Squire, 1987). The different hippocampal sectors amygdalar nuclei resemble cortical modules (Woolf, 1998). The plausibility of this type of organisation is supported by computer simulations. It is shown that a modular neural networks perform better than a non-modular neural network with the same amount of 'neurones' (Boers & Kuiper, 1992). The processing function of these cortical modules is supposed to be qualitatively similar in all cortical regions and each module is related to specialised cortical functions (Mountcastle, 1979). Bigl and colleagues (1982) showed that individual cholinergic neurones innervate cortical modules measuring approximately 1-2 $mm^2$.

4.1.2. Different aspects of a memory are stored separately

Memory is stored as changes in the same neural systems that ordinarily participate in perception, analysis, and processing of the information to be learned (Squire, 1986). Therefore, no single memory centre exists where an entire memory is stored. Instead, different aspects of an event are stored in separate modules. The information is stored in these modules. Thus memory is localised in the sense that particular brain systems represent specific aspects of each event, and it is distributed in the sense that many neural systems participate in representing a whole event. On each occasion that recall or recognition occurs, information is expressed by the activity of the same modules, involved in the storage of the information (Squire, 1986; figure 6).

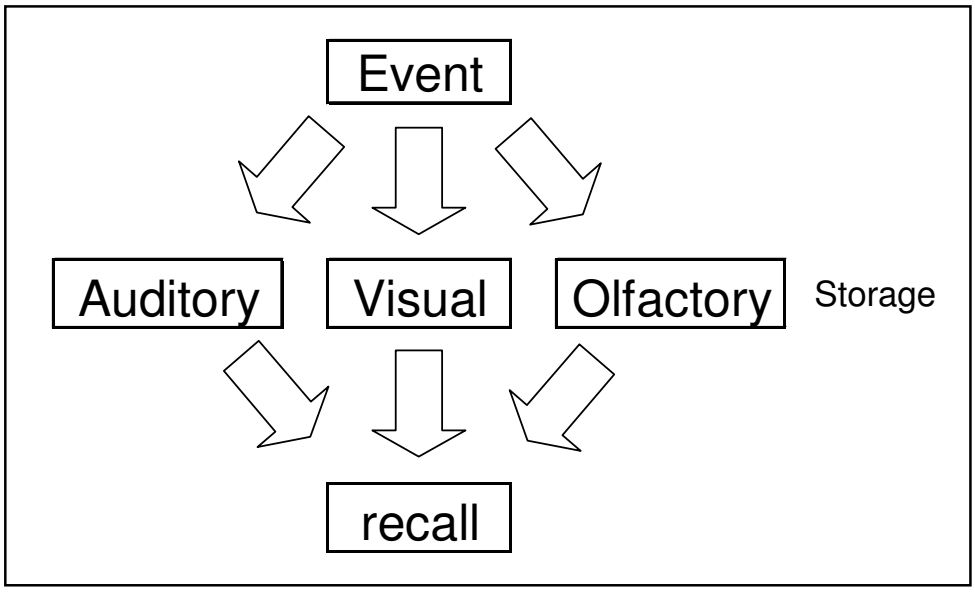


Figure 6. Schematic representation of distributed memory storage

**4.1.3. Location of some brainstructures important in memory processes.**

For a correct functioning of the brain all brainstructures are of importance. However, with respect to memory formation and storage some structures are more important. The anatomy of these structures is shown in the human brain (figure 7). One of these structures is the hippocampus (e.g. Bliss & Collingridge, 1993; Tsien *et al*., 1996; Squire, 1986). The hippocampus is a paired structure situated in the temporal lobe below the corpus callosum. Another structure involved in memory storage is the amygdala (Cahill & McGaugh, 1998). This structure is situated at the end of the tail of the caudate nucleus and is also paired. The amygdala is situated in the temporal lobe before the hippocampus. The thalamus consists mainly of relaying information (Kelly, 1991) and is situated below the lateral ventricles, on each side of the third ventricle. Finally, the neocortex is important in memory. This is the part of the cortex that is visible on the external surface of the cortex (Kelly, 1991) and serves as information storage sites (e.g. Hasselmo, 1999; Squire, 1987) and other memory processes in for example the prefrontal neocortex (Goldman-Rakic, 1995) and association neocortex (Hasselmo, 1999).

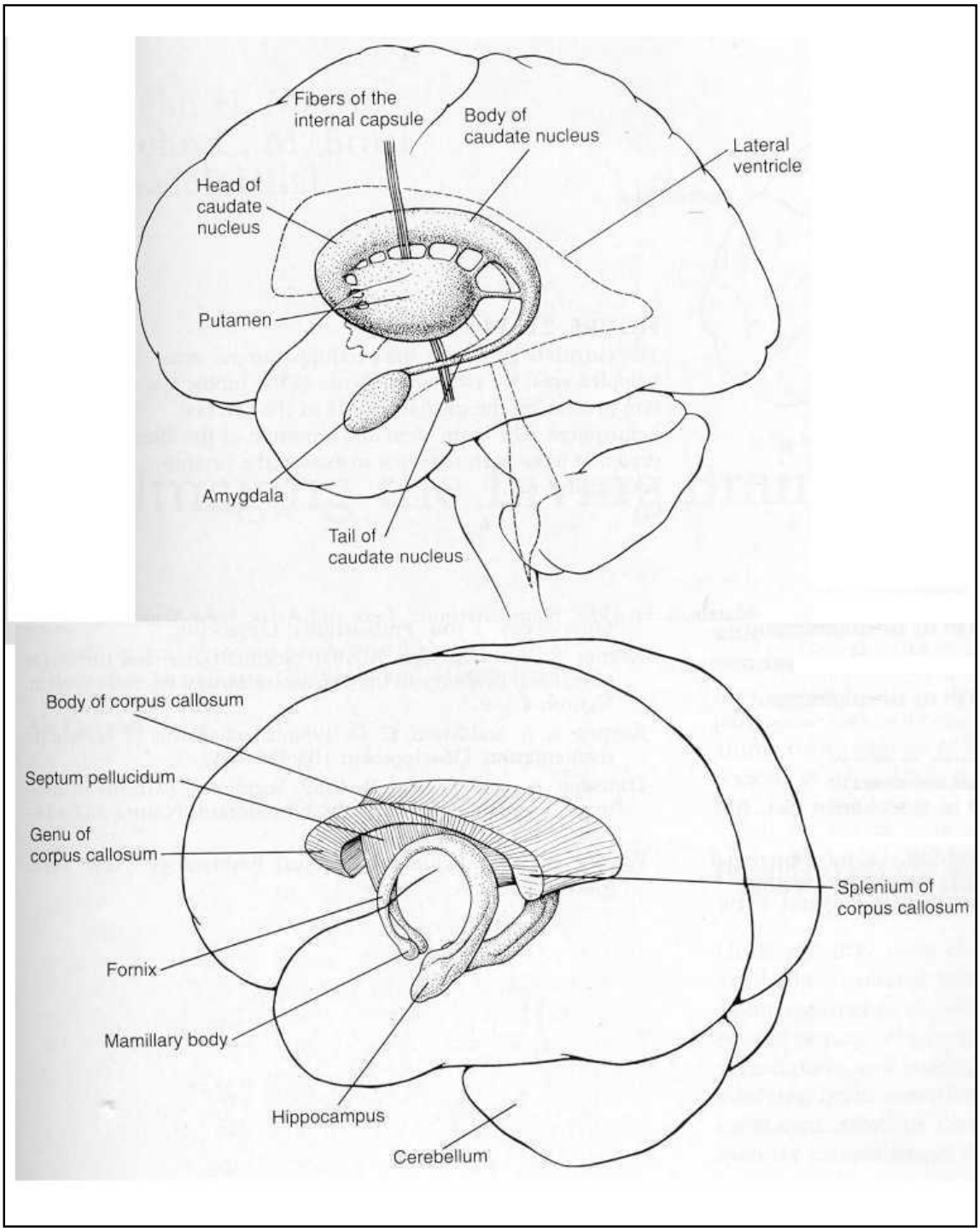


Figure 7a. Location of the amygdala and the hippocampus in a 3-dimensional image of the human brain (see text for explanation).

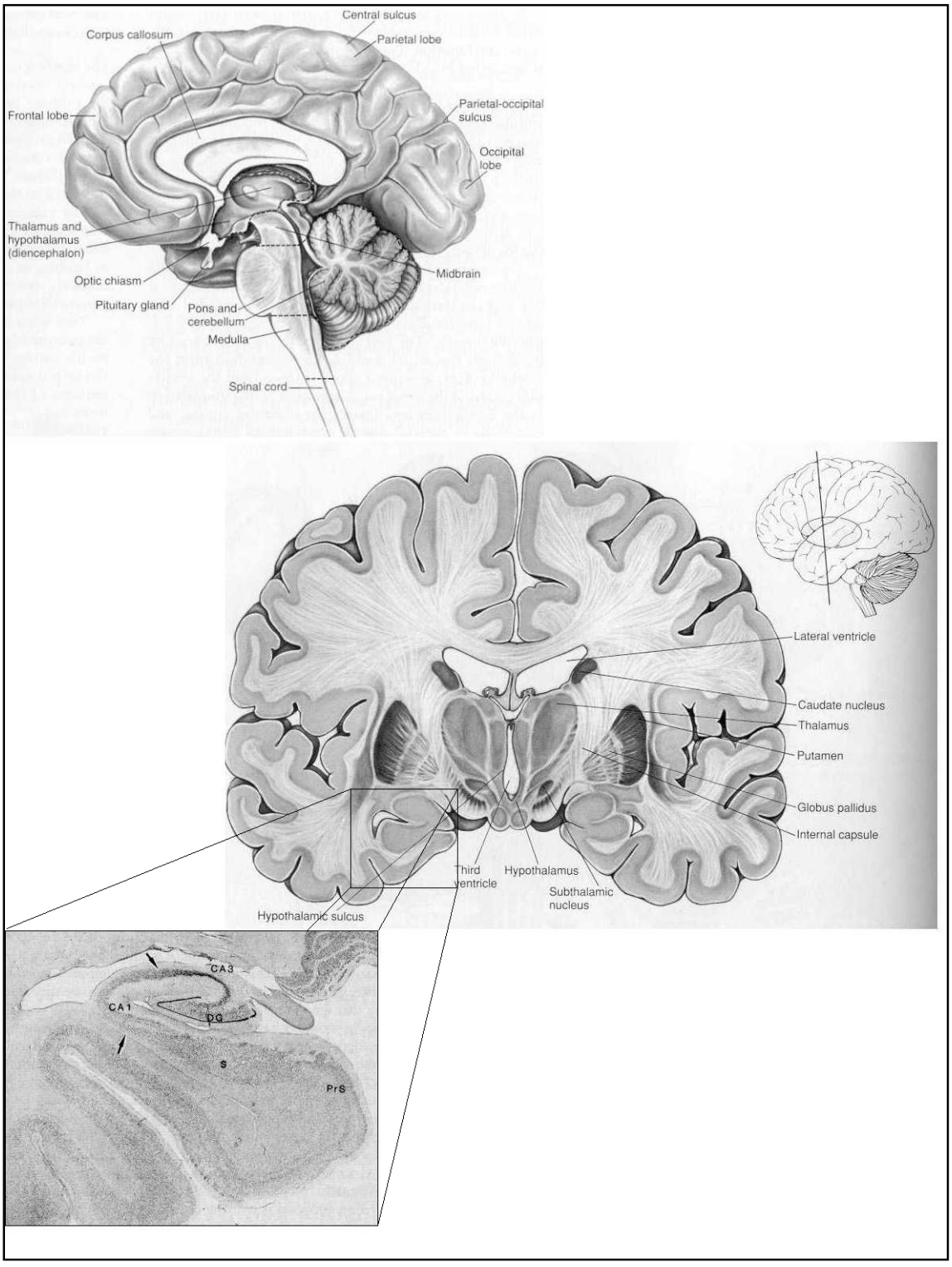


Figure 7b. Location of the cortex, thalamus and the different regions of the hippocampus amygdala in a midsagittal and a coronal section of the human brain (see text for explanation).

## 4.2. Short-term memory

The mammalian memory has complex workings and evidence for a subdivision of memory into short-term memory (STM) and long-term memory (LTM) comes from different approaches: cognitive psychology, neuropsychology and neurobiology (Baddeley, 1992; Squire, 1987; Squire, 1986; Squire & Cohen, 1984; Tulving, 1972).

### 4.2.1. Properties

STM or working memory is generally viewed as memory with a short time span ranging from seconds to minutes (Goldman-Rakic, 1995). It has a low storage capacity (Squire, 1987; Kupfermann, 1991) and requires no protein synthesis (Schwarts *et al*., 1971). In this regard, STM resembles STP very much, and these two terms are used synonymous. However, STP is a synaptic mechanism, which can bring about STM, but STM is much broader and reflects actual information storage over a short time, while STP involves only synaptic potentiation. The same goes for LTP and LTM. Another property of STM is that it is independent of the hippocampal damage (Squire, 1987 chapter 10).

### 4.2.2. Location of STM

Where is STM located? Monsell (1984) states capacities for temporal storage are distributed over diverse cognitive subsystems and should be seen as an intrinsic property of each processing module. Recent studies also state that critical substrates for STM are distributed in a modality specific fashion throughout the cortex (Goldman-Rakic, 1988; Wilson *et al*., 1993b). Since each part of the cortex has its own specialised form of information processing, STM is an umbrella for a heterogeneous array of short-term capacities, such as the short-term memory of numerical data or textual data. Temporary information storage may thus occur within each brain area where stable changes in synaptic efficacy (long-term memory) can eventually develop.

However, the neural substrates of specific short-term memory functions have more and more become identified with the prefrontal cortex (Fuster, 1989; Goldman-Rackic, 1987). Prefrontal lesions do not produce a straightforward, general memory impairment, but they can influence performance in memory tasks that go beyond the simple requirement to recall or recognise isolated events (see Squire, 1987). Lesions result in impairment when the order of contextually similar events must be remembered.

It is also suggested that it the prefrontal cortex is important in selective attentional processes (see Wenk, 1997) and this attention is mediated by cholinergic basal forebrain neurones (see Dunnet *et al*., 1991; Voytko *et al*., 1994). Cowan (1995) proposed an integrated framework for attention and memory, with selective attention as a component of STM. In support of the role of cholinergic neurones in attention and STM, acetylcholine antagonists have been reported to disrupt STM, but not LTM (see Woolf, 1998).

Prefrontal cortex presumably performs its computations on many kinds of information, which are analysed concurrently for other purposes by other regions of cortex. The prefrontal cortex can indeed be functionally subdivided: It has recently been shown that the prefrontal neurones that code visuospatial memoranda are located separately from those that code simple, complex, or categorical features of stimuli (Wilson *et al*., 1993a). Also, neurones coding for target location rarely if ever code for object qualities and vice versa. These memory centres are connected to the appropriate visual centres via relays in the parietal and temporal lobes (see Goldman-Rakic, 1995). Also, in rats, monkeys and humans it has been demonstrated that both prefrontal areas and hippocampal formations are activated during performance of STM tasks. The prefrontal cortex has strong anatomical connections with the

hippocampus, which itself is interconnected with frontal cortex as well as with other cortical association areas (Van Hoesen *et al*., 1972). This strongly suggesting a reentrant network organisation enabling the prefrontal cortex and hippocampal formation to operate with other cortical and subcortical structures as an integrated unit (for further discussion see Goldman-Rakic & Friedman, 1991). Thus, the prefrontal cortex allows information to be remembered in its appropriate context, that is, in the correct temporal order and with accurate reference to other spatially and temporally coincident events. Subsequently, the hippocampus operates upon this information, allowing it to endure in the organised form it has achieved in prefrontal cortex.

4.2.3. Two aspects of STM

From the above results two aspects of STM can be distinguished (figure 8). In the intact brain the different brainstructures are very much interconnected and therefore these different aspects should not be seen as strict and separate functions. The first aspect of STM is that it involved with recall and recognition of previously learned information. This process seems to be operational in patients with prefrontal lesions as well (see Squire, 1987 chapter 14) and may therefore be an intrinsic capacity of the cortical storage modules.

The second aspect of STM is that it provides structure and organisation to newly presented information. This process is probably mediated by the prefrontal cortex. The prefrontal cortex has also been proposed to function in the gating of information (Javitt *et al*., 1996). This gating, structuring and organising of information may in part be mediated by attentional processes, since when paid attention, the temporal order of new stimuli can be better remembered. The selected, structured and organised information may then move on to the hippocampus to allow it to be stored in the organised form in long-term storage. Indeed, it has been suggested that information that is retained by monkeys for more than tens of seconds enters intermediate or long-term memory stores and likely depends on mechanisms beyond STM, possibly involving long-term potentiation in the hippocampal formation (Goldman-Rakic, 1995). In addition, attentional processes may make it possible to keep memory stored in short-term memory for longer period by focussing attention and repeating it, for example to remember a telephone number until it is dialled.

If these views are to be reconciled, STM can be viewed as information that is on-line, whether it is recall of previously stored information or organising of new information. In this context, the contents of STM are as much on the output side of long-term storage sites as they are an important source of input to those sites (Goldman-Rakic, 1995).

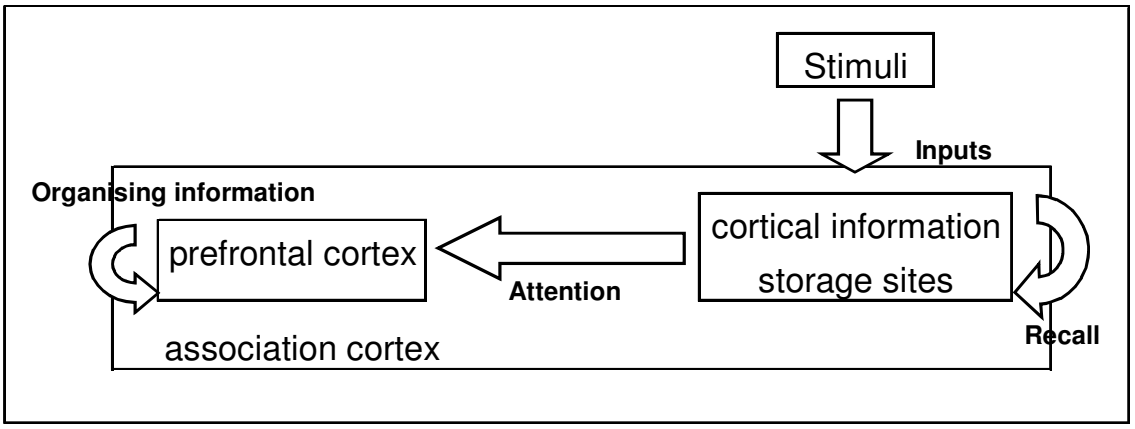


Figure 8. Schematic model for the interaction between different brainstructures and different processes in STM. Incoming stimuli are being processed and stored in the same place, termed the cortical information storage sites. One aspect of STM is the recall and recognition of previously learned information, stored in the cortical information storage sites. This is believed to be an intrinsic capacity of the cortex (see text for discussion). Another aspect of STM is the structuring and organising of incoming new information. This aspect is thought to occur in the prefrontal cortex and is possibly connected with attentional processes (see text).

4.2.4. Mechanisms of STM

Cortical information processing is dependent on the interplay between excitatory, glutamergic and inhibitory GABAergic neurotransmission (Javitt *et al*., 1996). In addition, acetylcholine also plays an important role as indicated by the disruption of STM through acetylcholine antagonists (see Woolf, 1998). How can these neurotransmitters exert an effect to produce STM? Although the specific actions of each transmitter are not discussed here, it has been shown that prefrontal neurones have memory fields.

The memory fields can be defined as maximal firing during the delay period of a neuron to the representation of a target in one or a few locations of the visual field with the same neuron always coding for the same location (Funahashi *et al*., 1989). Memory fields were found in delayed response tests in which spatial locations had to be stored in the STM for a number of seconds. In these tests, the subject is shown the location of a food morsel that is then hidden from view by an opaque screen. Following a delay period of several seconds, the subject chooses the correct location out of two or more choices. Thus the subject has to remember where the bait had been placed a few seconds earlier, and the correct response is guided by a representation of the prior stimulus rather than the stimulus itself.

Also prefrontal neurones have opponent memory fields; i.e. their rate of firing in the delay period is enhanced for one target location and inhibited on trails with target stimuli of opponent polarity (Goldman-Rakic, 1995; figure 9). The discovery of memory fields provides a valuable clue to how the neural circuitry subserving STM might be organised.

How do these memory fields arise? Recent studies (see Goldman-Rakic, 1995; Wilson *et al*., 1994) showed that the patterns of activity expressed by closely adjacent pyramidal and nonpyramidal neurones are often inverse, such that, as a nonpyramidal neuron increases its rate of discharge, a nearby pyramidal neuron decreases its rate (figure 9), suggesting that feed-forward inhibition may play a role in the construction of a memory field in prefrontal neurones (Goldman-Rakic, 1995). The inhibitory transmitter GABA is indeed crucial for the stimulus selectivity of memory fields in visual and somatosensory cortices. Infusions of

bicuculine, an antagonist of GABA receptors, transform the stimulus selectivity into non-specific responsiveness, suggesting that GABAergic inhibitory interneurones shape receptive fields (Wilson *et al*., 1994).

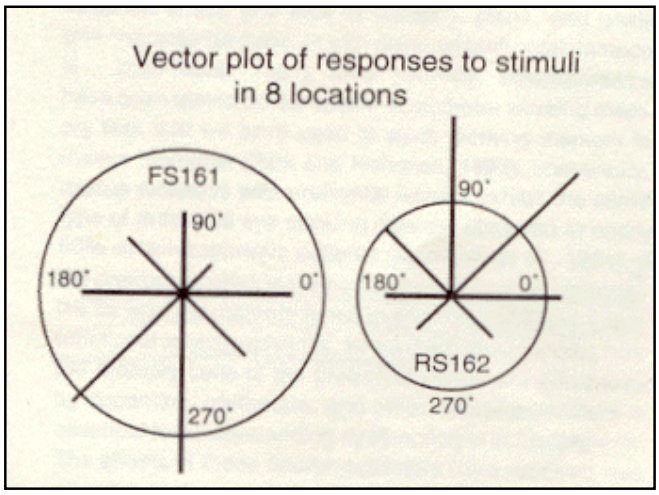


Figure 9. Memory fields and opponent memory fields of two adjacent neurones. The first neuron will have a maximal firing rate when stimuli will be presented at 270°, and the minimal firing rate is at about the opposite direction. The second neuron is adjacent to the first neuron and is inverse, having a maximal firing rate when stimuli are presented at 90° (from Wilson *et al.*, 1994).

Recent evidence suggest that NMDA receptors which have been predominantly studied in the hippocampus in relation to LTP, may also be important in echoic memory (a form of auditory memory with a very short time span) in the auditory cortex (Javitt *et al*., 1996). It was shown that NMDA antagonists did not significantly alter the intracortical response profile to standard stimuli, but did significantly decrease the mismatch negativity amplitude, an index of generation of auditory sensory memory. The unique molecular switch properties of NMDA receptors are suggested to make differentiation between standard stimuli and deviant auditory stimuli possible.

## 4.3. Long-term memory

4.3.1. Properties

LTM has a long time span lasting more than 1 hour and requires protein synthesis (Squire & Schlapfer, 1981). LTM can be further distinguished into information based on skills or procedures (procedural memory) and information based on specific facts and data (declarative memory) (Squire, 1986). Declarative memory is explicit and accessible to conscious awareness, and it includes the facts, episodes, lists, and routes of everyday life. Procedural memory is implicit, accessible only through performance, by engaging in the skills or operations in which the knowledge is embedded. In this paper the focus will be on declarative memory.

In contrast to STM, LTM does depend on the intactness of the hippocampus. The hippocampus consists of different regions (Figure 10). Damage to the hippocampus results in amnesia. Lesions of the hippocampal formation, as well as head trauma, electroconvulsive shocks and electroconvulsive therapy result in both impairment of storage of new declarative information (anterograde amnesia) as well as recall of previously stored information (retrograde amnesia). The retrograde amnesia is temporally graded: recent memories are more disrupted than distant memories (see Squire, 1987 chapter 13). This led to the suggestion of consolidation. This is the process by which memory gradually becomes resistant to disruption by an amnesiac agent. Several researchers have suggested that these properties of declarative memory might be the result of a two-stage process of long-term memory formation (e.g. Hasselmo, 1999; Buzsaki, 1989). However, it is important to realise that memory consists of a series of interdependent but potentially dissociable memory processes: encoding, storage, consolidation and retrieval (Riedel *et al*., 1999).

4.3.2. Two-stage model of memory storage

The role of the hippocampus during consolidation is not entirely clear. Different views exist. Extensive lesions, including the entorhinal cortex (EC) of the hippocampus cause long-term retrograde amnesia (also affecting distant memories), whereas limited lesion causes retrograde amnesia of short-term duration (affecting recent memories only) (Rempel-Clower, 1996). This led to the suggestion that consolidation does not necessarily result in formation of links in association neocortex, but could instead result in strengthening of representations within the hippocampus itself or within the EC (Hasselmo, 1999; Nadel & Moscovitch, 1997). However, since removal of the hippocampus does not effect the very distant memories, the hippocampus cannot serve as a final storage site and the deficit cannot be a general impairment in retrieval (Squire, 1986)

Alternatively, the hippocampus could serve as in initial storage site and during the consolidation phase the memory is slowly transferred to the cortex (Paulsen & Moser, 1998; Skaggs & McNaughton, 1996) making it more and more resistant to amnesiac agents. This can also explain the observation of Rempel-Clower: distant memories are best consolidated and (almost) wholly dependent on the cortex. Thus only extensive lesions in the hippocampus can result in disruption. Recent memories are more dependent on the hippocampus and limited lesions can already result in disruption. Also, this can explain the fact that retrograde amnesia is temporally graded. Anatomical studies confirm the existence of projections between the hippocampus and the association cortices exist (Van Hoesen, 1982), which are necessary for information transfer from hippocampus to the cortex. In addition, it has been recognised that the hippocampal system is able to change quickly, while the neocortical synapses change slowly (Hara & Kitajima, 1997). Consolidation occurs when the

hippocampal system repeatedly reactivates representation in the neocortex: this eventually leads to strong interconnections among cortical sites, which can support memory independently of the hippocampal system (Alvarez & Squire, 1994).

Finally, there is a view very similar to the previous one (see Squire 1987, chapter 13). In this view the hippocampus is important for maintaining the coherence of stored memory, by making contact with those distributed sites that together represent the different aspects that define an event. During consolidation the coherence of these sites increases. The hippocampus maintains organisation of these storage sites until they can be activated as an ensemble without the participation of the hippocampus. It is suggested that the CA3 region provides a mechanism for linking together disparate information from multiple regions of association neocortex (Hasselmo, 1999). In this view, the hippocampus also has a time-limited role in memory formation and is in accordance with all properties of declarative memory formation.

The last two views emphasise the time-limited role of the hippocampus and all three views suggest a two stages in memory formation: the first for the encoding and initial storage of new sensory information from the environment, and the second for consolidation by reactivating an old memory in order to form additional traces. The two-stage model of memory function requires very different dynamics during each stage. The formation of memory in two distinct stages may be another checkpoint to ensure that only salient information is stored, especially if the consolidation of memories is also a competitive process (see Squire, 1987, chapter 13). Hasselmo (1999) provides a model for acetylcholine in switching the activity of both hippocampus and neocortex between these two stages. At the cellular level, the switching between encoding and consolidation may require GABAergic interneurones (Paulsen & Moser, 1998)

4.3.3. The role of acetylcholine in the two-stage model

Hasselmo (1999) suggest that the first stage (encoding and storage) takes place during active waking and the second stage (consolidation) takes place during quiet waking and slow wave sleep. It is suggested that these dynamics are modulated by acetylcholine, which shows parallel fluctuations during the different stages of waking and sleep.

*active waking*: During active waking large amplitude theta oscillations occur in the hippocampus, whereas the neocortex displays high frequency, low amplitude activity (Lipton *et al.*, 1999). Acetylcholine levels are high and it is suggested that these contribute to the generation of the theta oscillations. The theta oscillations put the hippocampus in a plastic mode, facilitating storage of new information.

The high acetylcholine levels are also suggested to selectively inhibit the excitatory feedback from the CA3 region to the EC via the CA1 region, representing the output pathway, while having weaker inhibitory effects at many of the feedforward connections to the hippocampus, representing the input pathway (figure 10a). This general suppression of the output pathway reduces the influence of the hippocampus on the EC and other cortical areas, but no total suppression was shown. This means the hippocampal influence is reduced, but not removed so there still is enough connectivity of the hippocampus and the association neocortex to allow retrieval of stored information.

The reduced hippocampal influence may be necessary in order to prevent the output to dominate over incoming input and thus allow distortment of the initial sensory perception, causing hallucinations. These were indeed observed under the influence of cholinergic antagonists at high doses (Perry & Perry, 1995).

*quiet waking and slow-wave sleep*: During quiet waking and slow-wave-sleep, periodic brief, large amplitude events termed sharp waves originate in hippocampal region CA3 and spread back through region CA1 to the EC. Interestingly, during slow-wave sleep, the neocortex engages in burst-relate, slow oscillatory activity (Wilson & McNaughton, 1994), leading to the proposal that synaptic plasticity might also be linked to slow, synchronised oscillations in the neocortex. It is suggested that during sharp wave activity, a compressed version of information stored in the hippocampus is transferred to the neocortex.

Acetylcholine levels drop to 60% and 30% compared to active waking during quiet waking and slow wave sleep respectively. This results in stronger feedback from CA3 via CA1 to EC, the output layers of the hippocampus. This increase in output activity can in combination with the sharp waves activate neurones in the association neocortex. Thus during these stages, information can flow from hippocampus to the neocortical storage sites (figure 10b). Indeed, a correlation was found between ripples observed during hippocampal sharp waves and the spindles observed in prefrontal cortex EEG (Siapas & Wilson, 1998).

In addition, cells which fired during the previous waking period (Skaggs & McNaughton, 1996; Wilson & McNaughton, 1994) have a greater tendency to fire together during slow-wave sleep (Skaggs & McNaughton, 1996; Wilson & McNaughton, 1994). This can provide the appropriate dynamics for the formation of additional traces within regions CA3 and CA1, allowing the hippocampus to 'train' the EC or association neocortex on the basis of previously encoded associations (Buzsaki, 1989).

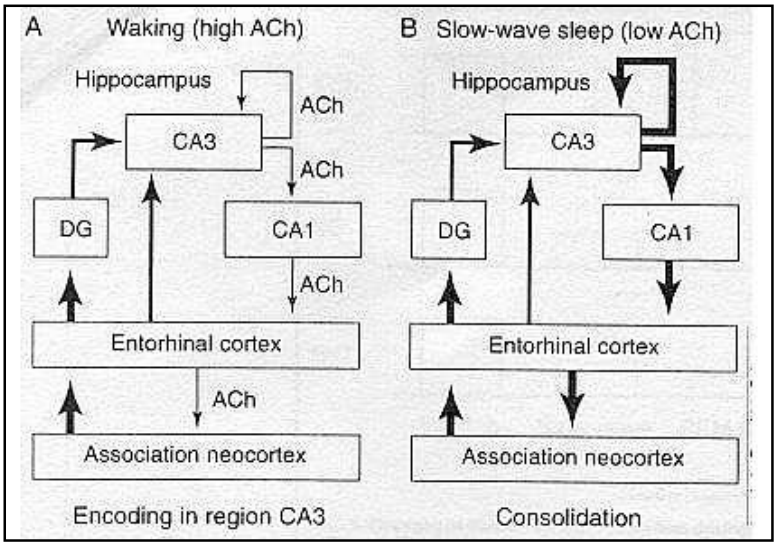


Figure 10. Two-stage model of long-term formation.(A) During active waking, information coded by neocortical structures flows through the EC and dentage gyrus into hippocampal region CA3. Connections less sensitive to acetylcholine are shown in thick arrows. Connections suppressed by acetylcholine (thin arrows) to region CA1, entorhinal cortex and association cortex are stong enough to mediate immediate retrieval. (B). During quiet waking or slow-wave sleep, memories are reactivated in region CA3 during sharp-waves (see text). These waves of activity flow back through region CA1 to EC and neocortex. This enables the process of consolidation of long-term declarative memory (from Hasselmo, 1999).

*REM sleep*: During REM sleep The EEG is similar to that during active waking, with higher-frequency and lower-amplitude waveforms than during slow-wave sleep (Marrosu, 1995). Acetylcholine levels in hippocampus are above those seen during active waking (e.g. Marrosu, 1995), while levels of acetylcholine in the frontal neocortex increase but only to somewhat lower levels, equivalent to those seen during quiet waking (Marrosu, 1995). Also, both noradrenergic and serotonergic neurones shown no activity during REM sleep.

The high levels of acetylcholine in the hippocampus might strongly supress feedback to the cortex. It is hypothesised that, during waking, high levels of both noradrenaline and acetylcholine shut down recurrent connections in neocortex, but during REM sleep, the somewhat lower levels of acetylcholine and the very low levels of noradrenaline in the neocortex might allow spread of activity within neocortical areas without a strong influence from the hippocampus. The REM sleep would allow neocortical structures to undergo a process of re-analysis, in which this declarative information would be re-interpreted in relation to previous learned information. Possibly this involves the prefrontal cortex, which is involved with organising information (see section 4.2.).

### 4.4. Short-term memory and long-term memory integrated

The schematic model for short-term memory can now be extended with the long-term memory (figure 11). It is uncertain whether the hippocampus contains the full information or that it provides a linkage between different aspects of the information which are separately stored in the neocortical storage sites. In my personal opinion, during active waking, new information is initially stored in the cortex as short-term memory, which has indeed been suggested as a intrinsic function of the cortical storage sites. At the same time, information would also be analysed for relevance. In this way the brain can select between relevant and non-relevant information and this may take place in the frontal cortex, possibly mediated by cholinergic fibres. When the information is judged relevant, the hippocampus is activated and entrained to link the different aspects of the information.

During quiet waking and slow-wave sleep the hippocampus then repeatedly actives the association cortex, which in turn activates the slow neocortical storage sites, where these memories were already stored in a short-term form. During this consolidation, the links between the different aspects will be strengthened. In time the links between the different aspects in the association cortex will be sufficiently strong for the ensemble to be activated together, without the participation of the hippocampus. Also during consolidation there is selection, possibly competition between the ensembles of neurones representing different memories to ensure that only the relevant and interesting information is stored. Other information will be forgotten. This may be accomplished by simple remembering, thus activating the ensemble again. This process may be independent of the prefrontal cortex. Alternatively, it may be accomplished by remembering and a subsequent re-analysis of information by the prefrontal cortex. This may occur either during active waking, in which case new consolidating signals will be sent to the hippocampus, or during REM sleep when there is little influence from the hippocampus.

This sequence of events seems likely since it appears most efficient. Information is initially stored at the same place where long-term memories will be stored. Thus there does not need to be a transfer of information to the hippocampus, which, subsequently, will have to be transferred to the cortex again. Rather, the hippocampus will be entrained to activate and thus provide a link between the different aspects of an event. Also, in comparison to the cortex, the hippocampus is a small brain structure, not capable of containing the multiple details of a memory, making the hippocampal role of linking information more likely.

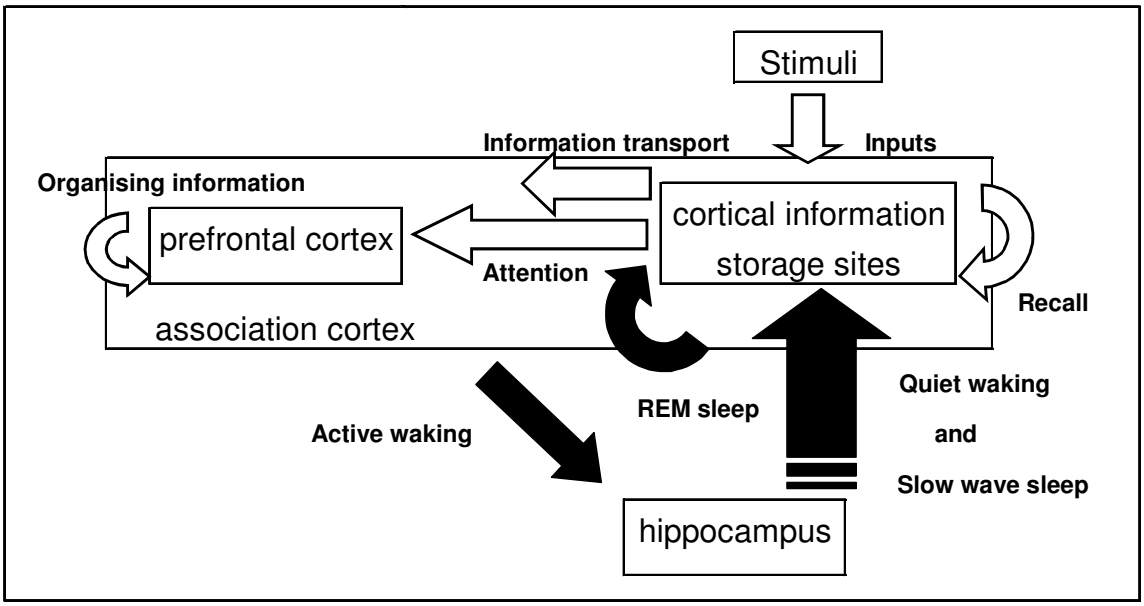


Figure 11. Schematic representation of the different stages involved in both short-term and long-term memory storage. Open arrows represent processes with a short-term duration, solid arrows represent processes with a long-term duration. See text for explanation.

### 4.5. Modulation of memory: the amygdala

There is extensive evidence that the amygdala mediates the memory-enhancing effects of stress hormones (e.g. Clark *et al*., 1999; Cahill and McCaugh, 1998; Roozendaal *et al*., 1996; McCaugh *et al*., 1996; Figure 12). Studies with humans who have received temporal-lobe surgery, including removal of the amygdala, conclude that removal of the amygdala does not cause memory impairment (Scoville & Milner, 1957). However, impairment of emotionally influenced long-term memory was reported in patients with selective amygdala damage (Cahill, 1995; Babinsky, 1994). In addition, in a recent positron emission tomography (PET) study, glucose levels in the right-amygdala were measured while subjects watched either emotional film sessions and neutral film sessions. Later, the number of films recalled was established. A correlation was found between glucose levels in the amygdala and number of films recalled, providing a link between amygdala activity and memory enhancement (Cahill, 1996; Figure 13). More evidence for a role of memory modulating comes from a study with microinfusion of amphetamine. It was shown that microinfusion of amphetamine in the hippocampus selectively enhanced retention of spatial training and injection in the caudate nucleus selectively enhanced retention of cue/response training. However, infusion into the amygdala enhanced both types of memory, reflecting a general enhancement of memory (Packard *et al*., 1995).

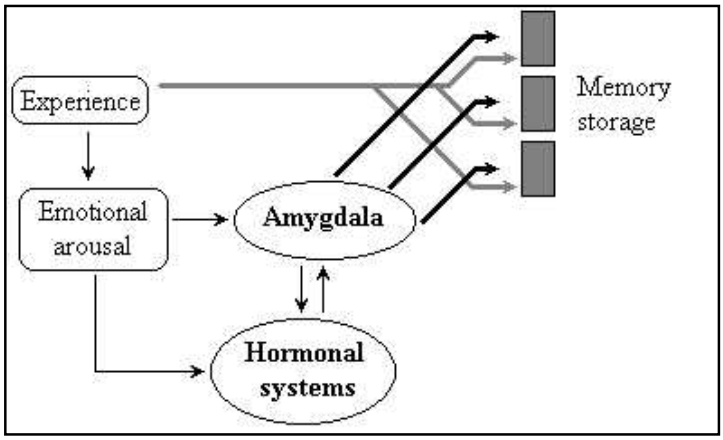


Figure 12. Hypothetical memory-modulatory mechanism for emotionally arousing events. Experiences can be stored in various brain regions with little or no involvement of either stress-hormone activation or the amygdala. During periods of emotional arousal, stress hormones systems interact with the amygdala to modulate memory-storage processes occurring in other brain regions. This modulation is enhanced by acetylcholine and inhibited by GABA (Modified from Cahill & McGaugh, 1998).

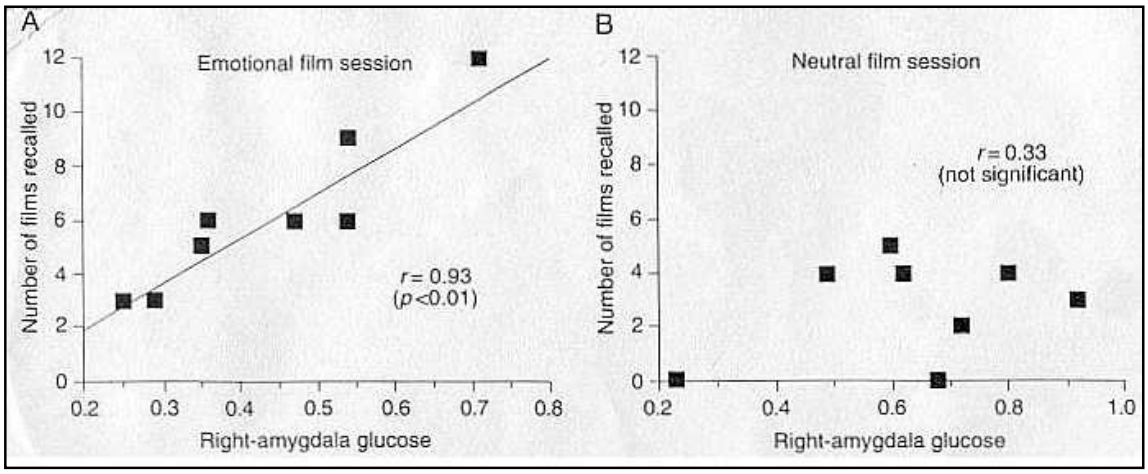


Figure 13. Amygdala activity in healthy humans selectively correlated with the formation of declarative memory for emotionally arousing information. Correlations between (A) glucose utilisation in the right amygdala while viewing a series of relatively emotionally arousing films and long-term recall of those films and (B) glucose utilisation in the right amygdala of the same subjects while viewing a series of relatively emotionally neutral films and long-term recall of those films. (Modified from Cahill *et al.*, 1996).

How does the amygdala enhance memory? The amygdala is a heterogeneous collection of distinct nuclei and a number of experiments indicate the basolateral amygdala as the nucleus involved in modulation of memory storage (see Cahill & McCaugh, 1998). The basolateral nucleus (BLN) projects prominently to the hippocampus and entorhinal cortex and pharmacological stimulation of the amygdala functionally activates both of these regions. The amygdala could also modulate memory storage processes in the neocortex via projections to various cortical regions.

Since LTP can be induced in the amygdala (Chapman *et al.*, 1990; Clugnet *et al.*, 1990) and blockage of LTP also attenuates fear-based learning, it has been suggested that neural changes mediating fear conditioning may be located within the amygdala (LeDoux, 1995; Franselow *et al.*, 1994; Miserendino *et al.*, 1990). However, it is suggested that the amygdala influences memory storage in other brain regions by activating those regions. Lesions of the stria terminalis (a major amygdala output pathway) do not block inhibitory avoidance learning, but do block memory modulation of stress hormones (Liang *et al.*, 1990; Liang & McGaugh, 1983) suggesting that the amygdala is involved in modulation memory storage, but is not the locus of neural changes mediating long-term memory of emotionally arousing experiences. In addition, inactivation of the amygdala prior to retention tests did not impair memory. Also lesions in the BLN block memory-modulating effects of infusion of glucocorticoid agonists and antagonists directly into the dorsal hippocampus. Additionally BLN lesions attenuate and BLN stimulation facilitates the induction of LTP in the dentate gyrus (see McGaugh *et al.*, 1996). These data indicate the amygdala itself is not the site of storage, but rather a brainstructure which enhances information storage in other brainstructures.

4.5.1. Stress hormones and interaction

There are two types of stress hormones: adrenal hormones and glucocortical hormones and both modulate memory (see Cahill and McCaugh, 1998). Adrenaline enhances memory in a dose-dependant way, and the effects are time-dependent; only doses administered shortly after training enhanced memory; at the time it would normally be released by aversive stimulation. Adrenocortical hormone release (cortisol in humans) is also activated by emotional arousal. This is the second wave of the autonomic response to an emotional event. With both adrenal catecholamines and glucocorticoids administered after training, the effects on memory are dose-dependent and time-dependent with moderate doses and administration immediately after training producing maximal enhancement (McGauch *et al*., 1996).

There is also evidence of interaction between these two stress hormones. Studies using adrenalectomized rats have shown that the level of circulating corticosterone is a major factor in determining the sensitivity of adrenaline in modulating memory storage (see Roozendaal *et al*., 1996). Also, adrenaline mediated enhancement of retention was abolished using metyrapone (an inhibitor of elevated corticosterone levels during stress) (Roozendaal *et al*., 1996).

Both adrenaline effects and glucocorticoid effects are blocked by β-adrenergic antagonists and it is therefore suggested that these compounds stimulate noradrenaline release in the amygdala (see McGaugh *et al*., 1996). Adrenaline poorly passes the blood-brain barrier and adrenaline effects appear to be mediated by the activation of peripheral β-adrenergic receptors (McGaugh, 1989), located on vagal afferents. Stimulation of the vagal nerve in humans caused a significant enhancement of word recognition (Clark *et al*., 1999).

4.5.2. GABAergic and cholinergic influences

An overview of GABAergic and cholinergic influences is presented by McGauch and colleagues (1996). GABAergic agonists and antagonists infused systemically impair and enhance retention. Comparable effects are produced by post-training intra-amygdalar infusions. GABA is believed to affect the noradrenaline release in the amygdala. At the subsequent step, activation of muscarinic receptors is thought to be involved, since cholinergic actions cannot be inhibited by propanolol, a $\beta$-adrenergic antagonist. It is well established that, when administered systemically after training, muscarinic cholinergic agonists and antagonists enhance and impair respectively, retention of a variety of tasks. Moreover, highly comparable results have been obtained with post-training intra-amygdalar infusions. All parts of the amygdala receive cholinergic input from the substantia innominata and nucleus basalis, located in the forebrain (Woolf, 1991). It is thought that acetylcholine fixates attention and thus plays a role in modulating memory (see section 4.2; Woolf, 1998).

An example of cholinergic influences involving the amygdala is that the cholinergic cells might provide the associative link between the frequency-related representation of the tone conditioned stimulus (CS) and the neural representation of the autonomic sensation related to the fear of the unconditioned stimulus (US) stored in the amygdala. This association requires three neural structures: the cholinergic basal forebrain, the auditory cortex and the amygdala. There is potential for dendritic and traditional synaptic contact to exist between the cholinergic cells projecting to the amygdala and those projecting to the auditory cortex (see Woolf, 1996a). Presumably, acetylcholine released from basal forebrain neurones causes sequentially focused attention upon these two associable representations. Evidence from recent human-brain imaging studies suggests that the amygdala and orbitofrontal cortex interact functionally during emotionally arousing situations (Cahill *et al*., 1996). Intervals between CS and US of 250-500 ms produce optimal training, which is consistent with the

storage of representations for approximately one half second (sensory memory; Woolf, 1998; Woolf, 1996b), since then the two stimuli would be successive and the representation would be the strongest.

## 5. CONCLUDING COMMENTS

In this literature search I have presented an overview of different aspects of the functioning of memory in order to gain a global understanding of how memory works. Memory is represented by neural activity patterns and these patterns can be altered through learning. During the formation of memory there is an emphasis on specialisation and selection. The brain is composed of different modules, which are each specialised for a particular function. On a larger scale there are different brainstructures, each specialised for a particular function.

In addition to this specialisation there is selection between relevant and non-relevant information. On a cellular level there are a number of checkpoints, inhibitory constraints which have to be removed in order for a synaptic connection to be strengthened. On a larger scale there are several brainstructures which ensure the storage of relevant information only. The prefrontal cortex analyses and gates information to the hippocampus. The hippocampus in turn, serves as a temporal storage site. During consolidation of information in the neocortical storage sites there is further selection and non-relevant information can still be discarded and forgotten.

In addition to these general mechanism there is a special mechanism which enhances memory during an stressful situation. There are clear evolutionary advantages in this mechanism, since an organism will be able to better remember under which circumstances the stressful situation took place and thus be better able to anticipate or avoid such a situation in the future.

There are several mechanisms and pathways which make the communication between the different brainstructures and modules possible such as oscillations and specific types of neurones such as GABAergic neurones and cholinergic neurones. This leads, together with the different forms of LTP and the different gene cascades to a very complex functioning of the brain. Although I have outlined the general principles of memory it will be a very hard, but challenging task to unravel the precise workings of memory.

There are some guidelines for further research. First, authors should stay critical against ruling hypothesis and at the same time be open to new possibilities and explanations as was advanced by Shors & Matzel (1997). Second, many authors focus on one specific transmitter or one specific brainstructure. They then find many possible links between their confined part of research and other parts and functions to stress the importance of their research. This leads to multiple explanations for the same phenomenon. While these studies provide necessary insights, comparison with other fields of research have to be made in order to determine the importance played by each part of research. For example, the formation of the synaptic tag (section 2.1.5.; Frey & Morris, 1997) in hippocampal pyramidal neurones provides an explanation why unimportant things are well remembered if they occurred during a well remembered event. But if this well remembered event was emotional, this can also be explained by an enhancement in memory formation due to actions of stress hormones on the amygdala. Thus, in order to unravel the precise workings of memory, besides specialised studies, there should be integrative studies, evaluating the importance played by each system and each transmitter.

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## 8. LIST OF ABBREVIATIONS USED

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|---|---|
| AMPA | α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate |
| apCAM | Aplysia cell adhesion molecules |
| BLN | Basolateral nucleus |
| CCh | Carbachol – a cholinergic agonists |
| CS | Conditioned stimulus |
| EC | Entorhinal cortex |
| EEG | Electroencephalogram |
| Fas II | Fasciclin II |
| GABA | γ-Aminobutyric acid |
| LTD | Long-term depression |
| LTM | Long-term memory |
| LTP | Long-term potentiation |
| MAP-2 | Microtubule associated protein 2 |
| MAPK | Mitogen-activated protein kinase |
| mGluRs | Metabotropic glutamate receptors |
| NCAM | Neural cell adhesion molecule |
| NMDA | N-methyl-D-aspartate |
| STM | Short term memory |
| US | Unconditioned stimulus |