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THE ROLE OF
THE SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM
IN COGNITIVE FUNCTIONS

Doctoral dissertation

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ABSTRACT

Cholinergic dysfunction is a hallmark of Alzheimer's disease (AD). This dysfunction is assumed to be mainly responsible for the cognitive defects in AD. However, the mechanism by which the cholinergic system regulates cognitive functions is elusive. This study was designed to find out the role of the septohippocampal cholinergic system in cognitive functions.

The methods used were behavioural testing (water maze, radial arm maze, passive avoidance and Y-maze) and electrophysiology (hippocampal EEG-recording and place cell recording). Apamin, a potassium channel blocker, was found to improve water maze spatial navigation of medial septal (MS) lesioned mice. The memory defect that is present in MS-lesioned mice was almost completely reversed by apamin. Apamin had no effect on the cognitive parameters of Y-maze and passive avoidance of MS-lesioned mice. The performance of intact mice was not affected. Apamin was also found to dose-dependently reverse the memory defect of hippocampal (HC) lesioned mice in radial arm maze. In the water maze, a similar observation was made: apamin improved the spatial navigation of HC-lesioned mice. Metrifonate, a cholinesterase inhibitor, was found to alleviate the memory defect of MS-lesioned mice. Metrifonate had no effect on the performance of intact mice. In contrast to apamin, metrifonate did not improve the water maze spatial navigation of hippocampal-lesioned mice. The effects of metrifonate and apamin were observed not to be mediated by modulation of the hippocampal theta rhythm in MS-lesioned mice. However, in intact mice, metrifonate induced changes in the hippocampal theta, and these changes were shown to be mediated by non-M₁-M₂ muscarinic receptors. In addition, a selective cholinergic lesion of the septum was found to impair the ability of the hippocampus to remap in response to a new visual environment. In a familiar environment, the place fields of lesioned and sham operated animals had similar characteristics. However, upon subsequent exposures to the new environment, the place cell response in controls evolved in the direction of pattern separation, whereas in the lesioned animals the pattern evolved in the direction of pattern completion. As a result, the final representation of the new environment still resembled the familiar environment in the lesioned rats whereas a totally new representation developed in the controls.

Taken together, the septohippocampal cholinergic system has at least two differential effects on the hippocampus: one, M₁-mediated effect, regulates the cognitive functions, and the other, M₃/M₅-mediated effect, regulates hippocampal EEG. The effect on cognitive functions is likely to involve inhibition of afterhyperpolarisation. These findings support the role of the cholinergic system in the regulation of the predominant mode of the hippocampus: cholinergic innervation acts as a switch between the information gathering and information processing modes of the hippocampus. Thus, degeneration of the cholinergic system induces a memory defect by reducing the occurrence of information gathering mode.

National Library of Medicine Classification: WL 314, WL 102, WT 155

Medical Subject Headings: Alzheimer disease; hippocampus; septum of brain; apamin; trichlorfon; cholinergic agents; receptors, muscarinic; spatial behavior; maze learning; memory; cognition; electroencephalography; theta rhythm; models, animal; mice; rats

Careful notes are the soul of science.

D. F. Duck, 1934-

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Sami Ikonen

ABBREVIATIONS

AChE	acetylcholinesterase
AD	Alzheimer's disease
AHP	afterhyperpolarisation
ANOVA	analysis of variance
ChAT	choline acetyltransferase
DB	diagonal band of Broca
EEG	electroencephalogram
GABA	gamma-aminobutyric acid
HC	hippocampus
hDB	horizontal limb of the diagonal band of Broca
IgG	immunoglobulin G
i.p.	intraperitoneal
LS	lateral septum
LTP	long-term potentiation
MS	medial septum
MSDB	medial septum / diagonal band of Broca
MSvDB	medial septum / vertical limb of the diagonal band of Broca
MTF	metrifonate
NB	nucleus basalis
s.c.	subcutaneous
vDB	vertical limb of the diagonal band of Broca

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications that are referred to in the text by the Roman numerals I-V.

I Ikonen, S., Schmidt, B., Riekkinen, P. Jr.: Apamin improves spatial navigation in medial septal -lesioned mice. *Eur. J. Pharmacol.* 347, 13-21 (1998).

II Ikonen, S., Schmidt, B., Riekkinen, P. Jr.: Characterization of learning and memory behaviors and the effects of metrifonate in the C57BL strain of mice. *Eur. J. Pharmacol.* 372, 117-126 (1999).

III Ikonen, S., Riekkinen, P. Jr.: Effects of apamin on memory processing of hippocampal-lesioned mice. *Eur. J. Pharmacol.* 382, 151-156 (1999).

IV Ikonen, S., Tanila, H.: Effects of metrifonate on the hippocampal theta rhythm of freely moving intact and MS-lesioned mice. *Pharmacol. Biochem. Behav.* In press.

V Ikonen, S., McMahan, R., Gallagher, M., Eichenbaum, H., Tanila, H.: Cholinergic system regulation of spatial representation by the hippocampus. Submitted.

This thesis includes also previously unpublished electrophysiological results.

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REFERENCES

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease that is characterised by a decline in cognitive functions, behavioural disturbances, impairment in daily functions and appearance of neuritic plaques and neurofibrillary tangles in the brain (Whitehouse et al. 1982, Blusztajn 1994, Albert 1996, Braak and Braak 1996). It affects generally people older than 60 years of age, but it can be diagnosed in individuals as young as 40 years of age. The prevalence of AD approximately doubles every 5 years after the age of 60 until more than 30 % of persons aged 85 years or older are afflicted (Jorm et al. 1987).

The cause of AD has long been unidentified, until in the end of the 1970's a degeneration of the cholinergic system of the AD patients was observed (Davies and Maloney 1976). Several studies have revealed evidence to support the theory that the loss of cholinergic function contributes to the cognitive deficits of AD. First, the amount of cholinergic neurons in the nucleus basalis (NB) and medial septum (MS) that project to the hippocampus, amygdala and cortex, is reduced in AD patients (Whitehouse et al. 1982). Second, the amount of choline acetyltransferase in the cortex and hippocampus is also reduced in AD patients (Perry et al. 1977). Third, drugs that block the effects of acetylcholine in the brain cause cognitive impairment in young control patients (Drachman and Leavitt 1974). Finally, and most convincingly, drugs that increase the effects of acetylcholine in the brain, acetylcholinesterase inhibitors, alleviate the symptoms of AD patients (Eagger et al. 1992).

The hippocampus is the most critical brain area for our ability to recollect everyday events and factual knowledge. This ability is what in everyday language is referred to as 'memory', although it should more precisely be called declarative memory, as opposed to other types, such as procedural memory, which is unconscious and hippocampus-independent recollection of information. The function of hippocampus is regulated by cholinergic innervation that arises mainly from the MS. Lesioning the MS removes the hippocampal cholinergic innervation and it induces a memory defect in experimental animals (Hagan and Morris 1988). MS-lesioning can

be used as a model for cholinergic hypofunction of AD. In addition, lesioning of the MS provides information about the role of the cholinergic innervation in a normal hippocampus. The cholinergic innervation has been proposed to have an important role in the proper functioning of the hippocampus, but so far, there is little available information about the direct mechanisms. This study was designed to investigate the role of the cholinergic system and in particular the MS in learning and memory.

2. REVIEW OF THE LITERATURE

2.1. ANATOMY OF THE SEPTO-HIPPOCAMPAL SYSTEM

In rats, the septo-hippocampal system includes the hippocampal formation, the septal area, their interconnections and the afferent and efferent pathways that connect them to other brain areas. The septum and the hippocampus are connected mainly by the fimbria and the dorsal fornix bundles (Fig. 1).

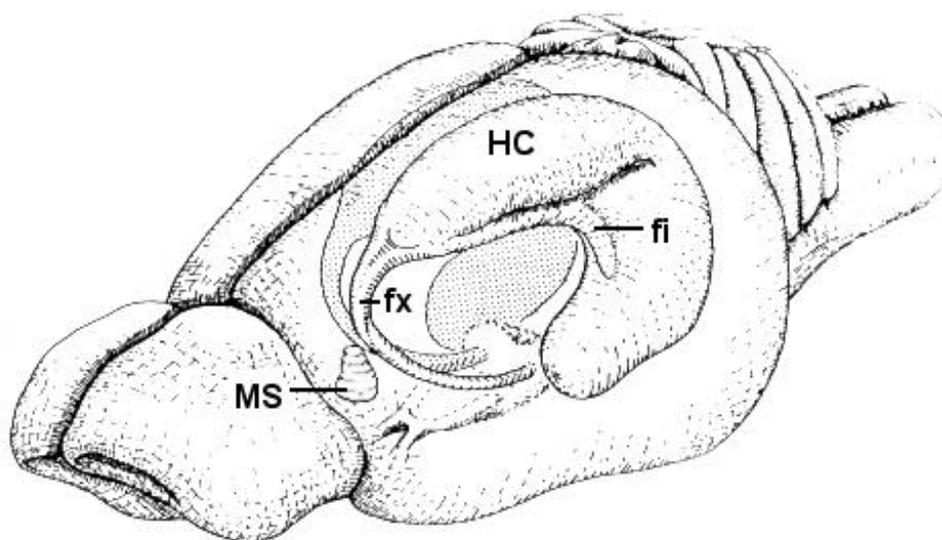


Fig. 1. A three-dimensional organisation of the septo-hippocampal system in the rat brain. The hippocampus is the C-shaped structure. Abbreviations: fx = fornix; fi = fimbria; HC = hippocampus; MS = medial septum (modified from Amaral and Witter 1995).

2.1.1. *The hippocampus*

The term hippocampal formation encompasses six subregions: the dentate gyrus, hippocampus proper, subiculum, presubiculum, parasubiculum and the entorhinal cortex (Amaral and Witter 1989, Amaral and Witter 1995). Often, as in this text, the word *hippocampus* is used to refer to a structure that is composed of hippocampus proper and dentate gyrus. The hippocampus is a C-shaped structure (Fig. 1) that has a characteristic laminar organisation: if the hippocampus is cross-sectioned at any septo-temporal level, it can be seen that the cells are

packed into distinct layers. In rats, the hippocampus proper comprises of three parts: CA1, CA2 and CA3. In humans, there are four parts: CA1, CA2, CA3 and CA4. The letters CA come from the Latin words *cornu ammonis*; “Ammon’s horn” in English.

The intrahippocampal connections form a *trisynaptic loop*, which is composed of the cells of dentate gyrus, CA3 and CA1 and their interconnections (Fig. 2) (Amaral and Witter 1995).

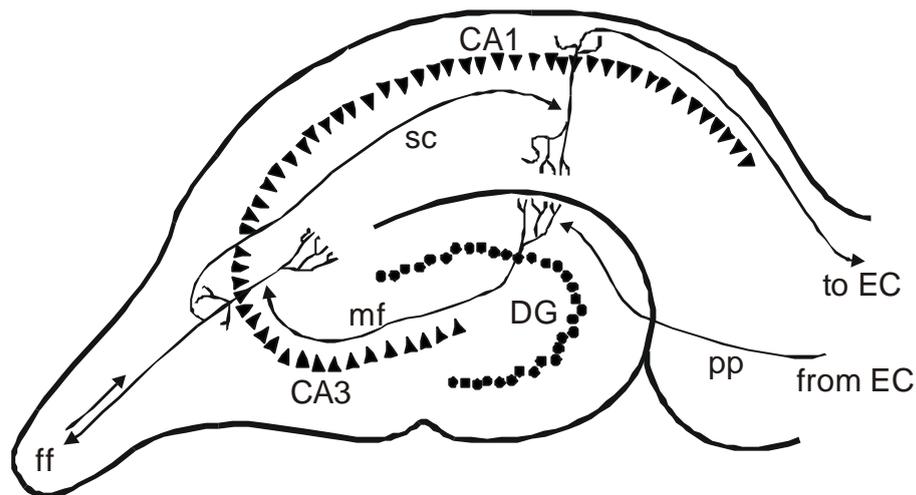


Fig. 2. The trisynaptic loop of the hippocampus. The filled triangles represent the pyramidal cell layer (CA1 and CA3) and the filled circles represent the granular cell layer of the dentate gyrus. Abbreviations: EC = entorhinal cortex; DG = dentate gyrus; pp = perforant pathway; mf = mossy fibers; sc = Schaffer collaterals; ff = fimbria fornix.

The first synaptic connections of the loop are formed between the entorhinal cortex and dentate gyrus. The cells in the superficial layers (mainly layer II) of the entorhinal cortex send their axons to the molecular layer of the dentate gyrus and they provide the hippocampus its main glutamatergic input. This pathway is called the perforant pathway. Collaterals of the same axons form also connections with CA3 pyramidal cells. The second synaptic connections are formed between the dentate gyrus and the CA3. The axons from the granular cells of the dentate gyrus innervate the dendrites of the CA3 pyramidal cells. These innervations are called *mossy fibers*. As in the case of the perforant pathway, also mossy fibers form connections with another cell population, namely the mossy cells of the dentate gyrus. These interneurons

provide feedback excitation back to the granule cells. In the third and last stage of the trisynaptic loop, the axons of the CA3 pyramidal cells form connections with the dendrites of the CA1 pyramidal cells in layers stratum radiatum and stratum oriens. These axons are called *Schaffer collaterals*, and again, they too branch to form connections with another cell population: the cells of the lateral septum and mammillary bodies. These axons pass through the fimbria/fornix. Thus, the trisynaptic loop has been closed, but the information that has been processed in the loop by the principal cells and the interneurons is projected back to the entorhinal cortex by the CA1 pyramidal cell axons, either directly or via the subiculum. While the input cells to the hippocampus were located in the superficial layers of the entorhinal cortex, the output axons from the hippocampus project to the deep layers of the entorhinal cortex (Amaral and Witter 1995).

While the trisynaptic loop is the main circuit of the hippocampus, it is still only one part of the entire circuitry. There are several other connections with important contributions to the function of the hippocampus: e.g. connections from the entorhinal cortex to the CA1 and the subiculum, connections between the two hippocampi via the commissures, and the subcortical connections via the fimbria/fornix, mostly with the septum. Other connections that pass via the fimbria/fornix are noradrenergic connections from the locus coeruleus, serotonergic connections from the raphe nuclei, histaminergic connections from supramammillary nucleus and dopaminergic connections from ventral tegmental area and the substantia nigra (see Dutar et al. 1995). A common feature of the other connections is that they provide sparse excitation, but massive inhibition of the pyramidal cells of the hippocampus. Therefore, only synchronisation enables the firing of the pyramidal cells (Freund and Gulyas 1997).

2.1.2. *The septum*

The septum was classified as a separate brain structure already in the second century by a Greek neuroanatomist Galen. It was determined as an area which is located between the anterior horns of the lateral ventricles (septum = from Latin, *saeptum*: a dividing wall or membrane especially between bodily spaces or masses of soft tissue). The first detailed

description of the anatomy of the septum was provided in 1901 by Cajal, who was also the first to classify it as part of the basal ganglia on the contrary to the previous generally accepted notion that it is a specialised part of cerebral cortex. Even today, a general agreement about the classification has not been accomplished. Furthermore, not even the exact boundaries defining the septum are generally agreed upon. What is generally accepted, then, is that the septum can be viewed as an interface or a relay station between the evolutionarily “old brain” (diencephalon) and “new brain” (telencephalon). It is assumed to maintain the balance between the endocrine and emotional components of the central nervous system.

The septal complex is usually divided into three parts: the medial septum / diagonal band of Broca (MSDB), the lateral septum (LS) and the posterior septum. The two subnuclei of MSDB are the medial septal nucleus (MS) and the nucleus of the diagonal band of Broca (DB). DB is further composed of two parts: the horizontal limb of DB (hDB) and vertical limb of DB (vDB). MS and DB are often classified as separate nuclei even though they are actually continuous and no anatomical boundary can be determined between them (Fig. 3). In fact, a more functional classification would be to combine MS and the vertical limb of the DB (as described in more detail in chapter 2.3.1.). Therefore, in this text, this complex is considered to be one functional unit and is called medial septum / vertical band of the diagonal band of Broca (MSvDB). The second part of the entire septal complex, lateral septum can be divided into three main parts: dorsal, intermediate and ventral parts. The third part, posterior septum is composed of two parts: the bilateral septofimbrial nucleus and triangular septal nucleus (Jakab and Leranath 1995).

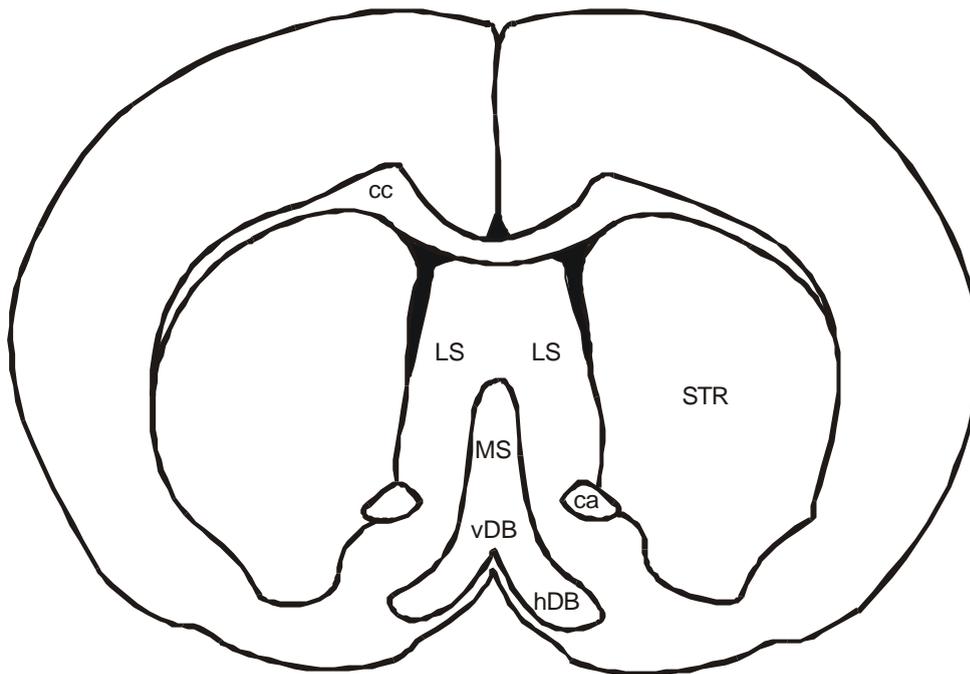


Fig. 3. Coronal section of the rat brain. Abbreviations: MS = medial septum; vDB = vertical limb of the diagonal band of Broca; hDB = horizontal limb of the diagonal band of Broca; STR = striatum; ca = commissura anterior; cc = corpus callosum. Adapted from Paxinos and Watson (1986).

As most brain structures, septum was originally defined on the basis of gross dissections rather than any rational structure-based principle. As a result, the septal complex is actually a group of structurally unrelated nuclei that are considered under one category only for historical reasons. For example, the functions of MSvDB and LS are quite different. MSvDB primarily relays the ascending information from the diencephalon to the telencephalic structures, whereas LS mediates the descending information from telencephalon to the diencephalon (Jakab and Leranath 1995). Furthermore, connections between the MSvDB and lateral septum are very sparse (Jakab and Leranath 1995).

The cells of MSvDB project mainly to the hippocampus and less extensively to the entorhinal cortex and cingulate cortex (Gaykema et al. 1990). Approximately 40-50 % of the cells that project to the hippocampus from this area are cholinergic and 10-20 % are GABAergic

(Linke et al. 1994). The cholinergic cells of the MSvDB have long been considered its functionally most important cell group, since it provides most of the cholinergic innervation of the hippocampus. However, data from recent studies have given the GABAergic cells a more prominent part in the function of the MSvDB (Lee et al. 1994, Wenk et al. 1994).

2.1.3. Interconnections

The interconnections between the septum and the hippocampus are reciprocal. The ascending connections from the septum to the hippocampus arise from the MSvDB. There are two types of connections: cholinergic and GABAergic. About 90 % of the cholinergic innervation of the hippocampus comes from the MSvDB. The cholinergic input provides a modulatory input to principal cells and GABAergic interneurons of the hippocampus (Wainer et al. 1984, Frotscher and Leranth 1985). The GABAergic projections terminate at the GABAergic interneurons of the hippocampus and thus provide a massive disinhibition of the pyramidal cells (Freund and Antal 1988). Both the cholinergic and the GABA-to-GABA input provide a synchronous orchestration of the entire hippocampal formation (see Chrobak 2000).

The hippocampus projects descending connections back to the septum. The main target area of the hippocampal projections is the LS. The CA1 pyramidal cells project to the entire LS whereas CA3 pyramidal cells project only to the caudal part of the LS (see Jakab and Leranth 1995). Although the LS is the main target of the hippocampal projections, there are also some connections to MSvDB from the (mainly GABAergic) interneurons of the hippocampus. These projections arise from the calbindin-containing interneurons and they terminate at both cholinergic and GABAergic cells of the MSvDB (see Jakab and Leranth 1995).

The fiber bundles that contain the main projections between the septum and the hippocampus are called the fimbria/fornix, the dorsal fornix and the supracallosal striae. A fourth ventral route passing through the amygdala has also been described (Milner and Amaral 1984). These bundles contain also other projections, for example noradrenergic and serotonergic projections from the brain stem to the hippocampus.

2.2. PHYSIOLOGY OF THE SEPTO-HIPPOCAMPAL SYSTEM

2.2.1. *The hippocampus: a spatial encoding device?*

The hippocampus is a specialised part of the limbic cortex, which is located in the temporal lobe in humans. It is known to function in the formation of memory. A classic example is a human patient H. M., whose both hippocampi were removed as an attempt to treat severe epileptic seizures. As a result, the patient completely lost his ability to form new memories. Some of the events immediately before the surgery were also lost from his memory, but old events remained intact (Scoville and Milner 1957). These observations along with later studies with experimental animals (Morris et al. 1982, Zola-Morgan and Squire 1986) led to a conclusion that the formation of new memories requires the function of the hippocampus, but eventually the memory trace is stored in another part of the brain and is no longer dependent on the hippocampus (Squire and Zola-Morgan 1991).

The discovery of the importance of the hippocampus in memory formation led to studies with experimental animals. It was discovered that removing the hippocampus or only damaging some parts of it, disrupted the ability of rats to form memory traces (see Jarrard 1986). However, there were some fundamental differences between humans and experimental animals in the effects of hippocampal lesions. In humans, hippocampectomy disrupted the memory formation in almost all kinds of tasks that require new learning, in particular declarative memory, leaving only implicit forms of long-term memory, such as procedural memory and priming intact (Scoville and Milner 1957, Warrington and Weiskrantz 1968). In rats, however, some tasks were relatively unaffected by the damage of the hippocampus, for example recognition memory (Aggleton et al. 1986) and fear conditioning (Phillips and LeDoux 1994). The most dramatic memory failure in animals was observed in tasks that required spatial memory. For example in water maze, a task that requires spatial learning, the rats with hippocampal damage were dramatically impaired (Morris et al. 1982).

The observation that the hippocampus functions in spatial learning was supported by studies demonstrating that hippocampal pyramidal cells can function as so-called “place cells” (O’Keefe and Dostrovsky 1971, Muller 1996). Electrophysiological recordings from freely moving animals showed that some CA1 and CA3 hippocampal pyramidal cells fire only in a restricted part of the environment. This part of the environment is called the “firing field” of the cell. This observation, together with the fact that hippocampal damage causes spatial learning deficits, led to hypothesis that the hippocampus implements an abstract representation of the environment, called a cognitive map (O’Keefe and Nadel 1978). Thousands of cells in the hippocampus are firing according to the location of the rat in a certain environment. By computing the information that can be obtained from these individual cells, the hippocampus can, by an unknown mechanism, determine the exact location of the rat in the environment. For example, in some studies, simultaneous recording of about one hundred hippocampal place cells enabled the researchers to theoretically compute the location of the rat with an accuracy of about 1 cm. The estimation was based on only the information that was obtained by recording the intensity of the firing of the place cells (Wilson and McNaughton 1993).

CA1 and CA3 cells are not the only ones in the hippocampal formation that are involved in spatial representation. The cells in the superficial layers of the entorhinal cortex (the input-area of the hippocampus) show location-selective firing, although the firing fields are larger and noisier than in CA1 and CA3 (Quirk et al. 1992). The dentate gyrus has also cells that fire selectively to location (Jung and McNaughton 1993). The selectivity of these cells is higher than in the entorhinal cortex. The output area of the hippocampus, the subiculum, also contains location selective cells, with low selectivity (Sharp and Green 1994). In addition, cells in pre- and post-subiculum contain so-called head-direction cells (Taube et al. 1990, Muller et al. 1996). These cells are active only when the head of the animal is in a certain angle to the environment. They provide more information for the hippocampal machinery that computes the location and position of the animal. Finally, also the cells of lateral septum have been observed to show location specific firing (Zhou et al. 1999).

The notion that the hippocampus is mainly involved in spatial encoding in rodents has been challenged by studies showing that the pyramidal cells of the hippocampus can have other than spatial functions (Bunsey and Eichenbaum 1996, Dusek and Eichenbaum 1997, Wood et al. 1999; for review, see Eichenbaum and Cohen 1988). These results indicate that the function of the hippocampus in animals is not limited to spatial encoding alone, but also non-spatial declarative processing takes place in the hippocampus, as does in humans (Scoville and Milner 1957). However, spatial memory is still the most universally accepted critical function of the hippocampus in rodents, monkeys and humans. In addition, the mechanisms of spatial learning are thought to be similar in rodents and humans. This makes the study of place cells a good model for the study of human diseases affecting cognitive processes.

2.2.2. *The septum: a generator of theta rhythm?*

Hippocampal theta rhythm is a regular electroencephalographic 4-12 Hz oscillation in the hippocampus and related structures. There are two types of theta rhythm: Type I theta has an overall frequency range of 6-12 Hz and it occurs mainly during walking and running. Type II theta has a lower frequency range of 4-9 Hz and it occurs during immobility (Kramis et al. 1975). The septum is considered the pacemaker of the theta rhythm since the discovery of pacemaker cells in the septum by Petsche et al. (1962). This view was confirmed by studies showing that lesions of the septal area completely eliminates the theta rhythm in the hippocampus of experimental animals (Andersen et al. 1979, Leung et al. 1994). Pharmacological studies of the theta rhythm revealed that the cholinergic innervation from the MS is the most important input that regulates the hippocampal theta rhythm. Indeed, various cholinergic agonists produce theta when administered systemically (Teitelbaum et al. 1975), when microinfused into the septum (Monmaur and Breton 1991) or hippocampus (Rowntree and Bland 1986, Colom et al. 1991) and even *in vitro*, when applied to the hippocampal slices (Konopacki et al. 1987). Conversely, cholinergic antagonists attenuate theta (Bennett et al. 1971, Kramis et al. 1975).

The role of theta in learning has been studied extensively since the discovery that septal lesions induce a memory defect (Stewart and Vanderwolf 1987, M'Harzi and Jarrard 1992, Leung et al. 1994; for review, see O'Keefe 1993). The crucial role of theta in learning and memory was supported by studies showing that muscarinic antagonists, that attenuate theta, also impair spatial memory (Bennett et al. 1971). Furthermore, basal forebrain grafts that reduce the spatial memory impairment (Dunnett et al. 1982), also partially restore behaviour-dependent theta rhythm (Buzsaki et al. 1987, Tuszynski et al. 1990). Consequently, theta has been considered essential to the proper function of the hippocampus (Winson 1978).

What, then, is the precise role of theta in the function of the hippocampus? It has been suggested that during theta oscillation, the hippocampus is in a 'feed-in' mode, i.e. it collects data from the entorhinal input. This mode is occasionally turned off by another mode, during which the hippocampus is processing the data and feeding the processed information back to the cortical areas. This mode is characterised by sharp waves and it is usually present when the animal is not actively moving (Buzsaki 1986). Thus, theta enables the cells of the hippocampus to gather data from the cortex in a controlled manner. It has been suggested that the mechanism by which theta is necessary for hippocampal function is that it provides a way of filtering out the hippocampal input signals. Depending on the phase of theta, the simultaneous input from the entorhinal cortex can either be strengthened (positive peak of theta) or dampened (negative peak of theta). Thus, theta is preventing interference with the information processing that takes place at the same time (see Vinogradova 1995).

The role of theta (or at least, the role of the cholinergic component of theta) in cognitive functions was challenged by studies which showed that lesioning of the septal area by 192-IgG-saporin, a selective cholinergic neurotoxin, which dramatically reduces the amount of theta in the hippocampus (Lee et al. 1994), does not induce a memory deficit in the water maze (Berger-Sweeney et al. 1994, Torres et al. 1994, Baxter et al. 1996). However, removal of the cholinergic input is not able to remove the theta oscillation from the hippocampus completely; a small but significant theta peak remains (Lee et al. 1994). Therefore, it is still possible that theta is important for the proper function of hippocampus, since this small theta

peak might be able to perform the most essential functions of theta in learning and memory. In conclusion, the most convincing view is that theta is crucial for the proper function of the hippocampus and that septum is crucial for the generation of theta but that the cholinergic cells alone do not account for the role of theta in learning. It is the complex circuitry of the entire septal area that orchestrates the function of hippocampal pyramidal cells and interneurons in a way that allows the hippocampus to function properly and that this orchestration can be recorded as an oscillation that can be called the hippocampal theta rhythm.

2.3. THE CHOLINERGIC SYSTEM OF THE CENTRAL NERVOUS SYSTEM

2.3.1. *Classification of the cholinergic nuclei*

According to the classification by Satoh et al. (1983), there are four main groups of cholinergic cells in the rat brain. The first group is composed of cells of the basal forebrain, that constitute the “rostral column”. The second group is composed of cells that are located in the pons and midbrain, and which are called the “caudal column”. The cells in the neostriatum, nucleus accumbens and olfactory tubercle constitute the third group, and the fourth group of cholinergic cells are in the spinal cord and the nuclei of the cranial nerves.

Another, more commonly used classification by Mesulam et al. (1983), classifies the cholinergic cells into six groups (Fig. 4). This classification is based on topographical variations in projection fields. The first group (Ch1) is composed of the cholinergic cells of MS, and they are thus distinguished from the cells in the vDB (Ch2). These two groups project mainly to the hippocampus. The hDB, that projects to the olfactory bulb, is classified as Ch3. The neocortex and amygdala are innervated by the fourth group of cells, Ch4. These Ch4 cells constitute a large group of cells that are located in the nucleus basalis (NB), preoptic magnocellular nucleus and some parts of the hDB. The thalamus is innervated by the two remaining groups of cells, Ch5 and Ch6. They are located in the pedunculopontine nucleus and laterodorsal tegmental nucleus. Although these nuclei (Ch1-6) are generally considered to be cholinergic, they contain also other types of cells. For example, only 10-20 % of the cells in

the Ch3 nucleus are cholinergic, whereas the proportion can be as high as 80-90 %, as in Ch4. Most of the studies concerning the role of the cholinergic system in learning and memory have concentrated on Ch1/Ch2 and Ch4, because they are supposed to be the most important ones based on their projection areas (hippocampus and cortex).

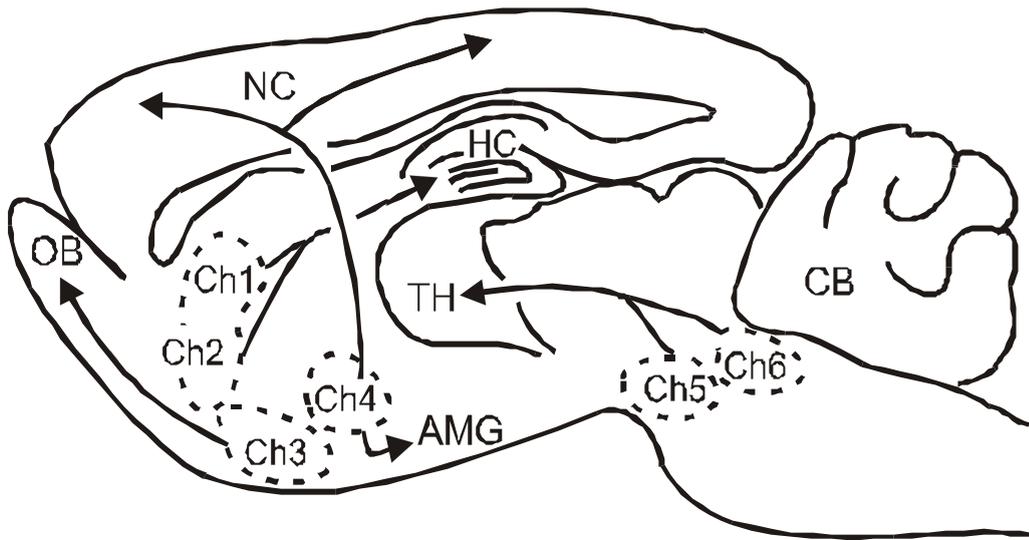


Fig. 4. The cholinergic nuclei of the rat brain (Ch1-6). Abbreviations: AMG = amygdala; CB = cerebellum; HC = hippocampus; NC = neocortex; OB = olfactory bulb; TH = thalamus. Modified from Mesulam et al. (1983).

2.3.2. Acetylcholine in a cholinergic synapse

Acetylcholine, the primary transmitter of the cholinergic system, is the only low-molecular-weight transmitter substance that is not an amino acid or derived directly from one. There is only one enzymatic reaction in the biosynthetic pathway. In that step, acetyl coenzyme A and choline are combined to form acetylcholine with the release of coenzyme A. The step is catalysed by the choline acetyltransferase enzyme (ChAT). The substrate acetyl coenzyme A is a very common substance in many metabolic pathways in all cells. Choline, on the other hand, cannot be synthesised by the nervous tissue, but it has to be derived from the diet and delivered to the nerve cells via the blood stream (Schwartz 1991).

In the synaptic terminals, acetylcholine that is stored in the synaptic vesicles, is released into the synaptic cleft as a result of an action potential. Acetylcholine diffuses to the pre- and postsynaptic membranes where it binds to the receptors. The cholinergic receptors in the mammalian central nervous system can be divided into two groups: muscarinic and nicotinic receptors, based on the ability of two natural alkaloids, muscarine and nicotine, to mimic the effects of acetylcholine. *Nicotinic receptors* are directly gated ion channels that are usually considered as mediators of acetylcholine in autonomic nervous system ganglia. However, they are present also in the forebrain (Arneric et al. 1995). In the hippocampus, they are present mainly in the stratum oriens and in the hilus, but their role in the cognitive processes is unclear. *Muscarinic receptors* are the main type of cholinergic receptors in the central nervous system (see Caulfield 1993). Presently, five muscarinic receptor genes (m_1 - m_5) are known, and they encode receptors M_1 - M_5 , respectively. All five subtypes are expressed in the hippocampus. All subtypes act via activation of G-proteins: M_1 , M_3 and M_5 receptors activate phospholipase C while M_2 and M_4 receptors act by inhibiting adenylate cyclase. A classification of acetylcholine receptors is presented in table 1.

	Nicotinic	Muscarinic				
		m_1	m_2	m_3	m_4	m_5
Brain	Two main classes, >10 subunits	Forebrain, Mainly post-synaptic	Whole brain, Mainly pre-synaptic	Whole brain, low levels	Forebrain, pre- and post-synaptic	Hippocampus, low levels
Periphery	1 type, 5 subunits	Autonomic ganglia	Heart, smooth muscle	Exocrine glands, smooth muscle	lung	heart

Table 1. The acetylcholine receptor family. From Arneric et al. (1995), Ehlert et al. (1995), Levey (1996) and Creason et al. (2000).

Removal of transmitters from the synaptic cleft is crucial for the proper function of the synapse. In the cholinergic system, the inactivation of acetylcholine is performed mainly by a specialised enzyme, acetylcholinesterase (AChE). These enzymes are located in the postsynaptic

membrane of the synapse, and they catalyse the conversion of acetylcholine molecule and water into acetic acid and choline, which can be returned back to the presynaptic terminal by a reuptake process. AChEs are found in central nervous system, red blood cells and motor endplates of the skeletal muscle, whereas butyrylcholinesterase, a related enzyme that hydrolyses butyrylcholine, can be found in the peripheral nervous system (Taylor 1991). Inhibitors of cholinesterases are the most commonly used drugs in the treatment of AD.

2.3.3. Pre- and postsynaptic effects of acetylcholine

In the hippocampus, all the known effects of acetylcholine are mediated by muscarinic receptors and they involve a decrease in four types of potassium currents: 1) a fast transient voltage-dependent current (I_A current) 2) a voltage-dependent current (I_M current) 3) a calcium-activated slow afterhyperpolarisation current (sI_{AHP}) and 4) a voltage independent “leak” current. Acetylcholine can also decrease Ca^{2+} -currents (see Dutar et al. 1995). A decrease in these currents can lead to four types of effects on the postsynaptic cell: 1) depolarisation of the postsynaptic cell, 2) enhancement of long term potentiation (LTP), 3) presynaptic inhibition of excitatory synaptic transmission and 4) suppression of neuronal adaptation. The depolarisation is associated with an increase in membrane resistance (Benardo and Prince 1982) and it may involve the voltage independent leak current. Enhancement of LTP has been observed in CA1 and dentate gyrus and it is mediated by M_1 -receptors (Blitzer et al. 1990, Burgard and Sarvey 1990). Presynaptic inhibition involves inhibition of Ca^{2+} -currents in the presynaptic part of an excitatory synapse leading to a decrease in the synaptic efficacy (Qian and Saggau 1997). The suppression of neuronal adaptation is mediated by a decrease in afterhyperpolarisation (AHP) which follows trains of action potentials, and it involves I_M - and sI_{AHP} -currents (Madison and Nicoll 1984). There are three types of AHP’s: fast (fAHP), medium (mAHP) and slow (sAHP), and in this study, mAHP is an important link connecting metrifonate (MTF) and apamin (the two main drugs used in this study), because it is inhibited by both drugs (cholinergic agonists decrease the I_M -current and apamin blocks the I_{AHP} -current, which both are major components of the mAHP). The fact that both muscarinic agonists (e.g. MTF) and SK-type Ca^{2+} -dependent K^+ channel blockers (e.g. apamin)

decrease mAHP, allows the use of apamin as a hypothetical “partial cholinergic agonist” that is selective to only one isolated action of acetylcholine instead of the wide range of effects produced by MTF.

2.3.4. Degeneration in Alzheimer’s disease

The so-called “cholinergic theory of Alzheimer’s disease” was born in the 70’s. First, it was demonstrated that the blockade of cholinergic receptors in young healthy subjects produced a memory deficit similar to that seen in AD (Drachman and Leavitt 1974). Soon after that, a cholinergic cell loss was observed in the brains of AD patients (Davies and Maloney 1976). Based on these observations, it was hypothesised that AD is a disease of the cholinergic system (Bartus et al. 1982). Later studies have shown this model to be too simplified: AD is too complex a disease to be caused by one neurotransmitter system alone. However, the cholinergic loss in AD is a major component of the neuropathology, which is strongly demonstrated by the fact that cholinesterase inhibitors are effective in alleviating the symptoms of AD (Eagger et al. 1991).

The degeneration of the cholinergic system in AD is strongest in the NB (Ch4). This area projects to the entire cortical mantle, and in AD brain tissue, a dramatic (up to 95 %) loss of cortical ChAT activity is a consistent finding (Davies and Maloney 1976). At the same time, also the number of cholinergic cells is decreased, the extent of the decrease reported varying from 15% to 95%, depending on the study (Whitehouse et al. 1981, Vogels et al. 1990, Geula and Mesulam 1999). Some studies have reported an atrophy in the remaining cell bodies (Mann et al. 1984, Rinne et al. 1987), but a pathological increase in cell size has also been reported (Iraizoz et al. 1991). Since NB-cortical connections are believed to be involved in attentional functions, the degeneration of NB neurons may account for the attentional deficit observed in AD patients (Lawrence and Sahakian 1995). Another cholinergic nucleus that is degenerated in AD is MSvDB (Ch1/Ch2). In the hippocampus, its main projection area, the amount of ChAT is reduced in AD patients (Perry et al. 1977). Cell loss in MSvDB is also present (Lehericy et al. 1993). Since the hippocampus is a crucial structure in memory

formation, the degeneration of the MSvDB cholinergic system may be related to the cognitive defect present in AD patients.

The cholinergic system is not the only neurotransmitter system that degenerates in AD. Other systems, such as the serotonergic and noradrenergic systems, are also affected by the disease (Mann 1983, Palmer et al. 1987). Indeed, the hallmarks of AD pathology, beta amyloid plaques and neurofibrillary tangles, are associated with most neuron types independent of the transmitter. Therefore, the basis of the cholinergic therapy for AD is mainly symptomatic. However, some reports have suggested that the cholinergic therapy may also reduce amyloid accumulation (see Roberson and Harrell 1997). Nevertheless, since the cell loss becomes very substantial at the later stage of the disease, the beneficial symptomatic effects of cholinergic therapy are likely to be achieved only at the early stages of AD. Even though the effects of cholinergic therapy are modest so far, they are still important as even small improvements in the symptoms can significantly improve the quality of life and postpone the institutionalisation.

The basic mechanisms by which the cholinergic therapy affects cognition are still poorly understood. Therefore, this study was designed to give valuable information about the mechanisms of cholinergic system and cholinergic therapy in cognitive processes.

3. AIMS OF THE STUDY

The aim of this study was to investigate the modulation of the hippocampal function by the cholinergic cells of the septum and pharmacological cholinergic therapy.

The specific aims of this study were to clarify:

- the effects of medial septal lesions on behaviour, hippocampal EEG and spatial encoding of hippocampal pyramidal cells and the effects of hippocampal lesions on behaviour (I-V).
- whether apamin, a SK-type K^+ -channel blocker, is effective in alleviating the memory defect in mice with a lesioned medial septum or hippocampus (I, III).
- whether metrifonate, a cholinesterase inhibitor, alleviates the memory defect of medial septal-lesioned and hippocampal-lesioned mice (II).
- whether apamin and metrifonate restore the theta rhythm defect present in MS-lesioned mice (IV).

4. MATERIALS AND METHODS

4.1. ANIMALS

Young (3-4-months-old) female C57BL/6J mice (study I: n=151; study II: n=184; study III: n=119; study IV: n=17) and young (5-6-months-old) male Long-Evans rats (study V: n=30) were used. The mice were housed five per cage, except the sham- and MS-lesioned mice, which were housed one per cage after the lesioning. The rats were housed in single cages. The environment conditions were controlled and constant (21 ± 1 °C, humidity at 50 ± 10 %, 12 hour light period). Food and water were freely available, except during the radial arm maze testing, when the mice were food-deprived. The study plans were approved by the Provincial Government of Kuopio, Finland.

4.2. LESIONING METHODS

Electrolytic medial septal- and hippocampal lesions for mice. The lesions were made by passage of an anodal DC current via tungsten electrodes. The hippocampal lesions (study III) were performed by applying current into two sites in each dorsal hippocampus (AP: -2.3, ML: ± 1.0 , DL: -2.4; AP: -2.9, ML: ± 1.8 , DL: -2.2) whereas only one site was used for MS-lesions (AP: 0.9, ML: ± 0.0 , DL: -4.7) (Studies I, II). Sham-lesioned mice were treated identically, but no current was applied. During the operations, the mice were deeply anaesthetised with a 1:1 mixture of Dormicum and Hypnorm (4 ml/kg, s.c.) and for analgesia the mice were given a 0.1 mg/kg injection of buprenorfin after the surgery. The mice were allowed to recover from the surgery for two weeks before starting the experiments.

Immunotoxic medial septal lesions for rats. 192 IgG-saporin (0.5 $\mu\text{g}/\mu\text{l}$, 0.2/0.3 μl) or phosphate-buffered saline were injected into two depths of the rat MS bilaterally (AP: 0.45, ML: ± 0.6 , DV -7.8/-6.2). The operation was performed at the Department of Psychology, UNC, Chapel Hill, USA, and were shipped to the Department of Neuroscience and Neurology, University of Kuopio, Finland for electrode implantations and recordings.

4.3. BEHAVIOURAL TESTING

4.3.1. *Water maze*

The water maze can be used as a measure for long-term spatial memory. In this study, a black circular pool, diameter 120 cm, and a black square platform (14 x 14 cm, 1 cm below the water line) were used. A computer connected to an image analyser (HVS Image, Hampton, UK) and a video camera monitored the swim pattern. If the mouse failed to find the platform in the maximum time (60/50s), it was placed there by the experimenter. The mice were allowed to stay on the platform for 5 s and a 30-s recovery period was allowed between the training trials. The extent of the learning can be measured by recording the ability of the animals to find the platform or by removing the platform after a training period and recording the time that the animal spends in that part of the pool which previously contained the platform (= probe trial).

In studies I and II, the training schedule of MS-lesioned mice consisted of eight consecutive days of testing. Four platform trials of 60 s were performed per day during the first five training days. On the sixth day, a probe trial of 50 s was performed. Immediately after the probe trial, the platform was placed in the opposite quadrant and five 50 s platform trials were performed. The schedule on the seventh day consisted also of five 50 s platform trials. The water maze experiment was finished on the eighth day with a probe trial.

In study II, the training schedule for hippocampal-lesioned mice was different from the one used with MS-lesioned mice, because HC-lesioning induces a more prominent impairment in the spatial memory. The training consisted of fifteen consecutive days of testing. Four platform trials of 60 s were performed per day during the first ten training days. During the last five days the testing took place in another room with clearly different visual cues. No drug treatments were administered at this part of the experiment. The location of the platform was changed to

another quadrant (relative to the experimenter) and four platform trials of 60 s were assessed per day.

In study III, the training schedule of hippocampal-lesioned mice in experiment 1 consisted of fifteen days of testing divided into three five-day blocks separated by two days. Four platform trials of 60 s were performed per day during the first ten training days. During the third five-day block, the training was performed in another room with different appearance, visual cues and location of the platform relative to the experimenter. At this stage, the treatment groups were changed. The training schedule in experiment 2 consisted of ten days of testing divided into two five-day blocks separated by two days. Four platform trials of 60 s were assessed per day.

During the platform training trials, the length and latency to find the submerged platform, swimming speed and the number of trials when the platform was found were recorded. During the probe trial, the number of former platform location crossings were recorded.

4.3.2. Radial arm maze

The radial arm maze can be used as a measure for both long-term reference memory and short-term working memory. This study employed a design similar to the one developed for rats (Olton et al. 1978) and later adapted for mouse (Crusio et al. 1987). In study III, during two days of pre-training (5 min per day), the mice were allowed to explore the baited radial arm maze. During the experimental phase, four of the arms were baited in a semi-random manner, with a unique combination of baited arms for each mouse. After each return to the center from an arm, the doors were closed for five seconds. The training was continued until all the baits were consumed or 15 min had passed. After each session, the droppings were removed from the apparatus. At the end of each day, the apparatus was cleaned thoroughly and rotated by 90°. A working memory error was defined as a visit to an arm that had been visited earlier during the session. A reference memory error was defined as a visit to an unbaited arm for the first time. Days 3-20 were used for the statistical analysis.

4.3.3. Passive avoidance

The passive avoidance can be used as a measure for long-term non-spatial memory. The passive avoidance box consisted of a lit and a dark compartment. During the training trial the mouse were placed in the lit compartment and 30 s later, the sliding guillotine door was opened. After the mouse entered the dark compartment (the latency was measured), the door was closed and a foot shock of 0.1 mA (0.5 s) was given. Then the mice were returned to their home cage and 24 h later, they were again placed in the lit compartment and the latency to enter the dark compartment was measured (900 s maximum latency). The memory effect was defined as a difference between the latencies on the first and the second day.

4.3.4. Y-maze

The Y-maze can be used as a measure for short-term memory, general locomotor activity and stereotypic behaviour. The Y-maze used in this experiment had black plastic walls that were 10 cm high. Its arms consisted of three compartments (10 cm x 10 cm) connected with 4 cm x 5 cm passages. The mouse was placed in one of the arm compartments and was allowed to move freely for 6 min without reinforcers. An arm entry was defined as the body of a mouse except for its tail completely entering into an arm compartment. The sequence of arm entries was manually recorded. An alternation was defined as an entry into all three arms on consecutive choices. The number of maximum spontaneous alternations was then the total number of arms entered minus 2, and the percent alternation was calculated as (actual alternations / maximum alternations) x 100. The test was run on two consecutive days.

4.4. PHARMACOLOGICAL AGENTS

4.4.1. Treatment groups

The drug doses and the treatment groups used in this study are shown in table 2. Apamin was dissolved in 0.9 % saline solution and injected intraperitoneally (i.p.). MTF was dissolved in 5

% sodium citrate (pH 5.5) and injected i.p.. Atropine solution was injected i.p.. BIBN-99 was dissolved in 0.9 % NaCl and injected subcutaneously (s.c.). AF267B was dissolved in H₂O and administered orally. AF150(S) was dissolved in PBS (pH 7.4) and administered orally. The drugs were administered 30-40 min before the behavioural testing or EEG recording .

			Apamin mg/kg	Metrifonate mg/kg	Other drugs mg/kg
Study I	Pre	Intact	0, 0.02, 0.06, 0.2	-	-
		Sham	0	-	-
		MS-lesioned	0, 0.02, 0.06, 0.2	-	-
	Post	Intact	0, 0.02, 0.06, 0.2	-	-
		MS-lesioned	0, 0.02, 0.06	-	-
Study II	Pre	Intact	-	0, 5, 15, 50	-
		Sham	-	0	-
		MS-lesioned	-	0, 15, 50	-
		Sham	-	0	-
		HC-lesioned	-	0, 15, 50	-
	Post	Intact	-	0, 5, 15, 50	-
		MS-lesioned	-	0, 50	-
Study III	Pre	Sham	0	-	-
		HC-lesioned	0, 0.06, 0.2, 0.4	-	-
Study IV	Pre	Intact	0, 0.02, 0.06, 0.2	0, 15, 50, 100	Atropine 25 BIBN-99 0.5 AF267B 1, 5, 25 AF150(S) 5
		MS-lesioned	0, 0.02, 0.06, 0.2	0, 15, 50, 100	Atropine 25

Table 2. The treatment groups and drug doses used in studies I-IV. Abbreviations: Pre = the drug was administered before the training; Post = the drug was administered immediately after the training.

4.4.2. Apamin

Apamin is a neurotoxin extracted from bee venom, which specifically inhibits a particular class (SK-type) of Ca^{2+} -dependent K^+ channels. These channels are characterised by their relatively high sensitivity to intracellular Ca^{2+} concentration, lack of voltage dependence and small conductance (Habermann 1984, Lazdunski et al. 1988, Dreyer 1990, Strong 1990), and they are involved in the generation of afterhyperpolarisation (AHP) that occurs subsequent to the action potential in many excitable cells (Kawai and Watanabe 1986). In mice and rats, apamin at very high doses produces signs of poisoning such as tremors, ataxia and lethal respiratory insufficiency (Lallement et al. 1995). There are many apamin binding sites in some of the brain areas implicated in learning and memory processing such as the septum, the hippocampal formation, cingulate cortex and the anteroventral thalamic nuclei (Mourre et al. 1986, Gehlert and Gackenheim 1993). Apamin has been shown to block the afterhyperpolarisation of many types of neurons and increase the firing of cholinergic neurons in a slice preparation of the medial septum-diagonal band region (Matthews and Lee 1991), suggesting that drugs acting via Ca^{2+} -dependent K^+ channels may modulate cholinergic function.

4.4.3. Metrifonate

Metrifonate (MTF) is a second generation cholinesterase inhibitor that acts as a prodrug for a long-acting metabolite and appears to have a broad therapeutic index (Schmidt et al. 1998). It has been used in the tropics as an antihelminthic (a drug for parasite infection) for more than 30 years. Earlier experimental reports have revealed that MTF treatment increases acetylcholine levels (Mori et al. 1995, Scali et al. 1997) and facilitates cognitive functioning in rats and rabbits (Schmidt et al. 1997). Trials with MTF-treated AD patients have shown improvement in tests that measure the severity of the symptoms (Morris et al. 1998).

Unlike some other cholinesterase inhibitors, MTF is not an active compound *per se*, but it has to be converted nonenzymatically to the active metabolite 2,2-dimethyl-dichlorovinyl

phosphate. The metabolite penetrates the blood-brain barrier, binds to the active site of the acetylcholinesterase enzyme and inhibits the enzyme irreversibly. The compound produces only mild side-effects in animals or humans compared to the high levels of side-effects produced by for example tacrine (tetrahydroaminoacridine) that is presently used as a drug for Alzheimer's disease (Knopman 1998). After the inhibition by MTF, the activity of the AChE-enzymes can be restored only by new synthesis of enzyme molecules because the binding of the compound to the receptor is irreversible (Hinz et al. 1996).

4.4.4. Other drugs

In study IV, several muscarinic agents were used to assess the subtype selective role of muscarinic receptors in the effects of MTF on theta. Atropine is a commonly used muscarinic antagonist used for blocking all types of muscarinic receptors. BIBN-99 is a selective M₂-antagonist (Doods et al. 1993); AF267B and AF150(S) are selective M₁-agonists (Brandeis et al. 1995).

4.5. ELECTROPHYSIOLOGICAL RECORDINGS

4.5.1. Surgery

Hippocampal EEG-electrode implantation. In study IV, the hippocampal electrodes were implanted during the MS-lesioning operation (described in chapter 4.2.). The hippocampal electrodes (100 µm in diameter, stainless steel) were implanted to the left hemisphere. The longer electrode was aimed at the hippocampal fissure (AP: -2.2, ML: -1.5, DV: -1.5) and the shorter at the CA1 stratum oriens. One posterior stainless steel screw served as a cortical screw electrode and another, above the frontal lobe, served as a ground and reference electrode. The implant was fixed to the skull with dental cement and two anchor screws.

Hippocampal tetrode implantations. In study V, each rat was implanted with two movable tetrodes (a bundle of four twisted 30 µm Nichrome wires), one tetrode aimed at CA1 (AP: -

3.3, ML: +1.8, DV:-2.2) and the other at CA3 (AP: -3.3, ML: -3.2, DV: -2.5). In addition, bipolar stimulation electrodes were implanted at the lateral hypothalamus for rewarding brain stimulation. The rats were deeply anaesthetised with pentobarbital and chloral hydrate (each 40 mg/kg; i.p.) and for analgesia they received 0.1 mg/kg of buprenorfine (s.c.).

4.5.2. EEG recordings

EEG recordings in study IV during movement took place in a cylinder (diameter 70 cm). The free movement of the mice was encouraged by novel objects. EEG was recorded only during walking between the objects. EEG recordings during alert immobility took place on an upside down bucket.

The recordings (1-s sweeps) were performed using the longer wire electrode located in the hippocampal fissure. The frontal screw served as the indifferent and ground electrode. A dual-channel JFET (junction field effect transistor) on the headstage acted as a source follower. The signal was amplified 1000-4000 times, filtered (1-100 Hz) and digitised at 1 kHz. The data sampling and analysis was performed by a commercial software (DataWave Technologies, Longmont, CO, USA). Four 1-s sweeps were combined and seven epochs of 4 s were sampled for Fast Fourier Transformation analysis which was performed so that the power of frequency bands 1-3.5 Hz, 4-7.5 Hz, 8.5-12 Hz and 4-12 Hz was measured and the ratio of each band to the total power (0.1-50 Hz) was calculated. The power and frequency at maximum power (5-11 Hz) was also determined.

4.5.3. Place cell recordings

The place cell recordings in study V took place 2 months after the lesioning and at least one week after the implantation surgery. The animals were trained to chase invisible randomly distributed target sites for medial forebrain bundle stimulation (Tanila et al. 1997). Neural activity was amplified 5000-10 000 times, bandpass filtered (0.3 - 5 kHz), and digitised at 25 kHz using the Enhanced Discovery software (DataWave, Longmont, CO, USA). The position

of the rat was determined by a video camera following system that tracked two incandescent light bulbs mounted on the headstage assembly (digitised at 60 Hz). The tetrodes were moved 80 μm a day until a good unit isolation was achieved. The stability of recorded units was verified by two days of recordings before the current experiment. Units were analysed offline using the Autocut software (DataWave Technologies Inc.). The theta recordings were performed between the two tetrodes of each hemisphere (one channel of each tetrode). For this purpose, the signal was amplified 1000 - 2000 times, bandpass filtered (0.1 - 50 Hz), and digitised at 1 kHz. On the first day of the experiment, two baseline recordings (Fam1, Fam2) were performed in a cardboard cylinder that was familiar to the rats. On the second day, the test session consisted of a sequence of five runs (Fam3 - New1 - New2 - New3 - Fam4). An illustration of the testing environments can be seen in figure 5 (p. 46). The familiar environment is the same cylinder that was used on the first day, and the New-environment was a plastic hexagon with all features (size, shape, colour, visual cues) different from the familiar cylinder. During both days, each run lasted 7 min, and between runs the rat was placed for 5 min in a round bucket that was suspended from the ceiling above the recording arena.

4.6. HISTOLOGY

The location of the MS- and hippocampal lesions (studies I-IV) was confirmed with a Nissl staining. The lesioned mice were decapitated and the brains were removed and immersed for 1-2 days in a 4 % formaldehyde solution. 50 μm sections were cut with a vibrating microtome and the sections were stained with cresyl fast violet to see the position of the lesion.

At the end of the study V, the rats were deeply anaesthetised and the recording sites were marked by passing anodal current (30 μA , 5 s) through the electrodes. The animals were perfused with buffered 4 % formaldehyde, and the brains were cut in 50 μm sections. The location of the electrode tips was determined histologically by Prussian blue reaction (Tanila et al. 1997). The immunotoxin lesions were verified by observing decreased AChE staining of the hippocampal slices (as described in Hedreen et al. 1985) and loss of ChAT-positive neurons in the MS (as described in Mikkonen et al. 1997).

4.7. STATISTICAL ANALYSIS

The radial arm maze performance and the water maze performance were analysed using analysis of variance for repeated measurements. The ability of the mice to find the water maze platform and the passive avoidance performance was analysed using Mann-Whitney U-test for two independent samples. For analysis of the activity in the Y-maze, a one-way analysis of variance followed by Scheffe's post-hoc multiple group comparison was used. The EEG-recordings of mice were analysed with analysis of variance for repeated measurements as a test of between-subjects effects and as a test of within-subjects effects. The effects of subtype selective compounds were analysed with a paired-samples t-test. The place cell correlations and field parameters were analysed with analysis of variance for repeated measurements and one-way analysis of variance.

5. RESULTS

5.1. LESION EFFECTS ON BEHAVIOUR, EEG AND PLACE CELLS

5.1.1. Behavioural testing

MS-lesioning impaired the water maze performance of mice. In all experiments using MS-lesioned mice and sham lesioned mice (studies I-II), a clear difference between the groups was observed in the training phase of the water maze paradigm. The impairment was observed also when the location of the platform was reversed. The MS-lesioned mice were also slightly impaired in the probe trials, but the differences did not reach significance. However, all the groups (including the control mice) were unable to develop a significant bias towards the previous location of the platform in the probe trial. Swimming speed was unaffected by the MS-lesion.

Also a hippocampal (HC) lesion impaired the performance of the mice in the water maze (study III). During the first ten training days in the water maze, all the HC-lesioned groups performed poorly when compared to the sham-lesioned group. The impairment was visible also after a complete change of the testing environment. The swimming speed was not affected by the HC-lesion.

In the Y-maze, all the MS-lesioned groups made significantly less total moves than the sham-lesioned group during the first day of training (studies I-II). However, the percentage alternation was not affected by the MS-lesion. The hypoactivity of the MS-lesioned mice was no longer present during the second day of testing. In the passive avoidance test, a major proportion of MS-lesioned mice refused to enter the dark compartment completely already on the training day, while the sham-lesioned mice had a latency of only about 40 seconds. In study II, the difference between the latencies to enter the dark compartment on the testing day and the training day was increased in the MS-lesioned mice. However, the difference was not observed in study I. HC-lesioned mice were not tested in the Y-maze or passive avoidance.

In the radial arm maze, hippocampal-lesioned mice were clearly impaired when compared to the controls in the number of working memory errors and reference memory errors (study III).

5.1.2. Hippocampal EEG

The EEG of the control mice during movement was characterised by a sharp theta peak of 8.5 Hz in frequency, which was nearly abolished by the MS-lesion (study IV). MS-lesioning also shifted the frequency of the maximum power from 8.5 Hz to 7.0 Hz. During alert immobility, the control mice showed a peak at 6.5 Hz which was much wider and less prominent than the one seen during movement. The MS-lesion dramatically reduced the total theta power. The analysis of the lower theta band (4-7.5 Hz) revealed that during immobility, the theta power of the control animals is mainly concentrated on this band, and the MS-lesioning markedly reduced its power. All animals had only a little power left in the upper theta band (8.5-12 Hz), but still a diminution of the power in the MS-lesioned animals was statistically significant. However, the ratio of the theta bands was unaffected by the MS-lesion.

5.1.3. Place cells

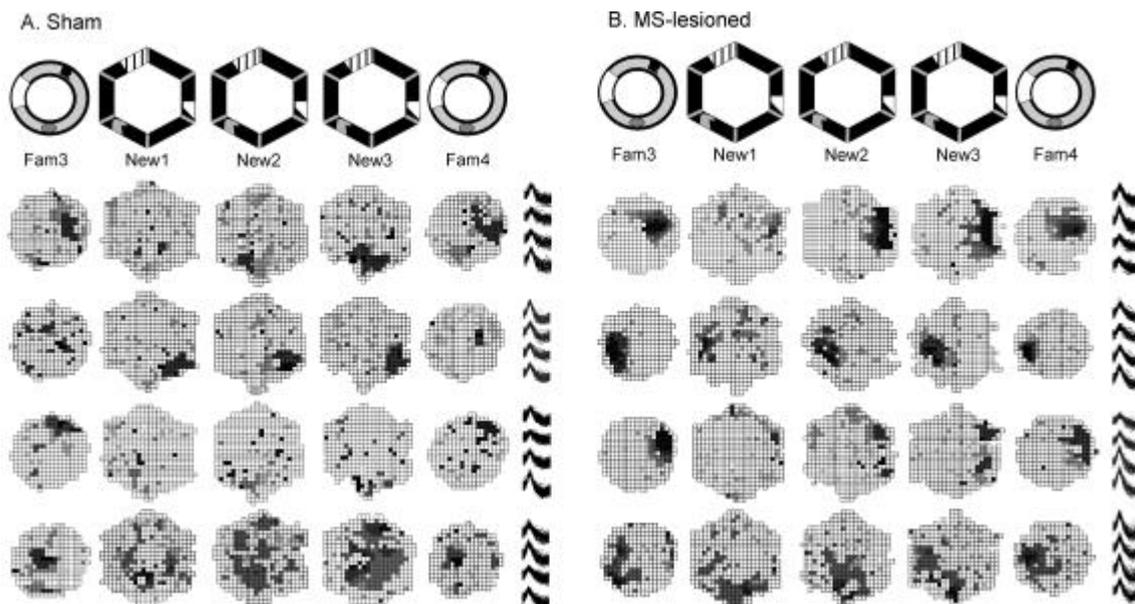


Fig. 5. The experimental protocol and four examples of firing fields from both groups.

Basic firing properties. The analysis of the basic firing properties of place cells in a highly familiar environment (Fam3) did not reveal any significant differences between sham-lesioned and MS-lesioned groups (Fig. 6). The mean spatial selectivity score did not differ significantly between the groups, and also the place fields of the cells of the MS-lesioned rats were shown to be as stable as those of control rats (study V).

During the first run in the novel environment, the place cells of both groups were similarly responsive to the new environment. In both groups, exposures to the new environment resulted in increases in grand mean rate and infield firing rate, the number of firing fields, the combined field area and also in the running speed. All of these properties recovered to their baseline levels when the rats were returned to the familiar environment (Fig. 6).

In contrast to the above described place field properties that were largely normal in the MS-lesioned rats, changes in the mean spatial selectivity differed between the groups during presentations of the new environment (Fig. 6). In control rats, spatial selectivity dropped initially in the new environment but returned back to baseline during the last run. In contrast, the spatial selectivity of the MS-lesioned rats did not react to the change of the environment.

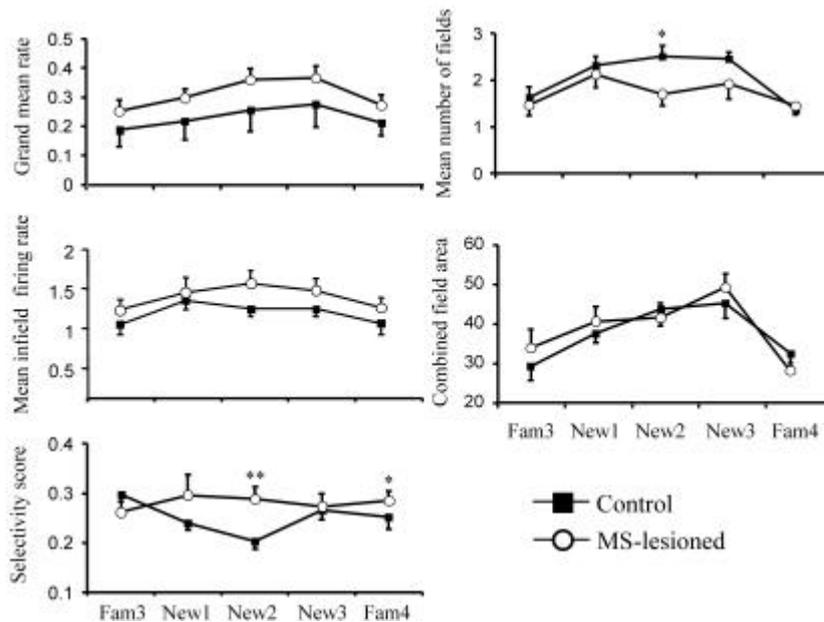


Fig. 6. Basic firing properties of the cells during the experiment.

Spatial correlations. MS-lesioning had no effect on the similarity scores of the place fields in the familiar environment or in the first exposure to the new environment (Fig. 7). However, upon repeated presentations of the novel environment, notable differences were observed. In the controls, the spatial correlations between successive runs in the new environment rose significantly above the chance level. However, the correlation between the final map in the new environment and the original map in the familiar environment (Fam3 x New3) was not above zero. This combination of findings indicates an ongoing remapping in hippocampal ensembles of intact rats. In the MS-lesioned group, the spatial correlations between successive runs in the new environment also rose. Indeed, the evolution of these correlations exceeded those of control rats and rapidly approached that observed between their two baseline runs in the familiar environment (Fam1 x Fam2). However, this was not simply due to a faster course of remapping in MS-lesioned rats, because the correlation between the final run in the new environment versus the original pattern in the familiar environment on Day 2 (Fam3 x New3) was significantly above zero in MS-lesioned rats (Fig. 7). Therefore, unlike in controls, the gradual rise in correlations with exposure to the new environment in MS-lesioned rats reflected a re-convergence to the original mapping.

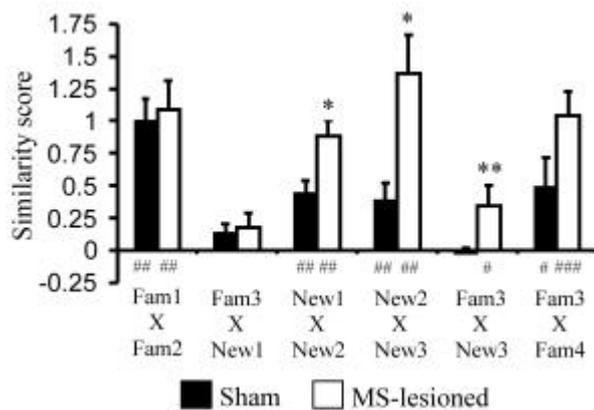


Fig. 7. Mean similarity between selected pairs of runs. Pound signs indicate significantly different correlations from zero: # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, one-sample t-test.

In contrast to normal overall firing rates and basic spatial firing patterns observed in hippocampal cells of MS-lesioned rats, the percentage of spikes in the bursts differed significantly between the groups (Fig. 8). The difference was due to an almost total absence of

movement-related bursting in all CA1 cells and CA3 cells of the controls but clear bursting in CA3 cells of the MS-lesioned rats. Notably, this difference in bursting was most obvious during the re-exposures to the new environment (Fig. 8).

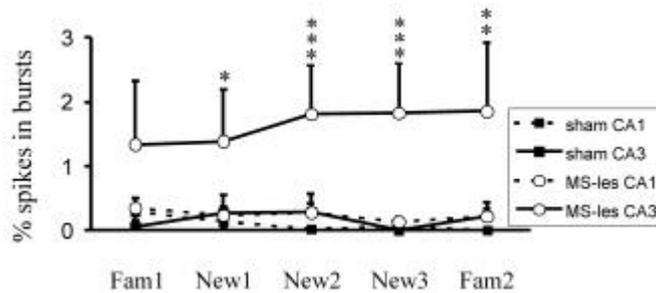


Fig. 8. Consistently higher occurrence of bursts in CA3 cells of the MS-lesioned rats compared to any other group of cells.

Analysis of hippocampal field potentials revealed substantial loss of theta activity associated with alert immobility in the MS-lesioned rats (Fig. 9). In periods of alert immobility in freely moving animals, the total power of theta was reduced in the MS-lesioned rats and the frequency at maximum power was increased in the MS-lesioned rats when compared to controls. Furthermore, during immobility associated with light restraint, control rats had long bouts of continuous theta, whereas the two lesioned rats given this test had no observable theta. During movement, the maximum power was marginally reduced in the MS-lesioned rats.

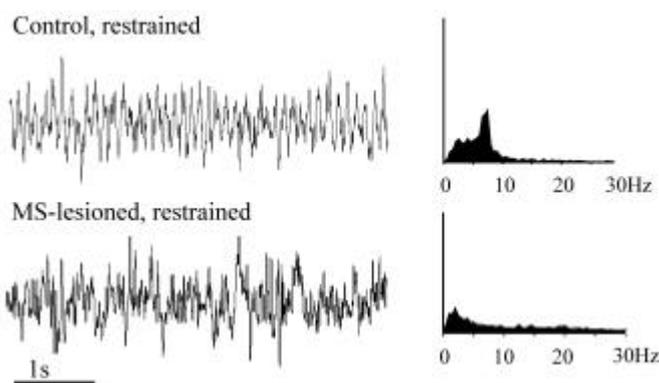


Fig. 9. Intrahippocampal theta recordings from a representative control and Ch-X rat during restraint in a towel.

5.2. IMPROVEMENT OF SPATIAL LEARNING BY APAMIN

Intact mice. In the water maze (study I), apamin treatment administered before or after the daily training had no effects on the performance of intact mice. There were no group differences in counter crossings during the probe trials, either. In the Y-maze, apamin administered before or after the daily training had no effect on total moves or percent alternation during either of the testing days in the Y-maze. In passive avoidance, apamin administered before or after the daily training had no effect on the performance.

MS-lesioned mice. Apamin alleviated significantly the memory impairment of MS-lesioned mice in the water maze (study I). The effective dose was 0.06 mg/kg (and 0.02 mg/kg, to a lesser extent). Apamin did not affect the swimming speed. In the spatial bias test, no group differences were observed in counter crossings. Also during the platform reversal, apamin treatment alleviated the memory defect, but again, the swimming speed was not affected. Apamin did not affect the probe trial performance. When apamin was administered after the training in the water maze, the performance of MS-lesioned mice was mainly unaffected. No differences were found during the probe trial.

In the passive avoidance test, apamin alleviated the hypoactivity of the MS-lesioned mice by decreasing the entry latency during the training day. However, the difference between the latencies to enter the dark compartment on the testing day and the training day was unaffected. In the Y-maze, a similar alleviation of hypoactivity was observed, but the difference between the groups did not reach significance. The cognitive parameters were unaffected in both tests.

HC-lesioned mice. In the radial arm maze, apamin reversed the defect present in the hippocampal-lesioned mice (study III). It decreased the number of working memory errors and reference memory errors dose-dependently.

In the water maze experiment 1 (study III), apamin decreased the escape length of the hippocampal-lesioned group during days 1-10. During days 11-15 (after the change of the

treatment of the groups), the group that was now treated with the same dose of apamin (0.2 mg/kg), showed an improved performance. However, also the swimming speed during days 1-10 of the group treated with apamin 0.2 mg/kg was reduced compared to the vehicle group. On the other hand, the swimming speed was not affected during days 11-15. In the water maze experiment 2, a higher dose of apamin (0.4 mg/kg) increased the escape length compared to the vehicle group, and it also increased the swimming speed.

5.3. IMPROVEMENT OF SPATIAL LEARNING BY METRIFONATE

Intact mice. During the initial training days in the water maze, MTF administered before or after the daily training had no effect on the intact mice (study II). There were no group differences in the probe trial, either. During the platform reversal stage, MTF treatment before or after the training had no effect on escape latency or swimming speed. However, MTF 15 mg/kg administered after the training did decrease the percentage of platform finding. During the second probe trial, there were no group differences. Furthermore, no differences between the groups were found in the Y-maze or in passive avoidance.

MS-lesioned mice. During the first five days in the water maze, MTF alleviated the memory defect present in the MS-lesioned mice (study II). The swimming speed and the probe trial performance were unaffected. During the platform reversal stage, MTF had no effect on the navigation failure, but it slightly increased the swimming speed. On the eighth day, during the probe trial, MTF failed to improve the probe trial performance. When MTF was administered after the training in the water maze, no group differences were observed during the training phase or in the probe trial.

In the Y-maze, during the first day, MTF alleviated the hypoactivity of the MS-lesioned mice by increasing the total moves (study II). The percentage alternation was not affected, however. In the passive avoidance testing, a similar alleviation of the hypoactivity was observed, but the differences did not reach significance. The cognitive parameters were unaffected in both tests.

HC-lesioned mice. MTF failed to have any effect on the performance of the hippocampal-lesioned mice in the water maze (study II). Swimming speed was not affected, either.

5.4. DIFFERENTIAL EFFECTS OF APAMIN AND METRIFONATE ON EEG

During movement, the total theta power was unaffected by MTF both in the sham and the MS-lesioned group (study IV). In the sham group, the frequency of the theta peak was shifted from 8.5 Hz to 7.5 Hz. In the MS-lesioned animals, MTF did not shift the frequency of the maximum power. Atropine decreased the total and maximum theta power in the sham group and in the MS-lesioned group, but it did not shift the frequency of the maximum power. Apamin had no effect on the movement-related hippocampal EEG.

During alert immobility, MTF increased the total theta power of the sham group, but not of the MS-lesioned group. MTF dose-dependently increased the power in the lower theta band of the sham lesioned animals but it had no effect on the lesioned animals. Drug by group interaction was also found for the higher theta band. MTF decreased the power of upper band theta in the sham group, but again, it had no effect on the lesioned group. The ratio of the theta bands 8.5-12 Hz to 4-7.5 Hz was decreased by MTF in the sham group while the lesioned group remained unaffected. The total theta power was decreased by atropine in the sham group, while the lesioned group with little remaining theta power was unaffected. Atropine markedly decreased the amount of theta in the 4-7.5 Hz band of the sham lesioned mice, but it had no effect on the lesioned group. Atropine had no effects in the theta band 8.5-12 Hz in either group. As a result, the ratio of the higher to lower theta bands was increased by atropine in the sham group, but not in the lesioned group. Apamin had no effect on the immobility-related hippocampal EEG.

In the second part of the immobility experiment, MTF was confirmed to increase the power of the theta band 4-7.5 Hz of intact animals. Atropine injection before the MTF (MTF) injection blocked the effect completely. BIBN-99 injection before the MTF injection did not block the effect of MTF as the theta power was still increased; on the contrary, it slightly potentiated the

effect. AF267B at the dose of 1 or 5 mg/kg had no effect, but a massive dose (25 mg/kg) induced a similar effect in the EEG as MTF: the power in theta band 4-7.5 Hz was increased. This effect was even stronger than the one achieved by MTF. AF150(S) induced no observable changes in the EEG.

5.5. HISTOLOGY

In studies I-III, the locations of the lesions in MS- and HC-lesioned mice were confirmed by studying the cresyl fast violet -stained sections. All lesioned mice accepted to these studies had lesions that encompassed the medial septum (MS-lesioned mice) or the dorsal hippocampus (HC-lesioned mice). The decrease of acetylcholine-containing fibers in the hippocampi of MS-lesioned mice was confirmed by acetylcholinesterase staining.

In study IV, the histology confirmed the location of the shorter hippocampal electrode in the alveus or stratum oriens and of the longer hippocampal electrode in the stratum lacunosum moleculare or the hippocampal fissure. The fact that the electrode pairs straddled the CA1 pyramidal cells was also observed by the characteristic 180° phase shift of theta between the two electrodes of the pair (Brankack et al. 1993).

In study V, the location of recording sites was confirmed to be the CA1 and CA3 pyramidal cell layers. All CA1 electrodes were located in the CA1b area with an exception of one electrode in the CA1a and three electrodes in the CA1c area. The CA3 electrodes were located in the CA3b area with an exception of two electrodes in the CA3a area and one electrode in the CA3c area. Inspection of the histological sections stained for AChE revealed a substantial loss of cholinergic innervation of the hippocampus in the MS-lesioned rats. In addition, sections stained for ChAT revealed a near total loss of ChAT positive neurons in the medial septum of the MS-lesioned rats.

6. DISCUSSION

6.1. VALIDITY OF MS-LESIONING AS A MODEL OF AD

There is no animal model for Alzheimer's disease *per se*, but experimental animals have been used in attempts to identify cognition-enhancing drugs that might be useful in AD. A widely used model has been lesioning of the MS. It is clear that MS-lesioning is not ideal for the purpose, but it does mimic at least some aspects of the disease. First, it produces a memory defect. Second, it produces a cholinergic defect in the hippocampus, a property that is the best described neurotransmitter-defect in AD. Therefore, it has for long been the best available animal model for the study of pharmacological agents that might be useful in AD.

The validity of MS-lesioning as a model for AD can also be questioned. The recent development of 192-IgG-saporin, a selective cholinergic neurotoxin, has revealed that a selective destruction of the cholinergic cells of the MS, unexpectedly, does not produce a memory defect (Berger-Sweeney et al. 1994, Torres et al. 1994, Baxter et al. 1996). Therefore, it can be argued that nonselective MS-lesioning is not a valid model for cholinergic hypofunction of AD. Moreover, a selective IgG-saporin-lesion cannot be used either, because a manipulation that does not impair memory is naturally useless as a model for developing cognition-enhancers. However, IgG-saporin-lesioned animals can be used as a model for attention defect, which is also present in AD patients (see Lawrence and Sahakian 1995).

Another confounding factor is that MS-lesioning produces also non-cognitive effects: changes in the emotional behaviour and reactions to stimuli (Fried 1973). Mice and rats with septal lesions are difficult to handle and show increased irritability and defensiveness (Albert and Chew 1980). MS-lesioned rats show also increased freezing behaviour and acoustic startle responses (Blanchard et al. 1979). However, the effects of MS-lesioning on anxiety are not entirely clear, because in some other tests, MS-lesioning seems to produce anxiolytic effects (Treit and Pesold 1990). Nevertheless, the non-cognitive effects of MS-lesioning can influence

the performance in cognitive tests and subsequently, a drug that alleviates the non-cognitive effects could be mistakenly identified as a cognition-enhancer.

In this study, both apamin and MTF decreased the hypoactivity of MS-lesioned mice in Y-maze and passive avoidance test. Therefore, it is possible that these compounds alleviate symptoms of MS-lesioning not by affecting memory or learning *per se*, but via non-cognitive measures, such as anxiety, apathy or attention. To separate the cognitive and other effects of a certain compound is extremely difficult, and often, it can have both types of effects. However, while in Y-maze and passive avoidance the activity effects on MS-lesioned mice can be interpreted as non-cognitive effects, in the water maze the drugs had no effect on swimming speed or thigmotaxis of intact or MS-lesioned mice. Thus, it can be assumed that the effects of apamin and MTF are only partly mediated by non-cognitive effects. The anti-apathy effect of MTF, which can be seen in Y-maze and passive avoidance, can be related to the ability of MTF to alleviate apathy in AD patients (Cummings et al. 1998, Morris et al. 1998). The results also suggest that K⁺ channel blockers might have a similar effect on AD patients.

6.2. DRUG EFFECTS ON BEHAVIOURAL TESTING

Based on earlier reports of the positive effects of MTF on MS-lesioned rats (Riekkinen et al. 1996), it was expected to have a similar effect in mice. Indeed, MTF was observed to improve the spatial learning of MS-lesioned mice. However, some differences between mice and rats were found. In the rat study, MTF improved the reversal learning as well as initial learning, but in the mouse study, it improved only the initial learning. This can be explained either by a fundamental difference between the cognitive functions of rats and mice or by a difference in the water maze search strategy. The latter is supported by an observable difference in the search strategy of the mice when compared to rats. Well-trained rats search for the platform by swimming small circles close to the area of the platform until they find it, while mice use a different strategy: they seem to choose a fixation point in the surrounding room and using this point, they perform long diagonal cuts across the pool. After a failure to find the platform, they swim to the same starting point along the pool wall, and repeat the

diagonal cut. This is a confounding factor also in the probe trial assessment, in which it is difficult to obtain a significant bias even for well-trained mice. The different search strategy can also explain the discrepancy in the results of the intact mice: the performance during the training days was clearly improved (almost to ceiling level), but the probe trial did not reveal a significant bias towards the location of the platform. In other words: the lack of a significant bias may simply be an artefact caused by a non-rat-like reaction of the mice to the removal of the platform. Another factor that complicates the measurement of mouse behaviour in the water maze is that mice in general learn slower than rats. Therefore, an extremely high contrast and clear appearance of the visual cues might be required for the mice to be able to perform more effectively. This might also improve the spatial bias during the probe trial.

MTF did not improve spatial learning of the HC-lesioned mice. This is a strong indication that the main target area of MTF is the hippocampus, more specifically the dorsal hippocampus, since the lesion comprised only the dorsal hippocampus. This is in agreement with the view that cholinesterases affect mainly by increasing the acetylcholine levels in the hippocampus (Scali et al. 1997).

Apamin has been widely used as a tool in muscle research since the 70's. The first report on the effects of apamin in learning and memory processes was published only a decade ago by Messier et al. (1991). In their report, apamin was observed to facilitate memory processing in appetitively-motivated bar-pressing response in mice. In another study, apamin improved learning in an object recognition test in rats (Deschaux et al. 1997). These results, which were obtained using intact animals, provided the basis for our hypothesis that apamin could improve the memory of MS-lesioned mice. This hypothesis proved to be correct, which suggests that blockers of K^+ channels may be useful in the treatment of memory impairment caused by cholinergic hypofunction, such as AD. It is noteworthy that apamin was more effective in alleviating the memory defect than MTF, which suggests that K^+ channel blockers have a good potential as drugs for AD. The basis of the effects of apamin on learning and memory is not clear. It is possible that apamin potentiates the function of the cells that remain alive in the MS after the lesioning. This idea is supported by a study by Matthews and Lee (1991)

demonstrating that apamin increases the firing of cholinergic cells in a slice preparation of the MSvDB. By increasing the activity of the remaining cells, apamin might compensate for the lesion-induced defect. Another possibility is that apamin affects directly the hippocampus. This hypothesis is supported by studies showing that apamin is effective in blocking the K⁺ currents in CA1 pyramidal cells (Stocker et al. 1999) and that apamin facilitates long-term potentiation (LTP) in CA1 area of rat hippocampus (Behnisch and Reymann 1998). However, the present results with HC-lesioned mice do not support this. Apamin was shown to improve learning also in HC-lesioned mice, which indicates that the site of action is elsewhere. Furthermore, it was shown that apamin has no effect on the hippocampal EEG of MS-lesioned mice. However, the HC-lesion used in this study was not complete, but it comprised only the dorsal hippocampus. The ventral hippocampus is not assumed to be essential in spatial learning (Moser and Moser 1998), but if it does have a significant role, the positive effects of apamin may be mediated through it. A third possibility for the effect of apamin is that it affects some other brain area, unrelated to the septohippocampal axis, such as the midbrain dopaminergic neurons (Morikawa et al. 2000), or, that it simply has no specific target area. The idea that apamin has a broad range of effects which are not limited to a certain transmitter system or brain area is supported by the fact that the tests in which apamin has been shown to facilitate memory processing have been heterogeneous, measuring different aspects of memory processing.

6.3. ELECTROPHYSIOLOGICAL RECORDINGS

6.3.1. Drug effects on hippocampal EEG

MS-lesioning was observed to abolish the theta rhythm of mice during both movement and immobility. MTF and apamin, drugs that reverse the spatial memory deficit induced by MS-lesioning, did not restore the theta oscillation in the hippocampus.

Theta rhythm has been studied extensively in rats, but the properties of theta in mice have been largely unexplored. The present study indicates that theta rhythm in mouse is not different from

that previously found in rat. During active locomotion, a sharp theta peak was observed around 8 Hz, while during alert immobility, theta peaked around 6 Hz. Consistent with previous studies with rats (Sainsbury et al. 1987), the latter type was eliminated by atropine, while the movement-related theta was only partly sensitive to atropine. In addition, the present results also indicate that the effects of medial septal lesioning on hippocampal EEG are similar in mice as earlier reported in rats (Kolb and Whishaw 1977, Sainsbury and Bland 1981, Buzsaki et al. 1983). The electrolytic lesion of the MS, damaging both cholinergic and GABAergic cells, markedly attenuated the frequency peak in theta band.

The effect of MTF on the hippocampal theta of intact mice in the present study resembled that of physostigmine in rats (Lee et al. 1994): during alert immobility, MTF at the dose of 100 mg/kg significantly increased the theta power at the range of 4-7.5 Hz. This effect was shown to be mediated through muscarinic receptors, since it was completely blocked by atropine. A selective M₂-antagonist did not block the effect of MTF (rather, the effect was potentiated), which indicates that the effect is not mediated through M₂-receptors. M₁ receptors were also ruled out by the observation that selective M₁ agonists AF150(S) and AF267B had no effect on hippocampal theta at the doses of 1 or 5 mg/kg which are 2-10 fold larger than the doses that produce behavioural effects (Fisher et al. 1998, Ruske and White 1999, Fisher A., personal communication). With a considerably higher dose (25 mg/kg), AF267B produced an effect which is identical to the one obtained with MTF. This suggests that with a very large dose, this agent agonised also M₃ (or M₅) receptors, which are structurally closely related to M₁ receptors. Indeed, M₃ receptors have been suggested to mediate the muscarine-induced excitations in the septohippocampal neurons (Liu et al. 1998), and recently, it has been shown that it is the GABAergic (and not cholinergic) neurons of the MSvDB that are responsible for the M₃-mediated effects in the function of the hippocampus (Alreja et al. 2000). This is because the cholinergic neurons of the MSvDB regulate directly the function of the MSvDB-GABAergic neurons (and not other cholinergic neurons), which regulate the function of the hippocampus by means of disinhibition. Therefore, M₃ (or M₅) receptors are likely to be mediating the effects of MTF on the hippocampal theta rhythm.

There is cumulative evidence suggesting that the cognitive effects of acetylcholine are mediated through M₁ receptors. For example, M₁ agonists have cognition enhancing properties (Vincent and Sepinwall 1992, Brandeis et al. 1995, Tecle et al. 2000), and more specifically, they can alleviate the memory impairment that is induced by a cholinergic depletion of the hippocampus (Hodges et al. 1999). Moreover, M₁ antagonists impair learning in general (Hagan et al. 1987) and more specifically, block the favourable effects of cholinesterase inhibitors on spatial memory (Murakami et al. 2000). On the other hand, such evidence does not exist for M₃ receptors. Collectively, these and the present findings suggest that the cognitive effects of cholinesterase inhibitors are mediated through M₁ receptors while the effects on EEG are separate and are mediated through M₃ (or M₅) receptors. The observation that the doses of MTF that improve spatial memory do not induce changes in the hippocampal theta can be explained by the fact that in the hippocampus, M₃ receptors are much less abundant than M₁ receptors (Levey et al. 1994) and therefore require a higher cholinergic tonus.

A lesion of the MS attenuates the theta rhythm in the hippocampus. A recent study (Kinney et al. 1999) showing that cognition-enhancers stimulate theta suggested that the mechanism of apamin and MTF in alleviating the spatial memory defect in MS-lesioned animals could be restoration of hippocampal theta. However, both compounds failed to have any observable effect on the theta rhythm of MS-lesioned mice. In the case of MTF, this study showed a double dissociation between EEG changes and cognitive processes: in intact mice, MTF induces EEG-changes but not cognitive effects, and in MS-lesioned mice, MTF induces cognitive improvement, but no EEG-changes. Therefore, the present study, showing that a spatial learning deficit of MS-lesioned mice can be reversed by a cognition-enhancing drug without stimulating theta rhythm, strongly challenges the notion that spatial learning and memory deficits after medial septal lesion are primarily due to the loss of hippocampal theta.

If apamin and MTF do not act by stimulating hippocampal theta after the MS-lesion, there have to be other mechanisms mediating their cognition-enhancing properties. One possibility is the reduction of afterhyperpolarisation (AHP). This effect, which is commonly associated with apamin, is also shared by cholinergic muscarinic agonists (Cole and Nicoll 1983). Therefore,

reduction of AHP can be the common mechanism of action of apamin and MTF to enhance spatial learning and memory in MS-lesioned mice. Reduction of AHP increases spiking of hippocampal neurons and enhances LTP induction (Behnisch and Reymann 1998, Norris et al. 1998). It is possible that restoration of these effects in the hippocampus after MS-lesion is much more important for learning and memory than restoration of the theta rhythm.

6.3.2. Lesion effects on spatial encoding

Hippocampal pyramidal cells of rats lacking the cholinergic innervation of the hippocampus were shown to be able to form selective and stable place fields. However, the ability to respond to a change in the environment is impaired.

In the familiar environment, all the field properties and spatial firing patterns were unaffected by the cholinergic lesion. This shows that the cholinergic input to the hippocampus is not necessary for the development and maintenance of a spatial representation in the hippocampus. However, it is impossible to tell if there was a difference in the speed of the development of the representation, because the rats were familiarised to the recording environment for 2-3 weeks before the correct location of the electrode in the pyramidal cell layer was achieved. Thus, it is possible that the cells of the MS-lesioned rats developed the stable representation more slowly than the cells of the control animals.

Also, the initial place cell response to a new environment in both groups was similar: a clear change was observed upon the first exposure to the new environment. This indicates that all the rats were aware of the change of the environment. In addition, the changes in the place field properties and the prominent exploratory behaviour of the MS-lesioned animals in the new environment indicate that there was no impairment in the ability of the lesioned rats to observe the change in the environment.

This study showed that reduction of cholinergic innervation affects the direction of the response of the hippocampal network upon subsequent exposures to the new environment. In

controls, an expected re-mapping was observed: the representation of the environment changed completely. In MS-lesioned animals, the representation of the new environment was similar to the one of the familiar environment. This indicates that the information processing in the hippocampus is somehow impaired in animals lacking the cholinergic innervation of the hippocampus. The reason for the impairment is unclear but some hints can be achieved from a separate field of science: computer modelling of neuronal networks. In a study by Hasselmo et al. (1995), it was predicted that the level of cholinergic modulation determines the extent to which storage of new afferent input patterns depends upon previously stored patterns in the hippocampus. Specifically, at low levels of cholinergic modulation, the network recalls previously stored patterns and remains relatively unaltered by the new input. At high levels of cholinergic modulation, by contrast, the network learns the new pattern and excludes elements from previously stored patterns (Hasselmo et al. 1995). Thus, loss of cholinergic innervation, according to this model, might be expected to reduce the development of a distinct encoding pattern for a novel environment as observed in the present results.

Another indication of the impaired function of the hippocampus in the lesioned animals is the abnormal burst firing of CA3 cells of the lesioned animals during movement. Normally, hippocampal pyramidal cells often fire complex-spike bursts during sharp wave state but only rarely during movement (Buzsaki et al. 1983). Therefore, the high percentage of bursts in CA3 neurons of lesioned rats during movement is clearly abnormal. This suggests that the cholinergic input of the hippocampus affects the balance of the two known states of the hippocampus. According to the two-stage model of memory by Buzsaki (1989), the hippocampus can be either in exploratory theta-state, during which the cortical information is transmitted to the hippocampus, or in immobile sharp wave state, during which the information is processed in the hippocampus, especially in the CA3 area. During the latter stage, the CA3 pyramidal neurons typically fire in population bursts, which are absent during the theta stage (Buzsaki 1989). The role of the cholinergic system in this system was proposed in a subsequent Hasselmo's model (Hasselmo et al. 1996), which suggests that during theta-stage, the cholinergic innervation suppresses the CA3 intrinsic connections and CA3-CA1-connections by means of presynaptic inhibition. In contrast, during non-theta stage, the lack of

cholinergic inhibition allows the CA3 to start the bursting activity, which causes sharp waves. Interestingly, in this study, the CA3 cells of the animals with a selective cholinergic MS-lesion were shown to express highly increased burst firing, which suggests that without the cholinergic innervation, the hippocampus is constantly in a sharp wave mode. The sharp waves present in this mode would enhance the CA3-CA1 synapses and thus strengthen the pattern in the CA3 network that has been acquired in the familiar environment. Therefore, the transmission of new cortical information about the new environment to the hippocampus would be prevented and instead, the hippocampus would continue to express the pattern that was prominent in the familiar environment. In conclusion, septohippocampal cholinergic innervation may act as a switch between learning and recall modes of the hippocampal circuitry.

6.4. SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM IN COGNITIVE PROCESSES

Explaining the role of the septohippocampal cholinergic system in learning and memory has become more difficult after the discovery that selective lesioning of the cholinergic cells of the MSvDB does not impair the performance of rats in the water maze (Berger-Sweeney et al. 1994, Torres et al. 1994, Baxter et al. 1996). Previously, it was assumed that the cholinergic regulation of the hippocampus is crucial for the general function of the hippocampus, but now, a more complex explanation is needed to interpret the findings. The cholinergic regulation is still known to be important, but probably the water maze is not sensitive enough to detect the impairment in the hippocampal function. Indeed, rats that are cholinergically depleted but have otherwise intact neurotransmitter systems, may be able to compensate the deficit if the task is not sensitive enough. Moreover, impairments have been found in several other measures, such as short-term memory (Torres et al. 1994), attention (Bushnell et al. 1998) and strategy selection (Janis et al. 1998).

This study supports the idea that the cholinergic regulation acts as a switch between the two states of the hippocampus. This idea can explain the unexpected findings in the water maze: if the hippocampus is constantly in the sharp wave mode and the hippocampal place cells are selective and stable, it is possible (and even probable) that the hippocampus is not impaired in

its spatial function. Remapping function may be necessary only when there is a large amount of environments that need to be differentiated (as in the natural environment). This is not needed if the rat is tested consecutively in the same room. Therefore, it is still possible that the impairment of the lesioned rats can be detected in the water maze, but the task should include a differentiation between several environments. Another fact that can be explained by the theory of cholinergic regulation of hippocampal states is the early symptoms of AD patients. In an early stage of AD, the patients can easily get lost in a new environment, while they have no impairment in daily functions that take place in the patient's home. This can be related to the function of place cells that are stable and reliable in a familiar environment, while in a new environment, the impaired remapping causes confusion and inability to orient oneself. Differences in the spatial abilities of lesioned rats and AD patients can be explained by the degeneration of other transmitter systems along with the cholinergic one in AD patients.

7. CONCLUSIONS

Apamin, a blocker of SK-type of Ca^{2+} -dependent K^+ channels, alleviated the acquisition defect during reference memory testing in MS-lesioned mice. It had no effect on inhibitory avoidance or spontaneous alternation behaviour in the Y-maze. Also, apamin reversed the memory defect caused by partial hippocampal lesions, while it had no effect on the performance of intact mice. These results suggest that blockade of Ca^{2+} -dependent K^+ channels alleviates the memory defect induced by a damaged septohippocampal axis. However, the site of action is probably not any separate brain area, but the actions of apamin are likely to be mediated by several brain areas or transmitter systems.

Metrifonate, a cholinesterase inhibitor, alleviated the water maze spatial memory defect induced by a MS-lesion, whereas the spatial memory defect induced by a partial lesion of the dorsal hippocampus was not improved. This suggests that the site of action of metrifonate is the dorsal hippocampus.

Since neither metrifonate nor apamin increased the power of theta in the MS-lesioned mice, theta rhythm *per se* is concluded not to be necessary for the proper function of the hippocampus. The effects of metrifonate on theta rhythm are likely to be mediated via M_2 muscarinic receptors, while its effects on cognition are likely to be mediated via M_1 receptors.

These findings support the idea that the role of the septal cholinergic modulation of the hippocampus is to act as a switch between its two functional modes. More specifically, the cholinergic regulation determines whether the hippocampus is in an information-collecting or in an information processing/retrieving mode.

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