Chapter 1

Introduction and scope of this thesis
Chapter 1

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I. The hippocampus

1.1. The hippocampal circuitry and its role in memory formation
One of the key structures in the brain that has a wide range of memory functions is the hippocampus (Squire, 1992). The hippocampus can be divided in several major areas: the dentate gyrus (DG), Cornu Ammonis 3 (CA3), Cornu Ammonis 1 (CA1) and subiculum, see figure 1 (Amaral and Witter, 1989). The DG granule cells receive input from the stellate cells in layer 2 of the entorhinal cortex via the perforant path (Nyakas et al., 1988; Witter, 1993). The granular cells of the DG project to the CA3 pyramidal cells via the mossy fibers. In addition, CA3 pyramidal neurons receive input from two other sources, namely the stellate cells in layer 2 of the entorhinal cortex and from the recurrent collaterals of the CA3 pyramidal cells themselves. The recurrent collaterals are the most numerous type of input to CA3 pyramidal cells (Ishizuka et al., 1990). Besides projections of the CA3 pyramidal cells via the Schäffer collaterals, the CA1 area receives input from layer 3 of the entorhinal cortex through the perforant pathway. The CA1 pyramidal cells project either directly or via the subiculum to layer 5 of the entorhinal cortex (Johnston and Amaral, 1997), as well as to a variety of other areas including the prefrontal cortex, lateral septum, anterior thalamus and mamillary bodies (Amaral and Witter, 1995).

Based on lesion studies, it has become apparent that the hippocampus plays a crucial role in the formation of memories in various learning paradigms including spatial tasks like the Morris water maze (Schenk and Morris, 1985), radial maze (Olton and Papas, 1979), Y maze (Jarrard, 1986; Etkin et al., 2006), as well as in various associative tasks including fear conditioning (Kimble, 1963; Phillips and Ledoux, 1992). The hippocampus can rapidly form spatial representations of the environment (also referred to as spatial maps). In addition, the hippocampus can develop an association of a specific context within which an important event occurred, for instance where an aversive stimulus was received (e.g. electrical shock was given) or the location of a food reward or a submerged and invisible platform.

1.2. The hippocampus as a comparator of previously stored and novel information
In addition to the formation of new memories, the hippocampus has been regarded to be the location where previously stored memories can be compared with actual sensory information from the environment (Gothenberg et al., 1996; Knight, 1996). Based on anatomical connectivity and computational modeling, both area CA3 and area CA1 of the hippocampus have been implicated in the detection of changes in a familiar environment, also known as match-mismatch processing (for review Rolls and Kesner, 2006; Lisman and Otmakhova, 2001). In case of a comparison between recently stored information (e.g. minutes old) and novel sensory information, area CA3 is of paramount importance in this novelty detection (Lee et al., 2004, 2005; Nakazawa et al., 2003). In case of longer time intervals (ranging from hours to days), area CA1 seems to react as a novelty detector, rather than area CA3 (Wan et al., 1999; Fyhn et al., 2002; Jenkins et al., 2004). In contrast to area CA1 and CA3, the DG is generally not seen as the location where novelty detection takes place. The DG plays an essential role in pattern separation (Gilbert et al., 1998; Goodrich-Hunsaker, 2005; Leutgeb et al., 2007; McHugh et al., 2007), a process important to distinguish relatively similar
environments or situations. Input coming from the entorhinal cortex is dispersed onto the broad layer of sparsely firing granule cells. Each granule cell therefore carries only a small and distinct fraction of information of the total input (McNaughton and Nadel, 1989). As suggested by Leutgeb and colleagues (2007), the segregation of inputs from the cortex might be retained due to the sparse firing levels of the granular neurons in combination with the relatively meager connections between granule cells and CA3-pyramidal cells.

**Figure 1.** The hippocampus is a brain structure crucial for learning and memory processes. (A) A representative picture of a coronal section of a mouse brain stained for the catalytic subunit of CaN. (B) Enlargement of the dorsal hippocampus stained for the catalytic subunit of CaN (See insert A). (C) A schematic representation of the most important subregions within the dorsal hippocampus: (DG) dentate gyrus; (CA3) Cornu Ammonis 3; (CA1) Cornu Ammonis 1; (SUB) Subiculum.
2. Memory formation and the ‘Hebbian rule’: a brief history

Only half a century ago, initial hypotheses about the neurobiological correlates underlying memory formation were postulated. In the 1950s, Donald Hebb hypothesized that synaptic efficacy increases when: 1) there is presynaptic activity at the synapse, and 2) a critical level of postsynaptic depolarization occurs (Hebb, 1949). This ‘Hebbian rule’ was confirmed by Bliss and Lomo (1973), who showed that brief and intense synaptic activation in the hippocampus indeed results in a long-term enhancement of synaptic efficacy of the stimulated synapses (a process referred to as long-term potentiation, LTP). From a theoretical point of view, there was a necessity for an ‘anti-Hebbian rule’ that explained a mechanism controlling the weakening of synaptic connections when presynaptic and postsynaptic activity did not occur together (Stent, 1973). In the early 1990s several investigators revealed that a weak but prolonged stimulation resulted in a weakening of synaptic strength, a process known as long-term depression (LTD) (Dudek and Bear, 1992; Mulkey and Malenka, 1992).

The discovery of bi-directional plasticity induced by LTP and LTD opened the possibility to elucidate basic cellular and molecular mechanisms of synaptic plasticity giving insight in the processes underlying the formation of memories. Nowadays, LTP is generally seen as a key mechanism underlying memory formation since it has been shown that learning induces LTP in vivo. In contrast to LTP, the function of LTD in (hippocampal) memory formation remains debated and is less well understood.

3. NMDA and AMPA receptors

One of the two glutamate receptor families involved in LTP and LTD is the ionotrophic receptor family that is ligand dependent. The ionotrophic receptor family can be subdivided into three classes: the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor, the N-Methyl-D-Aspartate (NMDA) receptor, and kainate receptors (Hollmann and Heinemann, 1994).

AMPA receptors are the main transducers of rapid excitatory transmission in the central nervous system (Derkach et al., 2007). They are tetramers comprised of four receptor subunits (GluR1-GluR4) and consist of a large extracellular N-terminal domain, three transmembrane domains and an intracellular C-domain. The subunit composition varies between brain regions and affects the functional properties of AMPA receptors and the trafficking of these receptors (Derkach et al., 2007). AMPA receptor functioning is further mediated by several phosphorylation sites on the intracellular C-domain of all four subunits (Song and Huganir, 2002). The GluR1 subunit has one currently known threonine site and various serine phosphorylation sites (Lee, 2006). Of these various phosphorylation sites, the serine-831 (S831) and serine-845 (S845) sites are of particular interest. Changes in the phosphorylation state of both sites have been implicated in the expression of LTP and LTD. Phosphorylation of S831 increases the single channel conductance (Derkach et al., 1999), is enhanced following LTP (Barria et al., 1997; Lee et al., 2000), and is reduced after de-potentiation (Lee et al., 2000). S845 phosphorylation increases the mean open probability
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of the channel (Banke et al., 2000), while dephosphorylation of the site occurs after LTD
(Kameyama et al., 1998; Lee et al., 1998; Lee et al., 2000; Brown et al., 2005). In addition,
enhanced phosphorylation levels of this site are found after de-depression (Lee et al., 2000).
Phosphorylation of S845 is also required for the insertion of GluR1 containing AMPA receptors
into synapses with LTP induction (Esteban et al., 2003), and regulation of AMPA receptor
recycling (Ehlers, 2000). A recent study by Whitlock et al (2006) assessed whether train-
ing in an inhibitory avoidance paradigm evoked changes in the phosphorylation state of both
GluR1 serine sites. They revealed that this training induced a rapid enhancement of S831 phos-
horylation which returned to baseline levels within an hour. In contrast, no changes in
phosphorylation levels were found for the S845 site. Recently, two additional phosphoryla-
tion sites of the GluR1 subunit were discovered. Boehm and colleagues (2006) revealed
that phosphorylation of the serine-818 site is necessary and sufficient for GluR1 synaptic
insertion by LTP. The threonine-840 site is known to be strongly phosphorylated under
basal conditions in the hippocampus (Lee et al., 2002), however its function with regard to
AMPA receptor functioning remains to be elucidated. Figure 2 shows a schematic survey of
the currently known relations between changes in the phosphorylation state of the GluR1
serine sites and AMPA receptor function. An overview of the phosphorylation sites of the
GluR1 subunit and the known functions are shown in table 1. For a detailed description of
the phosphorylation sites of the GluR2 and GluR4 subunits see, Lee (2006).

As mentioned above, it is widely accepted that AMPA receptor functioning is con-
trolled by changes in the phosphorylation state of the receptor subunits. However, studies
investigating alterations in receptor phosphorylation in relation to memory formation in vivo
remain scarce. Whitlock co-workers (2006) recently showed that one trial inhibitory avoid-
ance learning produced the same changes in hippocampal AMPA receptor phosphorylation
as induction of LTP. In addition, they showed that this learning paradigm indeed induces LTP
in the CA1 region of the hippocampus.

NMDA receptors stay silent at resting membrane potential, but play a crucial role in
the induction of LTP (Nicoll and Malenka, 1999) and LTD (Bear et al., 1999). The receptors
consist of two obligatory NR1 subunits and two NR2 subunits (Nakanishi, 1992; Hollmann
and Heinemann, 1994). Several isoforms of the NR2 subunit exist (NR2A- NR2D), that
control the properties of NMDA receptor channel, like the threshold for the removal of
the Mg2+ block (Cull-Candy et al., 2001). When the postsynaptic membrane is sufficiently
depolarized, the Mg2+ block is removed from the channel (Mayer et al., 1984). After the
removal of the Mg2+ block, the channel can be activated by glutamate resulting in a postsyn-
aptic influx of Ca2+. This Ca2+ influx triggers various signaling cascades that can lead to
either LTP or LTD depending on the duration and level of Ca2+ influx in the postsynaptic
cell. For a detailed review of known phosphorylation sites on NMDA receptor subunits, see
Figure 2. A model for the bidirectional changes in AMPA-receptor phosphorylation and NMDA-receptor-dependent plasticity in a post-synaptic element. High frequency stimulation (HFS) can result in a strong increase in Ca2+ influx leading to the activation of PKA and CaMKII. As a consequence, HFS can lead to a PKA-dependent phosphorylation of the S845 site as well as an enhanced open channel time (P0) when delivered to previously depressed synapses (LTD). Furthermore, HFS can in addition lead to an increase in channel conductancy of GluR1-containing AMPA receptors through the phosphorylation of the S831 site when delivered at a naive synapse resulting in LTP. Recently it was shown that phosphorylation of the S818 (by PKC) is sufficient for the incorporation of AMPA receptors into the membrane with LTP. It is currently unknown whether CaN or PP1 dephosphorylates this serine site. Low frequency stimulation results in a low Ca2+ influx resulting in the activation of the phosphatases CaN and as a consequence also PP1 activation. LFS applied to naive synapses results in exocytosis of GluR1 containing receptors through a CaN-dependent dephosphorylation of the S845 site and reduced open channel time. When applied to a previously potentiated synapse, LFS induces a PP1-dependent dephosphorylation of the S831 site resulting in reduced channel conductivity. Grey arrows indicate protein phosphatase-dependent mechanisms, black arrows indicate protein kinase dependent mechanisms. For abbreviations, see text.
### Table 1a: Phosphorylation of AMPA GluR1 subunits phosphosites

<table>
<thead>
<tr>
<th>site</th>
<th>Identified function</th>
<th>Protein kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>S818</td>
<td>Phosphorylation necessary for synaptic incorporation and LTP (Boehm et al., 2006).</td>
<td>PKC (Boehm et al., 2006)</td>
</tr>
<tr>
<td>S831</td>
<td>Increased single channel conductance of homomeric receptors (Derkach et al., 1999; Oh &amp; Derkach, 2005).</td>
<td>CaMKII (Barria, et al., 1997; Mammen et al., 1997) PKC (Roche et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Enhanced phosphorylation following LTP (Barria, Muller et al., 1997; Lee et al., 2000).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can substitute for S845 in mediating LTP (Lee et al., 2003; Lee et al., 2004).</td>
<td></td>
</tr>
<tr>
<td>T840</td>
<td>Highly phosphorylated in hippocampus under basal conditions.</td>
<td>PKC (Lee et al., 2002)</td>
</tr>
<tr>
<td>S845</td>
<td>Increased mean open probability of channel (Banke et al., 2000).</td>
<td>PKA (Roche et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>Increased phosphorylation after de-depression (Lee et al., 2000).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Necessary for insertion into synapses with LTP induction (Esteban et al., 2003).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can substitute S831 for mediating LTP (Lee et al., 2003; Lee et al., 2004).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enhanced AMPA receptor insertion (Ehlers et al., 2000)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 1b: Dephosphorylation of AMPA GluR1 subunits phosphosites

<table>
<thead>
<tr>
<th>site</th>
<th>Identified function</th>
<th>Protein phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>S831</td>
<td>Reduced channel conductance, depotentiation of previously potentiated synapse (Lee et al., 2000), (Huang et al., 2001)</td>
<td>PP1 (Lee et al., 2000)</td>
</tr>
<tr>
<td>S845</td>
<td>Endocytosis of GluR1 containing AMPA receptors (Kameyama et al., 1998; Lee et al., 2000; Beattie et al., 2000; Smith et al., 2006).</td>
<td>PP1* (Lee et al., 2000) Calcineurin* (Beattie et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>Necessary for LTD expression (Lee et al., 2003; Lee et al., 2004).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Down regulation of GluR1 receptor current (Tavalin et al., 2002).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Necessary for LTD expression (Lee et al., 2003; Lee et al., 2004).</td>
<td></td>
</tr>
</tbody>
</table>

* Beattie and colleagues (2000) suggested that, based on the strength of synaptic activation of NMDA receptors, either CaN alone or both CaN and PP1 are needed for AMPAr endocytosis.

Adapted and modified from Lee, 2006.
4. Protein kinases and synaptic plasticity

Protein kinases are enzymes that phosphorylate numerous substrates including other protein kinases, receptor subunits, and phosphoproteins. They have different functions within the same cell depending on the subcellular location. The protein kinase families that have been studied most intensively in relation to LTP and memory formation are the calcium/calmodulin-dependent kinase family (CaMK family), cyclic adenosine 3’5’-monophosphate-dependent protein kinase (PKA) family, the mitogen-activated protein kinase (MAPK) family and the Ca2+/phospholipid-dependent protein kinase (PKC). The role of the PKA family and MAPK family will be discussed in paragraph 4.1-4.2.

4.1. PKA structure, cellular function and memory formation

PKA is one of the most intensively studied protein kinases in regard to synaptic plasticity and memory processing. The mammalian PKA family consists of four regulatory subunits (R\(\alpha\), R\(\beta\), R\(\alpha\), and R\(\beta\)), that each have two cAMP binding sites with different affinities for cAMP, and three catalytic subunits (C\(\alpha\), C\(\beta\), C\(\gamma\)). All these subunits show distinct expression patterns across mammalian brain regions (Cadd and McKnight, 1989), and are strongly expressed especially in areas that are essential for learning and memory (e.g. neocortex and hippocampus). In the absence of cAMP, PKA is a tetrameric complex consisting of two regulatory subunits and two catalytic subunits. PKA consisting of R\(\alpha\) dimers is referred to type I PKA, while PKA consisting of R\(\beta\) dimers is referred to as type II PKA (Tasken et al., 1993; Francis and Corbin, 1999; Skalhegg and Tasken, 2000).

Activation of PKA is initiated via two mechanisms. The first is by influx of Ca\(^{2+}\) through NMDA receptors or voltage-gated Ca\(^{2+}\) channels; Ca\(^{2+}\) (together with calmodulin) can activate adenylyl cyclases (AC) resulting in cAMP production (Eliot, 1989) (See Fig.3). Alternatively, the synthesis of cAMP can be initiated through the binding of transmitters and hormones to guanine nucleotide-binding regulatory protein (G-protein) coupled receptors (Tang and Gilman, 1991). G-proteins bind to adenylyl cyclases resulting in its activation, which starts the synthesis of cAMP. As a consequence of binding cAMP to the regulatory subunits, the monomeric catalytic subunits are released and can then phosphorylate various substrates including the GluR1 S845 site (see Fig.2). Besides the S845 of the GluR1 subunit, PKA targets many other substrates.

Among them the NMDA receptor subunits NRI (Tingley et al., 1997) and NR2A (Krupp et al., 2002). Activated PKA can phosphorylate inhibitor I (II), a phosphoprotein which, when phosphorylated, inhibits protein phosphatase I (PP1) activity (Huang and Glinsmann, 1976). The released catalytic subunit can also translocate to the nucleus to phosphorylate serine-133 on the cyclic AMP-response element-binding protein (CREB). As a result, transcription of CREB-associated genes (for instance Zif268 and c-Fos) is initiated (Yamamoto et al., 1988; Gonzalez and Montminy, 1989; Sheng et al., 1991). In case of CA1 neurons, MAPK activation is required to couple PKA activation to CREB phosphorylation (Roberson et al., 1999). In addition to CREB, PKA can also target the transcription factor Elk1 via the MAPK pathway (Roberson et al., 1999; Grewal et al., 2000; Morozov et al., 2003).
Various electrophysiological studies have recognized the role of PKA in L-LTP, a late form of LTP that can last for several hours (Frey et al., 1993; Huang and Kandel, 1994; Nguyen and Kandel, 1996; Abel et al., 1997; Matthies and Reymann, 1993; Woo et al., 2000, 2002, 2003). L-LTP requires NMDA receptor activation (Collingridge et al., 1983), protein synthesis (Frey et al., 1988) and gene transcription (Nguyen et al., 1994). In addition to its role in L-LTP, the role of the protein kinase PKA in the formation of long-term memories has been studied intensively using transgenic and/or pharmacological approaches. Mice lacking the RIβ subunit of PKA did not show any abnormalities in the learning paradigms assessing spatial memory formation, due to compensatory mechanisms for the loss of a given subunit (e.g. enhanced expression of the Rlα subunit) (Huang et al., 1995). Similarly to the RIβ subunit knock out mice, Cβ1 knock-out mice performed normally in spatial learning tasks (Huang et al., 1995; Qi et al., 1996). Despite the fact that both knock-out mouse lines did not show any reduction in PKA activity and normal performance in various learning tasks, marked defects were found in certain forms of synaptic plasticity including certain forms of LTP and LTD. Using a transgenic approach, Abel and colleagues (1997) generated a mutant mouse overexpressing a dominant negative form of the regulatory subunit of PKA R(AB) under control of the CaMKIIα gene (thereby restricting the expression to the postnatal forebrain). In contrast to the conventional knock-out mice, these transgenic mice showed reduced PKA activity, decreased L-LTP and memory deficits in learning paradigms requiring the hippocampus like the Morris water maze and contextual fear conditioning, suggesting that PKA is crucial for the consolidation of long-term memories. In line with these findings, several other studies have shown that pharmacological intervention of PKA activity in various brain regions inhibits the formation of long-term memories, but not short-term memories in other learning paradigms including tone cued and contextual fear conditioning (Schafe et al., 1999; Schafe & LeDoux, 2000), conditioned taste aversion (Koh et al., 2002) and inhibitory avoidance (Quevedo et al., 2004). While PKA is beneficial for the formation of long-term memories, it was recently shown that PKA functions as a molecular constraint in case of the extinction of previously formed contextual fear memories (Isiegas et al., 2006).

4.2. MAPK structure, cellular function and memory formation

Originally, the MAPK family was found to be essential in various cellular processes including cell division, differentiation and apoptosis (Schaeffer and Weber, 1999). MAPKs can be activated by a number of extracellular signals including growth and neurotrophic factors (Segal and Greenberg, 1996). During recent years, a significant role for MAPKs in hippocampal synaptic plasticity and memory formation has been recognized. The MAPKs consist of a family of a core of three kinases: MAP kinase kinase kinase (MAPKKK, also known as Raf-1 and B-Raf), MAP kinase kinase (MAPKK, also known as MEK) and MAP kinase (P44 MAPK = erk1, P42 MAPK = erk2). MAPKKK activates MAPKK through phosphorylation. Upon activation MAPKK phosphorylates MAPK at both a threonine and tyrosine residue. MAPK signaling can furthermore be regulated via PKA and PKC signaling mechanisms (Sweat, 2001). Activated MAPK can result in the activation of the transcriptions factor CREB and Elk-1 inducing gene transcription (Wang et al., 2007) (Fig.3). CREB-dependent gene transcription is shown to be essential for the maintenance of LTP and long-term memory (Silva et al., 1998). Recently, persistent LTD, a form of LTD that is protein-synthesis dependent
and can last for days, was suggested to be a consequence of altered gene expression (Kau-
derer and Kandel, 2000). Thiels and colleagues (2002) revealed that the MAPK cascade
contributes to persistent LTD, through the promoting of gene transcription via the serum
response element (SRE) (Fig.3). Pharmacological analysis revealed that inhibition of hippo-
campal MAPK activation resulted in impaired spatial memory formation and LTP (Blum et
al., 1999; Selcher et al., 1999). Similar impairments were found for fear motivated learning
(Atkins et al., 1998; Cammarota et al., 2000).

Figure 3. A schematic representation of a cell body with a nucleus (square) and a postsynaptic cell com-
ponent. Activation of protein kinase and protein phosphatase pathways by HFS and LFS in the postsynaptic
structure. Black arrows indicate the activation of protein kinase pathways via HFS which can lead to CREB
and Elk-1-associated gene transcription resulting in LTP. Grey arrows indicate the activation of protein phos-
phatase and protein kinase pathways as a result of LFS resulting in SRE-associated gene transcription and
LTD. For abbreviations, see text.
5. Protein phosphatases

Protein phosphatases counteract the actions of protein kinases by dephosphorylating the same substrates. The family of serine/threonine specific protein phosphatases is divided into three different groups of phosphatase that are highly homologous (PP1, PP2A, PP2B). The role of PP2B (and PP1) in relation to synaptic plasticity and memory formation is reviewed in detail in paragraph 5.1 Phosphatase 2C, a fourth known serine/threonine phosphatase belongs to an unrelated second phosphatase family. In addition to serine/threonine phosphatases, various tyrosine phosphatases are also known to be involved in synaptic plasticity (for detailed reviews see: Wagner et al., 1991; Nairn and Shenolikar, 1992).

5.1. Calcineurin structure, cellular function and memory formation

Calcineurin, also known as protein phosphatase 2B (CaN, PP2B), is a member of the serine/threonine serine phosphatase family. It is the only calcium-dependent phosphatase in the brain and has a very high affinity for calcium (Klee et al., 1979). It is a heterodimer composed of a catalytic A-subunit (CaNA) and a regulatory subunit (CaNB). There are two isoforms of the CaNA subunit that are present in the brain (CaNAα and CaNAβ), while CaNB subunit has only one known isoform in the brain (CaNBα1; Wera and Hemmings, 1995). CaN is strongly expressed both presynaptically and postsynaptically in principal neurons and largely absent in interneurons and glia (Sik et al., 1998). High concentrations are found in the hippocampus, caudate putamen and substantia nigra, while CaN is moderately present in the cerebral and cerebellar cortex (Goto et al., 1986). CaN is one of the most prominently expressed enzymes in the central nervous system, since it constitutes almost 1% of total protein in the brain (Klee et al., 1979).

The CaNA subunit can bind a complex of calcium and calmodulin. Both the catalytic and regulatory subunit are needed for full CaN activity. The CaNB subunit remains tightly bound to the catalytic subunit regardless of the binding of calcium. Binding of calcium to the regulatory subunit enhances CaN activity, which is further regulated by endogenous inhibitors, anchoring proteins, ions metals and oxidative processes (Rusnak and Mertz, 2000).

CaN has numerous functions in various cellular compartments including the cytoplasm, nucleus as well as in pre- and post-synaptic elements of synapses. For instance, it dephosphorylates the GluR1 S845 site (Banke et al., 2000), and it shortens the channel open time of NMDA receptors (Lieberman and Mody, 1994; Tong et al., 1995) via targeting of the NR2A subunit (Krupp et al., 2002). In addition, CaN can also activate a second protein phosphatase known as protein phosphatase 1 (PP1), through the dephosphorylation of the phosphoprotein I1 (Huang and Glinsmann, 1976) (Fig.3). PP1 can dephosphorylate numerous other substrates including the AMPA receptor GluR1 S831 site (Lee et al., 2000) and the transcription factor CREB (Bito et al., 1996). Overall, activity of CaN (and PP1) can result in suppressed protein synthesis and receptor dephosphorylation (and endocytosis), resulting in a depressed synapse. In agreement with this, various electrophysiological studies have emphasized the importance of these protein phosphatases for LTD and depotentiation.

In vivo analysis suggested that reducing CaN activity in the forebrain via the expression of
a CaN inhibitor facilitated LTP but had no effect on LTD (Malleret et al., 2001). Similar observations were done using a pharmacological approach (Ikegame and Inokuchi, 2000). Reduced CaN activity levels enhanced both short-term and long-term memory in various spatial and non-spatial learning tasks (Malleret et al., 2001; Ikegame and Inokuchi, 2000). Likewise, overexpression of forebrain-CaN resulted in defective long-term memory, evident in both a spatial task and a visual recognition task, although it did not affect short-term memory (Mansuy et al., 1998). Similarly, reduction of PP1 activity in the forebrain facilitated the formation of long-term memory and was accompanied by elevated phosphorylation levels of CREB (Genoux et al., 2002). Based on these studies, it was suggested that synaptic weakening negatively affects memory formation and promotes forgetting (assuming that LTP encodes memories). A second view is that both synaptic strengthening and weakening play a crucial role in memory formation (Willshaw and Dayan, 1990; Migaud et al., 1998). Using genetically engineered mice lacking CaN in distinct regions of the forebrain, Zeng et al (2001) showed that CaN is active in situations that require adaptation of previously stored information to match with changes in a familiar environment. In line with this, Runyan and colleagues (2005) showed that intervention of CaN activity in the prefrontal cortex impaired the acquisition of consecutive platform locations. Lin et al (2003) in addition revealed that blocking CaN activity in the basolateral amygdala impaired the extinction of previously acquired tone cued fear memories. Overall, these studies indicate that depending on the learning paradigm and approach used, phosphatases can function as a constraint or be necessary for memory formation.

6. AKAP150 regulates the cellular distribution of PKA and CaN

As mentioned above, protein kinases and phosphatases have selective targets in different cellular compartments ranging from receptor subunits in the synaptic ending to gene transcription factors in the nucleus. Therefore, it is very important to strictly regulate the intracellular distribution of the protein kinases and phosphatases. One mechanism to orchestrate the localization of kinases and phosphatase is via A-kinase anchoring proteins (AKAPs). The AKAP family consists of at least 50 members with different binding properties and subcellular distributions (Wong and Scott, 2004). One common feature of AKAPs is that they all bind PKA and target it to a specific location within neurons. One of the AKAPs that received particular attention is the mouse AKAP150 (a structural orthologue of the human AKAP79 and bovine AKAP75, Carr et al., 1992). In addition to PKA, AKAP150 binds and targets CaN to specific substrates like the GluR1 subunit and is strongly expressed in brain areas trivial for learning and memory (Glantz et al., 1992; Ostroveanu et al., 2007). AKAP150 is not merely a static protein, but plays an important role in synaptic plasticity since it (at least in part) controls the actions of PKA and CaN. In vitro studies have shown that blocking PKA binding to AKAP150 results in a loss of AMPA receptor surface expression as is observed under conditions of LTD (Snyder et al., 2005). Furthermore, it has been reported that, in hippocampal slices, brief NMDA receptor activation leads to persistent removal of AKAP150 and PKA, from post-synaptic membranes to the cytoplasm (Gomez et al., 2002; Smith et al., 2006). In contrast, the same treatment
does not evoke a significant translocation of CaN, suggesting that CaN dissociates from the AKAP150-PKA complex after brief NMDA receptor activation (Smith et al., 2006). Furthermore Moita and colleagues (2002) reported that injections with HT-31 (a peptide that inhibits the binding of PKA to AKAPs) in the basolateral amygdala reduced formation of long-term fear memories. Likewise, Nijholt and colleagues (submitted) showed that intra-hippocampal injections with HT-31 inhibited the formation of long-term memories for contextual fear. Overall these studies show that indeed AKAP-150 plays a crucial role in is required for the formation of long-term memories.

7. Sleep and hippocampal synaptic plasticity

Over the years it has become apparent that sleep has many functions including a role in processes underlying memory formation (Stickgold and Walker, 2005; Boonstra et al., 2007). Graves and co-workers (2003) revealed that sleep deprivation given 0-5 hours after training in a contextual fear conditioning paradigm impaired memory consolidation resulting in impaired performance during the retention test given 24 hours after the conditioning. In contrast, sleep deprivation given 6-10 hours after conditioning had no effect on memory consolidation. Furthermore, they showed that sleep deprivation did not interfere with tone-cued learning (a hippocampus-independent version of the task), suggesting that particularly the hippocampus is sensitive to sleep loss. Similar deficits have been found for another learning paradigm that requires the hippocampus (Palchykova et al., 2006a, 2006b). Looking at the effect(s) of sleep deprivation on hippocampal functioning at the molecular level, it has been suggested that loss of sleep affects LTP, NMDA receptor composition and surface expression in the hippocampus (Campbell et al., 2002; Davis et al., 2003; McDermott et al., 2003; McDermott et al., 2006; Kopp et al., 2006). In contrast to NMDA receptor function, the effect of sleep loss on AMPA receptor function has received scarce attention.

8. Aim and outline thesis

As described above, the contribution of hippocampal subregions in the detection of changes in a familiar environment has received considerable attention, but still remains unclear. The majority of these studies were based on subregion specific lesions or protein expression patterns of immediate early genes that can be activated via several cellular signaling pathways. Therefore, it is unclear which hippocampal signal transduction cascades play a role in the hippocampus in match-mismatch detection. The major aim of this thesis is to investigate whether cellular signaling cascades that are known to be involved in hippocampal memory formation are similarly involved in the detection of and adaptation to changes in a familiar environment (e.g., memory flexibility). Emphasis is given to the activity balance of the protein kinase PKA and the protein phosphatase calcineurin.

In the majority of the studies described in this thesis, we used a simple memory paradigm, the Y-maze reference task. The Y maze consists of a start arm and two test arms forming the Y. During training, mice could visit both accessible arms, but
only one of the two arms was baited. With ongoing training, mice progressively learned to locate the baited arm. After the animals learned to discriminate the baited arm, the food reward was relocated to the previously non-baited arm and subjects had to learn that the previously non-baited arm was now baited (reversal training).

In chapter 2, we explored the role of hippocampal CaN in the formation of new memories and the adaptation to changes in a familiar environment: Do Y-maze training and reversal training induce changes in hippocampal CaN expression, activity levels and protein levels? In the next set of experiments, we used a transgenic approach that allowed us to temporally suppress CaN activity in forebrain neurons. In chapter 3A, we tested whether reducing neuronal CaN activity in the forebrain altered the rate of acquisition during training and reversal training in the Y maze. In chapter 3B, the same transgenic mouse model was used as in chapter 3A, but in this study we used an alternative learning task namely contextual fear conditioning paradigm to investigate the role of CaN in learning and memory. Does reducing forebrain CaN activity affect consolidation and extinction of contextual fear memories?

Since the phosphorylation state of many substrates is dependent on the balance between PKA and CaN activity, the question remained whether training and reversal training induced changes in hippocampal PKA. Therefore, in chapter 4 we focused on hippocampal PKA and explored if training and reversal training in the Y maze changed hippocampal PKA expression and protein levels. Secondly, we investigated if training and reversal training evoked changes in the phosphorylation state of the AMPA receptor GluR1-S845 site that is targeted by both PKA and CaN (Chapter 4).

As described in the introduction, the hippocampus is an essential brain structure for the formation of new memories. One of the other brain regions that is also known to be particularly important in reward-motivated learning is the striatum. For that reason, in chapter 5, we studied PKA and CaN protein concentrations as well as AMPA receptor phosphorylation levels at the GluR1-S845 site in the striatum during learning and reversal learning in the Y maze.

Although the hippocampus is particularly sensitive to sleep loss, the cellular mechanisms underlying sleep deprivation-induced hippocampal dysfunctioning are hardly understood. In chapter 6, we investigated the effect of 6 and 12h of sleep deprivation on hippocampal AMPA receptor function and the underlying mechanisms. By studying the effect of sleep loss on learning and reversal learning (chapter 7). In this chapter, we applied 5 hours of sleep deprivation directly after each training session and determined if sleep deprivation affected the acquisition rate during training and reversal training in the Y maze. In addition, we described changes in the hippocampal expression of the immediate early genes c-Fos, Zif268 and the phosphorylation state of P44/P42 MAPK after Y-maze training with or without sleep deprivation. To further investigate the role of sleep in memory consolidation, in chapter 8 we analyzed the behavioral performance of mice with disturbed sleeping patterns (due to the loss of the Cry1 and Cry2 genes that are a crucial component of the circadian system). In relation to this thesis, the main question of this chapter was: Do disturbed sleeping patterns affect the acquisition rate during learning and reversal learning in the Y-maze? Chapter 9 summarizes and discusses all findings of thesis and provides an overall conclusion and future perspectives.
Chapter 1

9. References


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Brown TC, Tran IC, Backos DS, Esteban JA (2005) NMDA receptor-dependent activation of the small GTPase Rab5 drives the removal of synaptic AMPA receptors during hippocampal LTD. Neuron 45: 81-94.


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Nijholt IM, Ostroveanu A, Luiten PGM, Van der Zee EA, Eiseb UL. Inhibition of PKA anchoring to A-kinase anchoring proteins impairs the consolidation of contextual fear memories. Submitted.
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