From the Department of Neuroscience
Karolinska Institutet, Stockholm, Sweden

Neurochemical Regulators of the Septohippocampal Pathway: role in Spatial and Aversive Learning

Elin Elvander Tottie
MSc

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To my mother
- the greatest fighter of us all

Rows and floes of angel hair
And ice cream castles in the air
And feather canyons everywhere
I've looked at clouds that way

I've looked at clouds from both sides now
From up and down, and still somehow
It's cloud illusions I recall
I really don't know clouds at all

Joni Mitchell

ABSTRACT

The aim of this thesis was to investigate the significance of major neurotransmitters in the septohippocampal pathway for hippocampal-dependent learning and memory. The cholinergic and GABAergic neurons in the medial septal/vertical limb of the diagonal band of Broca area (MS/vDB) projecting to the hippocampus, constitute the septohippocampal pathway, which has been implicated in a number of important functions such as attention, anxiety-like behavior and hippocampal-dependent learning and memory. Both extrinsic and intrinsic neuroregulators can influence the activity of septohippocampal neurons, including acetylcholine (ACh), serotonin (5-HT), glutamate and the neuropeptide galanin.

It has previously been reported that cholinergic muscarinic transmission within the MS/vDB has an excitatory role and that blockade of septal muscarinic transmission impairs hippocampal-dependent learning. To test this hypothesis, the muscarinic receptor antagonist scopolamine was infused into the MS/vDB. Intraseptal scopolamine produced only a minor impairment in spatial acquisition in the Morris water maze, a hippocampal-dependent task, and also caused an increase in basal hippocampal ACh release. Contrary to earlier findings, the present results indicate that the MS/vDB cholinergic neurons are under an inhibitory muscarinic tone and that the impairing effects of systemic scopolamine cannot be related to an inhibitory action on MS/vDB cholinergic neurons.

The neuropeptide galanin, which is co-localized with cholinergic septohippocampal neurons, has been proposed to have an inhibitory role in hippocampal-dependent cognition. In contrast, intraseptal galanin enhanced hippocampal ACh release combined with a facilitation in spatial learning, i.e. galanin appears to excite, not inhibit, septohippocampal cholinergic neurons. The combination of galanin and scopolamine produced a marked impairment in spatial learning concomitant with a profound increase in hippocampal ACh release. This finding suggests that the level of muscarinic activity within the MS/vDB is important for the role of galanin in septohippocampal functions.

The 5-HT_{1A} receptors are located presynaptically in the raphe nuclei, regulating the firing rate of serotonergic neurons, and postsynaptically on a number of target neurons involved in cognitive functions. Stimulation of 5-HT_{1A} receptors by systemic administration of the agonist 8-OH-DPAT impaired spatial learning in the rat and produced a biphasic effect in an aversive learning task, i.e. passive avoidance (PA). Hence, lower doses facilitated whereas higher doses impaired PA memory in both rats and mice. The learning impairments were abolished by the 5-HT_{1A} receptor antagonist NAD-299, which in itself facilitated PA memory but failed to affect spatial learning. Furthermore, 5-HT_{1A} receptor blockade could eliminate the impairment in PA induced by a reduction in muscarinic or glutamatergic transmission, caused by scopolamine or the NMDA receptor antagonist MK-801. These findings support the view that pre- and postsynaptic brain 5-HT_{1A} receptors play different roles in learning and memory, and that blockade of brain 5-HT_{1A} receptors can enhance cholinergic and/or glutamatergic transmission of importance for cognition.

The high density of glutamatergic fibers and the evidence for an intrinsic glutamatergic system within the MS/vDB suggest that medial septal glutamatergic transmission is important for septohippocampal cognitive functions. Blockade of glutamatergic transmission in the MS/vDB by local infusion of the NMDA receptor antagonist D-AP5 impaired spatial learning at a dose of 5 μg. This impairment appears not to be caused by sensorimotor disturbances or changes in anxiety-like behavior. In contrast to spatial learning, lower doses of D-AP5 (0.3, 1 and 5 μg) impaired PA retention, suggesting that NMDA receptors in the MS/vDB play different roles in spatial vs. aversive (emotional) learning.

The 5-HT_{1A} receptor is located on both cholinergic and GABAergic neurons in the MS/vDB. Intraseptal infusion of 8-OH-DPAT impaired PA memory but did not affect spatial learning, suggesting that the 5-HT_{1A} receptors in the MS/vDB play a more important role in aversive than in spatial learning. Since stimulation of 5-HT_{1A} receptors has been shown to inhibit NMDA receptor signaling in the hippocampus, 8-OH-DPAT was microinjected together with a subthreshold dose of D-AP5 (1 μg). This combination caused a marginal but significant deficit in spatial learning, but a profound impairment in spatial memory in the retention test. This finding suggests that there exists an interaction between NMDA and 5-HT_{1A} receptors in the MS/vDB of importance for the establishment of a stable, long-term memory.

Acquisition of the water maze task involves multiple types of memories, subserved by different neuronal substrates. Learning of the behavioral procedure by non-spatial pretraining (NSP) was shown to improve water maze acquisition in control rats, but only initially. NSP also attenuated, but could not completely abolish, the spatial impairments caused by systemic scopolamine. These results indicate that acquisition of non-spatial information is important for subsequent spatial learning. Importantly, in contrast to earlier suggestions, brain muscarinic receptors appear to be important for spatial learning and memory, while they seem to play a minor role in acquisition of the behavioral procedure.

In summary, these results give evidence for a role for ACh, 5-HT and glutamate in the MS/vDB for hippocampal-dependent learning and memory. ACh and galanin interactions as well as 5-HT_{1A} and NMDA receptor interactions appear to have major roles in septohippocampal functions. These findings may have important implications for the development of treatments for cognitive impairments.

Keywords: acetylcholine, muscarinic receptor, galanin, serotonin, 5-HT_{1A} receptor, glutamate, NMDA receptor, water maze, passive avoidance, septum, hippocampus, learning, memory, spatial, aversive, emotional, cognition
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LIST OF PUBLICATIONS

This thesis is based on the following publications,
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II. LÜTTGEN M., **ELVANDER E.,** MADJID N. AND ÖGREN, S.O. (2005) Analysis of the role of 5-HT_{1A} receptors in spatial and aversive learning in the rat. *Neuropharmacology* 48:830-852.

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ABBREVIATIONS

5-HT	5-hydroxytryptamine; serotonin
8-OH-DPAT	(±)-8-hydroxy-2-dipropylaminotetralin hydrobromide; a 5-HT_{1A} receptor agonist
ABC	Avidin-biotin-peroxidase
aCSF	Artificial cerebrospinal fluid
Acetyl CoA	acetyl coenzyme A
ACh	Acetylcholine
AChE	Acetylcholine esterase
AD	Alzheimer's disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid
ANOVA	Analysis of variance
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CA	Cornu Ammonis; part of the hippocampal formation
ChAT	Choline acetyltransferase
CNS	Central nervous system
D-AP5	D-(-)-2-amino-5-phosphonopentanoic acid; a competitive NMDA receptor antagonist
D,L-AP5	DL-2-amino-5-phosphonopentanoic acid; a competitive NMDA receptor antagonist
DNMTP	Delayed non-matching to position
DNMTS	Delayed non-matching to sample
DR	Dorsal raphe nucleus
ECG	Electrocardiogram
GABA	γ-aminobutyric acid
GAD	Glutamatergic acid decarboxylase
GAL-R	Galanin receptor
hDB	Horizontal limb of the diagonal band of Broca
i.p.	Intraperitoneally
ir	Immunoreactive
LS	Lateral septum
LTD	Long-term depression
LTP	Long-term potentiation
MK-801	(5R, 10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine hydrogen maleate; Dizocilpine; a noncompetitive NMDA receptor antagonist
mRNA	Messenger ribonucleic acid
MR	Medial raphe nucleus
MS	Medial septal nucleus
MS/vDB	Medial septum/vertical limb of the diagonal band of Broca nuclei
NAD-299	®-3-N,N-dicyclobutylamino-8-fluoro-3.4-dihydro-2H-1-benzopyran-5-carboxamide hydrogen (2R,3R)-tartrate monohydrate; Robalzotan; a 5-HT_{1A} receptor antagonist
NE	North east
NMDA	*N*-methyl-D-aspartate
NSP	Non-spatial pretraining
PA	Passive avoidance
PBS	Phosphate-buffered saline
(*R*)8-OH-DPAT	(*R*)-(+)-8-hydroxy-2-(di-n-propylamino)tertralin
s.c.	Subcutaneous
SUM	Supramammillary area
US	Unconditioned stimulus
VAChT	Vesicular acetylcholine transporter
vDB	Vertical limb of the diagonal band of Broca
WAY-100635	N-(2-(1-(4-(2-methoxyphenyl)piperazinyl))ethyl)-N(2-pyridinyl) cyclohexanecarboamide trihydrochloride; a 5-HT_{1A} receptor antagonist

INTRODUCTION

LEARNING AND MEMORY

GENERAL ASPECTS

Cognitive functions refer to higher order mental processes, such as awareness, attention, judgment, reasoning, comprehension and use of speech, as well as learning and memory. The word cognition is derived from the Latin verb cognoscere, meaning to learn, to perceive or to know. Learning and memory can be broadly defined as those processes by which individuals acquire knowledge, skills and experience, which is retained and results in adaptive behavior. Neurobiological theories generally assume that activity-dependent changes in synaptic plasticity are the critical components underlying learning and memory. Current research is, therefore, focused on characterizing the adaptive changes between neurons at synaptic sites and in neuronal networks, as they are modified by experience. Accumulating evidence suggest that the mechanisms of neuronal adaptations depend on the properties of the transmission network. A number of neurotransmitter and molecular systems are believed to regulate short- and long-term changes in neuronal plasticity and synaptic strength in neurons and neuronal networks (KANDEL, 2001).

Memory involves time-dependent processes that can be separated into a series of distinct events, i.e. encoding, consolidation, storage and retrieval. *Encoding* means literally to convert information into a code, i.e. the word refers to the way in which the received information is attended to, processed and prepared for subsequent storage in a memory. Naturally, more elaborate and detailed encoding, will result in increased memory strength (see SQUIRE & KANDEL, 2000). The concept of *consolidation* was first described more than a 100 years ago by George Elias Müller and Alfons Pilzecker (1900). These authors hypothesized that memory is not formed instantly but need time to be fixed, i.e. consolidated, and also proposed that memory during the consolidation process is vulnerable to disturbance (MÜLLER & PILZECKER, 1900). *Storage* of memories is the result of the consolidation process i.e. the final long-term memory. However, there is evidence that even long-term memories can be modified over time (BARTLETT, 1932). Maintaining information over time appears to be mediated through different brain areas depending on the type of memory, which are further discussed below. Finally, *retrieval* of memories, i.e. recollection or remembering, is the mnemonic process by which stored information is recalled and incorporated in ongoing behavior (see BYRNE, 2003).

The neurochemical and molecular mechanisms underlying the various stages in memory processing are still not well characterized. However, it is clear that different neurotransmitters play differential roles in encoding and consolidation of new memories (see below). Moreover, the molecular cascades involved in consolidation processes include new protein synthesis, changes in synaptic proteins and dendritic growth (see BYRNE, 2003; see SQUIRE & KANDEL, 2000).

MULTIPLE MEMORY SYSTEMS IN THE BRAIN

Studies of patients such as H.M., who suffered from severe amnesia as a result of medial temporal lobe resection (i.e. bilateral hippocampectomy), lead to the first insight that there are different memory systems in the brain (SCOVILLE & MILNER, 1957). Subsequent neuropsychological studies have indicated that memory can be divided into multiple psychological systems that process particular kinds of information. These memory systems are now classified into two major forms of long-term memory, declarative or nondeclarative (SQUIRE & ZOLA-MORGAN, 1988; TULVING, 1983) (Figure 1). Declarative (explicit) memory involves conscious recollection of facts and events and includes also knowledge about spatial and temporal contexts. The ability to recollect these types of memories is lost in amnesia (SEE POLDRACK & GABRIELI, 1997; SQUIRE & ZOLA-MORGAN, 1988). Nondeclarative (implicit) memory refers to a heterogeneous group of memories including motor/perceptual skills as well as priming, and is typically expressed as a change in

performance, e.g. increased accuracy or reduced response time. It is important to note that the same event can result in different types of memories involving both declarative e.g. place and time, as well as nondeclarative, e.g. fear and autonomic responses.

Of major importance is the finding that declarative and nondeclarative memory are dependent on different anatomical substrates (for reviews, see KIM & BAXTER, 2001; POLDRACK & GABRIELI, 1997; SCHACHTER & TULVING, 1994) (Figure 1). Studies in amnesic patients and lesion studies in monkeys, has shown that the medial temporal lobe and the diencephalon play a pivotal role in declarative memory (SCOVILLE & MILNER, 1957; SQUIRE, 1994; SQUIRE & ZOLA-MORGAN, 1991; ZOLA-MORGAN et al., 1986). In the medial temporal lobe, the hippocampal formation (see below) is of major significance and, if injured, causes severe damage to declarative memory function (SCOVILLE & MILNER, 1957). Nondeclarative memories engage in particular the basal ganglia, involved in motor and instrumental learning, and the cerebellum, which plays an important role in classical conditioning (see SQUIRE, 1994; THOMPSON & KRUPA, 1994) (Figure 1).

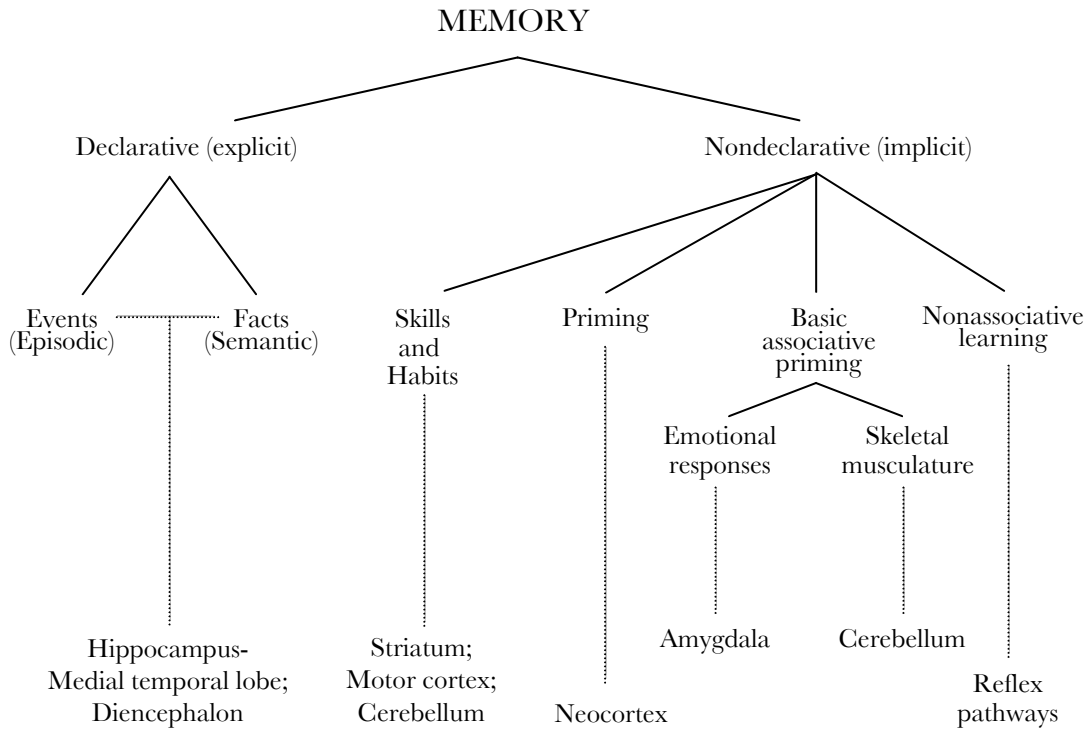


FIGURE 1. Classification of long-term memory systems and the brain structures involved. Long-term memory can be divided into declarative and nondeclarative memories. Declarative memory refers to the capacity for conscious recollection of facts and events, whereas nondeclarative memory involves e.g. changes in skilled behavior or conditional emotional responses. Modified from (MILNER et al., 1998; SQUIRE & ZOLA-MORGAN, 1988).

Time-dependent mechanisms of memory

The brain processes information in a time-dependent manner. Short-term memories refer to those processes that only temporarily handle information before it is retained and consolidated into long-term memory. The information held on-line in a short-term memory buffer seems to mainly involve the prefrontal cortex (FAW, 2003; LEE & KESNER, 2003). In contrast, the medial temporal lobe is essential for the conversion of short-

term memory into long-term declarative memories (SCOVILLE & MILNER, 1957; ZOLA-MORGAN et al., 1986), but its role seems to be time-limited. This hypothesis is based on the observation that there is a temporal gradient of retrograde forgetfulness in patients with amnesia (see POLDRACK & GABRIELI, 1997; SQUIRE & ALVAREZ, 1995). Moreover, early findings by Théodule Ribot (1839-1916), indicated that memory loss in amnesia was related to the age of the memory, i.e. to their grade of consolidation. Later studies also support the view that the medial temporal lobe plays an essential role in the encoding of new memories, while permanent memory storage occurs in the neocortex (see POLDRACK & GABRIELI, 1997). Recent findings, however, indicate a more complex role of different memory systems in the process of learning (see KIM & BAXTER, 2001). Thus, complex interactions between different memory systems seem to occur in any given memory task. This means that multiple memory systems are engaged even in learning tasks classified as "simple" (see KIM & BAXTER, 2001).

In contrast to earlier theories, more recent findings suggest that the hippocampus do play a role also in the retrieval of certain forms of memories, namely episodic and context-dependent memories (NADEL & MOSCOVITCH, 1997). This hypothesis, the "multiple trace theory", proposes that the spatial and temporal contextual information contributing to the episodic aspect of a memory will always, independent of time, engage the hippocampus (spatial) and frontal cortex (temporal) (NADEL & MOSCOVITCH, 1997). However, semantic memories (vocabulary, facts etc.) with no contextual setting appear to be retrievable without involving the hippocampus (see MOSCOVITCH et al., 2005; ROSENBAUM et al., 2001).

COGNITIVE DISORDERS

Cognitive impairment is the hallmark of many neurodegenerative disorders such as dementia and Parkinson's disease, as well as anxiety-related disorders, depression and schizophrenia. Moreover, cognitive dysfunctions are often seen following stroke, head-trauma or brain tumors (see KOPELMAN, 2002; see ZAKHAROV et al., 2001). Dementia is defined as a degenerative brain disease manifested by a progressive impairment of both short- and long-term memory functions as well as personality changes. Dementia consists of several pathological disorders, of which Alzheimer's disease (AD) is the dominant cause, accounting for about 50% of the cases. Magnetic resonance imaging studies of the brain in early AD cases have shown atrophy of the medial temporal lobe, in particular the hippocampus, compared to the control group (DE LEON et al., 1993). AD is also characterized by a progressive loss of neurotransmitter functions in areas of the brain associated with learning and memory, such as the hippocampus and cortex. Although several neurotransmitters are affected in AD, it is a general agreement that loss of cholinergic functions in the cortex and hippocampus are of importance for the cognitive impairment seen in this disease (DAVIES & MALONEY, 1976; PERRY et al., 1977; WHITEHOUSE et al., 1982). Drugs which increase the amount of acetylcholine (ACh) within the central nervous system (CNS) has until recently been the only medical alternative available for symptomatic treatment of the disease.

Cerebral hypoxia resulting from e.g. ischemic stroke can induce an amnesic syndrome. It has been shown that the hippocampus, in particular the CA1 area, is especially sensitive to hypoxia (ZOLA-MORGAN et al., 1986). Also head injuries can induce transient as well as permanent amnesia, while patients who suffers from mild concussion often report forgetfulness (see KOPELMAN, 2002). Moreover, subarachnoid haemorrhage as a result from aneurysm may cause memory impairments, e.g. ruptured aneurysms in the anterior communicating artery, resulting in damage of the septal nuclei (see below) (see CRAMON & MARKOWITSCH, 2000; GADE & MORTENSEN, 1990).

ANIMAL MODELS FOR STUDYING LEARNING AND MEMORY

Mechanisms underlying learning and memory can be studied by various approaches. One is based on trying to find correlations between changes in behavior as a result of training, and alterations in molecular and neurochemical mechanisms in the brain (see SQUIRE & KANDEL, 2000). The second approach, which is used in the present studies, is to train and test animals under the influence of manipulations of neurotransmitter systems linked to cognitive processes. In the latter approach, changes in synaptic plasticity are indicated by

the observed alteration in learned performance by the animal. This approach is based on two important assumptions, namely that it is possible to distinguish between learning and performance, and the selectivity of the manipulation. It is therefore preferable to select tasks which involve relatively distinct anatomical circuits such as spatial learning (see below) (MILNER et al., 1998).

One of the first scientists to study learning and memory through behavioral experiments was the Russian physiologist Ivan Pavlov (1849 – 1936) who, with his famous studies in dogs, discovered the psychological mechanisms underlying classical conditioning. There are today many behavioral models used to study different brain mechanisms of importance for learning and memory. However, even though many of these learning tasks appear simple, they represent a great challenge when trying to understand their biological mechanisms. Moreover, it must be emphasized that memory can only be inferred from a change in behavior, which means that molecular or neurochemical changes in the brain, without any behavioral correlate, cannot be interpreted.

SPATIAL BEHAVIOR

The ability of animals, including humans, to navigate and orient in the environment is of crucial importance for survival, e.g. for an animal to find the way to previously buried food or return to home. Spatial memory is critical for all species, since in the process of finding a way to a given place, the final destination is usually not visible and navigation must depend on memory of specific cues along the route (see HARTLEY ET AL., 2003). Spatial behavior has been classified in two major categories, namely taxon and local navigation, as defined by (O'KEEFE & NADEL, 1978). Taxon refers to the use of landmarks as cues, which an animal can move towards or away from. Local navigation involves the integration of several landmarks (cues) that together identify an area within which an animal can calculate a path. Another classification often used in the analysis of spatial behavior is egocentric versus allocentric (world-centered) processes. These processes refers to the way by which an animal navigate in the environment either by using cues and landmarks with respect to the location of its own body, or in relationship to each other, respectively (see JEFFREY, 2003).

Motivational systems in behavior

Learning and memory in rodents and in man depend on different drives or motivations. For instance, operant conditioning is based on positive reinforcement, i.e. the presentation of a reward, e.g. food, following a particular response, e.g. pressing a lever. Aversive conditioning and spatial learning, on the other hand, are based on negative reinforcement. In passive avoidance (PA), the acquisition of the behavioral response is motivated by the drive to avoid a test environment earlier associated with an aversive, unpleasant event, i.e. presentation of an electrical shock. In spatial learning, the rat is motivated to escape from a water maze, since rats experience swimming in water as slightly aversive. Aversively motivated behavior can therefore be defined as a behavior directed away from an object, context or situation, which has been, or is, associated with aversive experience. The adaptive behavior results in an escape from, or avoidance of, the context linked to the memory of the unpleasant event.

Cellular basis for spatial behavior

The biological and cellular basis for spatial behavior has been attributed to a group of cells in the hippocampus, which seems to code for features in the environment. In the early 1970's, O'Keefe and Dostrovsky discovered what they referred to as "place cells" (O'KEEFE, 1976; O'KEEFE & DOSTROVSKY, 1971), i.e. hippocampal pyramidal neurons that fire when the animal is in a specific spatial location in an environment. Assemblies of "place cells" that fire together in relation to environmental cues were suggested to provide a "cognitive map" of the environment. The "cognitive map" theory of hippocampal function suggests that the hippocampus acts as an allocentric mapping system (O'KEEFE & NADEL, 1978). It was later shown, that when an animal is placed in a novel environment, "place cells" forms rapidly and are maintained for a long period of time (WILSON & MCNAUGHTON, 1993). However, subsequent studies have suggested that hippocampal neurons fire not only in association with the animal's location, but also in relation to

ongoing behavior and the context of events the animal is in. Thus, hippocampal neuronal activity capture both spatial and non-spatial features, indicating that "place cells" appear not to have a unique role in spatial memory processes (EICHENBAUM, 2004; EICHENBAUM et al., 1999).

NEUROANATOMICAL BASIS FOR LEARNING AND MEMORY

THE HIPPOCAMPAL REGION

Anatomy

The hippocampal formation is regarded a phylogenetically old part of cerebral cortex (archicortex) and is shaped as an elongated structure, which in humans is located in the ventral-lateral wall of the lateral ventricles in each temporal lobe. In the rat, the hippocampal formation is a *C*-formed structure extending from the area of the basal forebrain, reaching over and behind the diencephalon and then down caudoventrally into the temporal lobes (Figure 2).

The hippocampal region, as defined by WITTER & AMARAL (2004), includes the hippocampal formation and the adjacent parahippocampal region. The hippocampal formation consists of three cytoarchitectonically distinct regions, namely the dentate gyrus, the hippocampus proper (which is subdivided into CA3, CA2 and CA1; CA = Cornu Ammonis) and the subiculum. The parahippocampal region, in turn, includes the entorhinal and perirhinal cortices, together with the postrhinal (in nonprimate mammals) or parahippocampal (in primates including humans) cortex (WITTER & AMARAL, 2004; WITTER et al., 2000). Information from association areas of the neocortex reaches the hippocampal formation mainly via the entorhinal cortex. The projection from the entorhinal cortex, the perforant path, terminates on the dendrites of the granule cells in the dentate gyrus, i.e. the molecular cell layer. The granule cells give rise to the mossy fibers that project to the CA3, from which the Schaffer collaterals projects to the CA1 area of the hippocampus. The pyramidal cells in CA1 then project out of the hippocampus via subiculum/entorhinal cortex to cortical areas (AMARAL & WITTER, 1989; WITTER & AMARAL, 2004). This pathway is referred to the trisynaptic pathway (Figure 3). The pyramidal and granule cells, which are glutamatergic, represent about 90% of the hippocampal neurons while the remaining 10% are GABAergic (γ-aminobutyric acid; GABA) interneurons. These neurons synapse on both other interneurons as well as principal cells to control and regulate the action of the neuronal firing within the hippocampus (see FREUND & BUZSAKI, 1996).

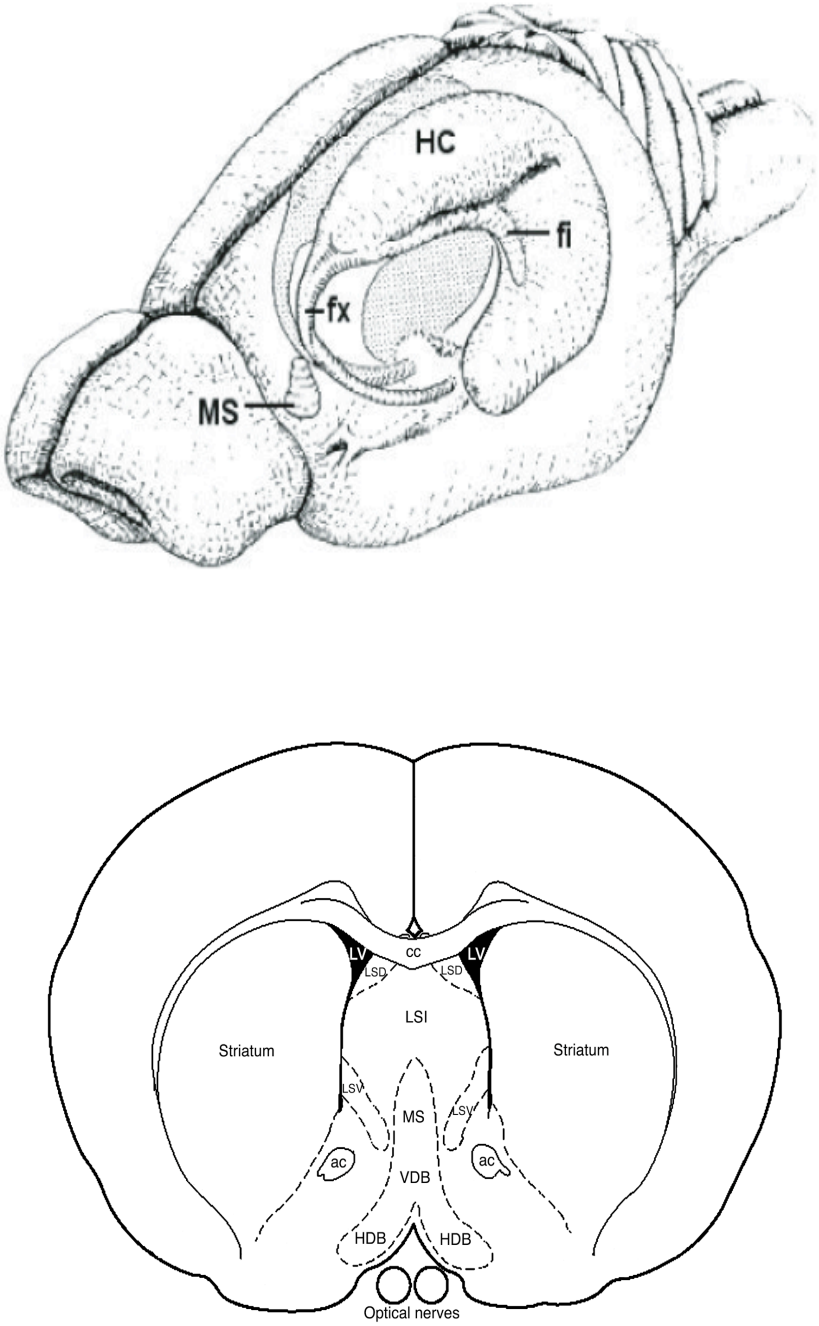


FIGURE 2. *Top*: A three-dimensional drawing of the localization of the MS/vDB and the hippocampus in the rat brain. The large, C-shaped structure is the hippocampus, the fi/fx represents the fimbria-fornix, i.e. the fiber bundle that carries the majority of information to and from the MS/vDB. Abbreviations: MS = medial septum; HC = hippocampus; fi = fimbria; fx = fornix. Modified from (AMARAL & WITTER, 1995).
Bottom: Coronal section of the rat brain at the level of the MS/vDB (+0.2 mm from bregma; (PAXINOS & WATSON, 1998)). Abbreviations: MS = medial septum; VDB = vertical limb of the diagonal band of Broca; HDB = horizontal limb of the diagonal band of Broca; LSD = lateral septum, dorsal part; LSI = lateral septum, intermediate part; LSV = lateral septum, ventral part; LV = lateral ventricle; ac = anterior commissure; cc = corpus callosum.

The cortical input to the hippocampus is the main source of information for hippocampal mnemonic functions. However, there are also afferents from subcortical areas, e.g. the septum, hypothalamus and brain stem, which have a modulatory role in hippocampal function. This input is suggested to inform about the "behavioral state" of the animal (WITTER & AMARAL, 2004). These afferents include noradrenergic fibers from the locus coeruleus, dopaminergic from the substantia nigra, serotonergic from the raphe nuclei and cholinergic from the medial septum (see below) (see AMARAL & KURZ, 1985; see AMARAL & WITTER, 1995; see DUTAR et al., 1995; see JAKAB & LERANTH, 1995; see WITTER & AMARAL, 2004).

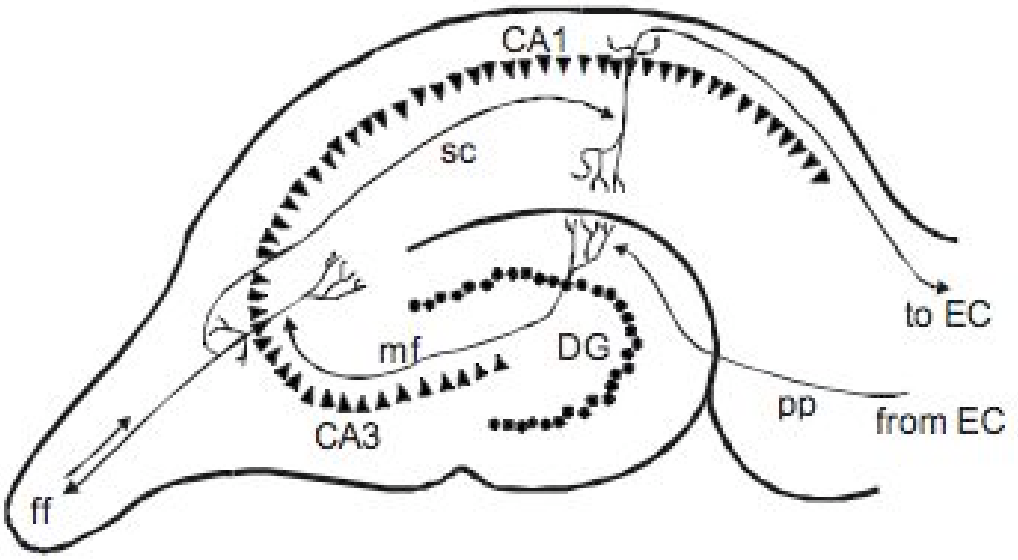


FIGURE 3. Schematic picture of the trisynaptic pathway in the hippocampus. The perforant path enters the hippocampus from the entorhinal cortex and synapse on the dendrites of the granule cells (black circles) in the molecular cell layer. These give rise to the mossy fiber pathway, which project to the CA3 pyramidal cells (black triangles) that in turn, via the Schaffer collaterals, synapse on pyramidal cells in the CA1, which project out from the hippocampus to the subiculum. Abbreviations: EC = entorhinal cortex; DG = dentate gyrus; pp = perforant path; mf = mossy fibers; sc = Schaffer collaterals; ff = fimbria fornix.

THE MS/VDB AREA

The septal region is an integrated part of the limbic brain system, located between the anterior horns of the lateral ventricles (septum = *saeptum* in Latin; a dividing wall or membrane), dorsal to the midline part of the anterior commissure and ventral to the anterior and middle regions of the corpus callosum (Figure 2). It can be regarded as an interface between the diencephalon and telencephalon with massive, reciprocal connections. This allows for an important role in a number of physiological and behavioral processes, which are related to higher cognitive functions such as learning, memory, emotions, fear and stress (see JAKAB & LERANTH, 1995).

Anatomy of the septal region

Ramon y Cajal was the first to describe the anatomy of the septum in detail, and classified it as being a part of the basal ganglia, in contrast to earlier suggestions that the septum is a part of the cerebral cortex. It is as of today no general agreement as to the classification of the septum, nor to the boundaries defining the septal area of the brain. According to Jakab & Leranth, the septal region can be divided into three parts, the lateral septum (LS), medial septum/diagonal band of Broca and the posterior septum (see JAKAB & LERANTH, 1995). Some authors also include the bed nuclei of the stria terminalis as a fourth, ventral group of the septal region (see RISOLD, 2004; see SWANSON & RISOLD, 2000).

The medial septum/diagonal band of Broca area consists of the medial septal nucleus (MS) and the adjacent, continuous diagonal band of Broca (DB), which includes two parts; the vertical (vDB) and the horizontal (hDB) limb of the DB (Figure 2). There are no clear, anatomical boundaries between the MS and the vDB and there are neurochemical and functional similarities between them, why they are referred to in this text as the medial septal/vertical limb of the diagonal band of Broca complex, i.e. MS/vDB. The LS, in turn, is divided into three parts, namely the dorsal, intermediate and ventral parts (see JAKAB & LERANTH, 1995). During development, the nuclear groups in the midline of the septum are formed first, followed by the lateral septal neurons. This medial to lateral gradient forms a laminated organization of septal neurons, appearing as an onion-skin-like pattern. MS/vDB can be further divided into a midline, a lateral and a MS-LS border zone as a result of its chronotopic development. Even though classified as one structure, the functional roles of the MS/vDB and the LS are different. Whereas the MS/vDB predominantly relays and integrates information ascending from the diencephalon to the telencephalon, the role of the LS is primarily to mediate information descending from the telencephalon to the diencephalon (see JAKAB & LERANTH, 1995).

Projections and neurotransmitters of the MS/vDB

The MS/vDB integrates and relays information originating from e.g. the brainstem, supramammillary area (SUM), hippocampus, entorhinal cortex and frontal cortex, which is conveyed by e.g. cholinergic, serotonergic, GABAergic and glutamatergic afferents (JASKIW et al., 1991; KÖHLER et al., 1982; LERANTH et al., 1999; LERANTH & KISS, 1996; TOTH & FREUND, 1992; WOOLF & BUTCHER, 1986) (Figure 5). In turn, the cells in the MS/vDB project primarily to the hippocampus but also, however less extensively, to the entorhinal and cingulate cortices.

The projection from the MS/vDB to the hippocampus is arranged in a clear mediolateral topographic manner. Hence, the lateral MS/vDB neurons predominantly projects to the ventral/temporal aspects of the hippocampus and the medial portion of the entorhinal cortex, whereas the MS/vDB cells located more medially innervate more septal/dorsal parts of the hippocampus and more lateral parts of the entorhinal cortex (GAYKEMA et al., 1990).

The two major neurotransmitters used by the principally projecting septohippocampal neurons in the MS/vDB are ACh and GABA (BRASHEAR et al., 1986; KISS et al., 1990; KÖHLER & CHAN-PALAY, 1983; KÖHLER et al., 1984; WOOLF, 1991). These two neurochemically identified neuronal populations are organized in a laminated pattern, where parvalbumin-containing GABAergic neurons are predominantly located in the midline zone, whereas choline acetyltransferase (ChAT)-positive neurons are primarily found in the lateral zone of the MS/vDB (see JAKAB & LERANTH, 1995; LÜTTGEN et al., 2005) (Figure 4). Within the MS/vDB, cholinergic axon collaterals terminate on GABAergic MS/vDB neurons (BRAUER et al., 1998) and vice versa, i.e. GABAergic axon collaterals terminate on cholinergic MS/vDB neurons (ALREJA et al., 2000; LERANTH & FROTSCHER, 1989)

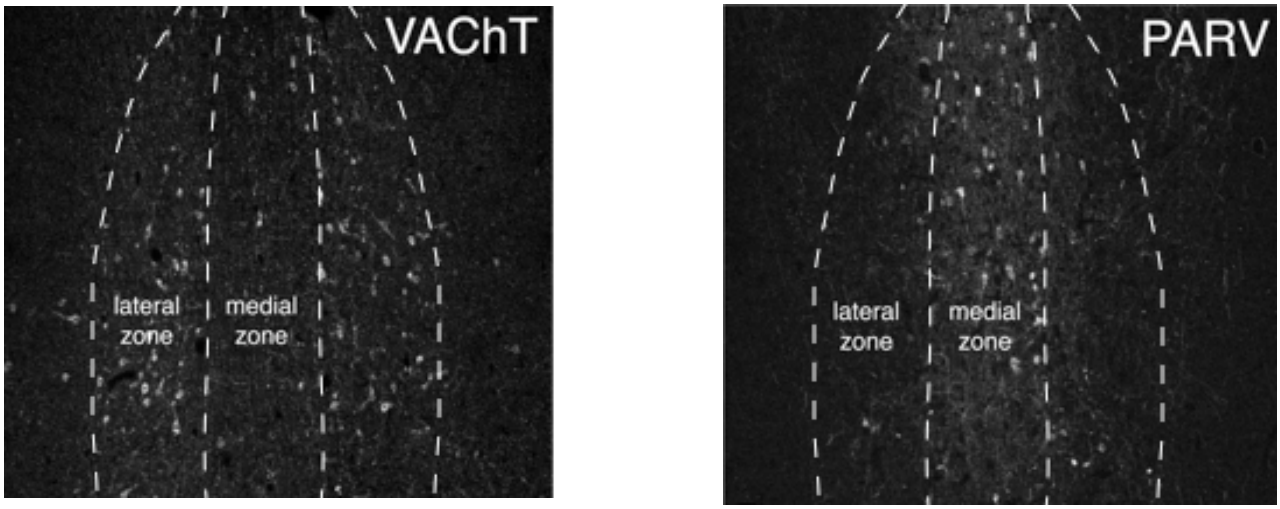


FIGURE 4. Immunofluorescence photomicrographs of sections from the MS/vDB illustrating the laminated pattern of neurochemically identified neurons. As seen in the left picture, vesicular acetylcholine transporter-immunoreactive (VAChT-ir) cells are predominantly seen in the lateral zone of the MS/vDB, whereas parvalbumin-positive cells primarily occupy the medial aspects of the MS/vDB (right). Modified from (LÜTTGEN et al., 2005).

The cholinergic MS/vDB neurons projecting to the hippocampus terminate on principal pyramidal cells as well as on GABAergic interneurons in the hippocampus (FROTSCHER & LERANTH, 1985; WAINER et al., 1984). In contrast, the GABAergic projecting cells terminate exclusively on hippocampal GABAergic interneurons, allowing for a disinhibition of the pyramidal cells (FREUND & ANTAL, 1988; KÖHLER et al., 1984; TÓTH et al., 1997). The projection between the MS/vDB and the hippocampus is reciprocal, i.e. there is a septo-hippocampo-septal loop (Figure 5). Thus, the hippocampus sends descending efferents to the MS/vDB as well as to the LS. The major part of the hippocampo-septal projection consists of glutamatergic neurons projecting to GABAergic neurons in the LS, which in turn project mainly to the hypothalamus and medial amygdala (JAKAB & LERANTH, 1995), but also to the MS/vDB even though this path seems to be sparse (see JAKAB & LERANTH, 1995; LERANTH et al., 1992; RISOLD & SWANSON, 1997). There are also connections from the hippocampus directly to the MS/vDB that are primarily GABAergic, which terminate on both cholinergic and GABAergic MS/vDB cells (ALREJA et al., 2000; GULYAS et al., 2003; see JAKAB & LERANTH, 1995; LERANTH & FROTSCHER, 1989).

In addition to the septo-hippocampo-septal connection, there exist also an entorhinal-septo-SUM reciprocal connection (Figure 5). Hence, glutamatergic neurons in the SUM innervate both MS/vDB septohippocampal cholinergic and GABAergic neurons, as well as hippocampal principal cells (BORHEGYI et al., 1998; LERANTH & KISS, 1996; MAGLOCZKY et al., 1994; see PAN & MCNAUGHTON, 2004; VERTES, 1992). Moreover, aspartate/glutamatergic cells in the entorhinal cortex synapse on MS/vDB GABAergic neurons located mainly at the border between MS/vDB and LS, from where GABAergic cells project back to the SUM (BORHEGYI & FREUND, 1998; LERANTH et al., 1999). As a consequence, excitatory (aspartate/glutamatergic cells) entorhinal cortical neurons are able to stimulate septo-SUM GABAergic cells that terminate on non-GABAergic (partly aspartate/glutamatergic) SUM neurons, which in turn ascend back and innervate MS/vDB and hippocampal neurons (Leranth et al., 1999) (Figure 5).

These subcortical and cortical reciprocal connections with the MS/vDB allow for groups of septal neurons to work as an ensemble, and blockade of synaptic transmission might interfere with the

synchronization of neuronal activity in the MS/vDB and thereby influence hippocampal functions (see HASSELMO et al., 2002; see VERTES & KOCSIS, 1997).

AMYGDALA

Amygdala is an almond-shaped structure (*amygdalum* = almond in Latin) located in the anteriomedial part of the temporal lobe and consists of four nuclei; the lateral, the basal, the accessory basal and the central amygdala nuclei (see ALHEID et al., 1995; see LEDOUX, 1993). Results from studies in humans as well as animals points towards an important role for the amygdala, in conjunction with closely related brain regions such as the hippocampus, in the acquisition, storage and expression of long-term memories for emotional events and in the response to environmental stimuli that signal threat (see DAVIS, 1997; see LEDOUX, 1993; see LEDOUX, 2000). Electrical stimulation of the amygdala in humans evokes feelings of fear and anxiety, and patients with damage to the amygdala show impairments in e.g. the recognition of facial expressions of fear (see BEGGS et al., 1999).

Anatomically, the lateral nucleus of the amygdala receives sensory information from e.g. thalamus, neocortex and olfactory cortex. This information is then projected via the basal nucleus to the central nucleus of the amygdala, which in turn mediate the emotional responses through projections to the brainstem (behavior) and hypothalamus (autonomic) (see LEDOUX, 1993; see LEDOUX, 2000). Moreover, there exist projections from the basal and lateral nuclei of the amygdala to the entorhinal cortex, subiculum, CA3 and CA1 areas of the hippocampal formation (PIKKARAINEN et al., 1999).

It is presently not clear whether the amygdala serves as the actual site of long-term memory storage, or whether its primary role is to modulate memory consolidation in other brain structures such as the hippocampus (see RICHTER-LEVIN, 2004). The observation that late phase long-term potentiation (LTP) (see below) can occur in the lateral and basal nuclei of the amygdala (DOYERE et al., 2003; YANIV et al., 2003) give evidence for a possible role of the amygdala in memory storage. However, a growing body of evidence implicates also a role for e.g. the hippocampus in some types emotional memory, i.e. when there exist spatial, contextual and/or relational processing. Damage to the hippocampus disrupts contextual conditioning and impairs acquisition of conditioned fear when the conditioned stimulus is a context (e.g. the dark compartment of a PA apparatus) (see MAREN, 2001; see WHITE & MCDONALD, 2002)

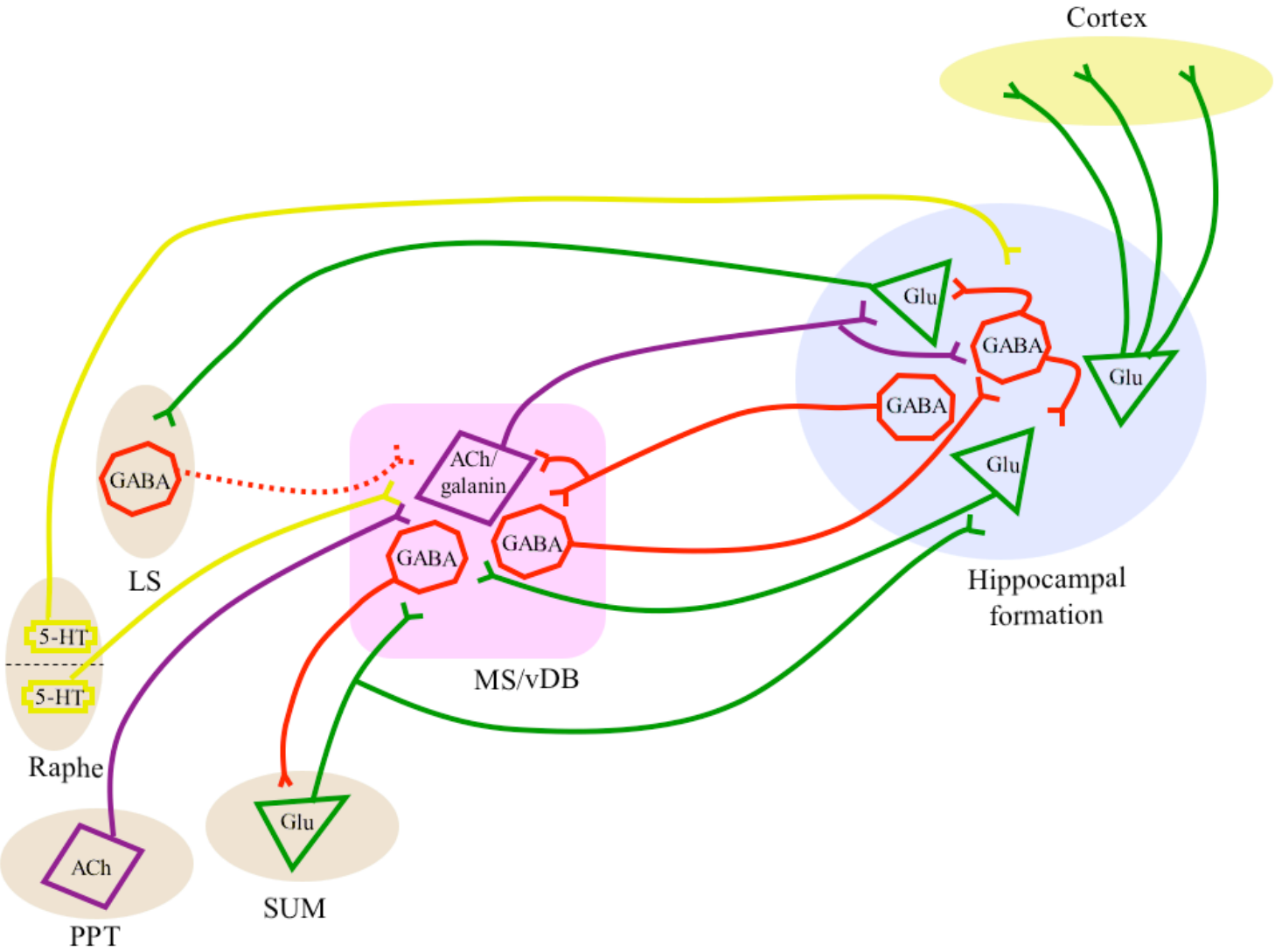


FIGURE 5. Simplified schematic figure illustrating the neurochemical projections connecting the hippocampus with the MS/vDB. In short, cholinergic and GABAergic neurons project to the hippocampus, which in turn projects back to the MS/vDB, forming a septo-hippocampal-septal loop. In turn, the MS/vDB receives glutamatergic input from the SUM, and sends GABAergic afferents back to the SUM. In addition, the SUM sends glutamatergic efferents directly to the hippocampal formation, allowing for a reciprocal hippocampo-septo-SUM loop. 5-HT neurons in the DR and MR projects to the hippocampus and MS/vDB, respectively. The MS/vDB also receives cholinergic input from the pedunculopontine tegmental nucleus (PPT). The information in the hippocampus is processed and conveyed to cortical areas. See text for further details. Modified from (JAKAB & LERANTH, 1995; PAN & MCNAUGHTON, 2004; RISOLD, 2004; VERTES et al., 2004)

NEUROTRANSMITTERS IN LEARNING AND MEMORY

Neurons in the brain communicate with each other mainly via chemical transmission. Neuronal activity results in the release of neurotransmitters from the axon terminal into the synaptic cleft, where they bind to receptors located on pre- or postsynaptic neurons and exerts their physiological effect. Chemical neurotransmission is tightly controlled both at the cell body level as well as at the terminal level by different pre- and postsynaptic autoregulatory mechanisms, which adjust transmission to the requirements of the neurons. This fine tuning of chemical neurotransmission can result in a change in neuronal activity indicated by an alteration in neuronal firing rate, a change in the amount of transmitter release per neuronal impulse or, finally, by changes in pre- or postsynaptic receptor functions and intracellular second-messenger systems. The available evidence suggest that changes in multiple neurotransmitter mechanisms underlie alterations in neuronal plasticity, expressed as a change in learning and memory (see KANDEL, 2001).

GLUTAMATE

Glutamate is a nonessential amino acid and is the predominant excitatory neurotransmitter in the mammalian brain. It plays a pivotal role in e.g. cognitive and motor functions (see GREENAMYRE & PORTER, 1994; see GRILLNER, 2003). It was first discovered in 1954 (HAYASHI, 1954), and subsequent studies have shown a relatively even distribution of glutamate in the brain, where numerous pathways uses glutamate as a transmitter (see BROMAN et al., 2003).

The synthesis of glutamate can occur in two ways: from glucose via the Krebs cycle and transamination of α-oxoglutarate, or from glutamine that is synthesized in glial cells and transported into the nerve terminal where it is converted to glutamate by the enzyme glutaminase. Once in the nerve terminal, glutamate is stored in synaptic vesicles, which upon depolarization will release glutamate into the synaptic cleft. Plasma membrane glutamate transporters on the presynaptic nerve terminal and/or on glial cells terminate the action of released glutamate through a high-affinity uptake process (see COOPER et al., 2003).

The hippocampus receives glutamatergic input from neocortical association areas, and it utilizes glutamate as the transmitter within the trisynaptic pathway (see below). There is also evidence for glutamatergic input to the MS/vDB from the enthorinal cortex, SUM, frontal cortex and nucleus reuniens thalami (BOKOR et al., 2002; JASKIW et al., 1991; LERANTH et al., 1999; LERANTH & KISS, 1996). Moreover, intrinsic glutamatergic neurons have been observed within the MS/vDB (HAJSZAN et al., 2004; MANSEAU et al., 2005) and a recent study have also suggested a glutamatergic septohippocampal projection (SOTTY et al., 2003).

Glutamatergic receptors can be divided into two groups, metabotropic and ionotropic receptors. The metabotropic receptors are G-protein coupled receptors linked to intracellular second messengers, mediating a slower and more modulatory transmission mechanism than that of the ionotropic. There are three classes of ionotropic receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptors, named after the agonists activating each specific class of receptor. Research on glutamate receptor function in learning and memory has mainly focused on AMPA and NMDA receptors, which are suggested to play distinct roles in synaptic plasticity (see above) (see RIEDEL et al., 2003). The AMPA receptors, which have a wide distribution in the CNS, are ligand-gated ion channels and mediate a fast, immediate postsynaptic response to glutamate release. The NMDA receptors are also ligand-gated ion channels and is distributed throughout the entire CNS with particular high receptor densities in the hippocampus and cerebral cortex (see COOPER et al., 2003; see RIEDEL et al., 2003). This receptor has a slower onset of action and seems to play an important role in synaptic plasticity.

Synaptic plasticity

The research on synaptic plasticity has been very much influenced by the innovative theories presented by the psychologist Donald Hebb in 1949. He hypothesized that connections between neurons increase in strength in proportion to the degree of correlation between pre- and postsynaptic activity. The phenomena of this synaptic change, "Hebb's synapse", was described as: "When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (HEBB, 1949). In addition, Hebb suggested that neurons that fire together can form cell assemblies, which serve to represent the sensory patterns that contribute to the sensory input, i.e. the "cell-assembly" hypothesis. These cell assemblies connect to each other when they are successively activated, and these connections are dependent on neuronal plasticity.

There is today a general agreement that changes in synaptic strength, and neuronal plasticity, have a pivotal role in mechanisms underlying learning and memory. Two forms of long-lasting synaptic plasticity have been described in the mammalian brain; long-term potentiation (LTP) and long-term depression (LTD), characterized by an increase or decrease in synaptic strength, respectively. Hippocampal LTP was first discovered in the dentate gyrus, indicated by an increase in the efficiency of synaptic transmission and in excitability of the granule cells following a brief, high frequency stimulation of the perforant path (BLISS & LOMO, 1973). LTP can be either associative or nonassociative, where the latter refers to LTP that is induced irrespective of the ongoing activity in other, neighboring synapses. Associative LTP, on the other hand, is characterized by three distinct properties, namely cooperativity, associativity and specificity. *Cooperativity* means that there exists a threshold for LTP induction in that the depolarization of the postsynaptic membrane needs to be strong enough to remove the Mg^{2+} block of the NMDA receptor (see below). *Associativity* refers to the phenomena that a "weak" input may be potentiated via neighboring synapses on the same neuron, i.e. a strong synaptic input may aid a weak input to induce LTP when occurring in close temporal contiguity. Finally, the term *specificity* states that LTP (i.e. associative LTP) is input-specific, meaning that LTP is only occurring in the activated synapses (see BLISS & COLLINGRIDGE, 1993).

The important discovery by Bliss and Lømo was later followed by the finding that tetanus-induced LTP could be blocked by glutamatergic NMDA receptor antagonists (COLLINGRIDGE et al., 1983), leading to the hypothesis that LTP is NMDA receptor-dependent. It is now known that the NMDA receptors are responsible for associative LTP, acting as a "coincidence" (cooperative) detector for LTP induction. The NMDA receptor channel contains a voltage-dependent channel-binding site for Mg^{2+}, which is relieved by postsynaptic depolarization (NOWAK et al., 1984), mediated by activation of another ionotropic receptor (predominantly AMPA receptors) (see RIEDEL et al., 2003) (Figure 7). The activation of the NMDA receptor leads to an increase in postsynaptic Ca^{2+} concentration that serves as a second messenger and activates signal transduction cascades (see LAMANTIA, 2004). LTP can be divided into an early phase, which lasts for less than 4 h and involves post-translational mechanisms. The maintenance of LTP for more than 4 h, i.e. late phase LTP, requires gene transcription and protein synthesis (see ABRAHAM & WILLIAMS, 2003; NGUYEN et al., 1994). The protein synthesis in neurons is regulated by transcription factors such as cAMP response element-binding protein (CREB), which have been related to the development of late-phase LTP lasting for days, even weeks (ABRAHAM et al., 2002; see ABRAHAM & WILLIAMS, 2003). Several protein kinases have been linked to late-phase LTP, including Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (Figure 7).

Although there is compelling evidence that changes in synaptic strength have an important role in mechanisms underlying learning and memory processing (see BLISS & COLLINGRIDGE, 1993; see MARTIN & MORRIS, 2002; see SILVA, 2003; TSIEN et al., 1996), the role of LTP for learning and memory in animals is still not conclusively proven. A crucial question is whether changes in synaptic strength, as indicated by LTP, encode memory itself or mainly play a supporting role in mnemonic functions (see MARTIN & MORRIS, 2002).

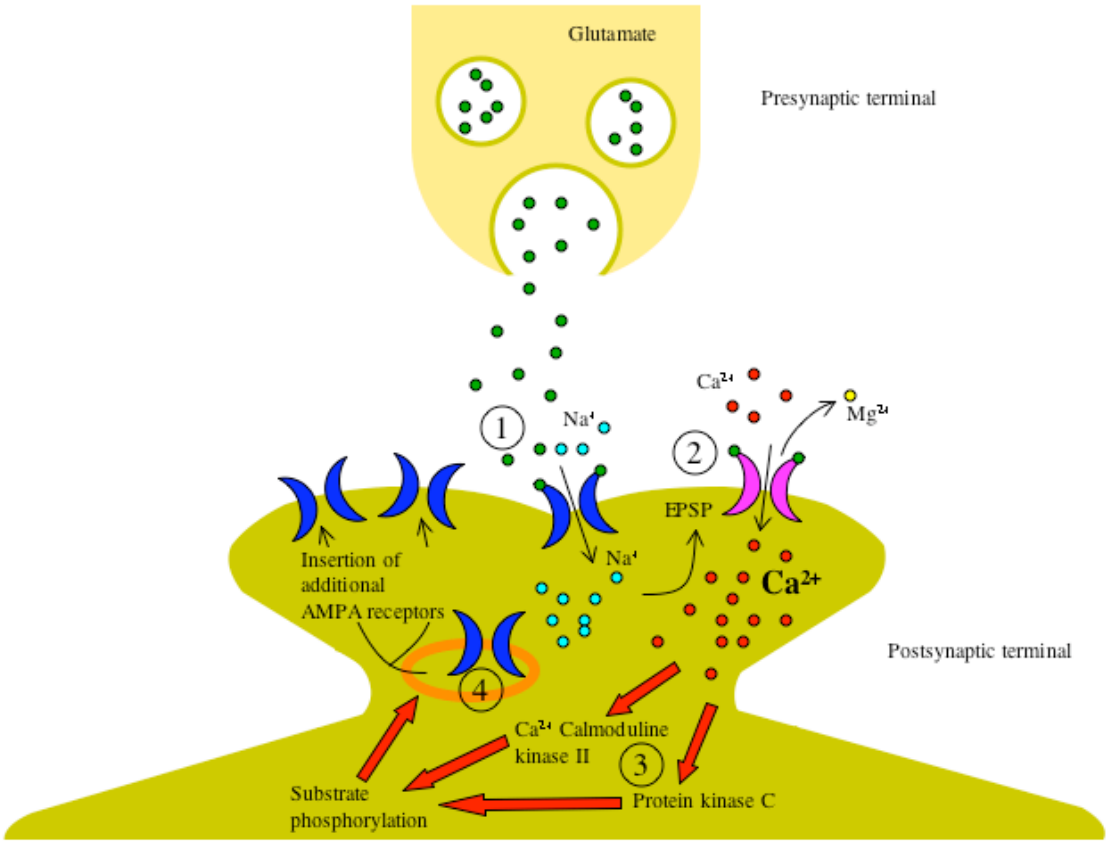


FIGURE 7. Schematic illustration of the mechanisms involved in LTP. Glutamate is released from the presynaptic terminal and binds to postsynaptic AMPA receptors (1). Upon activation, the AMPA receptor channel opens and Na^+ enters the cell. This results in an excitatory postsynaptic potential (EPSP) that depolarizes the postsynaptic membrane, which expels Mg^{2+} from the NMDA channel, allowing Ca^{2+} to enter (2). The Ca^{2+} ions activate postsynaptic protein kinases (3) that may act to insert additional AMPA receptors into the postsynaptic membrane (4), thereby increasing the sensitivity to glutamate. Modified from (PURVES et al., 2004).

GABA

GABA is an amino acid and serves as the major inhibitory transmitter in the mammalian brain by causing a hyperpolarization of the postsynaptic neuron. GABA is synthesized from the α-decarboxylation of L-glutamic acid, a reaction catalyzed by glutamic acid decarboxylase. After synthesis, GABA is stored in vesicles in the presynaptic terminal and upon depolarization, released into the synaptic cleft (see COOPER et al., 2003; see PURVES et al., 2004). GABA is removed from the synaptic cleft by plasma membrane transporters located on both neurons and glial cells.

GABA is present in high concentration in all regions of the mammalian brain. A vast amount of GABAergic neurons have shown to co-localize the calcium binding protein parvalbumin (HEIZMANN, 1984). In the MS/vDB, the parvalbumin-containing GABAergic cells are projecting neurons, ascending to the

hippocampus, whereas parvalbumin-lacking GABAergic cells seem to be local circuit neurons (BRAUER et al., 1991; FREUND, 1989).

There are two types of GABA receptors in vertebrates, the ionotropic $GABA_A$ receptor and the metabotropic $GABA_B$ receptor. They are both present in the mammalian brain, and are located pre- and postsynaptically on both GABAergic and non-GABAergic neurons (COOPER et al., 2003).

ACETYLCHOLINE

Acetylcholine represents a phylogenetically old molecule that is widely distributed in pro- and eukaryotic cells. The neurotransmitter function of ACh was discovered in the vagus nerve in 1921 by Otto Loewi (LOEWI, 1921). ACh is synthesized in one step from acetyl coenzyme A (acetyl CoA) and choline. The acetyl group from acetyl CoA binds to choline, a process catalyzed by the enzyme choline acetyl transferase (ChAT). Acetyl CoA is present in high amounts in the mitochondria of the cells, whereas choline is not produced by the body but has to be obtained from food and reaches the brain by the vascular system. Once synthesized, ACh is transported to and stored in synaptic vesicles by the vesicular acetylcholine transporter (VAChT) (ERICKSON et al., 1994). After ACh is released from the vesicles into the synaptic cleft, it is rapidly metabolized by the extracellular hydrolytic enzyme acetylcholine esterase (AChE), whereafter choline is transported back into the nerve terminals and re-used to synthesize new ACh (see PURVES et al., 2004).

Within the CNS, ACh-containing neurons are found in at least 10 relatively well defined groups of cells (MESULAM et al., 1983) (Figure 6). Among these, the cholinergic nuclei in the basal forebrain have been extensively studied since these cells degenerate in Alzheimer's disease. In the basal forebrain, cholinergic neurons in the nucleus basalis magnocellularis send ascending axons to the neocortex, while the MS/vDB sends cholinergic (and GABAergic, see below) axons to the hippocampal region (FIBIGER, 1982; MESULAM et al., 1983; WOOLF, 1991) (Figure 6).

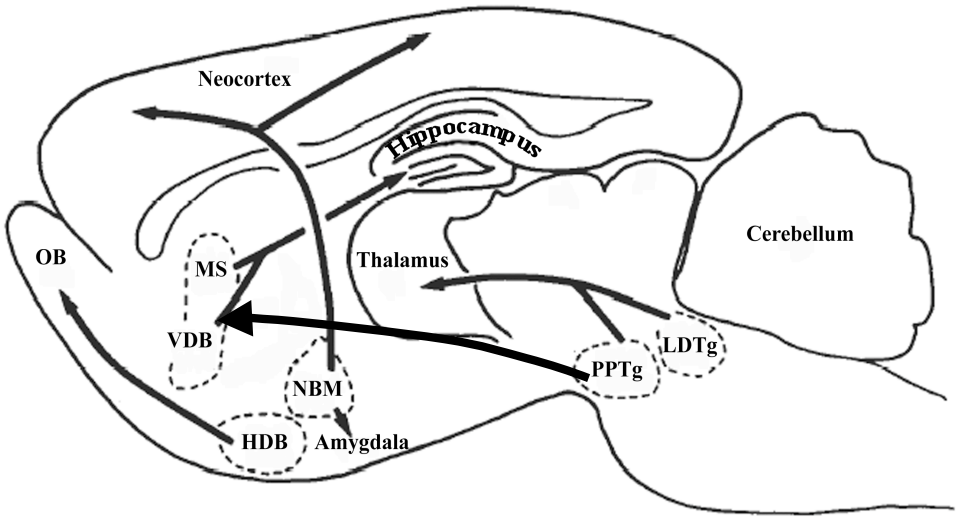


FIGURE 6. Schematic overview of the cholinergic nuclei and their projections in the rat brain. Abbreviations: MS = medial septum; VDB = vertical limb of the diagonal band of Broca; HDB = horizontal limb of the diagonal band of Broca; NBM = nucleus basalis magnocellularis; OB = olfactory bulb; PPTg = pedunculopontine tegmental nucleus; LDTg = laterodorsal tegmental nucleus. Modified from Mesulam *et al.* (1983).

Cholinergic receptors are divided into two classes, the ionotropic nicotinic receptors and the metabotropic muscarinic receptors, where the latter one is the main class of cholinergic receptors in the CNS (see CAULFIELD, 1993). There exist at least five subtypes of the muscarinic receptor, M_1 – M_5, identified through both pharmacological and molecular biological techniques. The subtypes can be further divided in two groups, based on activating of different G-proteins. Activation of M_1, M_3 and M_5 receptors results in stimulation of phosphatidyl-inositol turnover, whereas activation of M_2 or M_4 receptors leads to inhibition of adenylate cyclase and a decrease in cytosolic cAMP levels (see CAULFIELD, 1993; see CAULFIELD & BIRDSALL, 1998). All five receptor subtypes are expressed in the brain, and both cholinergic and GABAergic neurons in the septal area as well as neurons within the hippocampus express muscarinic receptors of the M_1 – M_5 subtypes (LAWSON & BLAND, 1993; LEVEY et al., 1995; LIU et al., 1998; ROUSE & LEVEY, 1996; VAN DER ZEE & LUITEN, 1999). The M_2 receptor can act as a presynaptic auto- or heteroreceptor, as well as a postsynaptic receptor within the septohippocampal pathway (HOSS et al., 1990; ROUSE et al., 2000).

In the 1970's, the "cholinergic theory of Alzheimer's disease" was formed. The theory was based on observations that cholinergic receptor blockade in young, healthy humans produced memory impairments similar to that seen in patients suffering from AD, and also that a loss of cholinergic neurons was observed in brains of AD patients, which correlated with cognitive impairments (DAVIES & MALONEY, 1976; DRACHMAN & LEAVITT, 1974). Based on these findings it was hypothesized that AD is a disease of the brain cholinergic system (BARTUS et al., 1982; DAVIES & MALONEY, 1976; PERRY et al., 1977; WHITEHOUSE et al., 1982). However, although AD today is defined as a neurodegenerative disorder related to abnormal protein (Aβ) accumulation in the brain, it is clear that ACh plays an important role for the symptoms seen in AD. For instance, AChE inhibitors are beneficial in the symptomatic treatment of patients suffering from AD. Moreover, recent studies suggest that there exist important interactions between loss of cholinergic neurotransmission in the brain and the accumulation of Aβ (see KAR et al., 2004).

SEROTONIN

The monoamine serotonin (5-hydroxytryptamin; 5-HT) was discovered in the 1940's by Rapport et al. (1949; 1947; 1948). A decade later, 5-HT was found to be present in the rat brain (TWAROG & PAGE, 1953), and suggested to serve as a neurotransmitter (BOGDANSKI et al., 1956; MARRAZZI & HART, 1955). 5-HT is synthesized from the essential amino acid tryptophan. Tryptophan is hydrolyzed by tryptophan hydroxylase to 5-hydroxytryptophan, which is then decarboxylated to 5-HT by amino acid decarboxylase (COOPER et al., 2003). Synaptically released 5-HT is primarily removed from the synaptic cleft by reuptake into the presynaptic terminal, a mechanism mediated by the 5-HT-transporter (ROSS & RENYI, 1977).

Using fluorescent histochemistry, the serotonergic system in the rat brain was mapped by Dahlström and Fuxe (1964). The nine groups of 5-HT neurons, identified and named B1 – B9, are predominantly located within the raphe nuclei in the brainstem (DAHLSTRÖM & FUXE, 1964). Subsequent studies have shown that the 5-HT neurons in the dorsal and medial raphe nuclei (DR and MR, respectively) innervate all areas of the rat forebrain (AZMITIA & SEGAL, 1978; VERTES, 1991; VERTES et al., 1999). However, the two 5-HT cell groups innervate different, complementary sites of the forebrain, where the DR projects primarily to lateral regions such as the amygdala, striatum, LS and to some degree to the ventral hippocampus. The MR, on the other hand, innervates more medially located areas, e.g. dorsal hippocampus, SUM and the MS/vDB (AZMITIA & SEGAL, 1978; LERANTH & VERTES, 1999; VERTES, 1991; VERTES et al., 1999) (Figure 5).

Seven 5-HT receptor families have been identified, comprising a total number of 14 pharmacologically and structurally distinct receptor subtypes (see BARNES & SHARP, 1999; see HOYER et al., 2002). All receptor families are G-protein coupled except for the 5-HT_3 receptor, which is a ligand-gated ion channel. Among these receptors, the 5-HT_{1A} receptor subtype is one of the most extensively studied and it has been implicated in cognitive functions (BUHOT, 1997; BUHOT et al., 2000; ÖGREN, 1985). Activation of the 5-HT_{1A} receptor induces neuronal hyperpolarization through G-protein-coupled K^+ channels and inhibits cell firing (see AGHAJANIAN, 1995). It is widely distributed throughout the CNS and expressed both pre- and postsynaptically. In the raphe nuclei, somatodendritic 5-HT_{1A} receptors are located on serotonergic neurons,

serving as autoreceptors. In limbic structures such as the cerebral cortex, hippocampus and basal forebrain, 5–HT_{1A} receptors are located postsynaptically (heteroreceptors) (CHALMERS & WATSON, 1991; POMPEIANO et al., 1992). In the MS/vDB, the 5-HT_{1A} receptor has been shown to be localized postsynaptically on both cholinergic and GABAergic cell bodies (AZNAR et al., 2003; KIA et al., 1996; LÜTTGEN et al., 2005). In addition, within the hippocampus, both excitatory pyramidal and granule cells, as well as GABAergic inhibitory interneurons express 5-HT_{1A} receptors (AZNAR et al., 2003; GULYÁS et al., 1999).

GALANIN

The peptide galanin was first isolated from the small intestine of the pig by Viktor Mutt and his colleagues at the Karolinska Institutet (TATEMOTO et al., 1983) and shortly thereafter, galanin-like immunoreactivity was demonstrated in the rat brain (RÖKAEUS et al., 1984). Galanin is a 29 amino acid (30 in humans) long peptide and is a part of the 123 amino acid-long precursor protein preprogalanin, which is cleaved into galanin and galanin message-associated peptide (RÖKAEUS & BROWNSTEIN, 1986; SCHMIDT et al., 1991; SILLARD et al., 1992; TATEMOTO et al., 1983). Studies in the rat have shown that galanin is widely distributed in the brain (SKOFITSCH & JACOBOWITZ, 1985). In the MS/vDB, there is a moderate number of galanin-ir neurons (SKOFITSCH & JACOBOWITZ, 1985), of which a subpopulation are co-localized with ChAT and project to the hippocampus (MELANDER et al., 1985). However, later studies have shown that the number of septohippocampal cholinergic neurons that co-localize with galanin appears to be fewer than previously suggested (see CORTES et al., 1990; HÖKFELT et al., 1998). It has been hypothesized that in the basal state, galanin is only to a small degree co-released with ACh into the hippocampus (MILLER et al., 1998). Galanin-ir in the hippocampus of the rat is mainly distributed in the ventral hippocampus (SKOFITSCH & JACOBOWITZ, 1985), which is the area of the hippocampus that is densely innervated by cholinergic afferent from the MS/vDB (GAYKEMA et al., 1990).

The first galanin receptor (GAL-R) was cloned in 1994 by Habert-Ortoli and collaborators (HABERT-ORTOLI et al., 1994) and since then, two more receptors have been cloned (see BRANCHEK et al., 2000). All three, GALR1-GALR3, are G-protein coupled receptors. Galanin receptor mRNA have been demonstrated for all three receptor subtypes in the MS/vDB (MENNICKEN et al., 2002; MILLER et al., 1997; O'DONNELL et al., 1999; O'DONNELL et al., 2003). A study on the cellular localization for the GAL-R1 receptor revealed that a few septal cholinergic neurons but most GABAergic neurons express GAL-R1 mRNA in the rat (MILLER et al., 1997). Within the hippocampus, galanin receptor mRNA for the GAL-R1 and GAL-R2 has been shown. GAL-R1 mRNA was found in the ventral subiculum, CA3 and CA1, whereas GAL-R2 mRNA was mainly distributed in the dorsal and ventral dentate gyrus and to a lesser extent in the ventral CA3 and CA1 (O'DONNELL et al., 1999; O'DONNELL et al., 2003). The exact localization and role in neuronal transmission of the three galanin receptor subtypes in brain areas of importance for learning and memory is still unclear, due to the lack of subtype-specific receptor ligands and/or antibodies (see HÖKFELT, 2005).

THE SEPTOHIPPOCAMPAL PATHWAY IN LEARNING AND MEMORY

BACKGROUND

There is a growing literature supporting a role for the septohippocampal pathway in cognitive functions. Early, unspecific lesions of the septum was associated with changes in emotional behaviors, described as the "septal rage" (BRADY & NAUTA, 1953) which was, however, later mainly associated with the lateral septal nucleus. Lesions of the MS/vDB have provided conflicting results as to its role in cognitive functions, probably due to the differences in the type of lesion as well as the choice of task used to test the animals (Table 1). Nonspecific lesions, such as electrolytic lesions, results in disruption in both reference and working memory functions while selective lesions of the cholinergic and/or GABAergic MS/vDB neurons have generally resulted in minor impairments or no effect (Table 1). In addition to its role in learning and memory, the septohippocampal pathway is also implicated in the modulation of fear and anxiety (see GRAY

& MCNAUGHTON, 2003). More specifically, it is suggested that "excitation" of the septum is necessary for normal fear responses (MENARD & TREIT, 1998; see TREIT & MENARD, 2000). It has been hypothesized that the MS/vDB integrates subcortical information (emotional, motivational and autonomic) of "biological significance" related to the "behavioral state" of the animal. This input in turn results in modulation of hippocampal "responsiveness" (see JAKAB & LERANTH, 1995; see WALSH, 2000).

Neurons in the MS/vDB can modulate hippocampal functions through different mechanisms including the theta rhythm. Hence, the cholinergic and GABAergic MS/vDB neurons projecting to the hippocampus contribute to orchestrate hippocampal theta rhythm (BLAND & BLAND, 1986; CHROBAK, 2000), which is a 4-12 Hz rhythmic pattern reflecting the periodic excitations of the hippocampal circuitry mediated by the entorhinal input. The generation of the theta rhythm occurs via rhythmically discharging cells in the MS/vDB, i.e. these neurons acts as a "pace-maker" for hippocampal theta (VERTES & KOCSIS, 1997). It is hypothesized that generation of theta is mediated through GABAergic septohippocampal neurons whereas the cholinergic input is modulatory (HENDERSON et al., 2004; VINOGRADOVA, 1995). Theta appears when the rat moves, explores new environments, or during sleep characterized by rapid eye movement (BLAND & BLAND, 1986). Moreover, this specific pattern of hippocampal neuronal firing has been proposed to be critical for spatial and contextual learning in rodents (HASSELMO, 2005; HASSELMO et al., 2002; see VERTES & KOCSIS, 1997; WINSON, 1978).

Table 1. Summary of the effects on learning and memory in a variety of tasks, as well as effects on hippocampal theta rhythm after non-selective (A) or selective (B) lesioning of neurons in the MS/vDB area of the rat

A. NON-SELECTIVE LESIONS OF THE MS/VDB

TYPE OF LESION	TASK	EFFECT	REFERENCE
Lesion of the MS/vDB using ibotenic acid	Spatial reference memory in the water maze	Impairment	(HAGAN et al., 1988)
Lesion of the MS/vDB using ibotenic acid	Trial-dependent discrimination in a T-maze	Impairment	(HEPLER et al., 1985)
Electrolytic lesion of the MS/vDB	Reference and working memory in the water maze and radial maze, resp.	Impairment	(MIYAMOTO et al., 1987)
Electrolytic lesion of the MS/vDB	Analysis of hippocampal theta rhythm	Elimination of theta rhythm in some animals	(WINSON, 1978)
Electrolytic lesion of the MS/vDB	Analysis of hippocampal theta rhythm	Elimination of theta rhythm	(RAWLINS et al., 1979)

B. SELECTIVE LESIONS OF CHOLINERGIC AND/OR GABAERGIC MS/VDB NEURONS

TYPE OF LESION	TASK	EFFECT	REFERENCE
Lesion of cholinergic neurons using IgG-192 saporin	Spatial reference memory in the water maze	Mild initial impairment	(BERGER-SWEENEY et al., 1994)
Lesion of cholinergic neurons using IgG-192 saporin	Spatial reference memory in the water maze and short-term memory in a delayed non-matching to position test (DNMTP)	No effect on spatial learning Impairment in DNMTP	(TORRES et al., 1994)
Lesion of cholinergic neurons using IgG-192 saporin	Spatial learning and memory using a place-discrimination paradigm in the water maze	No effect on acquisition Mild impairment in memory	(BAXTER et al., 1995)
Lesion of cholinergic neurons using IgG-192 saporin	Working memory assessed in a variable-delay radial arm maze	Impairment	(WALSH et al., 1996)
Lesion of cholinergic neurons using IgG-192 saporin	T-maze alternation Reference and working memory in the water maze Working memory in the radial maze	Reduction in T-maze alternation, impairment in reference memory and working memory in the radial maze No effect on working memory in the water maze	(LEHMANN et al., 2002)
Lesion of cholinergic neurons using IgG-192 saporin	Spontaneous alternation in a plus-shaped maze	Impairment	(CHANG & GOLD, 2004)
Lesion of cholinergic neurons using IgG-192 saporin	Spatial working memory in a radial arm maze placed in a water tank	No effect	(MCMAHAN et al., 1997)
Lesion of cholinergic neurons using IgG-192 saporin	Spatial working memory in the water maze	No effect	(FRIELINGSDORF et al., 2006)
Lesion of cholinergic neurons using IgG-192 saporin and/or lesion of GABAergic neurons using kainic acid	Spatial learning and memory in an 8-arm radial maze and in the water maze tasks	Kainic acid: No effect IgG-192 saporin: No effect in 8-arm, mild impairment in water maze Combination: Impairment in both task	(PANG et al., 2001)
Lesion of cholinergic neurons using IgG-192 saporin or lesion of GABAergic neurons using kainic acid	Analysis of hippocampal theta rhythm under urethane anesthesia or during locomotion	Kainic acid or IgG-192 saporin: Elimination of induced theta under anesthesia and attenuation, but not elimination, of theta during locomotion	(YODER & PANG, 2005)

ACETYLCHOLINE IN THE MS/vDB AND GALANIN

Cholinergic neurotransmission within the MS/vDB is sustained by ACh release from cholinergic fibers derived from the mesopontine region, as well as release from axon collaterals (BIALOWAS & FROTSCHER, 1987). There exist both electrophysiological and behavioral evidence that ACh within the MS/vDB regulates the activity of the septohippocampal pathway. Electrophysiological findings have demonstrated that ACh within the MS/vDB could mainly act via excitation of cholinergic neurons (DUTAR et al., 1983; LAMOUR et al., 1984). Also the facilitation in memory observed after intraseptal or systemic administration of muscarinic agonists such as oxotremorine or carbachol has been related to an increase in septohippocampal cholinergic neuronal activity (see Table 2), which is correlated with an increase in hippocampal ACh release (BLAND & ODDIE, 1998; GIVENS & OLTON, 1994; GIVENS & OLTON, 1995; GORMAN et al., 1994; MONMAUR & BRETON, 1991). This finding is also consistent with the observation that intraseptal injection of carbachol increased hippocampal theta rhythm, while lesions of the cholinergic input to the hippocampus from the MS/vDB profoundly decreased theta amplitude (LEE et al., 1994; YODER & PANG, 2005). Consistently, local administration of muscarinic antagonists into the MS/vDB produced both impairments in reference and working memory functions (see EVERITT & ROBBINS, 1997; GIVENS & OLTON, 1995; GIVENS & OLTON, 1990). These results lead to the hypothesis that muscarinic transmission in the MS/vDB has an excitatory role on cholinergic neurons. Moreover, the memory deficit caused by systemic administration of muscarinic antagonists was related to a blockade of this excitatory effect, resulting in a decrease in hippocampal ACh release and cholinergic transmission (GORMAN et al., 1994). However, recent electrophysiological findings have re-interpreted the role of ACh within the MS/vDB. Thus, ACh has only a minor effect on cholinergic neurons while its major effects is to excite septal GABAergic neurons via stimulation of muscarinic receptors (ALREJA et al., 2000). This indicates that the physiological role of cholinergic muscarinic transmission within the MS/vDB must be re-assessed.

Galanin has been suggested to play a role in hippocampal learning and memory functions, partly based on its colocalization with ACh in the septohippocampal projection (see above). Galanin inhibits ACh transmission both pre- and postsynaptically *in vitro* (FISONE et al., 1987). Moreover, galanin, perfused through a microdialysis probe, reduced basal ACh release in the ventral hippocampus, suggesting that it reduces ACh transmission via an inhibitory action on axon terminals from septal cholinergic neurons (ÖGREN et al., 1996). The reduction in ACh release correlated with an impairment in spatial learning in the water maze at a dose of 3 nmol/rat infused into the hippocampus (SCHÖTT et al., 1998a; SCHÖTT et al., 1998b; SCHÖTT et al., 2000; ÖGREN et al., 1996). Also other studies have indicated that galanin has an inhibitory effect in hippocampal-dependent learning mechanisms (see CRAWLEY, 1993; see CRAWLEY, 1996).

In view of the co-existence of ACh and galanin in septohippocampal neurons, as well as the presence of galanin binding sites within the MS/vDB, it was hypothesized that galanin could produce learning deficits by an inhibitory action on MS/vDB cholinergic neurons projecting to the hippocampus. In support of this hypothesis, intraseptal galanin administration impaired working memory in the T-maze and enhanced the deficit in the delayed non-matching-to-sample task (DNMTS) caused by systemic scopolamine (GIVENS et al., 1992; ROBINSON & CRAWLEY, 1993). These findings were in line with the observation that galanin infused into the MS/vDB, reduced scopolamine-induced increase in hippocampal ACh (ROBINSON et al., 1996). However, there was no report on the effects on hippocampal ACh release after intraseptal administration of galanin alone (ROBINSON et al., 1996).

Table 2. Summary of the effects of intraseptal administration of the muscarinic agonists oxotremorine or carbachol, the muscarinic antagonist scopolamine or the AChE inhibitor tacrine on learning and memory in different behavioral tasks.

DRUG	TASK	EFFECT	REFERENCE
Oxotremorine 1-10 μg/rat	Delayed non-matching-to-sample (DNMTS) radial maze	Dose-dependent impairment	(BUNCE et al., 2003)
Oxotremorine 0.5-5 μg/rat	T-maze spatial alternation	Reversed impairment in aged (22 months) rats	(MARKOWSKA et al., 1995)
Oxotremorine 2 μg/rat	Spatial reference memory in the water maze	(Mildly) improved reference memory	(FRICK et al., 1996)
Oxotremorine 0.5-2 μg/rat	T-maze alternation	Impairment in both naïve and saporin-lesioned rats	(PANG & NOCERA, 1999)
Carbachol 12.5-125 ng/rat	DNMTS radial maze	Dose-dependent impairment	(BUNCE et al., 2004a)
Carbachol 0.5 μg/rat	T-maze spatial alternation	No effect alone, but attenuated the effect of systemic scopolamine	(GIVENS & OLTON, 1995)
Scopolamine 5-30 μg/rat	Continuous conditional discrimination (CCD) (working memory)	Impaired choice accuracy	(GIVENS & OLTON, 1994)
Scopolamine 5-30 μg/rat	CCD and sensory discrimination (reference memory)	No effect	(GIVENS & OLTON, 1994)
Scopolamine 15 μg/rat	T-maze spatial alternation	Impairment, which was enhanced by concomitant syste mic scopolamine administration	(GIVENS & OLTON, 1995)
Scopolamine 2 μg/rat	T-maze alternation	No effect in naïve rats, but impaired choice accuracy in saporin-lesioned animals	(PANG & NOCERA, 1999)
Tacrine 12.5-25 μg/rat	DNMTS radial maze	No effect on performance in aged (15-16 months) rats	(SABOLEK et al., 2004)

SEROTONIN AND THE 5-HT$_{1A}$ RECEPTOR IN THE MS/vDB

There is a growing support for the role of 5-HT in cognitive functions. Serotonin is believed to mainly play a modulatory role by acting through interactions with glutamatergic and/or cholinergic neurotransmitter systems (see BUHOT et al., 2000; see FRANCIS, 1996; see STECKLER & SAHGAL, 1995; see ÖGREN, 1982; see ÖGREN, 1985). In addition to the proposed role of ACh in AD, is has been reported that serotonergic neurons and their receptors are affected in this disease (see MELTZER et al., 1998). Moreover, a number of symptoms often seen in AD patients, e.g. depression, anxiety and irritability, are improved by treatment with selective serotonin reuptake inhibitors (SSRIs) (GOTTFRIES et al., 1992).

Accumulating evidence suggest an involvement of the 5-HT$_{1A}$ receptor subtype in learning and memory functions (BUHOT et al., 2000; MISANE & ÖGREN, 2003; ÖGREN, 1985). This receptor is abundantly expressed in brain areas important for cognition, e.g. cerebral cortex, hippocampus and the MS/vDB (AZNAR et al., 2003; CHALMERS & WATSON, 1991; KIA et al., 1996; LÜTTGEN et al., 2005; POMPEIANO et al., 1992). Administration of a 5-HT$_{1A}$ receptor agonist before training disrupts learning and memory in PA (MISANE & ÖGREN, 2000), fear conditioning (STIEDL et al., 2000b) and spatial learning (CARLI et al., 1992; KANT et al., 1998; MCNAUGHTON & MORRIS, 1992). This impairment has been attributed to stimulation of postsynaptic 5-HT$_{1A}$ receptors, most likely located in the hippocampus, since intrahippocampal administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT impaired spatial and aversive learning (CARLI et al., 1992; STIEDL et al., 2000b). In contrast, low doses of 8-OH-DPAT facilitated learning and memory in an operant delayed matching-to-position task (COLE et al., 1994), presumably by stimulation of presynaptic autoreceptors located in the DR and MR nuclei (CARLI et al., 1998).

Alternatively, the impairing effects observed after systemic 8-OH-DPAT administration may be mediated via a stimulation of 5-HT$_{1A}$ receptors in the MS/vDB, which are located on both GABAergic and, to a lesser extent, cholinergic neurons (AZNAR et al., 2003; KIA et al., 1996; LÜTTGEN et al., 2005). This could modulate serotonergic transmission within the MS/vDB and thereby affect septal input to the hippocampus. This hypothesis is supported by the finding that hippocampal theta rhythm is modulated by serotonergic inputs from the raphe nuclei, which innervate the MS/vDB (see VERTES & KOCSIS, 1997). In support, inhibition of serotonergic MR neurons by local infusion of 8-OH-DPAT activated MS/vDB cells and generated theta in the hippocampus (KINNEY et al., 1996). Additional evidence for the involvement of the 5-HT$_{1A}$ receptor in hippocampal theta rhythm is based on the recent observation that 5-HT$_{1A}$-deficient knock-out mice display an increased magnitude in hippocampal theta oscillations (GORDON et al., 2005). It is, therefore, seemingly paradoxical that these mice are impaired in spatial learning (GORDON et al., 2005). However, there exist limited information concerning the involvement of septal 5-HT$_{1A}$ receptors in learning and memory functions, and the reported findings are contradictory. In one study performed in mice, intraseptal 8-OH-DPAT facilitated spatial discrimination (MICHEAU & VAN MARREWIJK, 1999), while two other studies in the rat reported either a small impairment in water maze acquisition or an impairment in working memory, respectively (BERTRAND et al., 2000; JELTSCH et al., 2004). However, information on the role of the medial septal 5-HT$_{1A}$ receptors in emotional learning is still lacking.

GLUTAMATERGIC INPUT: INTERACTIONS WITH THE 5-HT$_{1A}$ RECEPTOR

Studies on the role of glutamate and glutamatergic receptors in cognitive functions have primarily focused on the hippocampus. Hippocampal glutamate, acting via the NMDA receptor, is believed to play a pivotal role in synaptic plasticity. The finding that intracranial administration of the NMDA receptor antagonist D,L-AP5 impaired spatial learning and memory at a dose, which also blocked hippocampal LTP (MORRIS et al., 1986) gave the impetus to a large number of both molecular and behavioral studies that support an important role for hippocampal NMDA receptors in memory mechanisms (see LYNCH, 2004; see MORRIS et al., 2003; see NAKAZAWA et al., 2004).

Even though the role of hippocampal NMDA receptors in learning and memory have been studied extensively, there exist little information on the role of NMDA receptors in brain areas sending afferents to

the hippocampus, such as the MS/vDB. As discussed earlier, glutamatergic input to the MS/vDB originates from e.g. entorhinal and frontal cortices and the SUM. There are also evidence for intrinsic glutamatergic neurons in the MS/vDB (HAJSZAN et al., 2004; MANSEAU et al., 2005). Both cholinergic and GABAergic septohippocampal neurons are innervated by VGLUT2-ir glutamatergic boutons, which have been shown to express the NMDA receptor subunit NMDAR1 (PETRALIA et al., 1994; WU et al., 2003; WU et al., 2004). Changes in NMDA-related transmission can affect MS/vDB neurons, since intraseptal administration of the NMDA antagonist D-AP5 decreased the amplitude of hippocampal theta rhythm (LEUNG & SHEN, 2004). However, there exist limited information on the possible role of medial septal NMDA receptors in learning and memory.

5-HT_{1A} receptor agonists have shown to inhibit hippocampal pyramidal cell firing, (TADA et al., 1999) and interfere with NMDA receptor-mediated excitation and the induction of LTP in the visual cortex and hippocampus, respectively (EDAGAWA et al., 1999; SAKAI & TANAKA, 1993). Moreover, 5-HT_{1A} receptor activation was reported to suppress NMDA receptor function in the prefrontal cortex, partly mediated through a CaMKII- mediated action (YUEN et al., 2005). Activation of 5-HT_{1A} receptors also abolished a rise of extracellular glutamate caused by the NMDA receptor antagonist 3-[(R)-2-carboxypiperazin-4yl]-propyl-1-phosphonic acid (CPP) as measured by *in vivo* microdialysis in the prefrontal cortex (CALCAGNO et al., 2006). In the MS/vDB, the 5-HT_{1A} receptors are located on GABAergic and cholinergic septohippocampal neurons (LÜTTGEN et al., 2005) which are innervated by VGLUT2-ir glutamatergic boutons (see above). Taken together, both anatomical and neurophysiological findings indicate the possibility for important interactions between the NMDA and the 5-HT_{1A} receptors also in the MS/vDB.

AIMS

GENERAL AIM

The overall aim of this thesis was to investigate the role of major neurotransmitters in the septohippocampal pathway, and their significance for hippocampal-dependent learning and memory (see Figure 8).

SPECIFIC AIMS

- To investigate the interaction between muscarinic receptors and galanin in the MS/vDB and its influence on hippocampal ACh and spatial learning.
- To determine the role of 5-HT_{1A} receptors in spatial and aversive learning in rats and mice, and the interaction with cholinergic and glutamatergic systems.
- To study the role of the 5-HT_{1A} receptors in the MS/vDB in spatial and aversive learning.
- To examine whether the NMDA receptors in the MS/vDB play a role in hippocampal-dependent cognition, and to study possible interactions between NMDA and 5-HT_{1A} receptor function.
- To determine whether the impairing effects of scopolamine on spatial learning involves learning mechanisms or result from impairments in non-spatial functions.
- An important aim in all experiments was to investigate whether effects on spatial and/or aversive learning could be dissociated from possible effects on sensorimotor disturbances, motor functions and anxiolytic-like behavior.

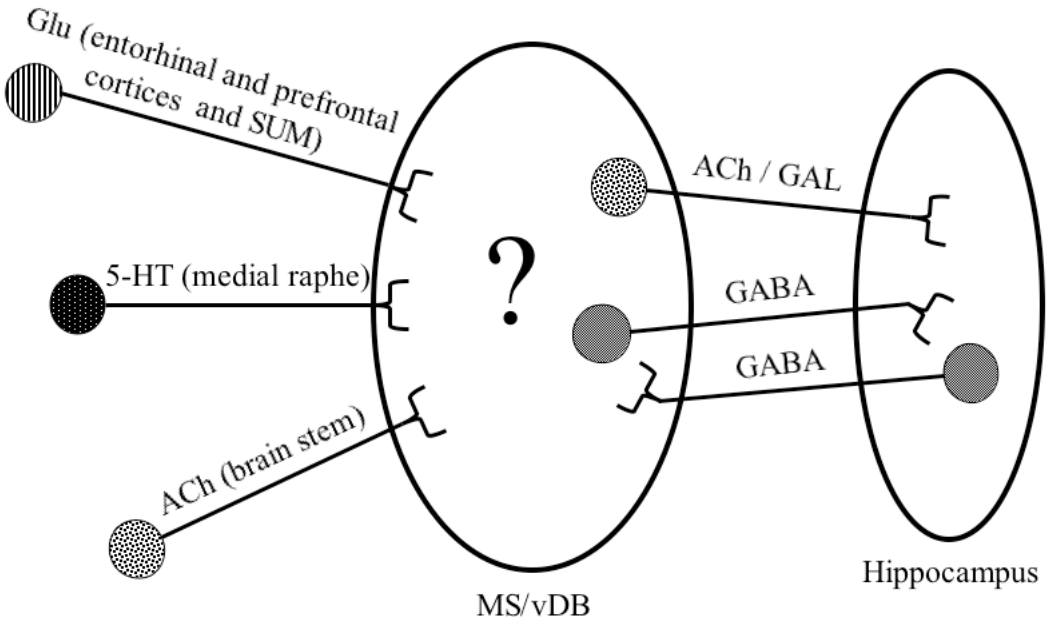


FIGURE 8. Schematic picture of the major converging input to the MS/vDB, which affect the cholinergic/galaninergic (ACh/GAL) and GABAergic neurons projecting to the hippocampus, and thereby influence hippocampal functions. ACh afferents to the MS/vDB originates from the pedunculopontine nucleus in the brain stem; 5-HT neurons ascend from the MR nucleus and glutamatergic (Glu) from e.g. cortical areas and the SUM (see BUTCHER & WOOLF, 2004; see RISOLD, 2004; VERTES et al., 1999). The recent suggestion of a glutamatergic projection from the MS/vDB to the hippocampus is not shown.

MATERIALS AND METHODS

METHODOLOGICAL CONSIDERATIONS

When studying the neurobiological basis for learning and memory, it is important to select a task that the animals can acquire and recall, and that this task is dependent on learning and memory mechanisms, which process particular kinds of information. Moreover, the performance of the animals, or the read-out of the experiment, must be possible to measure both quantitatively and qualitatively. In addition, it is of outmost importance that the results obtained from the experiments represent valid and reliable measures of learning and memory.

Ever since the observation that lesions of the hippocampus profoundly disrupted learning and memory in the water maze (MORRIS et al., 1982), this task has been widely used in studying hippocampal learning functions. In addition to the water maze, the radial maze, T- and Y-mazes and area mazes such as the hole board maze, are all based on the idea to study spatial learning, where a correct response requires that the animal navigates to a specific location. The advantage with these mazes is that the experimenter, by controlling the spatial information presented to the animal, limits the number of variables, which can influence the learning process. However, it is important to acknowledge that there are a number of potential cues which the animal can process and use but that the experimenter cannot control, such as sound, tactile cues and olfactory cues (see JEFFREY, 2003). One of the main differences between the water maze and other mazes is the type of reward used to *motivate* the animal to learn the task (see below). The dry mazes, e.g. the radial maze, are mostly based on food-reward, which requires food-deprivation of the animal in order to motivate the animal to learn. The deprivation of food may lead to confounding factors interfering with learning, since pharmacological compounds can affect neurotransmitters involved in food motivation, i.e. hunger (SIMANSKY, 1996). Another major problem with dry mazes is the difficulty to completely eliminate olfactory cues, which the animal can otherwise use to navigate to the correct location. In this respect the water maze is preferable, since it is difficult for the animal to use odors as local cues in the water.

Passive avoidance (PA) is mostly defined as an aversive or emotional memory task, which involves both hippocampal and amygdala functions (LEDOUX, 1993; STIEDL et al., 2000b). Unlike the water maze, which is based on repeated training, PA is a one-trial learning task. The test procedure exploits natural tendency of rodents to explore new environments, but at the same time they tend to avoid contexts that are experienced as "fearful", such as bright open areas. The term "passive avoidance" refers to the suppression of the natural tendency to enter a context, e.g. a dark compartment, associated with an aversive experience, e.g. exposure to a weak electric current when entering the preferred, dark compartment. Thus, the training creates a conflict between the motivation to enter the preferred, dark compartment and the motivation to avoid the "aversive" context. The compartment in which the animal receives the aversive electrical current (unconditioned stimulus; US) provides the contextual cue for the aversive (emotional) memory that is established by Pavlovian or fear conditioning (see ÖGREN, 1985). Passive avoidance performance, like all learning tasks, is sensitive to several non-cognitive factors, such as changes in locomotor activity, alteration of nociceptive threshold and changes in anxiety-related behavior, which are important to rule out in order to obtain reliable results. Since PA differs from the water maze task in several aspects, studies of the same manipulation in both tasks will strengthen the conclusions that the observed alterations in behavioral performance involve learning and memory mechanisms.

In addition to the actual learning mechanisms, it is apparent that all learning requires several higher order processes such as brain motivational systems, as well as the ability to pay attention to the environment and collect proper information of the learning context in order to perform the task. Selective attention requires functional sensory systems so that the animal can see, feel, hear and smell the various cues needed to solve the task. For instance, if a pharmacological treatment results in disturbed visual abilities, the animal will have

difficulties in developing the proper learning strategies. It is, therefore, critical to exclude possible sensorimotor and attentional disturbances in which can interfere with the actual learning process.

In the water maze, the animal must learn the behavioral procedures of the task, e.g. to swim, detect the platform and stay on it until "rescued" by the experimenter. The initial reaction of a rat placed in the water maze is to swim along the wall of the maze, i.e. thigmotaxic swimming, which the animal must suppress in order to find the location of the platform. Several studies have shown that learning the behavioral procedures of the water maze, i.e. "learning how", is pivotal for the subsequent spatial learning of the hidden platform, i.e. "learning what" (BANNERMAN et al., 1995; HOH & CAIN, 1997; MORRIS, 1984; WHISHAW & TOMIE, 1987). This indicates that the water maze task requires the engagement of multiple types of memories in order to solve the problem of navigating to the correct platform position (see KIM & BAXTER, 2001; MORRIS, 1984; ÅHLANDER et al., 1999). It has been shown that knowledge of the basic behavioral procedure is of importance in the subsequent spatial learning (Packard, 2003). Non-spatial pretraining (NSP) can be performed in different ways that probably gives different information to the animal, for instance by using a visible platform and/or by omitting spatial cues using a curtain around the water maze (CAIN, 1997; CAIN, 1998; MORRIS, 1984).

ANIMALS

Male Sprague-Dawley rats, weighing 280-310 g (about 8 weeks of age) where obtained from Scanbur (former B&K Universal; Sollentuna, Sweden). At the time of testing, the animals were 10-12 weeks old. In paper III, male NMRI mice (8 weeks of age; 25-30 g; Scanbur) were used in the majority of the experiments. In some PA experiments and in the elevated plus-maze experiment, male C57BL/6J mice (8 weeks of age, 30 g, Scanbur) were used and heart rate experiment was performed using male C57BL/6J mice (12 weeks of age, 25-30 g, Charles River, Sulzfeld, Germany). Rats were housed in groups of three or four in standard plastic cages (Macrolon® Type IV; 55 x 32 x 20 cm) throughout the study or until time of surgery, whereafter they were housed separately (see below). NMRI mice and the C57BL/6J mice that did not undergo surgery for heart rate measurements were housed in groups of four to six, and the operated C57BL/6J mice in the heart rate experiment were housed individually in standard Macrolon®; Type II; 22 x 16 x 13 cm). All animals were housed in temperature- and humidity-controlled rooms with a 12 h light/dark cycle (lights on at 7 a.m.) with free access to standard food pellets and tap water. Animals were allowed to habituate to the animal facility for at least five days before starting the experiment. In all PA experiments, animals were handled daily for a period of four to five days during the pre-experimental period to reduce variations in their responses. Cages were changed twice a week. Animal housing and experimental procedures followed the provisions and general recommendations of the Swedish animal protection legislation, and all experimental procedures were approved by the local Animal Ethics Committee (Stockholm Norra Djurförsöksetiska Nämnd).

STEREOTAXIC AND MICROINFUSION PROCEDURES

STEREOTAXIC SURGERY (PAPERS I, IV & V)

Rats were anaesthetized with enflurane (5% for induction and 3% for continuous anesthesia, driven by a mixture of 50% N_2O and 50% O_2), pentobarbital (60 mg/kg intraperitoneally; i.p.) (paper I) or isoflurane (induction 4.7%; maintenance 2.1-3.4%; airflow 364-380 mL/min) (paper IV and V). When deeply anaesthetized, they were placed in a stereotaxic frame (David Kopff Instruments, Tujunga, California, USA) in a flat skull position, and body temperature was maintained at 37 °C using a temperature-controlled heating pad (CMA/105, CMA/Microdialysis, Stockholm, Sweden). The skull surface was exposed after which a guide cannula (26 GA; 6.9 mm; diameter 0.45 mm; Plastics One, Roanoke, VA, USA) was inserted into the MS/vDB area using the following coordinates from bregma (PAXINOS & WATSON, 1986): AP: +0.2,

ML: 0.0, DV: -7.4 (injection site) from the skull surface. The hole was drilled 1.0 mm lateral to the sagittal midline and the stereotaxic arm was tilted 8° to avoid puncturing the azygos pericallosal artery. After insertion of the cannula, it was fixed to the skull using dental cement (Dentalon® AgnTho's, Lidingö, Sweden), with three microscrews serving as anchor points (AgnTho's). Finally, a dummy cannula was inserted and secured with a dust cap.

In the microdialysis experiment, a microdialysis guide cannula (CMA Microdialysis) was implanted into the ventral hippocampus; AP: –5.0, ML: +4.8, DV: -6.7 mm relative to bregma (PAXINOS & WATSON, 1986). At the same time, an infusion guide cannula (Plastics One) was inserted into the MS/vDB (see details above).

After surgery, animals received an injection of sterile saline (2 ml) (CCS AB, Borlänge, Sweden) subcutaneously (s.c.) in the neck, to compensate for possible loss of body fluid during surgery (Paper I). In paper IV and V, the rats received post-surgical treatment consisting of s.c. injections of 1 ml sterile saline (CCS AB) and s.c. or intramuscular buprenorphin (Temgesic®, Shering-Plough AB, Stockholm, Sweden) at a dose of 0.05 mg/kg, as well as the topical anesthetic lidocaine (10 mg/dose spray; Xylocain®; AstraZeneca, Sweden), which was sprayed onto the wound. Following surgery, animals were housed in pairs in Macrolon® Type IV cages, separated by a Plexiglas wall, which allowed them to maintain social contact by visual and olfactory stimuli, but prevented them from licking each other's wounds and chewing each other's dummy cannulae. A recovery period of five to seven days under daily observations was allowed before starting the behavioral experiments. Any sign of postoperative distress, such as weight loss or touch vocalization before or during the experiments, excluded the animal from further testing.

INTRACEREBRAL MICROINFUSIONS (PAPERS I, IV & V)

Microinfusions of pharmacological compounds was conducted using a microinfusion pump (CMA/100, CMA/Microdialysis) and a Hamilton syringe (25 µl), which was connected via a plastic tube to an injection cannula (33 GA; 7.4 mm long with a diameter of 0.2 mm; Plastics One) that was 0.5 mm longer than the guide cannula. The infusion rate was 0.5 µl/min and the duration 60 s, after which the cannula was left inside the guide for an additional 60 s to avoid back-flow. Drugs were infused 10 min (paper I and V), 15 min (paper IV) or 20 min (paper I) before training/testing and the animals were lightly held by the experimenter during the infusion procedure.

COMPOUNDS

The test compounds or peptides used in the present studies were obtained from commercial sources: porcine galanin (Peninsula Laboratories Europe, Merseyside, UK; lot. No. 035599, 801943, 802080; and Bachem, Bubendorf, Switzerland; lot. No. 545173 and 518144), scopolamine hydrobromide (Sigma, St. Louis, MO, USA or Sigma-Aldrich, Stockholm, Sweden), carbachol (Sigma-Aldrich), 8-OH-DPAT [(+/-)-8-hydroxy-2-(di-n-propylamino)tertralin] (RBI, Natick, MA, USA, (*R*)8-OH-DPAT [(*R*)-(+)-8-hydroxy-2-(di-n-propylamino)tertralin] (Tocris, Bromma, Sweden), MK-801 [(+)-10,11-dihydro-5-methyl-5H-dibenzo[a,d]-cyclohepten-5,10-imine hydrogen maleate] (Sigma), NAD-299 [(R)-3-N,N-dicyclobutylamino-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide hydrogen (2R,3R-tartrate monohydrate] (AstraZeneca R&D, Södertälje, Sweden),
WAY-100635 [N-(2-(1-(4-(2-methoxyphenyl)piperazinyl))ethyl)-N-(2-pyridinyl) cyclohexanecarboamide trihydrochloride] (Wyeth Research Laboratory, Taplow, UK), physostigmine hemisulphate (Eserin, Fluka, Switzerland) and D-AP5 (D-(-)-2-amino-5-phosphonopentanoic acid (Tocris). Doses and time of injection/microinfusion relative to testing were based either on previous publications or on pilot studies. The compounds were dissolved in sterile saline, which also served as control (0.9% NaCl; CCS AB), and injected s.c. in the scruff of the neck at a volume of 2 ml/kg (rats) or 10 ml/kg (mice). The compounds or galanin that were microinfused were dissolved in freshly made artificial cerebrospinal fluid (aCSF) (ÖGREN et al., 1996),

which was also used as control. The chemical substances were stored in a refrigerator (4 °C) or on ice until use, and the concentration of galanin was calculated on the basis of the purity of the peptide. The different compounds and time intervals for the behavioral studies are summarized in Table 3.

TABLE 3. Summary of the various compounds, route of administration, time interval and supplier used in the different experiments. S.c. = subcutaneously; wm = water maze experiments; PA = passive avoidance

PAPER	COMPOUND/ PEPTIDE	ROUTE OF ADMINISTRATION	DOSE RANGES	TIME INTERVAL (MIN)	SUPPLIER
I	Carbachol	Intracerebral	0.5-1 μg/rat (wm)	20 min	Sigma-Aldrich
I	Galanin	Intracerebral	0.3–3 nmol (wm)	20 min	Peninsula/Bachem
I	Scopolamine	Intracerebral	10-15 μg/rat (wm)	20 or 10 min	Sigma
II, III	MK-801	S.c.	0.1-0.3 mg/kg (PA)	30, 20 or 10 min	Sigma
II, III	NAD-299	S.c.	0.05-1.5 mg/kg (wm) 0.1-3 mg/kg (PA)	30, 20 or 15 min	AstraZeneca
II, III	8-OH-DPAT	S.c.	0.03-0.3 (wm) or 0.01-1 mg/kg (PA)	15 min	RBI
III	Physostigmine	S.c.	0.0125-0.3 mg/kg (PA)	20 min	Eserin
II, III	WAY-100635	S.c.	0.3-1 mg/kg (wm) 0.03-3 mg/kg (PA)	50 or 30 min	Wyeth
IV, V	D-AP5	Intracerebral	0.3-5 μg/rat (wm and PA)	15 or 10 min	Tocris
V	(*R*)8-OH-DPAT	Intracerebral	1-4 μg/rat (wm) 4 μg/rat (PA)	10 min	Tocris
VI	Scopolamine	S.c.	0.3 mg/kg	10 min	Sigma-Aldrich

BEHAVIORAL EXPERIMENTS

EXPERIMENTAL PROCEDURES

Only one experimenter conducted each experiment. All behavioral tests were performed between 8 a.m. and 4 p.m. Before testing, animals were brought to the experimental room in transport cages and allowed to habituate to the room for at least 60 min prior to testing. In all experiments except for the elevated plus-maze, locomotion and cued platform tests in paper IV, the animals were experimentally naïve and used in only one experiment to avoid possible "carry over" effects between tasks.

THE WATER MAZE TASK (PAPER I, II, IV-VI)

In this task, an escape platform is located just beneath the water surface in a water tank, and thus invisible to the animal. The animal is placed in the water facing the wall and allowed to swim around to find the hidden platform. Over several trials, the animal will learn to navigate to the platform by the use of visual cues placed outside the maze, i.e. extramaze or distal cues (Figure 9). In the standard procedure, the location of the platform is constant, resulting in learning that involves trial-independent information, i.e. reference memory. This procedure is in contrast to working memory paradigms, where the platform is moved between sessions and, thus, the learning process involves trial-dependent information.

The water maze setup used in the present studies has been described in detail earlier (ÖGREN et al., 1996). In short, a circular tank (Ø 180 cm; 45 cm in height) was placed in the center of a room and surrounded by several extramaze cues, which were kept constant at all times (Figure 9). The tank was filled with tap water (22±1 °C) up to a height of 28 cm and the water was changed daily. A circular escape platform, 15 cm in diameter and in the same color as the water maze (paper I and II) or 10 cm in diameter and made out of Plexiglas (paper IV-VI) was placed in the center of one of the imaginary quadrants (north east; NE) in the water maze, and submerged one cm below the water surface and thus invisible to the rat. The smaller platform was used in some of the studies to make it more difficult for the animal to find the platform and, thus, easier detect a possible learning facilitation. Video tracking of the animal was performed using a digital TV system attached to the ceiling above the center of the maze. The camera was in turn connected to a computer that monitored parameters such as escape latency, i.e. time to find the platform, swim distance and swim speed (Water Maze Software, Edinburgh, UK). Moreover, analysis of thigmotaxic swimming was conducted (paper II, IV-VI), a parameter indicative of altered sensorimotor performance (CAIN et al., 1996) and defined as the percentage of time that the rat spent swimming in the peripheral annulus of the water maze, within 10 cm from the walls.

Animals were injected or microinfused with drug or solvent before the first trial on each day, i.e. prior to each training session. One training session consisted of four trials and animals were trained for five (paper I and II) or six (paper IV-VI) consecutive days. In paper I and II, the starting positions were rotated clock-wise, one quarter of a turn per trial (SCHÖTT et al., 1998a) over the days of training. In paper IV-VI, the starting positions were semi-randomized. As mentioned previously, the platform was placed in the center of the NE quadrant (target quadrant) and the location of the platform remained the same throughout the training period. On each trial, the animal was gently placed in the water facing the wall, and then released. It was allowed to search for the platform for 60 (paper I and II) or 90 s (paper IV-VI) and if the animal failed to locate the platform within this time, it was guided to it by the experimenter. It is important to point out, that the animal was not put on the platform by the experimenter, but guided to it using the experimenter's hand. This procedure allows the animal to learn that the platform is in the water and thus possible to swim to, in contrast to if the rat is lifted up from the water and put directly on the platform. Once on the platform, the animal was allowed to stay on it for 30 s before being removed from it and the next trial commenced.

The retention test, i.e. test of long-term memory, was conducted 2 h (paper VI), 24 hours (paper I, II, IV and V) or seven days (paper I) after the last training session. The platform was not present during the retention test and the animals did not receive any drug treatment before testing. All other parameters remained the same as during training. In the retention test, the parameters, which were measured included latency to first cross the area of the former platform location, total number of crossings of this area, time spent in target (NE) quadrant and target zone (defined as the circular area with a radius of 20 cm from the center of the former platform position), swim speed and thigmotaxic swimming. In the retention test, animals started from the two starting positions furthest away from the target quadrant.

FIGURE 9. Photographs of the water maze setup used in the present studies. The left photograph shows the water tank filled with water. On the right hand side is a photograph of one of the extramaze cues surrounding the water maze.

Non-spatial pretraining (Paper VI)

Non-spatial pretraining was performed with a visually cued platform consisting of a brightly painted cylinder, 3 cm in diameter and 5 cm high, which was attached to a circular platform (15 cm in diameter). The platform was submerged 1 cm below the water surface, and the cylinder extended 4 cm above the surface. A total number of six different platform locations were defined, where each location was 45 cm from the center of the pool. During NSP an opaque curtain, which occluded visible extramaze cues, surrounded the water maze. After being placed in the water, the rat was allowed to swim for 60 s, and if the rat failed to find the platform within this time, the experimenter guided it there. Six trials were run consecutively during one day, and the platform was moved to a new location between every trial. The platform and starting positions were semi-randomized. Spatial training was initiated 48 hours after completion of the NSP.

Cued platform test (Paper II and IV)

This test was performed to exclude possible sensorimotor disturbances induced by the pharmacological treatment, which can interfere with performance, such as impaired vision or decreased motivation. The design of this test is basically the same as for the NSP, with a cued platform that was moved semi-randomly between trials. The difference is that in this test, a total of eight trials were conducted during two consecutive days, i.e. four trials per day but there was no subsequent spatial training in the water maze.

STEP-THROUGH PASSIVE AVOIDANCE (PAPERS II, III-V)

The PA procedure was modified compared to standard experimental procedures in the field, since the animals were always handled prior to training and in some experiments (paper II, III and IV) habituated to the testing procedure before PA training. The change in procedure resulted in a reduced variability in PA responses, probably due to a reduction in stress (paper III). The electrical current varied between 0.2-0.4 mA, dependent on species, strain and strain factors. For instance, the response to the electrical current differed between mouse strains, i.e. the C57BL/6J was found to be more sensitive than the NMRI strain. Moreover, a

low electrical current was used to allow for detection of possible facilitation of PA memory, while a higher electrical current was used to study memory impairments. After the electrical shock (US) the animal was left in the dark compartment for an additional 30 s to strengthen the association between the US and the context, thus providing for improved contextual learning.

The PA apparatus consisted of a two-compartment shuttle box with a stainless steel bar floor (25 x 50 x 25 cm for rats; 10 x 16 x 18 cm for mice; Ugo Basile, Comerio-Varese, Italy) where the compartments were separated by a sliding door (7 x 7 cm for rats; 4 x 4 cm for mice). The two compartments were of equal size and the dark compartment (i.e. conditioning compartment) consisted of black plastic, while the light compartment was made out of white plastic and illuminated by a light bulb attached to the top Plexiglas cover.

Prior to training, animals were injected or microinfused with the test compound (for time intervals between drug administration and training, see Table 3) and after the selected time interval, the animal was placed in the light compartment with the door to the dark compartment closed. Following 60 s of exploration, the door was opened automatically and the animal could enter the dark compartment. The latency to cross from the light to the dark compartment was recorded. After entering the dark compartment with all four paws, the sliding door closed and a weak electrical current (0.2-0.4 mA) scrambled current, duration 2 s) was delivered through the grid floor, serving as the US (Figure 10). After an additional 30 s in the dark compartment, the animal was removed from the PA apparatus and returned to its transport cage. The retention test was conducted 24 h after training (Figure 10). The animal was placed in the light ("safe") compartment and after 10 s, the door opened and the animal had access to the dark compartment for 300 s. Retention latency, i.e. the latency for the animal to enter the dark compartment with all four paws, was recorded. If the animal did not enter within this time, it was removed from the apparatus and assigned a maximum retention latency score of 300 s.

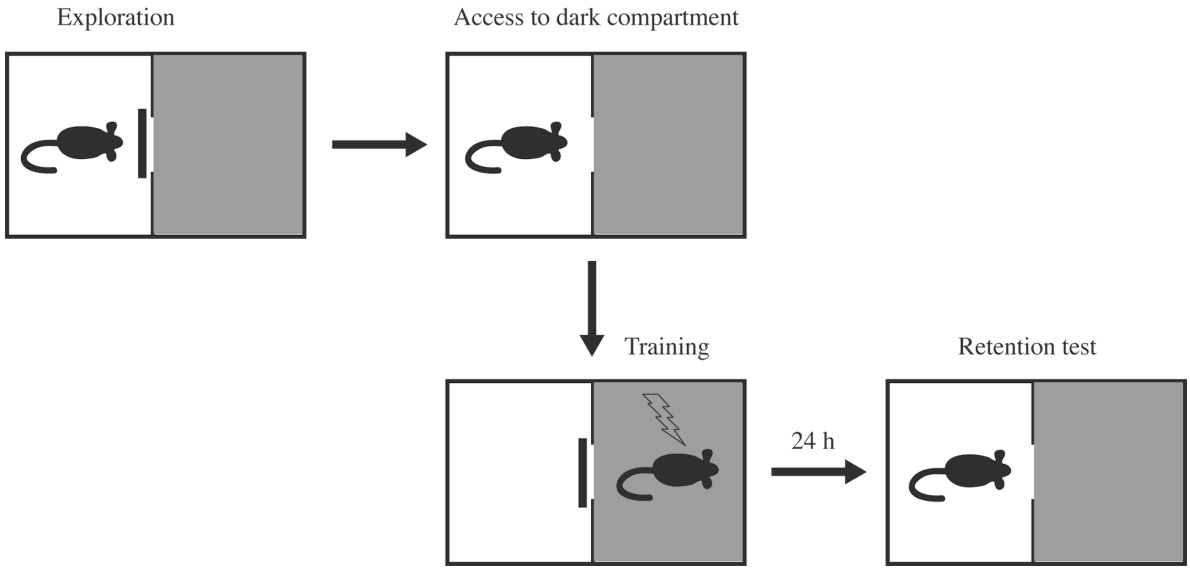


FIGURE 10. Schematic picture of the PA procedure. This is a hippocampal-dependent, aversive learning task based on Pavlovian fear conditioning. The task is based on the natural tendency for rodents to avoid brightly lit areas and simultaneous preference for dark spaces. During training on day 1, entrance into the dark, preferred compartment is associated with a weak, aversive electrical current, which serves as the unconditioned stimulus. Test compounds are injected or microinfused before training. On the second day, 24 h after training, memory is examined in a retention test where the animal is again placed in the light compartment and the latency, i.e. retention latency, to enter the dark compartment is recorded. See text for further details.

LOCOMOTOR ACTIVITY (PAPER I, II, IV AND V)

Analysis of exploration and spontaneous locomotor activity can be used to rule out possible disturbances in motor function, which may interfere with learning and memory performance. In addition, a test of locomotor activity can give information of the functional role of a number of neurotransmitters in brain regions, which subserve motor functions.

The system used to study locomotor activity is a fully computerized multicage system that uses infrared and red motion detection (ÖGREN et al., 1979). After habituation to the experimental room, animals were injected or microinfused with a test compound (except for the post-swim locomotor experiment in paper IV) and, after a selected time interval, placed individually in locomotor cages (25 x 40 x 30 cm) for 30-45 min. Both horizontal (motility and locomotion) and vertical (rearing; the animal stand on its hind limbs) activity was simultaneously recorded. The horizontal movements were detected by 48 photosensors placed in the floor in 4 by 4 cm squares covering the entire floor of the locomotor box. Motility was defined as a movement covering one photosensor, i.e. 4 cm, and locomotion was defined as a movement covering eight photocells, or 32 cm. Vertical movements were recorded by detectors placed in rows, 4 cm apart, located 13 cm above the cage floor. Each cage contained 50 ml of wood shavings and both the cage and the wood shaving was changed between each animal. Recording was initiated when the animal was placed in the locomotor box. The recording interval (30-45 min) allows for examination of the initial exploratory phase (lasting for approximately 20 min) as well as the subsequent habituation period.

ELEVATED PLUS-MAZE (PAPER III AND IV)

The elevated plus-maze is one of the most used models for studying anxiety-like behavior in rats and mice. It has been used extensively as a tool for testing anxiolytic-like effects of pharmacological compounds and has more recently also been used to study the relationship between emotionality and learning and memory (see BANNERMAN et al., 2004; see CAROBREZ & BERTOGLIO, 2005; LAMPREA et al., 2000; PELLOW et al., 1985). The elevated plus-maze is an ethologically-based animal model of anxiety (LISTER, 1990; RODGERS et al., 1997), which does not require conditioning. Rodents have an innate tendency to avoid elevated open arms as part of their safety-assessment strategies.

The elevated plus-maze apparatus consisted of four arms (open arm 30 x 5 cm, closed arm 30 x 5 x 10 cm for mice; open arm 46 x 15 cm, closed arm 46 x 15 x 23 cm for rats) and a central area (5 x 5 cm for mice; 15 x 16 cm for rats) (TSE Systems, Bad Homburg, Germany), elevated 1 m (mice) or 70 cm (rats) above the floor. Following drug administration, the animal was gently placed in the center area and allowed to explore the maze for 5 min. The parameters measured were time spent in open arms, closed arms and central region and number of entries to the open and closed arms. For mice, the total distance traveled (locomotion index) was also recorded. Between each animal, the maze was thoroughly cleaned with 70% ethanol.

HEART RATE MEASUREMENT (PAPER III)

Heart rate measurements were conducted to evaluate the effects of drug treatment on autonomic regulation (changes in heart rate and/or variability of heart rate), which can be altered by e.g. fear. Electrocardiogram (ECG) transmitters and electrodes were implanted i.p. or subcutaneously, respectively, as described earlier (STIEDL & SPIESS, 1997). Following recovery, telemetry measurements were performed 15 min after drug administration, under brief isoflurane anesthesia, necessary due to the aggressiveness of the C57BL/6J mice (STIEDL et al., 2000a). ECG was recorded during a period of 18 min and the ECG signal (lead II) was digitized at 4 Hz and stored for later off-line analysis. Artifacts were excluded and unrecognized beats in the recording were edited from the analysis. Heartbeat intervals (ms) derived from successive R-waves of the ECG-signal were converted into instantaneous heart rate, and for the statistical analysis, heart rate and heart rate variability were calculated in 2 min-intervals. Heart rate variability was determined by the root-mean-square of the sum of successive RR interval differences.

NEUROCHEMICAL EXPERIMENTS

MICRODIALYSIS (PAPER I)

The microdialysis technique was used to monitor the levels of extracellular neurotransmitters *in vivo*. The principle of microdialysis sampling is based on diffusion of molecules against the concentration gradients existing between the perfusate and the extracellular fluids. These concentration gradients result in substance entering, or leaving, the microdialysis probe. The microdialysis samples are collected at regular intervals and analyzed by suitable analytical techniques. In the present thesis, the microdialysis experiments were carried out in order to investigate the effects of intraseptal infusion of pharmacological compounds on the septohippocampal pathway. The extracellular levels of hippocampal ACh were measured in awake rats. Drugs were infused locally into the MS/vDB and the changes in ventral hippocampal ACh release were monitored simultaneously by microdialysis sampling (Figure 11). This design makes it possible to monitor the activity of the cholinergic neurons projecting to the hippocampus, indicated by changes in ACh release in the hippocampus.

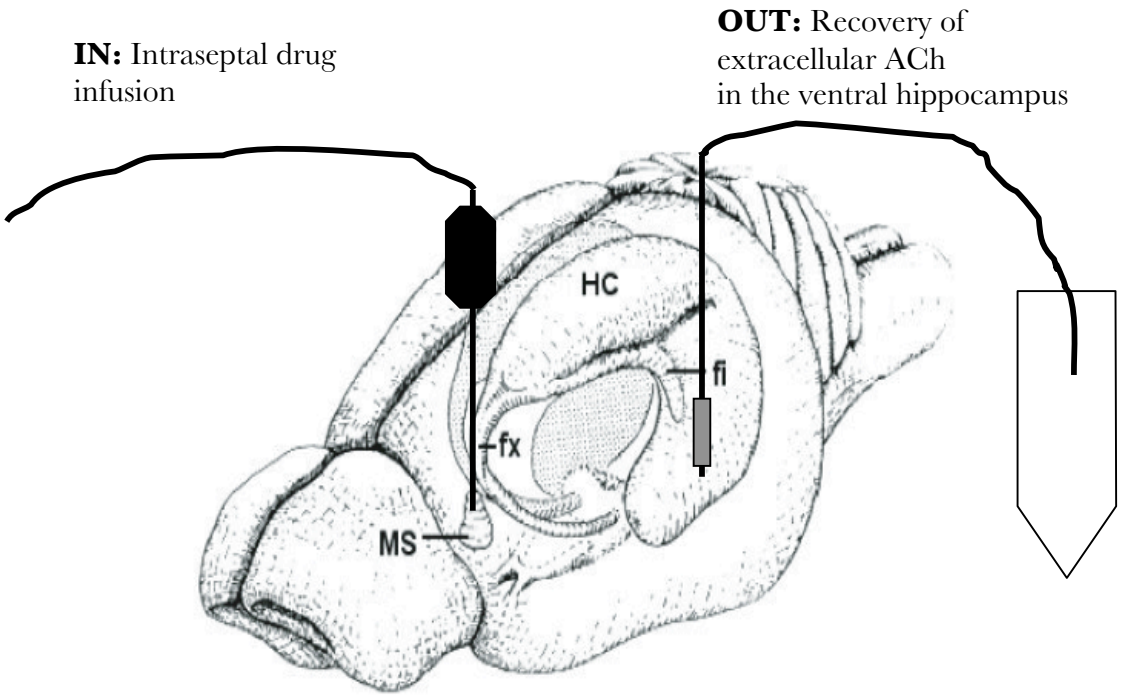


FIGURE 11. Schematic drawing of the microdialysis setup, where the effects of intraseptal drug infusions on extracellular ACh release in the hippocampus was measured. Drugs were infused through an injection cannula placed in the MS/vDB, and the extracellular concentration of ACh was simultaneously sampled via a microdialysis probe located in the ventral hippocampus. The ACh content was analyzed using high-performance liquid chromatography. Abbreviations; MS = medial septum; fx/fi = fimbria-fornix; HC = hippocampus. Modified from (AMARAL & WITTER, 1995).

The microdialysis surgery and experiments were carried out following a slightly modified protocol described elsewhere (KEHR et al., 1998; ÖGREN et al., 1996). Following recovery from surgery (see above), a microdialysis probe (2 mm membrane; Eicom, Kyoto, Japan or CMA/12; CMA Microdialysis) was inserted through the guide cannula and perfused with Ringer solution containing 0.5-2 μM neostigmine at a flow rate of 1.25-2 μl/min. After 3 h of habituation, fractions were collected every 10 or 20 min. The concentration of ACh in the microdialysis samples was determined using microbore column liquid chromatography with postcolumn immobilized enzyme reactor (IMER) and electrochemical detection on redox polymer-coated electrodes as described earlier (KEHR et al., 1998). The basal levels of ACh (not corrected for in vitro recovery) in the microdialysates were calculated as the average of three consecutive fractions collected before the intraseptal administration of the drug

HISTOLOGICAL TECHNIQUES

Both immunohistochemistry and *in situ* hybridization techniques were used to provide an anatomical correlate to the behavioral changes seen after administration of the various pharmacological compounds. In paper I, immunohistochemistry was used to determine the distribution of the neuropeptide galanin 5 and 20 min after infusion into the MS/vDB. In paper III, *in situ* hybridization was performed in order to study the anatomical substrates in the mouse septum for possible interactions between the 5-HT_{1A} receptor and cholinergic/glutamatergic neurons.

IMMUNOHISTOCHEMISTRY (PAPER I)

Immunohistochemistry is a method that allows for demonstration of the cellular/subcellular localization of proteins/peptides in tissue sections by the use of antibodies or antisera (COONS, 1958).

Tissue preparation

Animals were deeply anaesthetized (pentobarbital sodium) and transcardially perfused with 50 ml of sodium chloride (0.9%) containing 500 IE heparin, followed immediately by 500 ml 4% formaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7.1). The brains were removed and kept in the fixative for 2 h after which they were moved to 10% sucrose in 0.1 M PBS and stored in a refrigerator (+4 °C) until they were cryosectioned (14 μm) and mounted on chrome-alum-coated slides, dehydrated and processed for immunohistochemistry.

Immunohistochemical procedure

The tissue sections were incubated overnight in room temperature with primary rabbit antisera directed against galanin; diluted 1:400 in 0.1 M PBS for the avidin-biotin-peroxidase (ABC) complex staining procedure and a antiserum (rabbit) raised against human and rat neuropeptide Y (diluted 1:400 for the ABC staining procedure). After the second antisera, a biotin-conjugated antisera for ABC staining was added and the ABC technique was used with 3,3'-diaminobenzidine as the chromogen. The sections were coverslipped with Mountex (Göteborgs Termometerfabrik, Göteborg, Sweden) and analyzed in a light microscope and photographed for detection of galanin-ir.

IN SITU HYBRIDIZATION (PAPER III)

In contrast to immunohistochemistry, *in situ* allows for detection of cellular expression of a specific gene transcript using radioactively labeled probes complementary to the mRNA of interest. Thus, this technique results in visualization of mRNA and not the actual protein or peptide, which the mRNA is coding for.

Tissue preparation and oligonucleotide probes

Male NMRI mice were decapitated and the brains removed and frozen followed by cryosectioning (14 μm) and mounting onto pretreated glass slides (ProbeOn; Fisher Scientific Inc; Pittsburg, USA). The sections were stored at −20 °C until use. By the use of MacVector software (IBI, New Haven, CT), oligonucleotide probes were selected based on optimum ratio of guanosine + cytosine/total nucleotide numbers (50-65%) and minimal homology (not greater than 80%) with other GenBank entered nucleotide sequences. Oligonucleotide probes were made reversed and complementary to nucleotides and synthesized by CyberGene (Stockholm, Sweden) (Table 4).

TABLE 4. Oligonucleotide probes used for the *in situ* hybridization

PROBE	NUCLEOTIDES	GENBANK ACCESSION NO
5-HT_{1A} receptor	97-144	U39391
Parvalbumin	349-396	NM013645
VAChT	595-642	NM021712
VGLUT2	760-807	AF324864

Hybridization procedure and autoradiograms

The probes were labeled with ^{35}S-dATP (BioNuclear AB, Stockholm, Sweden) at the 3'-end by the use of deoxynucleotidyltransferase (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and purified using ProbeQuant G50 micro columns (GE Healthcare). *In situ* hybridization was conducted as previously described (DAGERLIND et al., 1992). Briefly, sections were hybridized at 42 °C overnight with 0.5 ng of labeled probe per slide in a cocktail, after which they were rinsed in 1x SSC buffer for 4 x 15 min at 56 °C. After cooling down in room temperature they were rinsed in distilled water and dipped in 60 and 95% ethanol.

Following air-drying, the labeled tissue sections were apposed to β_{max}autoradiography film (GE Healthcare), which were then exposed for varied time periods and then developed with LX 24 and fixed with AL4 (Eastman Kodak, Rochester, NY, USA). The films were scanned at 2000 dots per inch (UMAX PowerLook 3000) using the UMAX Magic Scan DA 4.2 software (UMAX Technologies, Fremont, CA, USA). Images were processed using the Adobe Photoshop 6.0 software (Adobe Systems, San José, CA, USA).

HISTOLOGICAL VERIFICATION OF THE INFUSION SITE

Each cannulated animal was decapitated and the brains were removed and frozen. Brains were cryosectioned (50 μm) and mounted on gelatin-coated (Paper I) or uncoated (Paper IV and V) glass slides. In paper I, thionine-staining was used to confirm the cannula position, whereas in paper IV and V, sections were immediately examined in a light microscope for cannula confirmation, and some sections were counterstained with nuclear fast red and computer scanned (Epson, Sollentuna, Sweden). Animals with an incorrect cannula position, i.e. outside the MS/vDB, were excluded from the final statistical analysis.

STATISTICAL ANALYSES

Overall effects of treatment in the behavioral experiments were analyzed using a repeated measures two-way analysis of variance (ANOVA) (water maze acquisition, microdialysis, locomotor activity) or a one-way ANOVA (water maze retention, PA, elevated plus-maze). When only two groups were compared in PA, a two-tailed t-test was used. In the quadrant and zone analyses from the retention test in paper IV and V, possible significant difference from chance was analyzed using a 95% confidence interval from the mean. For each significant F-ratio, Fisher's protected least significant difference test or Student-Newman-Keuls test were used (KIRK, 1968). A significant level of $P < 0.05$ was accepted as statistically significant and all post-hoc tests were two-tailed.

RESULTS AND DISCUSSION

STUDIES ON THE ROLE OF MUSCARINIC RECEPTORS IN LEARNING AND MEMORY (PAPER II, III AND VI)

EFFECTS OF SYSTEMIC MUSCARINIC BLOCKADE ON SPATIAL LEARNING (PAPER II AND VI)

The non-selective muscarinic antagonist scopolamine is widely used as a pharmacological tool for investigating the role of brain ACh in learning and memory mechanisms (BARTUS et al., 1985; BEJAR et al., 1999; BLOKLAND, 1995; DEUTSCH, 1971; HASSELMO & WYBLE, 1997; ÅHLANDER-LÜTTGEN et al., 2003; ÖGREN et al., 1996). However, there are a number of problems in using systemic administration of compounds to analyze specific psychological of physiological processes in the brain, since they can produce a number of unspecific effects. In this context, there are some important variables to consider, such as the affinity of the compound for the receptor system to be studies, the brain kinetics of the compound including the lipid-water coefficient, as well as the duration of the pharmacological action. Nevertheless, there are both advantages as well as disadvantages in using systemic administration of muscarinic receptor antagonists in studying the role of ACh in mnemonic functions. Unlike local administration of e.g. muscarinic antagonists, systemic administration has the advantage to block all muscarinic receptors within areas of the brain important for learning, such as the cortex and the hippocampus. On the other hand, scopolamine will produce behavioral effects by interfering with cholinergic muscarinic transmission in areas such as the striatum, which can interfere with the performance of the learning task. The behavioral effects of scopolamine are dose-related and it is therefore critical to use very low doses of the compound to minimize behavioral and physiological disturbances such as peripheral and central anticholinergic effects. In this context, it is notable that the published literature on scopolamine is mostly based on high or very high doses of the compound.

In view of this background, the present studies have used relatively low doses of scopolamine. Importantly, scopolamine, even at a dose of 0.1 or 0.3 mg/kg (s.c; 40 or 10 min prior to training, respectively) impaired spatial learning and memory in the water maze task, supporting earlier findings (ÅHLANDER-LÜTTGEN et al., 2003; ÖGREN et al., 1996). However, even at these very low doses, systemic administration of scopolamine also increased motor activity as measured by swim speed which could involve blockade of muscarinic receptors in both the hippocampus and striatum (ÖGREN et al., 1996). It is also well known that scopolamine increases locomotor activity, probably mediated by striatal mechanisms (TOIDE, 1989). Animals receiving systemic scopolamine also displayed an increase in thigmotaxic swimming, which, however, is dependent on the time interval between injection and spatial training (ÅHLANDER-LÜTTGEN et al., 2003). Together, these observations suggest that scopolamine induces behavioral effects at the same doses, which impair spatial learning. In view of these findings, it is critical to examine whether it is possible to dissociate the effects of scopolamine on non-cognitive factors from those related to learning mechanisms.

In order to successfully acquire the water maze task, the rat must learn the location of the hidden platform and also the general behavioral procedure (non-spatial components) of the task such as swimming, climbing onto the platform, remaining on the platform and associate the platform with rescue from the water. In addition, the animal has to suppress the natural tendency to swim along the pool wall (i.e. thigmotaxic swimming) (BANNERMAN et al., 1995; HOH & CAIN, 1997; MORRIS, 1984; WHISHAW & TOMIE, 1987). To investigate the role of non-spatial components for spatial learning, the animals received NSP in the water maze prior to the actual spatial learning sessions. NSP was conducted using a visually cued platform, which provides the animal with knowledge about the behavioral procedures and strategies needed for successful performance of the task. This training procedure also allows for investigation of possible disturbances in non-

mnemonic processes such as vision, motivation or sensorimotor coordination. NSP has previously been reported to prevent the spatial learning impairments seen after systemic muscarinic blockade (BEIKO et al., 1997; see CAIN, 1998; CAIN et al., 2000).

Analysis of the behavioral effects of saline- and scopolamine treated animals revealed profound differences during NSP. Saline-treated rats reduced their thigmotaxic swimming to a large extent, as well as their escape latency during the six trials (Figure 12B and 13A). Scopolamine (0.3 mg/kg; 10 min prior to NSP), on the other hand, markedly disrupted NSP performance, resulting in no change in escape latency or thigmotaxic swimming during training (Figure 12A and 13A) (paper VI). However, a further analysis using two training sessions instead of one, revealed that saline- and scopolamine-treated animals did not differ with regard to escape latency and thigmotaxic swimming on the second day (Figure 13B) (paper II). These findings indicate that scopolamine does not affect visual ability or swim motivation.

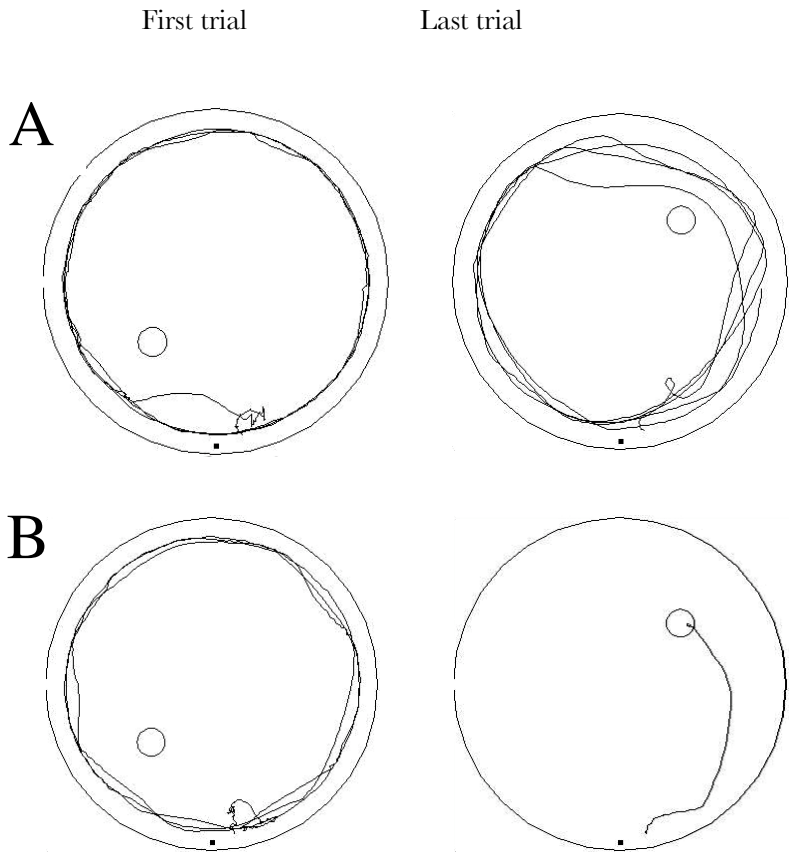


FIGURE 12. Swim paths from two representative animals in the NSP trials (paper VI). The first (left) and last (right) NSP trial is shown from a scopolamine (A) and saline (B) treated animal, respectively. In the saline-treated animal (B), the thigmotaxic swimming is clearly reduced over the six trials compared to the scopolamine-treated rat (A), which display a high degree of thigmotaxis on both the first and the last trial. The round circle and the little black dot represent the platform and starting position, respectively. Both the location of the platform and the starting positions were semi-randomized over the six consecutive trials. The water maze was surrounded by an opaque curtain, in order to occlude any extramaze cues.

Familiarity to the behavioral procedures prior to training is clearly important since saline-treated rats subjected to NSP performed better compared to non-pretrained control rats. NSP also reduced thigmotaxic behavior during the first two training sessions. However, this improvement was only seen during the first two training days (sessions), indicating that the non-pretrained rats are able to compensate for the initial

impairment, since no difference was seen at the time of retention. Rats that were given scopolamine before NSP but given saline before spatial training did not show any overall impairment in water maze learning, as compared to the control group receiving saline prior to both NSP and spatial training. This finding suggest that rats given scopolamine before NSP, which were markedly impaired during NSP, appear to have acquired certain elements of the task that are important for subsequent hidden platform acquisition.

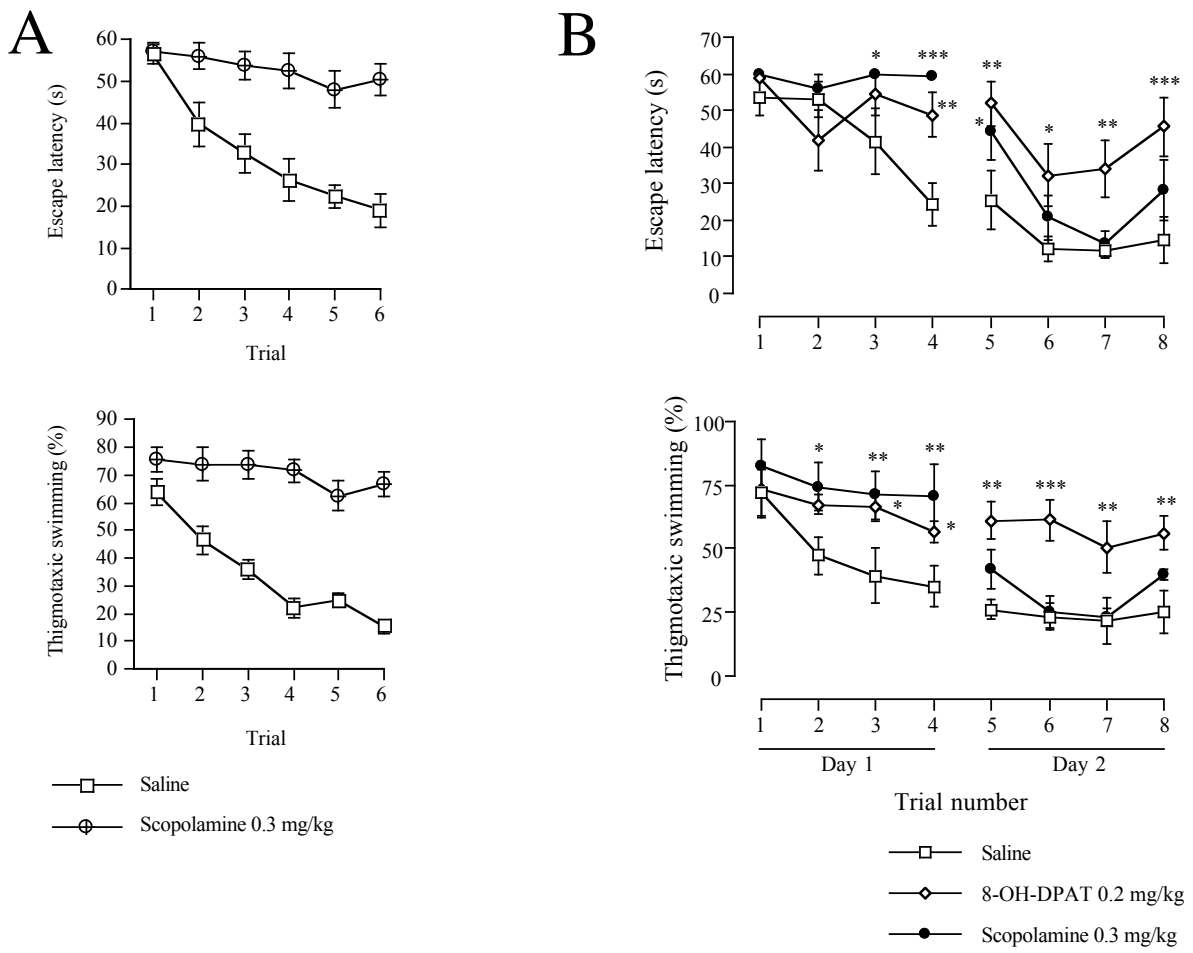


FIGURE 13. Comparison of the effects of scopolamine treatment on the visually cued platform tests in paper VI (A) and paper II (B). In the left panel (A), animals received scopolamine (0.3 mg/kg; 10 min before NSP; n=16), which consisted of six consecutive trials during one single day. The right panel shows animals that received scopolamine (0.3 mg/kg; 10 min before training) or 8-OH-DPAT (0.2 mg/kg; 15 min before training) prior to the visually cued platform test, which was conducted during two consecutive days with four trials per day. As shown in panel B, the initial impairment in performance seen on day 1 is clearly attenuated on day 2, indicating that animals, despite receiving scopolamine, can acquire certain elements of the task that are of importance for performance. Thigmotaxic swimming was defined as percentage of total swim time spent within a distance of 10 cm from the walls of the pool. *P<0.05; **P<0.01; ***P<0.001 vs. saline.

NSP also markedly reduced the scopolamine-induced impairment in spatial learning and memory observed in non-pretrained rats, in line with previous findings (BEIKO et al., 1997; see CAIN, 1998; CAIN et al., 2000). Furthermore, NSP eliminated thigmotaxic swimming in scopolamine-treated rats. However, importantly, the animals which were subjected to NSP and receiving scopolamine prior to spatial training showed a significant acquisition impairment, and also a highly significant memory impairment in the retention test. The failure of the scopolamine-treated rats to recall the former platform position can be due to an actual loss of memory or possibly a disturbance in memory retrieval. One way to test this hypothesis is to give a reminder cue to the animals before the retention test. Interestingly, animals with partial hippocampal lesions, which have deficient memory performance, are able to remember the location of a platform when subjected to a "reminding" procedure, suggesting that the impairment is probably related to a retrieval deficit (DE HOZ et al., 2004; MARTIN et al., 2005).

Taken together, the results from paper VI confirm the previous results showing that NSP can attenuate the spatial learning impairments induced by systemic muscarinic blockade. However, unlike previous proposals, these results support a role for brain muscarinic receptors in spatial learning and memory (BEIKO et al., 1997; CAIN, 1998; CAIN et al., 2000). The results are consistent with the view that successful performance in the water maze task involves not only spatial learning but also procedural learning, i.e. "learning how" (see Introduction) (PACKARD, 2003), which is acquired during NSP and thereby improves subsequent spatial performance. Importantly, brain ACh appears to play a minor role in the NSP effect on spatial learning.

EFFECTS OF SYSTEMIC MUSCARINIC BLOCKADE ON PASSIVE AVOIDANCE (PAPER II AND III)

In the PA experiments a weakly aversive foot-shock was used, to allow for detection of both decreases (i.e. memory impairment) and increases (i.e. memory improvement) in PA retention. Administration of scopolamine (0.3 mg/kg; 10 min before training) significantly impaired PA retention in the rat, confirming previous data (BAMMER, 1982; MISANE & ÖGREN, 2003; ÅHLANDER-LÜTTGEN et al., 2003) (paper II). The same pattern of results was obtained in mice. Pretraining administration of scopolamine (40 min prior to training) caused an impairment at 0.1 and 0.3 mg/kg, but not at 0.03 mg/kg. Importantly, the dose-range where scopolamine impaired PA retention in rats and mice is the same in a spatial learning paradigm in rats. This implies that systemic scopolamine affects similar mechanisms in the two species and the two tasks. Increases of brain ACh by systemic administration of the AchE inhibitor physostigmine prior to training produced facilitation at low doses and impairment of PA retention at higher doses. This bell-shaped effect may be explained by the occurrence of peripheral muscarinic side effects (tremor and salivation) and/or central cholinergic overstimulation produced by AChE inhibitors at high doses (YOSHIDA & SUZUKI, 1993).

Most studies are based on administration of drugs before training. However, there are results indicating that muscarinic receptors are involved in the consolidation of aversive memories. The present results showed that scopolamine (0.1 mg/kg) administered immediately after training failed to affect PA retention, suggesting that blockade of muscarinic receptors does not interfere with the immediate consolidation of memory.

The present data support a role for brain muscarinic receptors in emotional learning. Scopolamine profoundly increase hippocampal extracellular ACh due to blockade of presynaptic M_2 receptors (STILLMAN et al., 1996; ÖGREN et al., 1996). However, at the same time, scopolamine blocks postsynaptic muscarinic receptors, which results in memory impairment (MISANE et al., 1999). This hypothesis is supported by the finding that although M_2/M_4 knockout mice display an increase in basal hippocampal ACh release compared to wild type mice, they are impaired in the PA test (TZAVARA et al., 2003).

THE ROLE OF MEDIAL SEPTAL MUSCARINIC LIGANDS AND GALANIN IN SPATIAL LEARNING (PAPER I)

EFFECTS OF INTRASEPTAL MUSCARINIC LIGANDS ON LEARNING AND MEMORY; RELATION TO HIPPOCAMPAL ACETYLCHOLINE RELEASE.

A number of studies indicate a pivotal role for hippocampal ACh in cognitive processes such as spatial learning (EVERITT & ROBBINS, 1997; GIVENS & SARTER, 1997; HASSELMO, 1999; ÖGREN et al., 1996). As discussed previously, the role of the cholinergic septohippocampal neurons in learning and memory is presently not clear. It has been proposed that systemic scopolamine exerts its action via septohippocampal cholinergic neurons (GIVENS & OLTON, 1995). Medial septal neurons express a number of cholinergic receptors, which are potential targets for compounds affecting cognitive functions.

It has been suggested that the cholinergic neurons in the MS/vDB are regulated by excitatory cholinergic inputs (see Introduction). To test these hypotheses (paper I), scopolamine (10-15 μg/rat) was infused into the MS/vDB to produce a blockade of muscarinic transmission. This treatment only produced a marginal impairment in water maze acquisition at the highest dose administered, indicating that blockade of muscarinic transmission in the MS/vDB have little effect on spatial reference memory. Contrary to previous hypothesis, intraseptal scopolamine produced an increase in basal hippocampal ACh release, as measured by a microdialysis probe located in the ventral hippocampus. This finding is very important, since it indicates that the MS/vDB cholinergic neurons projecting to the hippocampus are under an inhibitory (ALREJA et al., 2000; WU et al., 2000), and not excitatory muscarinic tone, as previously hypothesized (DUTAR et al., 1983; see GIVENS & SARTER, 1997; LAMOUR et al., 1984; SEGAL, 1986) (Figure 15). Moreover, these findings do not support the previous proposal that the MS/vDB is an important area for the memory impairments observed after systemic scopolamine administration (GIVENS & OLTON, 1995).

Direct intraseptal infusion of cholinomimetic drugs have shown to influence hippocampal physiology and produce memory facilitation. To enhance cholinergic transmission, the muscarinic receptor agonist carbachol (0.5 and 1 μg/rat) was infused into the MS/vDB. Carbachol produced only a weak impairment in water maze acquisition at the 1 μg dose, which dose also increased locomotor activity but not swim speed, and produced a transient loss of balance in some animals. These findings indicate that the marginal water maze impairment could be related to a minor disturbance of sensorimotor functions. The results obtained with carbachol are in general agreement with recent findings that intraseptal carbachol given prior to training did not alter performance in a DNMTS radial maze task in the rat (BUNCE et al., 2004b).

Similar to scopolamine, intraseptal carbachol produced an increase in basal hippocampal ACh release. These findings are seemingly inconsistent with the results obtained with scopolamine, but consistent with the findings that carbachol activates the septohippocampal projection evidenced by an increase in hippocampal theta rhythm (MONMAUR & BRETON, 1991). The finding that both blockade and stimulation of MS/vDB muscarinic receptors result in similar effects can be explained by an electrophysiological study, showing that muscarinic cholinergic transmission is mainly transmitted via the septal GABAergic neurons. Moreover, muscarinic receptor antagonists were found to inhibit the firing rate of septohippocampal GABAergic neurons, presumably via blockade of muscarinic M_3 receptors (ALREJA et al., 2000). This would result in a disinhibition of septal cholinergic neurons through GABAergic axon collaterals, thereby increasing hippocampal ACh release. The effect of carbachol, on the other hand, is probably related to several different factors, including a decrease in muscarinic tone via stimulation of M_2 receptors, or via direct action on GABA transmission via M_2 and M_3 receptors, which allows for an indirect modulation of septal cholinergic neurons (paper I) (Figure 15). A recently published electrophysiological study provides an alternative interpretation. Thus, cholinomimetics such as carbachol can activate intrinsic glutamatergic neurons in the MS/vDB, which in turn can activate septohippocampal cholinergic and GABAergic neurons (MANSEAU et al., 2005). This means that the putative intrinsic glutamatergic network is an important target for the cholinergic input to the MS/vDB (Figure 14).

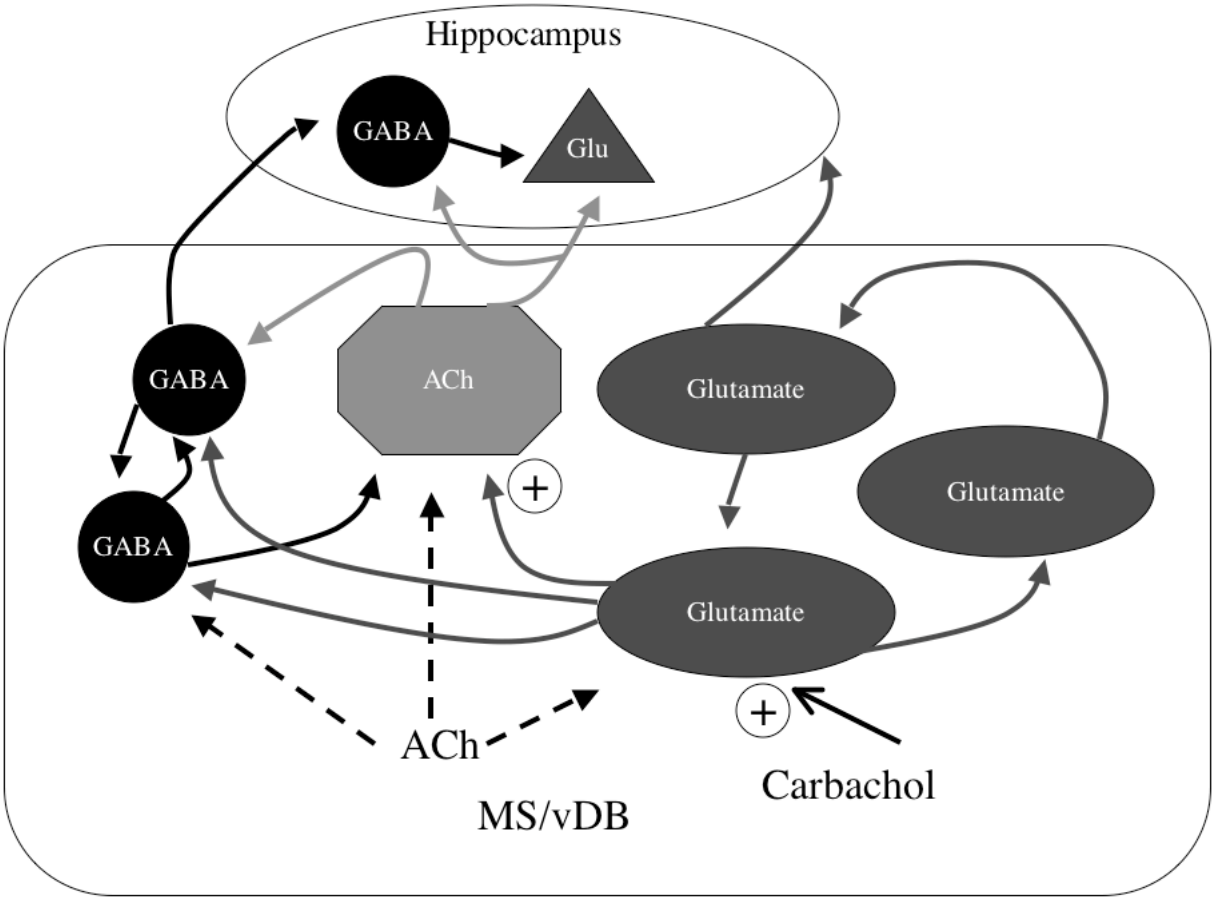
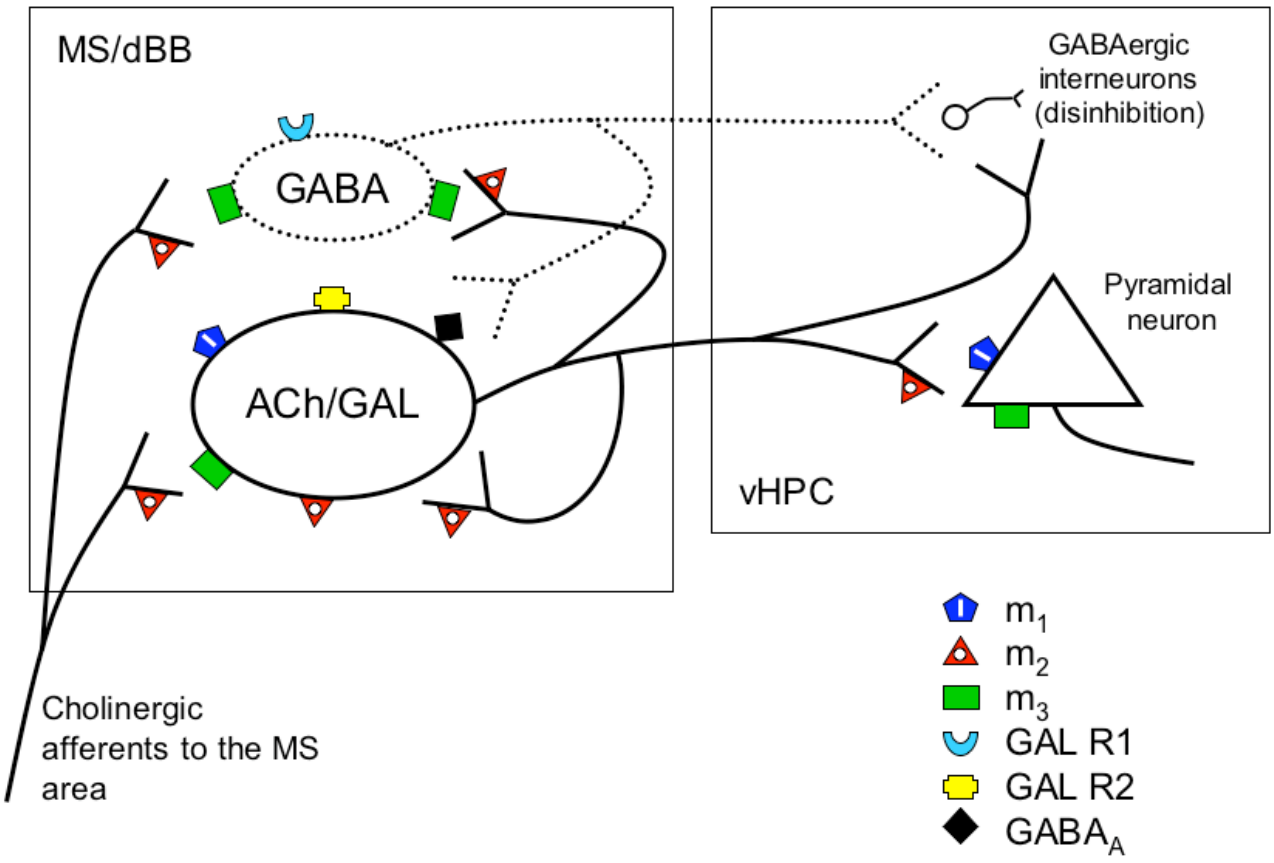


FIGURE 14. Schematic picture illustrating the interactions between the putative intrinsic glutamatergic neuronal network and the cholinergic and GABAergic septohippocampal pathway. The glutamatergic network can activate both cholinergic and GABAergic neurons projecting to the hippocampus. In addition, a subpopulation of the glutamatergic neurons presumably projects to hippocampus. ACh, originating in the brainstem, can activate all three neuronal types within the MS/vDB. In the present study, carbachol may activate glutamatergic interneurons, which in turn activates cholinergic neurons projecting to the hippocampus, resulting in an increase in basal hippocampal ACh release (activation/increased firing is represented by **+**). Adapted from (Manseau et al., 2005; Sotty et al., 2003)

MEDIAL SEPTAL GALANIN AND SEPTAL CHOLINERGIC NEURONS

On the basis of previous findings, galanin has been proposed to have an inhibitory role in memory functions and on hippocampal cholinergic transmission (see Introduction). Furthermore, it has been assumed that galanin also has an inhibitory function on cholinergic neurons of the septohippocampal projection (CRAWLEY, 1996). However, previous reports on the effects of intraseptal infusion of galanin have provided conflicting results. Both impairments (GIVENS et al., 1992) and no effects (ROBINSON & CRAWLEY, 1993) on working memory have been demonstrated following intraseptal galanin infusion. In contrast, our results suggest that galanin (0.3-3 nmol/rat) facilitates spatial acquisition when infused into the MS/vDB. Moreover, the doses of galanin, which improved spatial learning, also activated the septohippocampal projection since they produced an increase in basal hippocampal ACh release. This contrasts with the results obtained in the hippocampus, i.e. terminal areas of the cholinergic neurons, since galanin perfused through the microdialysis probe produced a marked decrease in ACh release in the ventral hippocampus (ÖGREN et al., 1996). These findings indicate opposite roles for galanin in its regulatory action on cholinergic neurons at the cell body and terminal levels, respectively.



The mechanism underlying the putative stimulatory effects of galanin on septohippocampal cholinergic neurons is difficult to interpret since the distribution of the galanin receptor subtypes in the MS/vDB is not known. Galanin could stimulate excitatory GAL-R2 receptors located on septohippocampal cholinergic neurons. Moreover, only a limited number of cholinergic septohippocampal neurons but most GABAergic neurons have been shown to express GAL-R1 mRNA in the rat (MILLER et al., 1997). Since the GAL-R1 receptor is believed to be mainly inhibitory (PARKER et al., 1995), stimulation of this receptor would result in an inhibition of the GABAergic neurons. This would, at least theoretically, cause a disinhibition of cholinergic septohippocampal neurons and subsequent increase in hippocampal ACh release, in analogy with the interpretation of the results obtained with scopolamine (see above) (Figure 15). This interpretation is supported by the finding that galanin markedly enhance the effects of scopolamine in the MS/vDB. Hence, the combination of galanin and scopolamine infused into the MS/vDB caused a profound increase in basal ACh release, combined with a marked impairment in spatial learning and memory. This finding indicates that the level of muscarinic tone within the MS/vDB is important for the effects of galanin on hippocampal cognitive functions.

Based on postmortem studies of AD brains, which have shown an overexpression of galanin and galaninergic fibers in cholinergic cell body areas associated with this disease e.g. the nucleus basalis magnocellularis and the MS/vDB, galanin has been implicated in AD (CHAN-PALAY, 1988; see COUNTS et al., 2003; MUFSON et al., 1993). Moreover, galanin receptor binding have been shown to be increased in terminal areas such as the hippocampus in AD patients (RODRIGUEZ-PUERTAS et al., 1997). Since galanin inhibits hippocampal cholinergic functions, (see CRAWLEY, 1996; ÖGREN et al., 1996), it has been proposed that galanin receptor antagonists could provide a novel therapeutic approach for the cognitive impairments seen in AD (CRAWLEY, 1996; HÖKFELT et al., 1987). However, our findings point towards a more complex role of galanin and, together with electrophysiological findings demonstrating that galanin can stimulate cholinergic neurons in the nucleus basalis magnocellularis (JHAMANDAS et al., 2002), suggest that the increases in galanin observed in cholinergic cell body areas of AD patients (CHAN-PALAY, 1988; MUFSON et al., 1993) may represent a way for the brain to "save" the cholinergic neurons (see COUNTS et al., 2003). In this context it is important to note that galanin also has a neurotrophic role (ELLIOTT-HUNT et al., 2004). These findings lead to the intriguing hypothesis that both galanin receptors agonists (to improve the functions of cholinergic neurons at the cell body level) and antagonists (to block the impairing effect of galanin on cholinergic transmission at the terminal level), may serve as novel therapeutic targets for the treatment of AD symptoms (see HÖKFELT, 2005). It is therefore of outmost importance to determine which galanin receptor subtypes that mediate the action of galanin at the cell body and terminal levels, respectively.

FIGURE 15. Schematic illustration showing the possible modulatory sites at which muscarinic ligands and galanin (GAL) may influence the activity of septohippocampal cholinergic and GABAergic neurons. Scopolamine blocks the muscarinic tone and decreases cholinergic septohippocampal neuronal activity. However, at the same time, scopolamine increases cholinergic septohippocampal impulse flow through a disinhibition of GABAergic neurons, concomitantly with a blockade of postsynaptic inhibitory M_2 receptors located on a subpopulation of septohippocampal cholinergic neurons. Carbachol, which stimulates M_1-M_4 receptors, enhance impulse flow in the septohippocampal GABAergic pathway through stimulation of M_3 receptors. Simultaneously, it enhances activity in the septal cholinergic neurons via stimulation of a subset of cholinergic neurons expressing excitatory M_1 receptors and by reducing the muscarinic tone within the septal area induced by stimulation of M_2 autoreceptors localized on cholinergic afferent input and axon collaterals. These two mechanisms are together sufficient to result in an increase in hippocampal ACh release. Galanin, in turn, mediates a disinhibition of GABAergic neurons through GAL-R1 (inhibitory) receptors, which are expressed by GABAergic neurons. This is combined with a concomitant stimulation of GAL-R2 (excitatory) receptors located on cholinergic septohippocampal neurons. The figure and proposed mechanisms are partly based on studies demonstrating receptor mRNA in the MS/vDB neurons (ALREJA et al., 2000; MILLER et al., 1997; O'DONNELL et al., 2003; ROUSE & LEVEY, 1996). Reprinted with permission from Elsevier.

Concluding remarks

In summary, the findings in paper I emphasize the important role of the level of muscarinic activity in the MS/vDB for the control of hippocampal cholinergic transmission as well as for hippocampal-dependent learning. The results also stress that there exist dynamic changes in cholinergic tone, which are important for hippocampal cognitive functions. There is no evidence for a monotonic relationship between an increase in hippocampal cholinergic transmission and cognitive functions, implying that complex dynamic variables determine the mnemonic effects of septohippocampal activity. Our results rather suggest that an increase in cholinergic transmission will not necessarily result in enhanced cognitive function. In other words, improvement of cognitive function may only be possible if the cholinergic transmission is readjusted to the optimal physiological range. If hippocampal cholinergic transmission is reduced, or there exist an overstimulation (Figure 16), cognitive function will be impaired. In this analysis it is clear that further understanding of the dynamic interactions between GABAergic, cholinergic and glutamatergic neurons is necessary for a better understanding of the complex role of the septohippocampal projection in cognition.

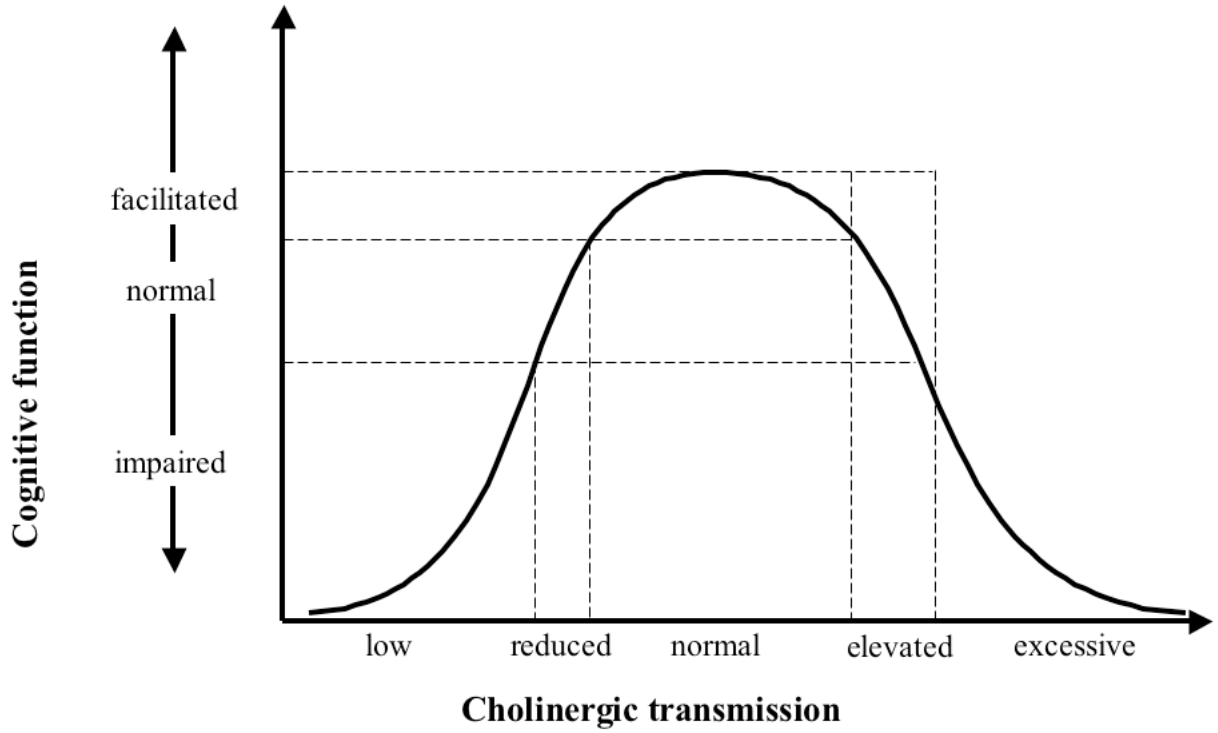


FIGURE 16. Schematic figure representing the theory of an optimal cholinergic transmission for cognitive function. We suggest there exist an optimal level of cholinergic transmission in the brain for cognitive performance, and if the cholinergic transmission is reduced, it will result in cognitive impairment. However, the same thing will occur if the cholinergic transmission is increased markedly above normal levels. Modified from (ELVANDER & ÖGREN, 2005) with permission.

ANALYSIS OF THE ROLE OF THE 5-HT$_{1A}$ RECEPTOR IN LEARNING AND MEMORY (PAPER II, III AND V)

EFFECTS OF SYSTEMIC ADMINISTRATION OF 5-HT$_{1A}$ LIGANDS

Accumulating results from anatomical, physiological and behavioral studies point towards a role for brain serotonergic 5-HT$_{1A}$ receptors in cognitive functions (BARNES & SHARP, 1999; BUHOT et al., 2000; ROTH et al., 2003; ÖGREN, 1985). Pretraining administration of 5-HT$_{1A}$ receptor agonists such as 8-OH-DPAT have in most studies resulted in learning and memory deficits (CARLI et al., 1995; CARLI & SAMANIN, 1992; KANT et al., 1998; MENDELSON et al., 1993; MISANE & ÖGREN, 2000; STIEDL et al., 2000b). On the other hand, the precise role of the cognate 5-HT$_{1A}$ receptor in cognition is presently not clear, since the results with 5-HT$_{1A}$ receptor antagonists have provided conflicting results. Studies using different 5-HT$_{1A}$ receptor antagonists have reported facilitation (BELCHEVA et al., 1997; PITSIKAS et al., 2003; SANGER & JOLY, 1989; SCHNEIDER et al., 2003), impairment (GALEOTTI et al., 2000) or no effects (CARLI et al., 1997; MANUEL-APOLINAR & MENESES, 2004; MISANE & ÖGREN, 2003; STIEDL et al., 2000b) on cognitive performance in different rodent tasks. Paper II and III, therefore, aimed at elucidating the role of the 5-HT$_{1A}$ receptor in both spatial and aversive (emotional) memory. Water maze experiments were performed in rats, whereas PA was conducted using both rats (paper II) and mice (paper III) to allow for species comparisons. To investigate whether the effects obtained after systemic administration of 8-OH-DPAT involves septohippocampal mechanisms, a 5-HT$_{1A}$ receptor agonist was locally infused into the MS/vDB and the effects in spatial and aversive learning was examined (paper V).

Water maze experiments (paper II)

Pretraining s.c. administration of the 5-HT$_{1A}$ receptor antagonists NAD-299 (0.05-1.5 mg/kg) and WAY-100635 (0.3 and 1 mg/kg) failed to alter spatial acquisition in the water maze. On the other hand, an impairment in spatial acquisition was observed after s.c. injection of 8-OH-DPAT (0.1-0.3 mg/kg). Notably, a low, presumably presynaptic dose of 8-OH-DPAT (0.03 mg/kg) did not affect water maze performance (Table 5). The impairment caused by 8-OH-DPAT (0.2 mg/kg) was completely blocked by NAD-299 (0.05 and 0.5 mg/kg), indicating that the deficit in acquisition caused by 8-OH-DPAT was mediated via postsynaptic 5-HT$_{1A}$ receptors.

An analysis of possible sensorimotor effects demonstrated that 8-OH-DPAT caused an increase in swim speed. This finding is in line with the observed increase in locomotor activity after 8-OH-DPAT administration, probably mediated via postsynaptic 5-HT$_{1A}$ receptor stimulation (JACKSON et al., 1998). In contrast, a decrease in rearing was observed, indicative of reduced exploratory behavior, in line with the findings in the water maze (see below). Further analysis of the 8-OH-DPAT-treated group showed that these rats displayed a marked increase in thigmotaxic swimming and difficulties in following the experimenter's hand, as well as to climb onto and stay on the platform. In addition, 8-OH-DPAT-treated animals showed a profound deficit in navigating to the platform in the visually cued platform test. Interestingly, while the scopolamine-treated animals improved on the second day of testing in the visually cued platform test, the rats receiving 8-OH-DPAT failed to alter their performance and remained impaired throughout testing (Figure 13B). Notably, NAD-299 or WAY-100635 did not alter swim speed or locomotor activity.

In summary, these findings suggest that stimulation of postsynaptic 5-HT$_{1A}$ receptors by 8-OH-DPAT impairs spatial learning at the same doses, which also produced sensorimotor disturbances and increased thigmotaxic swimming. This suggests that the poor spatial navigation strategies following 5-HT$_{1A}$ receptor stimulation probably contribute to the deficits in spatial learning. In this context, it is notable that the effects of 8-OH-DPAT in the visually cued platform test differs from that of scopolamine, indicating that stimulation of 5-HT$_{1A}$ or blockade of muscarinic receptors result in different consequences for the ability of the animal to acquire non-spatial information of importance for spatial learning.

TABLE 5. Effects of 5-HT_{1A} receptor ligands on water maze performance in the rat (paper II)

TREATMENT (MG/KG S.C.)	WATER MAZE ACQUISITION	WATER MAZE RETENTION
8-OH-DPAT (0.03 - 0.3)	0.03: No effect 0.1, 0.3: Impairment	0.03: No effect 0.1, 0.3: Impairment
NAD-299 (0.05 - 1.5)	No effect	No effect
WAY-100635 (0.3, 1)	No effect	No effect
NAD-299 (0.05, 0.5) and 8-OH-DPAT (0.2)	Blockade of the impairment induced by 8-OH-DPAT	Blockade of the impairment induced by 8-OH-DPAT
Scopolamine (0.1) and NAD-299 (0.05, 0.5)	No blockade of the impairment caused by scopolamine	No blockade of the impairment caused by scopolamine
NAD-299 (1) and scopolamine (0.3)	No blockade of the impairment caused by scopolamine	No blockade of the impairment caused by scopolamine
Scopolamine (0.1) and WAY-100635 (1)	No blockade of the impairment caused by scopolamine	No blockade of the impairment caused by scopolamine
WAY-100635 (0.3, 1) and scopolamine (0.1)	No blockade of the impairment caused by scopolamine	No blockade of the impairment caused by scopolamine

There are a number of behavioral studies indicating a putative interaction between 5-HT and cholinergic neurons of importance for learning and memory functions (see CASSEL & JELTSCH, 1995; LEHMANN et al., 2002; MILLAN et al., 2004; see STECKLER & SAHGAL, 1995). The complexity of this interaction is highlighted by the findings that both stimulation and blockade of brain 5-HT_{1A} receptors cause an increase in hippocampal ACh release (FUJII et al., 1997; HU et al., 2003; IZUMI et al., 1994). Moreover, the impairment induced by systemic scopolamine in the PA test was attenuated by the 5-HT_{1A} receptor antagonist NAD-299, supporting an important functional interaction between muscarinic and 5-HT_{1A} receptors in emotional memory (MISANE & ÖGREN, 2003).

The possible functional role for an interaction between 5-HT_{1A} and muscarinic receptors in spatial learning was studied in the water maze using selective 5-HT_{1A} receptor antagonists in combination with scopolamine. Neither NAD-299, nor WAY-100635, which by themselves failed to alter spatial learning, could attenuate the spatial learning and memory impairment induced by scopolamine. Moreover, the 5-HT_{1A} receptor antagonists also failed to affect the increase in swim speed produced by scopolamine administration, indicating that the changes in non-cognitive factors caused by scopolamine cannot be reversed by 5-HT_{1A} receptor blockade. Since the impairment in spatial learning induced by scopolamine seems to be partly related to non-cognitive factors, it will be important to examine whether 5-HT_{1A} receptor blockade, can block the scopolamine-induced spatial memory impairment seen in rats, which receives NSP before spatial acquisition.

In the interpretation of the present data, it is important to consider that interactions between 5-HT and ACh in the brain is of greater importance for spatial working rather than for spatial reference memory. This hypothesis is supported by the observation that the impairment in radial maze working memory induced by 192 IgG-saporin-induced lesions of the septal cholinergic neurons in the rat, was significantly attenuated when combined with a concomitant lesion of the serotonergic innervation of the hippocampus, unlike the impairment in water maze reference memory (LEHMANN et al., 2002).

Passive avoidance experiments (paper II and III)

In contrast to spatial learning, 5-HT_{1A} receptor stimulation produced a biphasic effect on PA retention in both rats and mice (Table 6). A low dose of 8-OH-DPAT (0.01 mg/kg in rats; 0.01 and 0.03 mg/kg in mice) facilitated PA retention, while higher doses caused an impairment. Moreover, in contrast to the results obtained in the water maze test, 5-HT_{1A} receptor antagonists dose-dependently facilitated PA retention in both species (Table 6). NAD-299 completely blocked the impairment, but failed to block the facilitation caused by 8-OH-DPAT, indicating that the impairing effect of 8-OH-DPAT involves stimulation of postsynaptic 5-HT_{1A} receptors, in line with the results obtained in the spatial learning task.

The facilitation of PA memory caused by low doses of 8-OH-DPAT is most likely mediated via stimulation of 5-HT_{1A} autoreceptors located in the raphe nuclei (WARBURTON et al., 1997), resulting in a reduction of neuronal firing rate (SPROUSE & AGHAJANIAN, 1988) and a reduction in serotonergic transmission. Since the facilitatory effects of NAD-299 on PA retention most likely is due to a blockade of postsynaptic 5-HT_{1A} receptors, NAD-299 could theoretically block the mnemonic effects caused by a low dose of 8-OH-DPAT. However, the fact that the combined administration of NAD-299 and a low dose of 8-OH-DPAT did not further facilitate PA retention compared to each drug alone, indicates that there exists an optimal physiological range in which a reduction in tonic 5-HT transmission may facilitate PA memory.

Consistent with earlier observations, (MISANE & ÖGREN, 2003), pretreatment with NAD-299 completely prevented the impairment in PA memory induced by scopolamine in both rats and mice. In contrast, when scopolamine was administered prior to NAD-299 in mice, the impairment was only partly attenuated, suggesting that the temporal kinetics of 5-HT_{1A} and muscarinic receptors are important, as shown previously in the rat (MISANE & ÖGREN, 2003).

It has been proposed that 5-HT_{1A} receptors are involved in memory consolidation (MANUEL-APOLINAR & MENESES, 2004; MENESES & TERRON, 2001). However, immediate post-training administration of 8-OH-DPAT, NAD-299 or WAY-100635 did not affect PA retention (paper III), supporting the notion that 5-HT_{1A} receptors primarily play a role in memory acquisition (encoding) rather than consolidation processes (MISANE et al., 1998).

TABLE 6. Effects of 5-HT_{1A} receptor ligands on PA retention in rats (left) and mice (right) (paper II and III). The doses in the PA retention columns represent doses of the 5-HT_{1A} receptor antagonists, i.e. WAY-100635 or NAD-299.

TREATMENT (MG/KG S.C.)	PA RETENTION RATS	TREATMENT (MG/KG S.C.)	PA RETENTION MICE
8-OH-DPAT (0.01 - 0.3)	0.01: Improvement 0.03: No effect 0.1, 0.3: Impairment	8-OH-DPAT (0.01 - 1)	0.01, 0.03: Improvement 0.1 - 1: Impairment
NAD-299 (0.3 - 3)	0.3, 1: Improvement 3: No effect	NAD-299 (0.1 - 3)	0.1, 3: No effect 0.3-2: Improvement
WAY-100635	---	WAY-100635 (0.03 - 3)	0.03, 0.3: No effect 1 - 3: Improvement
NAD-299 (1) and 8-OH-DPAT (0.01)	No effect as compared to each drug alone	NAD-299 (0.3) and 8-OH-DPAT (0.03)	No effect as compared to each drug alone
NAD-299 (1) and 8-OH-DPAT (0.3)	Blockade of the impairment induced by 8-OH-DPAT	NAD-299 (0.3) and 8-OH-DPAT (1)	Blockade of the impairment induced by 8-OH-DPAT
NAD-299 (0.3, 1) and scopolamine (0.3)	0.3: No blockade 1: Blockade of scopolamine-induced impairment	NAD-299 (0.1 - 1) and scopolamine (0.1)	0.1: No blockade 0.3, 1: Blockade of scopolamine- induced impairment
Scopolamine and NAD-299	---	Scopolamine (0.1) and NAD-299 (0.1-1)	0.1: No blockade 0.3, 1: Partial blockade of scopolamine-induced impairment
NAD-299 (0.3, 1) and MK-801 (0.1)	0.3: Blockade of MK-801-induced impairment 1: No blockade	NAD-299 (0.3, 1) and MK-801 (0.3)	0.3: Partial blockade 1: Blockade of MK-801-induced impairment

5-HT_{1A} receptors are present on pyramidal neurons in the hippocampus (AZNAR et al., 2003) and stimulation of the 5-HT_{1A} receptor hyperpolarizes pyramidal neurons (BECK et al., 1992; TADA et al., 1999). On the basis of these observations it was hypothesized that a 5-HT_{1A} receptor antagonist could block or attenuate the impairment in PA caused by a decrease in glutamatergic signaling. In line with this hypothesis, administration of the NMDA receptor antagonist MK-801 produced an impairment in PA memory that was completely blocked by pretreatment of NAD-299 in both rats and mice (Table 6). This finding gives evidence for important interactions between 5-HT_{1A} receptors and glutamatergic transmission mediated by the NMDA receptor. This hypothesis is further supported by recent findings showing that a 5-HT_{1A} receptor antagonist could enhance evoked glutamate release in the hippocampus of the rat, and reverse learning deficits in a visual spatial discrimination task caused by MK-801 in the marmoset (SCHECHTER et al., 2005).

In summary, the 5-HT_{1A} receptors appear to play a role in cognitive functions, and that 5-HT_{1A} receptor antagonists can facilitate some aspects of cognitive function, probably via modulation of cholinergic and/or glutamatergic neurotransmission.

Elevated plus-maze and heart rate measurements (paper III)

It is generally believed that changes in emotional state could influence aversive learning processes, and an increase in anxiety-like behavior is predicted to enhance hippocampal-dependent learning (see CAHILL & MCGAUGH, 1998; FREY & MORRIS, 1997; see RICHTER-LEVIN & AKIRAV, 2003). Since the compounds were administered before training, they could cause changes in emotional states of the animal, thereby affecting autonomic functions and cognitive performance. However, the 5-HT_{1A} receptor antagonist NAD-299 failed to affect anxiety-related behavior as tested in the elevated plus-maze. This finding contrasts with observations in 5-HT_{1A} receptor knockout mice, which display increases in anxiety-like behaviors (HEISLER et al., 1998; RAMBOZ et al., 1998). The differences between mice treated with 5-HT_{1A} receptor antagonists and 5-HT_{1A} receptor knockout mice could be explained by compensatory mechanisms in 5-HT transmission following 5-HT_{1A} receptor knockout (RAMBOZ et al., 1998), or that 5-HT_{1A} receptors are essential for the development of normal anxiety-related circuits in the brain during post-natal development (see GROSS & HEN, 2004).

Blockade of 5-HT_{1A} receptors did not produce any changes in heart rate or heart rate variability compared to control animals, as measured by telemetry. In this experiment, the C57BL/6J strain was used, since it is considered to be a highly emotional strain (GRIEBEL et al., 2000; STIEDL et al., 1999) in which possible changes in the emotional state would be more easily detected.

Taken together, these results indicate that the effects of 5-HT_{1A} receptor blockade on PA memory is most likely not related to alterations in the emotional state of the animal.

Expression of 5-HT_{1A} receptor mRNA in the MS/vDB of the mouse (paper III)

Since the number of behavioral studies performed in mice is steadily increasing, it is important to study the localization of various receptors in this species. With respect to the 5-HT receptors, the cellular localization of the 5-HT_{1A} receptor is not well characterized in mice. Therefore, an *in situ* hybridization study was conducted, to examine the localization of the 5-HT_{1A} receptor in the MS/vDB of the mouse. Since the 5-HT_{1A} receptor protein is reported to have a somatodendritic localization (KIA et al., 1996), 5-HT_{1A} receptor mRNA can be used to identify neurons expressing this receptor. 5-HT_{1A} receptor mRNA was shown to be co-distributed with VAChT, parvalbumin and VGLUT2 in the MS/vDB. This gives anatomical support for the hypothesis that 5-HT_{1A} receptor-mediated transmission can influence cholinergic, GABAergic and glutamatergic neurons in the MS/vDB in a manner which is probably very similar to the situation in rats (LÜTTGEN et al., 2005).

EFFECTS OF INTRASEPTAL INFUSION OF A 5-HT_{1A} RECEPTOR AGONIST (PAPER V)

So far, systemic administration of the 5-HT_{1A} receptor agonist and antagonists have provided evidence for a role of this receptor in both spatial as well as aversive learning. However, it is not clear whether the effects of

systemic administration of 5-HT_{1A} receptor ligands involve the septohippocampal projection. Based on the limited knowledge of the role of the septal 5-HT_{1A} receptor in cognition, we examined the effects of intraseptal microinjections of the 5-HT_{1A} receptor agonist 8-OH-DPAT on spatial and aversive learning.

Water maze

8-OH-DPAT (1 or 4 μg/rat) did not produce any significant changes in spatial learning or memory when infused into the MS/vDB prior to water maze acquisition. Moreover, no effects on swim speed or thigmotaxic swimming was observed. These results indicate that the impairment in spatial acquisition as well as the sensorimotor deficits caused by systemic administration of 8-OH-DPAT is most likely mediated by 5-HT_{1A} receptors in other brain structures than the MS/vDB. Previous results have shown that intrahippocampal administration of 8-OH-DPAT produce impairments in spatial acquisition (CARLI et al., 1992; CARLI et al., 1995; EGASHIRA et al., 2006), indicating that the hippocampal 5-HT_{1A} receptor most likely is an important target for 8-OH-DPAT given systemically. However, the present data cannot exclude a role for the 5-HT_{1A} receptor in the MS/vDB in other cognitive functions such as working memory. Thus, 8-OH-DPAT given in the same dose range as in the present study, produced working memory deficits when injected into the MS/vDB (JELTSCH et al., 2004).

Passive avoidance

In contrast to the results obtained in the water maze task, intraseptal infusion of 8-OH-DPAT (4 μg/rat) prior to training produced a pronounced impairment in PA memory, without any apparent changes in the responsivity to the electric shock (US). This indicates a major difference in the role of septal 5-HT_{1A} receptors in the control of spatial versus emotional memory.

Based on the localization of the 5-HT_{1A} receptors in the MS/vDB, it is predicted that 8-OH-DPAT would inhibit both GABAergic and cholinergic neurons, resulting in a decreased activity of septohippocampal neurons. However, the effect of intraseptal 8-OH-DPAT on septohippocampal transmission is presently not known. Since the septohippocampal pathway is believed to play a role also in anxiety, intraseptal stimulation of 5-HT_{1A} receptors could result in alterations in the emotional state of the animal, seen as a change in anxiety-like behavior. However, the available results are contradictory, since both an increase, decrease and no effect on anxiety-like behavior have been reported after local infusion of 8-OH-DPAT into the septal area in rats and mice (MENARD & TREIT, 1998; MICHEAU & VAN MARREWIJK, 1999).

THE ROLE OF THE MEDIAL SEPTAL NMDA RECEPTORS IN LEARNING AND MEMORY (PAPER IV AND V)

EFFECTS OF NMDA RECEPTOR BLOCKADE ON SPATIAL AND AVERSIVE LEARNING

At present, the knowledge of the role of septal NMDA receptors in cognitive functions is limited. The high density of glutamatergic fibers and the presence of an intrinsic glutamatergic system (see Introduction) support the hypothesis that glutamatergic transmission may be an important regulator of septohippocampal activity. In support of this hypothesis, intraseptal infusion of the NMDA receptor antagonist D-AP5 was found to attenuate the amplitude of hippocampal theta rhythm (LEUNG & SHEN, 2004), suggesting that blockade of septal NMDA receptors could interfere with hippocampal mnemonic functions.

Water maze experiments

D-AP5 (0.3-5 μg/rat), when infused into the MS/vDB, impaired both spatial acquisition and retention at the highest dose tested (5 μg-dose). This finding is consistent with results obtained after intrahippocampal administration of D-AP5, which caused an impairment in spatial learning (LIANG et al., 1994; STEELE & MORRIS, 1999).

It is well known that systemic administration of NMDA receptor antagonists cause sensorimotor disturbances (CAIN et al., 1997; ÅHLANDER et al., 1999), which probably interfere with spatial learning in the water maze test. Animals receiving intraseptal D-AP5 did not display any changes in behavior indicative of sensorimotor disturbances such as deflections, swim-overs, change in swim speed or motor stereotypies. Moreover, the transient effect seen in the visually cued platform test further implies that intraseptal D-AP5 did not produce visual or motivational disturbances. Interestingly, D,L-AP5 infused i.c.v. also did not cause any apparent changes in sensorimotor performance in a visual discrimination task (MORRIS, 1989). However, the deficit in spatial learning in our study was accompanied by an increase in thigmotaxic swimming, indicating that the learning deficit at least partly could be due to a failure of the animals to develop the necessary behavioral strategies to solve the task. It would, therefore, be of interest to analyze whether NSP could influence the impairing effects of medial septal D-AP5 on spatial learning.

The present results support a role for medial septal NMDA receptors in spatial learning and memory. Moreover, the deficit in spatial learning following intraseptal D-AP5 cannot be explained by changes in sensorimotor functions.

Passive avoidance experiments

To investigate the role of septal NMDA receptors in emotional memory, D-AP5 (0.3-5 μg/rat) was infused into the MS/vDB. All doses of D-AP5 impaired emotional memory in the PA task. This finding indicates that NMDA receptors within the MS/vDB are important for this type of aversive learning. Also hippocampal NMDA receptors have been shown to be involved in both PA (STIEDL & ÖGREN, unpublished data), as well as in fear conditioning in both rats and mice (BAST et al., 2003; STIEDL et al., 2000a; YOUNG et al., 1994).

Our results point towards a more important role for NMDA receptors within the MS/vDB in emotional than in spatial memory. It is possible that glutamatergic NMDA receptors play differential roles in the multiple types of information that is transmitted to the septal neurons by glutamatergic neurons, from e.g. the SUM or other lower brain structures. Differences in the activation of the distinct glutamatergic subsystems within the MS/vDB (MANSEAU et al., 2005) could result in different septohippocampal neuronal output. This could influence postulated different functional domains within the hippocampus, which are involved in different classes of behavior (RISOLD & SWANSON, 1996).

Analysis of anxiety-like effects in the elevated plus-maze

To study the potential role of glutamatergic mechanisms in the MS/vDB for emotional behavior, the effects of intraseptal D-AP5 on anxiety-like behavior was investigated in the elevated plus-maze. Intraseptal infusion of D-AP5 caused an anxiolytic-like behavior at the highest dose tested (5 μg), as shown by an increase in time spent in the open arms as well as number of entries to the open arms. This finding suggests that the NMDA receptor within the MS/vDB play a role in anxiety-related behavior. Our finding is consistent with the hypothesized role for the septohippocampal pathway in anxiety, suggesting that activation of this pathway is critical for anxiety states (DEGROOT & TREIT, 2003; see GRAY & MCNAUGHTON, 2003; see TREIT & MENARD, 2000). Accordingly, blockade of this increase in septohippocampal activity would reduce the animal's fear/anxiety response, which could explain the effects of D-AP5 on anxiety-related behavior in the elevated plus-maze. On the other hand, in view of the dose-dependent effect of D-AP5 on PA retention, this impairment cannot simply be explained by an anxiolytic effect. Thus, intraseptal D-AP5 caused an increase in anxiety-like behavior only at the 5 μg-dose, but an in PA memory was observed already at the 0.3 μg-dose.

In summary, based on the present findings it is evident that glutamatergic neurotransmission in the MS/vDB, mediated via the NMDA receptor, play an important role for hippocampal-dependent learning and memory and also have a role in anxiety-related behaviors.

INTERACTIONS BETWEEN NMDA AND 5-HT$_{1A}$ RECEPTORS IN THE MS/vDB: POSSIBLE IMPORTANCE FOR MEMORY FUNCTIONS

As shown previously, the results obtained with systemic administration of 5-HT$_{1A}$ and NMDA receptor antagonists imply interactions between these two receptors of importance for learning and memory. Stimulation of 5-HT$_{1A}$ receptors may inhibit NMDA receptor signaling and/or glutamatergic release, as previously suggested (CALCAGNO et al., 2006; EDAGAWA et al., 1999; YUEN et al., 2005). To investigate if such an interaction exists in the MS/vDB, subthreshold doses of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (4 μg) and the NMDA receptor antagonist D-AP5 (1 μg) were infused simultaneously into the MS/vDB before spatial training in the water maze.

The combination of 8-OH-DPAT and D-AP5 produced a weak impairment in spatial acquisition, as shown by a longer swim distance compared to control, without any changes in swim speed or thigmotaxic swimming. In contrast to acquisition, the results from the retention test conducted 24 h after the last training session revealed a marked memory impairment in the rats receiving the combination of 8-OH-DPAT and D-AP5. The increase in thigmotaxic swimming during the retention test most likely reflects a disruption in spatial memory, and not in sensorimotor disturbances, in view of the absence of thigmotaxis during acquisition.

The deficit in spatial memory following the combination of 8-OH-DPAT and D-AP5 was unproportionally large in relation to the marginal impairment observed in spatial acquisition. These results are intriguing and suggest that the interaction between septal NMDA and 5-HT$_{1A}$ receptors may be of particular significance, either for long-term consolidation and/or retrieval processes. The interaction between 5-HT$_{1A}$ and NMDA receptors most likely results in a modulation of septohippocampal neurons in a manner, which is of importance for the establishment of a stable, long-term memory. It is possible, based on electrophysiological studies in hippocampal cells, that activation of 5-HT$_{1A}$ receptors inhibits the activity of septal glutamatergic pyramidal neurons (TADA et al., 2004), thereby enhancing the effects of NMDA receptor blockade. Moreover, 5-HT, acting via the 5-HT$_{1A}$ receptor, has been shown to inhibit NMDA receptor-mediated ionic and synaptic currents in pyramidal neurons in the prefrontal cortex (YUEN et al., 2005). Taken together, these findings suggest that changes in *in vivo* serotonergic transmission, via the 5-HT$_{1A}$ receptor, can result in significant alterations in NMDA receptor function in the MS/vDB.

CONCLUSIONS

The major finding in this thesis support the view that cholinergic, serotonergic and glutamatergic transmission within the MS/vDB have significant consequences for hippocampal cognitive functions, probably by changing the activity in the septohippocampal pathway. This projection appears to play differential roles in emotional and spatial learning, indicating that septal input activates subregions of the hippocampus involved in the processing of different types of information.

In contrast to earlier proposals, cholinergic muscarinic transmission within the MS/vDB appears to be mainly inhibitory, whereas the neuropeptide galanin activates the septohippocampal cholinergic pathway. The level of muscarinic activity within the MS/vDB seems to be important for the effect of galanin on cognitive functions. However, the relationship between hippocampal ACh and cognition is not linear. Thus, the present findings indicate the existence of a limited range of cholinergic (muscarinic) transmission in the hippocampus, which may contribute to optimal cognitive performance.

Systemic administration of 5-HT_{1A} agonists and antagonists give evidence for a role of 5-HT_{1A} receptors in both spatial and emotional memory, with differential roles for pre- and postsynaptic 5-HT_{1A} receptors. There is also support for interactions between 5-HT_{1A} receptors and cholinergic and glutamatergic systems in the CNS of importance for cognition. However, 5-HT_{1A} receptors in the MS/vDB appear to be mainly involved in emotional memory.

Of major importance is the finding that there exists interactions between serotonergic and glutamatergic transmission, mediated by the 5-HT_{1A} and NMDA receptors within the MS/vDB, which appears to be of great significance for the development of spatial memory. The interaction between the 5-HT_{1A} and NMDA receptors may represent a novel mechanism by which the septohippocampal pathway controls cognitive processes.

Together, the findings in this thesis have important implications for development of novel therapeutic approaches in the treatment of cognitive dysfunctions in neurodegenerative diseases such as dementias and Parkinson's disease, as well as in neurological and neuropsychiatric disorders.

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