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PLASTICITY IN THE ENTORHINAL-HIPPOCAMPAL PATHWAY

Influences of gene mutations and hormones

Doctoral dissertation

To be presented with assent of the Medical Faculty of the University of Kuopio for public examination in Auditorium L3, Canthia Building of the University of Kuopio, on Friday 31th May 2002, at 12 noon

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ABSTRACT

Many studies have demonstrated that following entorhinal cortex (EC) ablations, the dentate gyrus (DG) of the hippocampus shows degeneration of the lesioned axons and terminals, followed by a sprouting response of the surviving axons. This lesion model has frequently been used to elucidate the mechanisms underlying neuronal plasticity, it is also of particular interest since it plays a critical role in learning and memory, and because of the vulnerability of its circuitry to degeneration in Alzheimer's disease and during the course of normal aging.

The present studies characterize in detail the projections from the entorhinal cortex to the hippocampal formation in mice. The differences in these connections between mice and rats are shown, and the differences between mice and rats following EC lesion-induced sprouting are demonstrated. Moreover, the presence of mutated genes and the effects of estrogen depletion on the sprouting response in the mouse hippocampus are evaluated using synaptophysin and acetylcholinesterase (AChE) staining.

The main findings of these studies are: **I.** The projections of the EC to the hippocampus have a laminar and topographical distribution in the mouse. The projection from the EC to DG is unilateral, arising from layer II neurons, whereas the projections to CA3, CA1, and subiculum are bilateral, and it originates predominantly from neurons in layer III. Neurons in the lateral part of both LEA and MEA project to the dorsal part of the hippocampus, whereas the projection to the ventral hippocampus originates from neurons in medial parts of EC. **II.** Species differences are present in EC to hippocampal formation projection, the main differences are: 1) the lack of a contralateral projection to DG in the mouse, 2) layer III of the EC projecting to CA3 (compared to layer II in the rat) and 3) a more limited septotemporal extent of the labeling in the hippocampus of the mouse. **III.** The process of neuronal reorganization after EC ablation in mice is similar to that in rats, however, the process of neuronal degeneration is different. Both species show an increase in the density of staining for synaptophysin and AChE in the outer molecular layer of DG following the EC lesion. Further, it should be noted that, whereas rats demonstrate significant shrinkage of the molecular layer of the DG following the denervation, mice do not show this response. **IV.** It has been suggested that Alzheimer's disease-linked *ps1* mutations deregulate neurite growth. We demonstrate that the presence of both the normal human *ps1* gene or the mutated human *ps1** gene in mice, compared to the mouse *ps1* gene, enhances plasticity following EC lesions. **V**. Our studies with estrogen depletion (ovariectomy) demonstrate that in these mice axonal sprouting following lesions of EC is substantially reduced. Estrogen supplementation restores the sprouting response to the level of the non-ovariectomized, normal mouse.

Taken together, the present studies provide detailed information on the projections from EC to hippocampus in mouse, further, we show the differences between mice and rats in these projections. We have demonstrated differences in the hippocampus in the response to denervation between these two species, whereas we establish that the sprouting response is similar. We have analyzed the consequences of mutations (in the human *ps1* gene) in mice, it enhances plasticity following EC lesions, and finally, we show that hormone levels influence the sprouting response, low levels of estrogen diminish sprouting.

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We and other animals notice what goes on around us. This helps us by suggesting what we might expect and even how to prevent it, and thus fosters survival. However, the expedient works only imperfectly. There are surprises, and they are unsettling. How can we tell when we are right? We are faced with the problem of error.

W. V. Quine, *From Stimulus to Science*

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Kuopio, May 2002

Inga Kadish

ABBREVATIONS

Aβ	amyloid β protein
Aβ-40	amyloid β protein 1- 40 (40 amino-acids)
Aβ-42	amyloid β protein 1 - 42 (42 amino-acids)
AChE	acetylcholinesterase
AD	Alzheimer's disease
ANOVA	analysis of variance
app	amyloid precursor protein gene
APP	amyloid precursor protein
BDA	biotinylated dextran amine
CA1	field *cornu Ammonis 1*
CA2	field *cornu Ammonis 2*
CA3	field *cornu Ammonis 3*
CE	caudal entorhinal field
ChAT	choline acetyltransferase
CNS	central nervous system
DG	dentate gyrus
DIE	dorsal intermediate entorhinal field
DLE	dorsal lateral entorhinal field
EC	entorhinal cortex
ECL	entorhinal cortex lesion
FB	fast blue
FG	fluoro gold
GABA	gamma-aminobutyric acid
GAP-43	growth associated protein - 43
GFAP	glial fibrillary acidic protein
LEA	lateral entorhinal area
LEC	lateral entorhinal cortex
ME	medial entorhinal field
MEA	medial entorhinal area
MEC	medial entorhinal cortex
NDBB	nucleus of the diagonal band of Broca
NMDA	N-methyl-D-aspartate
PHA-L	*Phaseolus vulgaris* leucoagglutinin
ps1	presenilin 1 gene

PS1	presenilin 1 protein
ps2	presenilin 2 gene
PS2	presenilin 2 protein
SPSS	statistical package for social sciences
VIE	ventral intermediate entorhinal field

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. Van Groen T, Miettinen P, Kadish I. The entorhinal cortex of the mouse: Organization of the projection to the hippocampal formation. Hippocampus 2002;12: In Press.

II. Van Groen T, Kadish I, Wyss JM. Species differences in the projections from the entorhinal cortex to the hippocampus. Brain Res Bull 2002;57:553-556.

III. Kadish I, Pradier L, van Groen T. Transgenic mice expressing the human presenilin gene demonstrate enhanced hippocampal reorganization following entorhinal cortex lesions. Brain Res Bull 2002;57:587-594.

IV. Kadish I, van Groen T. Differences in lesion-induced hippocampal plasticity between mice and rats. Manuscript.

V. Kadish I, van Groen T. Low levels of estrogen significantly diminish axonal sprouting following entorhinal cortex lesions in the mouse. J Neurosci 2002: In Press.

CONTENTS

INTRODUCTION

In recent years a consensus emerged about the role of the mammalian hippocampal formation in learning and memory. However, the idea that the hippocampus is involved in learning and memory functions is not in itself new. Neuropsychological studies performed on the famous amnesic patient H.M. (Scoville and Milner, 1957) have demonstrated clearly that the medial temporal lobe (the hippocampus) is involved in memory. Many studies in animals and humans since that date have confirmed the hypothesis that the hippocampus is involved in memory functions (e.g., Eichenbaum et al., 1992). More recently it has been shown that the entorhinal cortex (another area of the temporal lobe) is also involved in memory (e.g., Zola-Morgan and Squire, 1990).

The entorhinal cortex (together with the perirhinal and parahippocampal cortices) receives a wealth of information from many cortical association areas (Squire and Zola-Morgan, 1991), and most of the projections from the association cortices to the entorhinal cortex are reciprocal. However, the main output of the entorhinal cortex is to the hippocampus, and part of the output of CA1 and subiculum goes back to the entorhinal cortex (Van Groen and Lopes da Silva, 1986; Van Groen et al., 1986; Witter et al., 1989a; Witter, 1993). Taken together, it seems obvious that the entorhinal cortex is at a crucial, nodal point in the circuitry connecting the hippocampus to the neocortex. Therefore it should play an important role in the functioning of the hippocampus, since it controls the flow of information to (and from) the hippocampus.

Alzheimer's disease is clinically characterized by a progressive loss of memory and other cognitive functions, resulting in dementia. The intellectual decline is accompanied by the accumulation of neurofibrillary aggregates in various cortical and subcortical regions of the brain (Braak and Braak, 1991). One of the primary changes seen in Alzheimer's disease (AD) is a loss of brain volume (first seen in the entorhinal cortex). Main hallmark lesions of the disease are the presence of large numbers of neurofibrillary tangles and neuritic plaques in the cortex. The temporal lobe, and especially the entorhinal cortex has been shown to be the first cortical area that displays the neuronal pathology of AD (Braak and Braak, 1990, 1993). The pathology (i.e., the fibrillary aggregates) appears intracellularly as neurofibrillary tangles, and extracellulary as amyloid plaques. Most early onset AD is caused by mutations of the amyloid precursor protein (*app)* gene and the presenilin1 (*ps1*) and presenilin2 (*ps2*) genes. Each of these affect APP metabolism and, therefore, amyloidβ protein production (e.g., Price and Sisodia, 1998). Further, relatively early in AD most neurons of the entorhinal cell layers

II/III that give rise to the perforant path are lost (Hyman et al., 1990) leading to the loss of synapses in the hippocampal formation (Cabalka et al., 1992) similar to an entorhinal cortex lesion (ECL). Thus, it has been suggested that ECL in rodents might induce morphological changes in the hippocampus similar to those observed in AD patients. Therefore the analysis of the ECL model could lead to a better understanding of the processes underlying the pathomorphological changes in AD (Cotman et al., 1990).

It has only recently been widely accepted that in mammals the CNS retains a lifelong capacity for rearrangement of neuronal circuitry. The physiological ability to modify synaptic connections is maintained by the adult brain (e.g., Lynch and Cotman, 1975; Cotman and Nadler, 1978; Cotman et al., 1981). Numerous studies of lesion-induced forms of neuronal plasticity have increased the hope that naturally occuring mechanisms exist which may lead to a functional recovery of the human brain following trauma (for review see: Steward, 1994) and an amelioration of functional deficits in the course of neurodegenerative disease (for review see: Moore and Zigmond, 1994).

Studies performed in animals in the 1970's suggested that estradiol is a trophic hormone in the brain during development. More recent evidence suggests that it also exerts these actions during adulthood (Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992). Trophic effects have been reported in regions of the brain that express estrogen receptor mRNA, protein, and binding, as well as in regions that appear to be receptor poor. The findings that estradiol maintains the integrity and plasticity of neurons suggests that estradiol-responsive neurons may have more resistance to injury and/or facilitated regeneration from injury. Increasing evidence demonstrates that estradiol influences cognition and the incidence and progression of Alzheimer’s disease (Fillit, 1994; Sherwin, 1997; Henderson et al., 2000).

2. REVIEW OF THE LITERATURE

2.1. Entorhinal cortex

The entorhinal cortex (EC) forms part of a larger set of structures that surround the brain stem (Figure 1). This belt of cortex has been called "le grand lobe limbique" (Broca, 1878) or "limbic system" (MacLean, 1952). For a long time it was thought that the entorhinal cortex was primarily related to olfactory functions, i.e., it was considered part of the "rhinencephalon", however more recently it has been shown to also be involved in functions such as emotional and cognitive processes (Squire, 1992). It has to be noted that the entorhinal cortex does receive major inputs from the olfactory bulb and cortex. Lately it has become clear from many studies in many species (e.g., O'Keefe and Nadel 1978; Squire, 1992; Jarrard, 1993) that the entorhinal cortex, together with the hippocampus, contributes importantly to learning and memory. Especially in the rat and monkey, the role of the entorhinal cortex in learning and memory functions has been extensively studied (e.g., Zola-Morgan and Squire, 1990; Hagan et al, 1992; Jaffard and Meunier, 1993; Holscher and Schmidt, 1994; Pouzet et al., 1999).

The entorhinal cortex functions as the gateway to the hippocampal formation, because its output, through the perforant pathway, it is the major cortical source of input to the hippocampus (Figure 2). Furthermore, together with the subiculum it also provides the major output of the hippocampus (e.g., Witter et al., 1989a). Ramón y Cajal was the first to clearly describe fibers arising from the entorhinal area going to the fascia dentata and hippocampus proper (i.e., cornu Ammonis; Ramón y Cajal, 1901). This description has been confirmed and extended by the studies of Lorente de Nó (1934). Using the newly developed Golgi stain, he gave a very detailed description of the types of neurons that are present in the entorhinal cortex (Figure 1B). The cells that give input to the hippocampus are predominantly present in layers II and III, most neurons in layer II are modified pyramidal cells, or stellate cells, whereas layer III predominantly consists of small pyramidal neurons (Figure 1B). The deeper layers of the entorhinal cortex (i.e., layers V and VI) contain slightly larger pyramidal neurons, but the neurons of layer VI are smaller than those of layer V. The neurons in these layers, in general, do not project to the hippocampal formation, but to the cortex and thalamus, respectively. After this early work that has been mainly based on Golgi and fiber

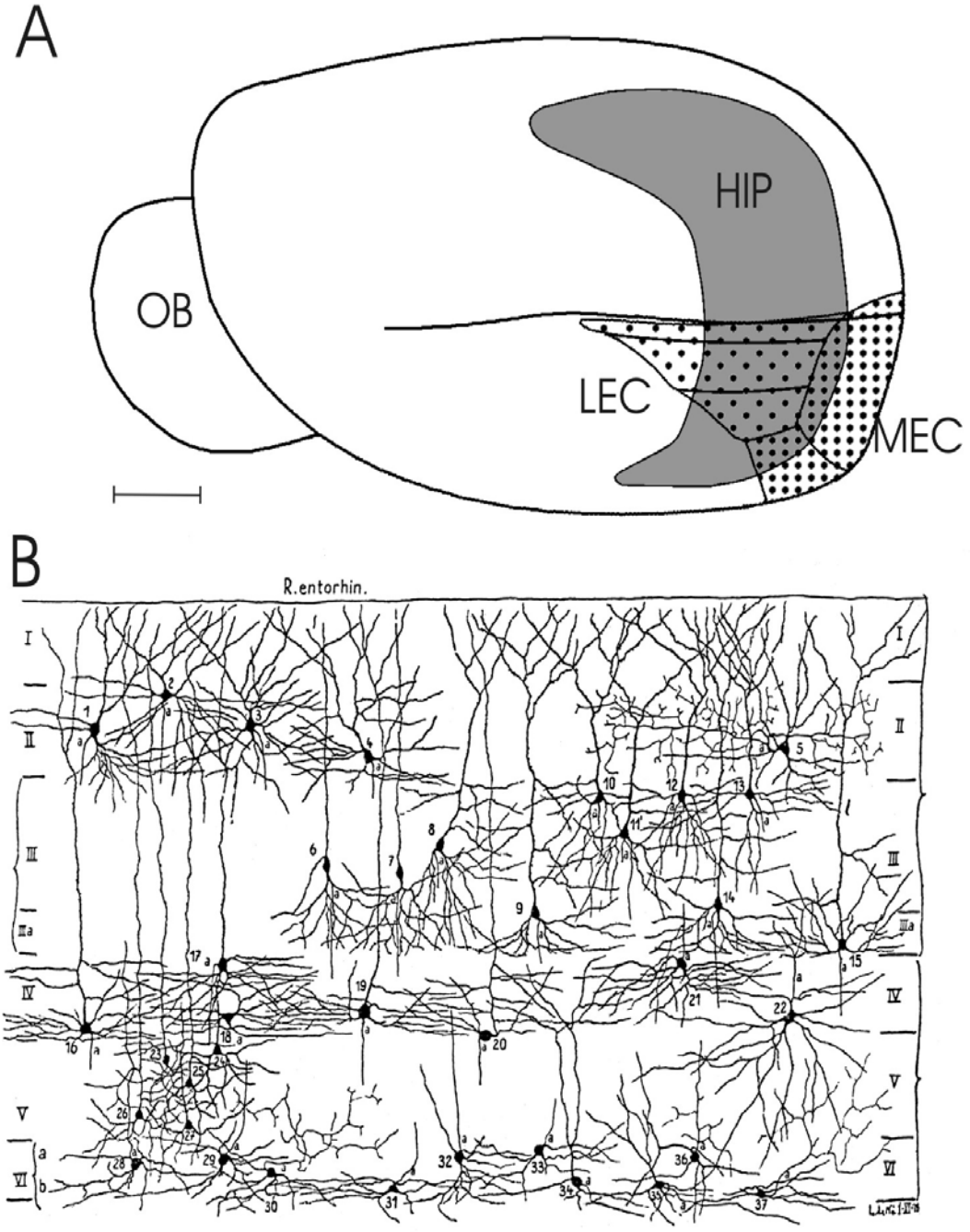

Figure1

A, schematic drawing of the mouse cortex, indicating the position of the entorhinal cortex and the hippocampus. HIP-hippocampus, LEC-lateral entorhinal cortex, MEC-medial entorhinal cortex, OB-olfactory bulb. Space bar equals 1000 μm.

B, schematic drawing of a section through the Golgi-stained entorhinal cortex of the mouse (modified after Lorente de Nó, 1934).

stains, it has not been until the development of the "new" silver degeneration technique(s) that new data have become available about these connections (e.g., Allen, 1948; Blackstad, 1958; Cragg, 1961; Raisman et al., 1965; Laatsch and Cowan, 1966; Nafstad, 1967; Hjorth-Simonsen and Jeune, 1972). Meanwhile, electrophysiological studies had demonstrated that the axons of the perforant path constituted a powerful excitatory pathway to the hippocampus proper (e.g., Green and Adey, 1956; Andersen et al., 1966). More recently, modern tracing studies performed in many species have confirmed that the entorhinal cortex projects by way of the perforant path to the dentate gyrus and hippocampus (e.g., Van Hoesen and Pandya, 1975; Steward and Scoville, 1976; Wyss, 1981; Witter and Groenewegen, 1984; Witter and Amaral, 1991; Dolorfo and Amaral, 1998b; Witter et al., 1989b). It has been demonstrated by a large number of studies in many species that the superficial layers of the entorhinal cortex project to the hippocampus (e.g., Ramón y Cajal, 1901; Van Hoesen and Pandya, 1975; Steward and Scoville, 1976; Ruth et al., 1982; 1988; Pohle and Ott, 1984; Witter and Groenewegen, 1984; Witter et al., 1989; Dolorfo and Amaral, 1998b). In the rat, the layer II cells of the EC have been shown to project primarily to the dentate gyrus (e.g., Steward and Scoville, 1976; Ruth et al., 1982; 1988; Tamamaki and Noyyo, 1993; Tamamaki, 1997; Dolorfo and Amaral, 1998b). The entorhinal cortex layer III cells have been shown to project predominantly to CA1 and subiculum, and this projection is bilateral (e.g., Steward and Scoville, 1976). Some of the early studies (Hjorth-Simonsen and Jeune, 1972; Steward and Scoville, 1976) showed evidence that a topographical organization likely existed in the entorhinal-hippocampal projection. Later studies have shown a topographical organization such that the projection originating in lateral parts of the entorhinal cortex predominantly terminated in septal (dorsal) levels of the dentate gyrus, whereas the axons arising from medial parts of the entorhinal cortex preferentially terminate in the temporal (ventral) part of the dentate gyrus (Ruth et al., 1982; 1988, Pohle and Ott, 1984; Witter and Groenewegen, 1984; Witter et al., 1989; Dolorfo and Amaral, 1998b). It is an important issue that the septotemporal distribution of entorhinal projections to the hippocampal formation is dependent on the lateral-to-medial location of the projecting cells in the entorhinal cortex and not on the origin in either medial or lateral entorhinal area. However, it should be noted that axons from the lateral entorhinal cortex terminate in the outer one-third of the dentate gyrus molecular layer, whereas the medial entorhinal cortex terminals are found in the middle

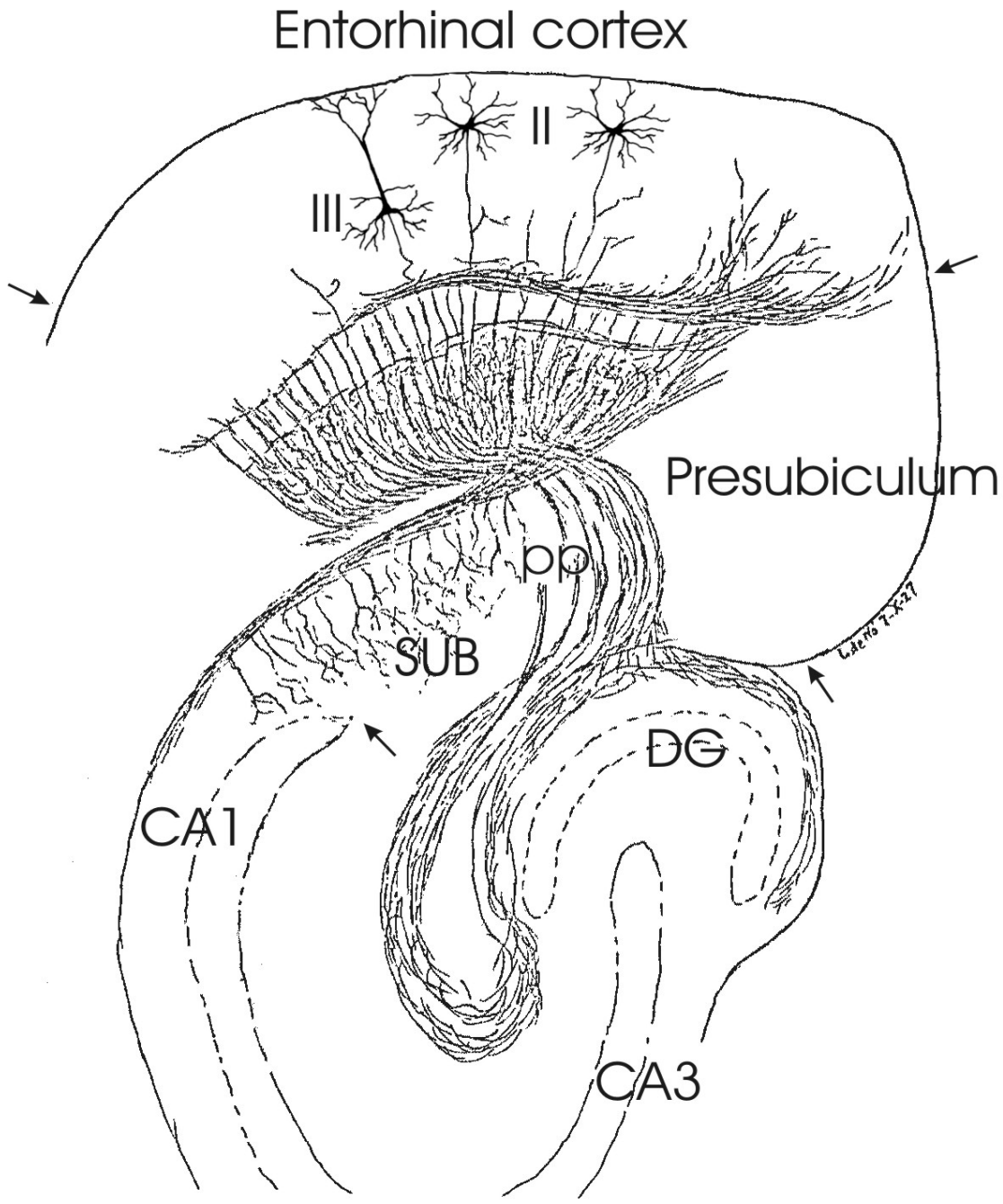

Figure 2

Schematic drawing of a horizontal section through the entorhinal cortex and adjacent hippocampus, indicating some of the interconnecting pathways. The perforant path (pp) arises from neurons in layers II and III of the entorhinal cortex, and passes through the subiculum (SUB). The axons from layer II neurons predominantly terminate in the outer two-thirds of the molecular layer of the dentate gyrus (DG), whereas the axons from layer III neurons project to stratum lacunosum-moleculare of CA3 and CA1, and the outer molecular layer of the subiculum. Arrows indicate areal borders, modified after Lorente de Nó, 1934.

molecular layer (e.g., Steward and Scoville, 1976; Wyss, 1981). Further, the entorhinal cortex innervates the whole hippocampal formation i.e., the dentate gyrus, CA3, CA2, CA1 and subiculum (Figure 2; Ruth et al., 1982; Ruth et al., 1988; Witter et al., 1989; Dolorfo and Amaral,1998a; Van Groen et al., 2002). Species differences in these connections (Hjorth-Simonsen, 1972 [rat]; Wyss, 1981 [rat]; Witter and Groenewegen, 1984 [cat]; Dolorfo and Amaral, 1988 [rat]) have been demonstrated.

2.2. Anatomy and connections of the hippocampal formation

The term hippocampal formation is commonly used to refer to the hippocampus proper and dentate gyrus, although it has to be acknowledged that the term is sometimes also used to include the subicular complex and sometimes even the entorhinal cortex (Amaral and Witter, 1995). We will use the term hippocampus referring to the dentate gyrus together with fields CA3, CA2 and CA1, whereas hippocampal formation includes hippocampus and subiculum (Figure 3). Ramón y Cajal (1893) divided the hippocampus into two components: dentate gyrus and Ammons horn, and Ammons horn was subdivided in an "upper" (also labled regio superior) and a "lower" region (regio inferior). This terminology has largely been displaced by the terminology that was introduced by Lorente de Nó, i.e., dentate gyrus and three subfields (CA1 [regio superior], and CA2 and CA3 [regio inferior]). But, especially in human and monkey literature the terms regio superior and regio inferior, and CA4 for hilus, are still used.

2.2.1. *Dentate gyrus*

The dentate gyrus consists of three layers: the molecular layer, the granule cell layer, and the polymorph zone (Figure 3). The principal cells of the dentate gyrus are the granule cells, whose dendritic tree is confined to stratum moleculare. The hilus, or polymorph zone of the dentate gyrus, is located below the granule cell layer and is bordered by CA3. The principal, and most abundant, cell types of the hilus are the mossy and basket cells. But many other interneurons are present in the dentate gyrus and hilus (and hippocampus proper, for review see Freund and Buzsáki, 1996), and these have projections that, in general, are related to the type of interneuron, e.g., basket cells primarily innervate the cell body layer. The granule cells of the dentate gyrus are considered to be the first stage of the *trisynaptic loop*.

The major extrinsic source of innervation to the dentate gyrus originates in the neurons of layer II of EC (e.g., Steward and Scoville, 1976; Figure 2). The EC projection accounts for about 90% of the synapses in the outer two-thirds of the molecular layer

(Steward, 1976; Steward and Vinsant, 1983). It should be noted, however, that these contacts are made on both the granule cell dendrites and the parvalbumin-positive interneuron dendrites (Nitsch and Frotscher, 1993). The perforant path is topographically organized such that lateral EC projects to the outer molecular layer, whereas medial EC projects to the middle molecular layer (Hjorth-Simonsen and Jeune, 1972; Wyss, 1981; Figure 3) and this projection has weak bilateral component in the rat (10 % of ipsilateral; e.g., Goldowitz et al, 1975; Steward and Scoville, 1976). The granule cells of the dentate gyrus also receive inputs from the contralateral (commissural) and ipsilateral (associational) hilar neurons. The commissural and associational (C/A) projections terminate in the inner one third of the molecular layer and do not overlap with the entorhinal afferents in the outer 2/3 of the molecular layer (Gottlieb and Cowan, 1973; Deller et al., 1995). This layer, the inner one third, also receives a relatively dense input from the supramammillary nucleus of the hypothalamus (Wyss et al., 1979). Another source of extrinsic innervation to the dentate gyrus originates in the septum and the nucleus of diagonal band of Broca. These areas send axons (containing acetylcholine or GABA as neurotransmitter) to the DG with relatively dense terminals in the outer two thirds of the molecular layer and the hilus, with less dense terminals in the areas just above and below the granule cell layer (Kohler et al., 1984; Freund and Antal, 1988; Dutar et al., 1995). Except for a small crossed projection, the septal projection is primarily ipsilateral (Segal and Landis, 1974; Peterson, 1989). Other sources of innervation originate in brain stem nuclei (the raphe nucleus and locus coeruleus) and (local) interneurons (Segal and Landis, 1974; Azmitia and Segal, 1978).

2.2.2. *Hippocampus proper*

The axons from the granule cells (the mossy fibers) leave the hilar region and synapse on the proximal dendrites of the CA3 pyramidal cells, and this represents the second stage of the *trisynaptic loop*. It should be noted that the mossy fibers also terminate densely on the mossy and basket cells of the hilus. The basal dendrites of the CA3, CA2, and CA1 pyramidal cells ramify in stratum oriens above the pyramidal cell layer, whereas the apical dendrites are present below the pyramidal cell layer. The area where the shafts of the apical dendrites are located (with little branching) is called stratum radiatum, and the apical

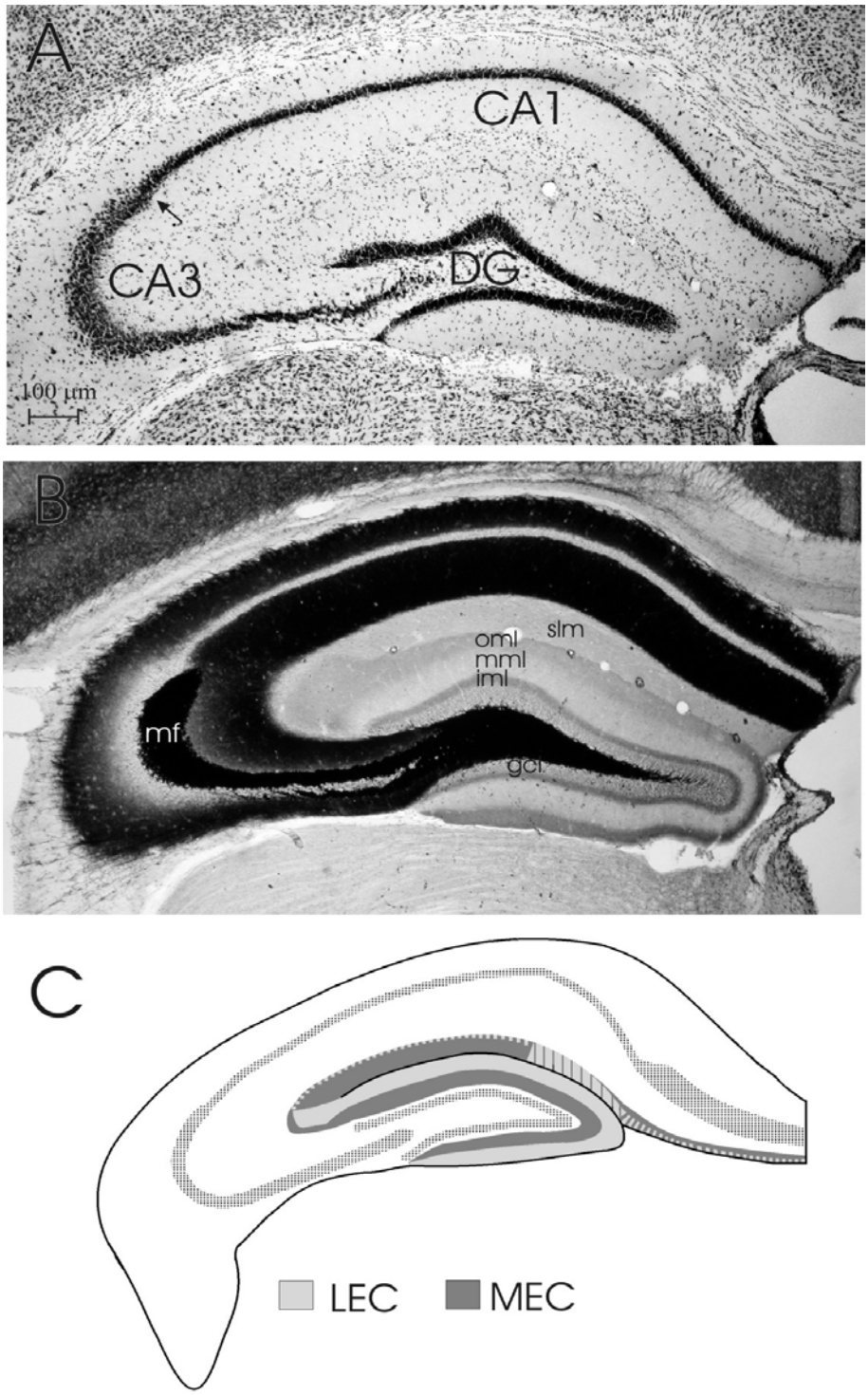

Figure 3.

A, photomicrograph of a Nissl stained coronal section through the dorsal hippocampus, B, the adjacent Timm's stained section. C, schematic drawing of the dorsal hippocampus indicating the terminal fields of the lateral (LEC) and medial entorhinal cortex (MEC). gcl-granule cell layer, iml-inner molecular layer, mf-mossy fibers, mml-middle molecular layer, oml-outer molecular layer, slm-stratum lacunosum moleculare.

dendrites branch extensively in stratum lacunosum-moleculare. In CA3 there is an additional layer called stratum lucidum located between the pyramidal cell layer and the stratum radiatum, within which the mossy fibers course and terminate. The axons of CA3 pyramidal cells project densely to CA1, as originally described by Schaffer (1892), but they also give rise to relatively dense axonal arborizations within CA3. The projection to CA1 represents the third stage of the *trisynaptic loop*. The CA3 projection to CA1 is bilateral and mainly innervates stratum radiatum, and the basal dendrites in stratum oriens. The ipsilateral inputs are referred to as the Schaffer collaterals and the crossed input is referred to as the commissural projection. The main output from CA1 is to the subiculum, but some axons also terminate in the entorhinal and prefrontal cortices (Amaral and Witter, 1989, Van Groen and Wyss, 1990). Local collaterals (and consequently the excitatory interactions among CA1 pyramidal cells) are extremely sparse (Lorente de No, 1934; Amaral and Witter, 1989; Van Groen and Wyss, 1990; Amaral et al., 1991). Many interneurons are present in CA3, CA2, and CA1 (Freund and Buzsáki, 1996), and these have projections that, in general, are related to the type of interneuron, e.g., basket cells primarily innervate the cell body layer. Classes of inhibitory cells exist in the CA fields that control the efficacy of different excitatory inputs, since their dendrites are limited to one sublayer, e.g., either stratum oriens or stratum lacunosum-moleculare, or with some interneurons, both.

The major extrinsic inputs to hippocampus proper arise from the EC, the septum, the nucleus of the diagonal band of Broca (NDBB), the nucleus reuniens and some brain stem nuclei (Wyss et al., 1979). The entorhinal input to the hippocampal formation is likely glutamatergic (Nadler et al., 1976; White et al., 1977; Colbert and Levy, 1992). In the rat, stratum lacunosum-moleculare of CA1 receives input from an entorhinal projection originating in the pyramidal cells of layer III (Steward and Scoville, 1976), whereas stratum lacunosum-moleculare of CA3 is innervated by layer II stellate cells in the entorhinal cortex (Steward and Scoville, 1976; Tamamaki and Nojyo, 1993). Cholinergic and GABAergic afferents emerging from the medial septum and the NDBB predominantly terminate in stratum oriens and stratum radiatum of CA1 and CA3 (Kohler et al., 1984; Nyakas et al., 1987; Freund and Antal, 1988). However, the GABAergic axons predominantly innervate interneurons in the hippocampus, thus disinhibiting the neurons. CA1 receives a dense, excitatory projection in stratum lacunosum-moleculare from the nucleus reuniens (Wouterlood et al., 1990; Dolleman-van der Weel and Witter, 1996). The hilus (below the granule cell layer), and both CA1 and CA3 (stratum lacunosum-moleculare) receive a dense serotonergic input from the dorsal and median raphe nuclei (Moore and Halaris, 1975). A

noradrenergic projection from the locus coeruleus densely innervates the hilus and stratum lucidum of CA3, where mossy fibers terminate, and less densely in stratum lacunosum-moleculare of CA3 and CA1 (Jones and Moore, 1977).

The main extrinsic projections of CA1 are to the subiculum and EC, but other limbic cortical areas, the lateral septum, the nucleus accumbens, and the olfactory bulb are also among the targets of the CA1 subfield (Van Groen and Wyss, 1990).

2.2.3. *Subiculum*

The axons from the CA1 pyramidal cells synapse on the dendrites of the subicular pyramidal cells, and this can be seen to represent the "fourth" stage of the *trisynaptic loop*. Unlike the pyramidal cells of CA3 and CA1, the pyramidal cells of the subiculum are not arranged in one layer, they are more dispersed and show differences between the deeper and more superficial neurons. The apical dendrites of the pyramidal cells of the subiculum ramify in the molecular layer. The neurons of the deepest layer predominantly project to the anterior nuclei of the thalamus, the more superficially located neurons project to the mammillary bodies and the septum, nucleus accumbens and prefrontal cortex (O'Mara et al., 2001). But a substantial projection to the deeper layers of the caudal medial entorhinal cortex is also present (Van Groen et al., 1987).

2.3. Hippocampal sprouting following entorhinal cortex lesion

The hippocampal formation has been shown to be a very suitable model system for the study of neuronal plasticity following lesions, because of its relatively simple organization, and its precise laminar pattern of terminal fields. The well defined, and confined, areas of axonal sprouting and synaptic remodeling make this cortical area a good model. Further, studies on lesions of the hippocampal formation have clinical relevance, for example, to Alzheimer's disease, epilepsy, and ischemic brain damage.

During development, the central nervous system (CNS) is highly plastic, and is characterized by massive circuitry rearrangement as neurons form appropriate connections and survive, or make inappropriate connections and are eliminated (Oppenheim, 1991; McConnel, 1995). For many years, neuroscientists believed that neurogenesis - the generation of new neurons - was restricted to early brain development. New findings have challenged this view and currently many neuroscientists believe that the capacity for circuitry rearrangement is maintained throughout life. The ability to reorganize synaptic connections in the adult brain is most readily observed in response to CNS injury.

The physiological ability to reorganize synaptic connections is retained by the adult brain even under pathological conditions (Lynch and Cotman, 1975; Cotman and Nadler, 1978; Cotman et al., 1981). After CNS trauma, surviving neurons form new synapses that replace those lost by the lesion. This reactive synaptogenesis was initially described by Liu and Chambers (1958) in the spinal cord and by Raisman (1969) in the brain. Since then, lesion-induced forms of neuronal plasticity have been demonstrated in numerous species and brain regions (Goodman et al., 1973; Steward and Messenheimer, 1978; Flohr and Precht, 1981). Together, these studies have increased the hope that mechanisms may exist which lead to a functional recovery of the brain following trauma, and, possibly, an amelioration of functional deficits in the course of neurodegenerative disease (Moore and Zigmond, 1994).

One of the most studied systems in brain plasticity following injury has been the regrowth of entorhinal axons in to the dentate gyrus of the rat following unilateral entorhinal cortex lesions (reviewed by e.g., Steward, 1989; Deller and Frotscher, 1997; Cotman, 1998). This pathway is also of particular interest because of its critical role in higher cognitive functions such as learning and memory, and because of its vulnerability to degeneration in AD as well as during the course of aging (e.g., Hyman et al., 1990). In this model, unilateral ablation of the entorhinal cortex results in nearly complete denervation of the outer 2/3 of the molecular layer of the dentate gyrus, and it is followed by sprouting and reinnervation of the molecular layer by undamaged fibers in the denervated area. Following unilateral ablation of the entorhinal cortex, the outer two-thirds of the ipsilateral dentate gyrus molecular layer loses 80-90% of its input, while no denervation occurs in the inner one-third of the molecular layer that contains the associational and commissural axons that are not lesioned. The early, "degeneration" phase is characterized by degenerating axons and terminals in the molecular layer of the dentate gyrus, that are removed rapidly by microglia and astrocytes (McWilliams and Lynch; 1979; Jensen et al., 1994). In the rat, the degeneration is relatively rapid, and complete, between 4 and 9 days postlesion, but signs of degeneration are present one month after the lesion, and even 240 days after the lesion degenerating terminals can be found (Matthews et al., 1976a). Degenerating terminals are being removed from the first day after the lesion by activated microglia (Gall et al., 1979); microglia has been shown to become activated within 24 h postlesion and it is maximally activated by 3 days postlesion (Jensen et al., 1994). A similar time course for the microglial reactivity in the dentate gyrus following entorhinal cortex lesions has been demonstrated in the mouse (Kadish et al., 1999). Astrocytes react slightly more slowly to the denervation, the first signs of activated astrocytes are found two days postlesion, with the period of maximal activation around 6-7 days

postlesion (Jensen et al., 1994). The later, "regeneration" phase (which, in the rat, starts approximately one week after the lesion) is characterized by reinnervation of the denervated zone, i.e., the sprouting of new axons and axon collaterals, and the formation of new terminals and synapses in the denervated part of the molecular layer of the dentate gyrus (Matthews et al., 1976b; Steward and Vinsant, 1983). Approximately 60-80% of the synapses lost after denervation in the outer two-thirds of the molecular layer are subsequently replaced by the new formation of synapses from remaining fibers in the denervated and adjacent areas (Cotman and Berchtold, 1998). In the rat, axons which sprout into the denervated zone arise from three major surviving afferent fiber systems which normally project to the dentate gyrus: projections from the contralateral entorhinal cortex, and the more sparse cholinergic (and possibly the GABAergic) projections from the septum and NDBB, and the commissural-associational pathway of the hilar neurons of the DG. In addition to the compensatory sprouting which occurs in denervated regions, study of the unilateral ECL model in the rat has revealed that there is also a rearrangement of neural circuitry in nondenervated areas of the CNS, i.e., in areas which are not primarily associated with the lesion (Hoff et al., 1981). Following EC lesions, they observed rearrangements in the inner molecular layer of the dentate gyrus, a region which does not receive input from the EC. Despite the absence of degenerating synaptic terminals in the inner molecular layer following ECL, there is a significant decline in synaptic density in this region and a subsequent recovery of synaptic density to control levels over a long period of time (Hoff et al., 1981).

It has been proposed to differentiate between "axonal regeneration" and "regenerative sprouting" as two distinct axonal responses to axotomy (Moore and Zigmond, 1994; Steward, 1994). "Axonal regeneration" implies the successful and specific reconnection of an axotomized neuron with its target structure, while "regenerative sprouting" describes the response of the proximal axon stump to regenerate, leading to multiple local axon collaterals without the restoration of normal connectivity. Axonal regeneration and regenerative sprouting are processes that occur at the proximal stump of an injured axon. In contrast, sprouting, terminal proliferation and reactive synaptogenesis occur in the area deafferented by the lesion and involve surviving fibres in or near the denervated zone. These processes lead to the reinnervation of the denervated target cells and to a restoration of the number of synapses. Based on electron microscopic observations, the formation of new synapses has been termed "reactive synaptogenesis" (Raisman, 1969; Matthews et al., 1976a,b; Lee et al., 1977; Cotman and Nadler, 1978; Steward and Vinsant, 1983).

2.4. Species comparison

Most of our knowledge of the organization and connections of the hippocampal formation has been derived from studies in the rat, as a cursory glance at the literature will show. Generally it has been assumed that the pattern of connections of the hippocampus exhibited in the rat is indicative of that in most mammals. However, careful studies on the intrinsic and commissural connections have shown that relatively large species differences are present, even in this "old, archicortical" part of the brain, Van Groen and Wyss (1988). The internal organization of the hippocampal fields, such as the hilar region has also been shown to be quite different between species (Van Groen and Wyss, 1988). Similarly the physiology and organization of the limbic system are dissimilar in different species (Lopes da Silva et al., 1990). Whereas the main transmitters in the limbic system are similar, i.e., glutamate and GABA, many of the neuropeptides and calcium-binding proteins show quite clear species differences (e.g., Lopes da Silva et al., 1990). Furthermore, it should be noted that clear differences in learning and memory have been demonstrated between different mouse strains (e.g., Lipp et al., 1987). Taken together, these data indicate that studies on different species are a necessity before similarity in either the anatomy or physiology is assumed. The differences in pathology between Alzheimer's disease mouse models and human AD patients are another example.

2.5. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of senile dementia. It is characterized by a progressive cognitive impairment and a brain specific neuropathology such as the abundant occurrence of amyloid β (Aβ) plaques, neurofibrillary tangles, and neuronal and synaptic loss (Selkoe, 1991). In AD some brain areas, primarily temporal cortex and hippocampus, exhibit substantial loss of nerve terminals (and proteins associated with synapses), and it has been suggested that this decrease may be the primary pathological change or may accompany neuronal loss, neurofibrillary tangle formation, and/or neuritic plaque formation (DeKosky and Scheff, 1990; Masliah et al., 1989; 1991). The pattern of pathological changes in the temporal cortex in AD is relatively consistent between individuals, with, in general, the first neurofibrillary changes affecting primarily layers II and IV of EC and the CA1/subicular fields (Braak and Braak, 1985; Hyman et al., 1988; 1990). Furthermore, neuritic plaques are commonly found in the perforant pathway terminal zone in the outer two thirds of the dentate molecular layer in AD patients (Gibson, 1983; Hyman et al., 1988). Amyloid deposits in these regions are an invariant pathological feature of AD and

result from the aggregation of a 39 to 42 amino acid long protein known as amyloid β protein (Selkoe, 1996).

Whilst many investigators believe that increased Aβ levels and Aβ plaque formation are critical to the pathogenesis, others argue that Aβ and plaque formation are an epiphenomenon (e.g., Neve and Robakis, 1998; Joseph et al., 2001).

Autosomal dominant early onset AD is caused by mutations of the amyloid precursor protein (*app)* gene and the presenilin1 (*ps1*) and presenilin2 (*ps2*) genes. Each of these affect APP metabolism and, therefore, Aβ protein production (e.g., Price and Sisodia, 1998). APP is processed by three secretases, α-, β-, and γ-secretase; the action of α-secretase leads to the production of α-APPs, and when followed by γ-secretase lead to the production of the P3 fragment. β-secretase activity produces β-APPs, however, when this is followed by the γ-secretase, Aβ is produced (Esler and Wolfe, 2001). PS1 has been shown to be either the γ-secretase itself, or to be part of the enzyme complex. The aberrant metabolism of APP may be causally related to the development of dementia by leading to the formation of neuritic plaques, and loss of synapses and neurotransmitters in the medial temporal lobe memory areas and cortical associational regions (Braak and Braak, 1990; Price and Sisodia, 1998). Abnormal APP function has been suggested to lead to neurodegeneration in AD by: 1) direct toxicity of elevated levels of aggregated Aβ (Selkoe, 1993), 2) disruption of synaptic function (Masliah, 1995), 3) deficient neuroprotective activity against excitotoxicity (Mattson et al., 1993a,b; Mucke et al., 1995), and/or any combination of above mentioned. The direct toxic role of the fibrillar Aβ protein alone *in vivo* is controversial. Mutations in the *ps1* gene have been shown to alter APP processing, resulting in an increase of the more amyloidogenic $A\beta_{1-42}$ vs $A\beta_{1-40}$ (e.g., Borchelt et al., 1996), the 42-amino acid peptide form of Aβ, which is the major component of the amyloid plaques that are present in the brains of AD patients (Storey and Cappai, 1999). PS1 not only plays a role in APP processing, but it has also been shown to be involved in cortical development, i.e., PS1 has been shown to play a major role in the Notch signalling pathway (e.g. Haass, 1997; Saftig et al., 1999; Zhang et al., 2000). Other recent studies have shown that PS1 could play a more direct role in brain plasticity, e.g., mice expressing a human *ps1* gene containing an AD mutation display increased hippocampal long-term potentiation compared to mice expressing the normal gene (Pigino et al., 2001). Transfected neuroblastoma cells expressing *ps1* mutations demonstrate impaired neurite outgrowth compared to non-transfected cells (Dowjat et al., 1999). In contrast, a recent study has demonstrated that *ps1* mutations deregulate neurite growth, i.e., in differentiated neurons a *ps1* mutation promotes a marked increase in total neurite length (Pigino et al., 2001).

Further, increases in neuronal plasticity appear to contribute importantly to the development of the pathological hallmarks of AD (i.e., to the development of plaques and tangles [Mesulam, 1999]).

2.6. Estrogen

Studies, performed in animals, suggests that estrogen is a trophic hormone in the brain not only at the time of fetal development, but it exerts its neurotrophic effects in the adult as well, i.e., the adult brain remains highly plastic and hormone-modulated (e.g., Beyer, 1999). For instance, Toran-Alerand et al. (1976; 1984) demonstrated that estrogen elicits selective enhancement of neurite growth and differentiation (both axons and dendrites), using cultured slices of the developing rodent brain. This growth-promoting property of estrogen has been confirmed subsequently in steroid target regions of the adult brain (Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992). Trophic effects have been reported in regions of the brain that express estrogen receptor mRNA, protein, and binding as well as regions that appear to be receptor poor.

Shughrue et al. (1997) has shown the distribution of the α and β isoforms of estrogen receptor mRNA in the adult rat brain, and he has demonstrated that there are significant differences between the distributions of the two isoforms, with the α isofrom predominantly present in brain areas that are related to sexual functioning. The colocalization of estrogen receptors, neurotrophins, and their cognate receptors as shown by Gibbs et al., (1994) suggests potential interactions between estrogen and neurotrophins. Several studies suggest that estradiol may show growth promoting effects by influencing the expression of the neurotrophins, nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and neurotrophin 4/5 (NT4/5). In addition, estradiol may influence their receptors and the pan-neurotrophin receptor, $p75^{NTR}$. During development estradiol receptor mRNA colocalizes with NGF, BDNF and NT3 in subsets of cells in the cortex and hippocampus (Miranda et al., 1993b).

Previous studies have shown that estradiol induces structural and functional changes in the excitatory input to hippocampal CA1 pyramidal cells of the adult female rat. Removal of endogenous estrogen by ovariectomy decreases the density of dendritic spines on the apical dendritic tree of CA1 neurons (Gould et al., 1990; Woolley and McEwen, 1993; McEwen et al., 1999). Treatment with estradiol can either prevent (Gould et al., 1990) or reverse (Woolley and McEwen, 1993) the ovariectomy-induced decrease in spine density. Estrogen rapidly potentiates CA1 pyramidal cells' response to excitatory input evoked either by

synaptic stimulation (Wong and Moss, 1992; Foy et al., 1999) or by glutamate receptor agonists (Gu and Moss, 1996). Treatment with estradiol in vivo increases synaptic excitability of CA1 neurons with a delayed time course that parallels the changes in dendritic spine/synapse density. Estrogen has been shown to regulate neuronal function through at least two distinct mechanisms with different time courses.

The rapid effects of estrogen occur too quickly to involve genomic action of classical estrogen receptors, but delayed effects that could involve genomic mechanisms have been shown. Estradiol may exert trophic and protective effects by acting via genomic mechanisms on a variety of genes including the neurotrophins and their receptors, cell death proteins and structural proteins that control maintenance of synapses and neurite outgrowth. Estradiol may further exert tropic and protective effects by influencing the expression of genes that code for survival factors in the brain. Pike (1999) found that in hippocampal cells, estrogen increased Bcl-x_L in a paradigm of amyloid toxicity. Bcl-2 and Bcl-x_L can act upstream in cellular signalling to prevent the pro-apoptotic actions of bax, inhibit free radical production and may regulate calcium sequestration. The induction of this family of genes may have multiple downstream effects, which together suppress apoptosis and favour cell survival (MacManus and Linnik, 1997).

There is accumulating evidence that estrogen exerts effects by novel mechanisms that are different from those involving transactivation, nuclear receptor dimerization and binding to consensus estrogen response elements. Multiple different second messenger pathways, including cAMP (Zhou et al., 1996; Murphy and Segal, 1997) or MAP kinases (Watters et al., 1997) may be involved. Some of these novel mechanisms of estrogen action may require cross-talk with the estradiol receptors. Other trophic and protective effects of estrogen occur in the absence of any known intracellular estrogen receptor (Green et al., 1998) and are detectable within minutes. This strongly suggests that estrogens may act either on a membrane receptor or may act by influencing membrane receptors that are linked to ion channels (Gu and Moss, 1996; Moss et al., 1997).

Several lines of evidence suggest that a mechanism involving an estrogen-signalling pathway may contribute to a lowered risk for AD. It is recognized that there is a gender difference in the prevalence of AD in women which cannot be solely accounted for by longevity. After menopause, a declining estrogenic stimulus, either from dramatically reduced levels of circulating estrogen (or from aromatizable androgen levels), both of ovarian origin in the female, might make estrogen target neurons in the brain more susceptible to age- or disease-related processes such as AD. It has been reported that the risk of developing AD

and cognitive decline is lower in women with a history of hormone replacement therapy (HRT) use during the postmenopausal period (Paganini-Hill and Henderson, 1994; Tang et al., 1996). Furthermore, women with AD exibit beneficial responses to cholinesterase inhibitor medications only if they are also receiving estrogen replacement therapy (Schneider et al., 1996). However, recently, it has been reported that short term estrogen therapy does not improve cognitive symptoms after AD is established (Henderson et al., 2000), but a neuroprotective role of estrogen or benefits of long term administration of estrogen remain possibilities that are supported by epidemiological studies. Several mechanisms may contribute to the relation of estrogen state to AD. Estrogen has been shown to act as a neuroprotectant and neurotrophic agent, it can also enhance s-APP secretion and it can prevent $A\beta_{1-42}$ aggregation *in vitro*. Steroid hormones such as 17β-estradiol are signal transduction compounds which can regulate APP metabolism in vitro (Jaffe et al., 1994; Chang et al., 1997; Xu et al., 1998). From a therapeutic standpoint the modulation of APP metabolism via signal transduction pharmacology might be beneficial in individuals with, or at risk for, AD (Gandy et al., 1994) and 17β-estradiol via its possible ability to elevate protein kinase C activity could be identified as a potential modulator of Aβ metabolism. Estrogen can exert antioxidant effects (Behl et al., 1997), and modulate expression of a number of growth-related proteins, including apolipoprotein-E, GAP-43, neurotrophic factors and neurotrophic factor receptors (Gibbs et al., 1994; McMillan et al., 1996; Singer et al., 1996; Gibbs, 1998).

Finally, estradiol may exert protective effects by acting on second messenger pathways such as the nitric oxide pathway. Pelligrino et al. (1996) have reported that estradiol appears to enhance neuronal NOS expression and activity, which leads to an improved vasodilation capacity. Further, estrogen may also regulate glial reactivity to disturbances in the milieu, thereby constituting another potential mechanism of estrogen mediated neuroprotection in AD (Blurton-Jones and Tuszynski, 2001). Therefore, these trophic and protective actions of estrogen may provide insight into clinical observations of improved cognitive function and decreased neurodegeneration in hormone-replaced women.

3. AIMS OF THE STUDY

The present study was designed to characterize the projections from the entorhinal cortex to the hippocampal formation in mice, to describe the differences in hippocampal sprouting following entorhinal cortex ablation between mice and rats, and to evaluate the effects of (AD-related) mutations and hormones on lesion-induced synaptic plasticity in the hippocampus of mice.

The specific aims were:

1) to study in detail the connections between the entorhinal cortex and the hippocampal formation in mice by using anterograde and retrograde tracing methods, and to describe both the origin and the laminar distribution of the projections from the entorhinal cortex to the hippocampal formation in the mouse, and compare these findings to the rat data. **(I, II)**

2) to describe the similarities and differences between mice and rats in lesion-induced sprouting in the hippocampus. **(IV)**

3) to examine the effects of the presence of the mutated human *presenilin 1* (*ps1*) gene on lesion-induced sprouting in the hippocampus of the mouse. **(III)**

4) to evaluate the effects of estrogen depletion and supplementation on axonal sprouting in the mouse hippocampus. **(V)**

4. MATERIALS AND METHODS

4.1. Animals (I-V)

Male, adult C57BL/6J mice (n=69) were used in publication I and II, in publication II also 70 adult male rats (Sprague Dawley; Charles River, Wilmington, MA) were used. In publication III, transgenic mice (n=41, C57xCBA) overexpressing wild type or mutated (M146L mutation) human *ps1* were used, these lines of animals were generated as described previously (Leutner et al., 2000). F2 animals of these lines were shipped to Kuopio three months before the experiments. The transgene expression is under the control of the human HMG-CR-Promotor that represents a housekeeping-type promotor which showed a strong and ubiquitous expression pattern with high expression in neurons (Czech et al., 1997). Adult, female mice (n=15, C57BL/6J strain; National Laboratory Animal Center, Kuopio) and adult female rats (n=15, Wistar; National Laboratory Animal Center, Kuopio) were used in publication IV. For publication V 45 female C57BL/6J mice (National Laboratory Animal Center, Kuopio) were used.

Prior to surgery all animals were housed 2 to 3 per cage, at constant humidity (60 ± 5%), temperature (22 ± 1° C) and light cycle (06:00-18:00). Following surgery all animals (including sham controls) were housed individually. All procedures were approved by the Animal Use and Care Committee of the University of Kuopio, the State Provincial Office of Eastern Finland, and followed the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC).

4.2. Anterograde tract-tracing with BDA (I, II)

Mice were anaesthetized with a mixture of a sodium pentobarbital and a chloral hydrate solution (50/50;i.p.; 36 mg/kg); the head was shaved and placed in a stereotaxic frame such that the skull was horizontal. A small hole was drilled in the skull at the appropriate coordinates. Glass micropipettes (10-20 μm tip) filled with a 10% solution of biotinylated dextran amine (BDA; Molecular Probes, 10,000 MW, Eugene (OR)) in phosphate buffer (pH 7.4) were lowered into the entorhinal cortex, and a positive pulsed DC current was applied for 15-20 min to the solution. Coordinates for the injection site were adapted from the atlas of Franklin and Paxinos (1997). Seven to fourteen days later, the mice were reanesthetized, perfused, and the brain was removed from the skull.

4.3. Retrograde tracing with fluorescent dyes (I, II)

For confirmatory, retrograde transport experiments, a small (10-30 nl) amount of a fluorescent dye (fast blue [FB], 4% in DH_2O, Illing, Germany; or fluorogold [FG], 4% in dH_2O, Fluorochrome, Inc. Englewood, CO) was injected by pressure into the hippocampus in the animals. In a small number of animals two retrograde tracers were injected at different positions on the septotemporal axis of the hippocampus so as to be able to estimate the degree of overlap between different populations of entorhinal cortex neurons projecting to the hippocampus. Similarly, in a few animals the two retrograde tracers were injected into one side of the hippocampus each, to be able to estimate the degree of overlap between ipsi- and contralateral projecting entorhinal neurons. After a 7-10 day survival period mice were reanesthetized, perfused, and brain was removed from the skull.

4.4. Histological methods (I-V)

All mice were deeply anaesthetized with a mixture of a sodium pentobarbital and a chloral hydrate solution (50/50;i.p.; 50 mg/kg), and transcardially perfused with 50 ml of buffered saline, followed by 100 ml of a 4% buffered (pH 7.4) paraformaldehyde solution to which picric acid (0.5%) was added. The brains were removed from the skull and stored in the fixative for 4 hours, thereafter they were transferred to a 30% sucrose solution. Rats were deeply anaesthetized with sodium pentobarbital (80 mg/kg) and transcardially perfused with 100 ml of buffered saline, followed by 200 ml of a 4 % buffered (pH 7.4) paraformaldehyde solution to which 0.5 % picric acid was added. The brains were removed from the skull and stored in the fixative for 4 hours, thereafter they were transferred to a 30% sucrose solution. The brains were sectioned in the coronal plane using freezing, sliding microtome (35μm thick, one-in- six series).

We used a modification of the Timm's sulphide silver method to illustrate the regional architecture of the hippocampus and entorhinal cortex. This method is based on the precipitation of endogenous metals (e.g., zinc) with sulphide, and the visualization of these metal sulphides with silver. For Timm's staining mice were anesthetized, and transcardially perfused with 0.1 % Na_2S in 0.1 M phosphate buffer (pH 7.4) for two minutes, followed by 10 min with a 4 % paraformaldehyde solution, and continued with another 10 min of the Na_2S solution. After perfusion the brain was removed from the skull, and following cryoprotection, two series of 1 in 6 transverse sections were cut on a freezing microtome. The sections were collected in TBS and rinsed overnight prior to staining with the sulphide silver method according to Danscher (1981). In short, the sections were mounted on slides, and developed

with silver amplification (i.e., in the dark the sections were developed in a solution containing: gum arabic, citrate buffer, hydroquinone, and silver lactate). Following development, the sections were rinsed, postfixed, and coverslipped.

Detailed descriptions for immunohistochemical staining procedures are presented in publications (I- V). The primary antibodies were monoclonal mouse anti-synaptophysin (dilution 1:1000; Sigma) and mouse anti-GAP43 (1:1000; Sigma). The monoclonal Anti-Synaptophysin (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. A synaptosome preparation from rat retina was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). Monoclonal Anti-Synaptophysin localizes the 38 kD band of synaptophysin in rat brain extracts using an immunoblotting technique. The antibody reacts with human, guinea pig, and pig synaptophysin. The product localizes synaptophysin in neurons, neuromuscular junctions, paraganglia cells, hypophysis, pancreatic islet cells and adrenal cells (Sudhof et al., 1986). Monoclonal Anti GAP-43 (mouse IgG2a isotype) is derived from the GAP-7B10 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with HPLC-purified GAP-43 from neonatal rat forebrain membranes. The isotype is determined using the Sigma ImmunoType TM Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). Monoclonal Anti GAP-43 (Growth-Associated Protein-43) recognizes an epitope present on both kinase C phosphorylated and dephosphorylated forms of GAP-43. The epitope detected by the antibody seems to be a tertiary configuration, existing in all 3 isoforms of GAP-43 (Meiri et al., 1987). The biotinylated secondary antibodies were goat anti-mouse (1:400; Sigma) and goat anti-mouse IgG* HRP (1:500; Pierce). After primary and secondary antibody incubations, the sections were reacted with ExtrAvidin (1:1000; part of the Mouse ExtrAvidin Peroxidase Staining Kit, Sigma). The immunoperoxidase reaction was developed in a Ni-enhanced DAB solution (12.5 mg DAB in 25 ml 0.1 M phosphate buffer pH 7.4, 30 µl H_2O_2 (30%), with 1 ml of a 15% ammonium Ni-sulfate solution added). The stained sections were mounted on gelatinized slides, dried overnight and coverslipped. Control sections in which the primary antisera were omitted or in which normal serum was used revealed no detectable staining. An adjacent series of sections was stained with Nissl and one series of sections was histochemically stained with acetylcholinesterase (AChE).

4.5. Entorhinal cortex lesions (III-V)

Mice were anaesthetized with a mixture of a sodium pentobarbital and a chloral hydrate solution (50/50;i.p.; 36 mg/kg); the head was shaved and placed in a stereotaxic frame such that the skull was horizontal. The skull was surgically exposed and a hole was drilled at the appropriate coordinates, and the needle (90 μm tip diameter) of a Hamilton 0.5 μl microsyringe was stereotaxically lowered to the entorhinal cortex. The coordinates for the entorhinal cortex injection site were: A-4.3, L+4.1, and V-5.5 (Franklin and Paxinos, 1997). Two times 150 nl of ibotenic acid (10 mg/ml, Sigma) was injected, i.e., 2 injections (at V-5.5 and V-4.5, respectively) were made into the entorhinal cortex at a rate of 50 nl/min. Adult rats were anaesthetized with sodium pentobarbital (50 mg/kg); the head was shaved, and placed in a stereotaxic frame such that the skull was horizontal (i.e., bregma and lambda were at the same level). The skull was surgically exposed and a hole was drilled at the appropriate coordinates, and the needle (90 μm tip diameter) of a Hamilton 0.5 μl microsyringe was stereotaxically lowered to the entorhinal cortex. The coordinates for the two injection sites were: A-6.0 , L+6.2 , and V-7.3; and A-7.3 , L+5.0 , and V-7.8 (Paxinos and Watson, 1982). At each injection site, 400 nl ibotenic acid was injected into the entorhinal cortex at a rate of 50 nl/min.

To assess the lesion size, the entorhinal cortex was digitized using a Nikon Coolpix 990 camera, the images were converted to Grey Scale images with the Jasc Paint Shop Pro 7 program, the resulting pictures were analyzed using the ScionImage (NIH *Image*) program. The extent of the entorhinal cortex lesion was defined as the area that displayed a significant loss of layer II and III neurons.

4.6. Ovariectomy (V)

For ovariectomy the mice were anaesthetized with a mixture of a sodium pentobarbital and chloral hydrate solution (50/50; i.p., 36 mg/kg). The animal was placed on its abdomen and the skin on the back was shaved and sterilized. A small, dorsal, midline incision was made in the skin, approximately in the middle (antero-posterior) of the back, then a muscle incision was made approximately halfway down the side of the body. The ovaries were visualized in the abdominal cavity and retracted from the body using a forceps (they were removed by holding on to the periovarian fat). The junction between the Fallopian tube and the uterine horn was cut, the ovary removed and the uterine horn was replaced in the abdominal cavity, and the wound was closed. Following surgery (i.e., at the same day) a

pellet containing 17 β estradiol (Innovative Research of America, Sarasota, FL, USA) was placed under the skin at the back of the neck. These pellets release a steady flow of the hormone for the duration of the experiment (i.e., 60 day release pellets). The moderate (0.18 mg/p) dose pellets resulted in a moderate level of 75-100 pg/ml of estrogen in the blood, the high (0.72 mg/p) dose pellets resulted in a high level of 300-400 pg/ml of estrogen in the blood (Innovative Research of America, Sarasota, FL, USA). To control for the efficacy of the ovariectomy, and estrogen supplementation, following sacrifice the weight of the uterus was measured.

4.7. Measurements

The appropriate sections, and areas of the hippocampus were digitized using a Nikon Coolpix 990 camera, and the images were converted to grey scale using the Paint Shop Pro 7 program. The optical density in the appropriate bands of the molecular layer (outer or middle molecular layer) of the ipsilateral (to the lesion) and contralateral dentate gyrus and in ipsi- and contralateral CA1 (in the same sections) of the hippocampus were measured using the ScionImage (NIH, NIMH, Bethesda, MD, USA) program. These measurements were done on the same section, or on the adjacent, simultaneously stained, section. Density measurements were performed by an investigator who was blind to the treatment of the animals. For analysis the optical density measurements were converted to relative densities, i.e., the density of synaptophysin staining of the molecular layer of the contralateral dentate gyrus was set as 100%. Further, the width of the molecular layer (and inner molecular layer) of the dentate gyrus and of stratum lacunosum-moleculare and stratum radiatum of CA1 was measured. All measurements were done in triplicate, i.e., measuring the same areas at three different places in the dorsal hippocampus, but at the same lateromedial position in the dentate gyrus and in CA1. This was necessary since the width of these layers varies with the lateromedial position of the measurement in the hippocampal formation. Data were analyzed by ANOVA (SPSS version 10.0), and post-hoc tests (Tukey and Scheffe) were carried out to determine the source of a significant main effect(s) or interaction.

For the two dimensional reconstruction **(I, II)**, three 1 in 6 series through the entire extent of the hippocampus were digitized using the Neurolucida program (MicroBrightField, USA). The outlines of the cortical hemisphere, the dentate gyrus, CA3 and CA1, and the subiculum were plotted and the plots were aligned. A two-dimensional unfolded map of the dentate gyrus and hippocampus and entorhinal cortex was constructed following the procedures of Swanson et al. (1978) and Dolorfo and Amaral (1998a). The data were plotted

on the two generic unfolded maps for al cases, since the variability between animals was minimal (it should be noted that C57BL/6J mice are an inbred strain of mice, and are thus geneticaly identical).

5.RESULTS

5.1. Organization of the projection from entorhinal cortex to the hippocampal formation in the mouse (I)

The origin and the terminations of the projections from the entorhinal cortex to the hippocampal formation of the mouse (C57BL/6J strain) have been studied using anterogradely and retrogradely transported tracers. The entorhinal cortex was principally divided into two areas, i.e., the lateral entorhinal area (LEA) and the medial entorhinal area (MEA). LEA was the origin of the lateral perforant path that terminated in the outer one-third of the molecular layer of the dentate gyrus, and MEA was the origin of the medial perforant path that ended in the middle one-third of the molecular layer of the dentate gyrus. This projection was mostly to the ispsilateral dentate gyrus, only a few labeled axons and terminals were found in the contralateral dentate gyrus. The projection to the dentate gyrus originated predominantly from neurons in layer II of the entorhinal cortex. The entorhinal cortex also projected to CA3 and CA1, and to subiculum, in both CA3 and CA1 the terminals were present in stratum lacunosum-moleculare, whereas in the subiculum the terminals were in the outer part of the molecular layer. The projection from the entorhinal cortex to CA3, CA1, and subiculum was bilateral, and it originated predominantly from neurons in layer III, but a small number of neurons in the deeper layers of the entorhinal cortex contributed to this projection. The projection of entorhinal cortex to the hippocampus was topographically organized, neurons in the lateral part of both LEA and MEA projected to the dorsal part (i.e., septal pole) of the hippocampus, whereas the projection to the ventral (i.e., temporal pole) hippocampus originated from neurons in medial parts of the entorhinal cortex.

5.2. Differences in the organization of the projection from entorhinal cortex to the hippocampal formation between the mouse and the rat (II)

Both differences and similarities exist among mammalian species in the projections from entorhinal cortex to hippocampal formation. We have analyzed these projections in mice and compared them to the projections in rats; in general, the entorhinal cortex-to-hippocampus projections are similar in mice compared to those in rats. In the mouse, axons from layer II neurons terminated in the outer and middle thirds of the molecular layer of the dentate gyrus, and axons from layer III neurons terminated bilaterally in the stratum lacunosum-moleculare of areas CA1 and CA3, and in the molecular layer of the subiculum. However, in contrast to rat, mouse entorhinal cortex neurons did not appreciably project to

the contralateral dentate gyrus. Also there was smaller septotemporal extent of axonal fields in the mouse. Rats, compared to mice, showed a similar topographical organization of the entorhinal-hippocampal projections, with neurons in the lateral part of both the lateral and medial entorhinal cortex projecting to the dorsal part or septal pole of the hippocampus, whereas the projection to the ventral hippocampus originated primarily from neurons in medial parts of the entorhinal cortex.

5.3. Differences in lesion-induced hippocampal plasticity between mice and rats (IV)

5.3.1. *Sprouting in the mouse*

In the EC-lesioned mice a large, significant (ipsilateral compared to contralateral; $p<0.001$) increase in synaptophysin staining density was present in the outer part of the molecular layer of the dentate gyrus four weeks following the lesion. It should be noted that the axons of the EC also terminate in stratum lacunosum-moleculare of CA1, however no change in synaptophysin staining density was measurable in this layer in the mouse, and no change in staining density was present in any other area of the hippocampus. Our data also showed that the mouse did not display any significant shrinkage in the width of the molecular layer of the dentate gyrus following partial ECL, neither did the outer molecular layer show a change in width in a mouse, and none of the mice showed a change in the width of stratum radiatum and stratum lacunosum-moleculare of ipsi- or contralateral CA1. The change in density of staining for synaptophysin was also reflected in material stained for AChE. The change in AChE staining density was, however, not as consistent as the increase in synaptophysin staining.

5.3.2. *Sprouting in the rat*

Similarly, in the rats that received unilateral ECL a significant (ipsilateral compared to contralateral; $p<0.001$) increase in synaptophysin staining density was present in the outer molecular layer of the dentate gyrus. However, the rats also demonstrated a significant ($p<0.005$) increase in the staining density of stratum lacunosum-moleculare of CA1. In contrast to mouse, the rat did show a significant decrease (77% of the contralateral size; $p<0.001$) in the size of the molecular layer of the dentate gyrus. The outer molecular layer (where the lesioned lateral EC axons terminate) actually shrunk to 52% of the contralateral size ($p<0.001$), the middle molecular layer of the dentate gyrus also decreased in width. Further, the rat showed a small, but significant (approximately 10%; $p<0.05$), decrease in the size of stratum lacunosum-moleculare. None of the rats showed a change in the width of the inner molecular layer of the dentate gyrus after partial ECL.

In both species the lesions of the lateral EC lead to changes that were limited to the lateral perforant path terminal field in the dentate gyrus, and these density changes were confined to the septal, dorsal part of the hippocampus. However, in the rat the staining density for synaptophysin in the lateral perforant path terminal field in stratum lacunosum-moleculare of CA1 in the dorsal part of hippocampus was also upregulated, in contrast to the mouse where this upregulation in CA1 was not present. No significant changes were present in other, more ventrally located parts of the dentate gyrus or CA1 (areas that are not denervated by the lesion), either in the mouse or the rat.

5.4. Effects of the presence of the mutated human presenilin 1 gene (M146L; *hps1**) on lesion-induced sprouting in the hippocampus of the mouse (III)

In the normal **mPS1** mice (the negative litter mates of the human PS1* expressing animals), four weeks following the entorhinal cortex lesions, there was no change in the density of staining for synaptophysin in the molecular layer of the dentate gyrus ipsilateral to the lesion. In **hPS1*** mice a large, significant increase in synaptophysin staining density (i.e., a sprouting response) was present, and in the **hPS1** mice the response was similar in size. Both groups of mice demonstrated a significant increase ($F_{(2,18)} = 9.79$; $p < 0.001$) in the density of synaptophysin staining compared to the normal, **mPS1** mice. It should be noted that the axons of the entorhinal cortex also terminated in stratum lacunosum-moleculare of CA1, however no change in synaptophysin staining density was measurable in this layer.

The change in density of staining for synaptophysin was also reflected in the material stained for AChE, this material also showed an increase in the staining intensity. The change in AChE staining was, however, not as consistent as the increase in synaptophysin staining. Some animals showed a significant change in synaptophysin but not in AChE staining. Further, it should be noted that none of the animals showed an increase in AChE without an accompanying change in synaptophysin.

5.5. Effects of estrogen on axonal sprouting in the mouse hippocampal formation (V)

The data demonstrate that in control mice, four weeks following the lateral entorhinal area lesions there was a significant increase (from 100 % to 119.5 ± 4.4 %) in the density of staining for synaptophysin (i.e., sprouting) in the outer one-third of the dentate gyrus molecular layer ipsilateral to the lesion. Both the mouse groups that received ES displayed a

similar sprouting response following the entorhinal cortex lesion (M-ES from 100% to 121.3 ± 3.8 %, and H-ES from 100% to 126.0 ± 3.8 %, respectively). However, in mice that were ovariectomized and did not receive estrogen supplementation (i.e., the OE group) the sprouting response was substantially reduced (i.e., no change, 100 % and 100.1 ± 0.5 %, compared to all other groups, $F_{(3,30)} = 6,75$; $p < 0.001$).

Measurements of the synaptophysin staining density demonstrated that the change was confined to the ipsilateral dentate gyrus. No measurable changes in staining were present in ipsilateral CA1 (where the entorhinal axons also terminate) or in contralateral CA1, or in any other areas of the ipsi- or contralateral hippocampus.

Further, it should be noted that the change in staining density for synaptophysin was also reflected in material stained for AChE, i.e., this material also showed an increase in staining density. In control mice after EC lesion there was an increase (from 100% to 126.7 ± 5.9 %; $p< 0.05$) in the density of staining for AChE in the outer one-third of the dentate gyrus molecular layer ipsilateral to the lesion. The two mouse groups that received ES displayed a similar sprouting response following the EC lesion (M-ES from 100% to 127.9 ± 8.5 % [$p<0.05$] and H-ES from 100% to 118.8 ± 6.4% [$p<0.05$], respectively). However, in mice that were ovariectomized and did not receive ES, the sprouting response was substantially reduced (100% and 100.1 ± 1.2 %). Nevertheless, these changes in density were not significantly different between the groups ($F_{(3,26)} = 2,81$; $p <0.06$), since each group had a few animals where AChE staining was either not changed, or changed very little. Again, no visible changes in density of AChE staining were present in any other area of the ipsi- or contralateral hippocampus.

6. DISCUSSION

6.1. Methodological aspects

6.1.1. *Comments on the nomenclature of the mouse entorhinal cortex and hippocampal formation*

These studies employ the mapping of Van Groen (2001) of the mouse entorhinal cortex. The entorhinal cortex (EC) is subdivided into two main parts, i.e., the "classical" lateral entorhinal area (LEA) and the "classical" medial entorhinal area (MEA). LEA is further subdivided into three fields: dorsolateral entorhinal field (DLE), dorsal intermediate entorhinal field (DIE) and ventral intermediate entorhinal field (VIE), and MEA is subdivided into two fields: caudal entorhinal field (CE) and medial entorhinal field (ME; Van Groen, 2001).

The hippocampal formation has been classified according to Ramón y Cajal (1893) and Slomianka and Geneser (1991a,b, 1993). In the present studies the division of the hippocampal formation into a septal (i.e., dorsal) and a temporal (i.e., ventral) pole according to Blackstad (1956) is used.

We have employed a modified Timm's stain as a histochemical marker of the afferent systems of the dentate gyrus, three bands of staining can clearly be distinguished in the molecular layer of the dentate gyrus in Timm's stained material (Geneser et al., 1993; Figure 3). It should be noted that in the molecular layer of the dentate gyrus of the mouse, the two bands of the lateral and medial perforant path are wider than the inner band of labeling (which only occupies approximately 17 % of the total of stratum moleculare; West and Andersen, 1980). The inner band of the molecular layer primarily contains the terminals of the associational and commissural axons of the hilar neurons. However, for ease of reading we have employed the terms outer and middle one third of the molecular layer in our descriptions of the terminal fields of the lateral and medial entorhinal cortex, respectively. Further, the width of stratum lacunosum-moleculare in CA1 is comparatively larger in the C57BL/6J mouse than it is in the rat (West, 1990).

6.1.2. *Immunohistochemical controls*

The specificity of the antibodies used in the present series of the studies were previously described (Meiri et al., 1986; Sudhof et al., 1987; Calhoun et al., 1996).

6.1.3. *Synaptophysin immunoreactivity as a tool to study reactive synaptogenesis in the mouse hippocampal formation*

Sprouting, a form of neuronal plasticity, can be identified by staining for GAP-43 or synaptophysin - a 38kDa calcium-binding glycoprotein (Südhof, 1987) - found in the membranes of presynaptic vesicles. GAP-43 (growth-associated protein-43; another protein that is present in the presynaptic terminal) has been used as a marker for sprouting (e.g., Namgung and Routtenberg, 2000), but our results indicate that there are no differences in the upregulation of these two proteins (i.e., an increase in the staining density in the lesioned dentate gyrus molecular layer) following entorhinal cortex lesions. However, staining for GAP-43 is less reliable than staining for synaptophysin in the mouse (McNamara et al., 1996). Taken together with the fact that most other research in sprouting in the mouse has used synaptophysin as a marker of sprouting (e.g., Calhoun et al., 1996), we have used only synaptophysin immunocytochemical staining as an indicator of changes in synaptic density in the rest of the experiments. It has been shown that during development the expression of synaptophysin increases parallel with the formation of synapses, and synaptophysin immunoreactivity matches completely with the distribution of synaptic profiles. Although this technique does not allow for any differentiation between synapse type or neurotransmitter identity, it enables an estimate of presynaptic terminal density over a wide area of the neuropil (Masliah et al., 1990; Eastwood and Harrison, 1995). This method has been extensively used to demonstrate synaptic loss (characteristic of e.g., Alzheimer's disease; Terry et al., 1991) and synaptic loss following experimentally induced lesions such as perforant path transection and entorhinal cortex ablations (Masliah et al., 1991; Kirkby and Higgins, 1998).

6.1.4. *Use of transgenic animals*

Despite intensive efforts, a fully authentic transgenic mouse model of AD has not yet been created. Nevertheless, overexpression of mutant proteins or single AD-associated molecule in various transgenic mouse lines has produced a variety of phenotypes that, as a first approximation, are remarkably similar to the human AD condition, and these mouse models are providing an assay system in which selected details of pathogenesis and therapeutic intervention can be evaluated. We have used several mouse models of AD to study the effects of different mutations, i.e., transgenic mice overexpressing wild type (wt) or mutated (M146L mutation) human *ps1* that were generated as previously described (Czech et al., 1998). The presence of the transgene (wt or mutated transgene) was monitored by

polymerase chain reaction (PCR) using oligomers for the human *ps1* gene. Following the shipping of the animals to Kuopio, they have been kept in Kuopio.

6.2. Interpretation of results

6.2.1. *Organization of the projection from entorhinal cortex to the hippocampal formation in the mouse*

The perforant path is generally subdivided in a lateral and a medial component, arising in the lateral (LEA) and medial (MEA) entorhinal cortex, respectively (e.g., Hjorth-Simonsen, 1972). These two perforant paths show different staining densities in Timm's stained brain sections, with the lateral perforant path staining more densely than the medial perforant path (e.g., Haug, 1974; West and Andersen, 1980; Fredens, 1981; Figure 3). Further, it has been demonstrated that there are other physiological and histochemical differences in the lateral and medial perforant path (e.g., Witter et al., 1989a). It should be noted that the cat and rhesus monkey display a clear laminar division in medial and lateral perforant path endings in the dentate gyrus, whereas the macaque monkey does not show this division (e.g., Van Hoesen and Pandya, 1975; Witter et al., 1989b; Witter and Amaral, 1991). Similar to most other species, the mouse shows a division in lateral and medial perforant path endings in the dentate gyrus, demonstrated both by the Timm's stained material and in our anterograde tracing studies (Stanfield et al., 1979). It should be noted that in the mouse the entorhinal cortex terminals occupy a larger part of the molecular layer of the dentate gyrus than in the rat (West and Anderson, 1980), but there are also differences in the size of the area occupied by the perforant path endings between different mouse strains (Stanfield and Cowan, 1979; Fredens, 1981; unpublished observations).

Comparable to all other species, in the mouse the projection from the entorhinal cortex to the dentate gyrus originates exclusively in layer II neurons. A large number of studies in many species have demonstrated that predominantly neurons residing in layer II of the entorhinal cortex project to the dentate gyrus. In the rat, the layer II cells of the EC have been shown to project to the dentate gyrus (e.g., Segal and Landis, 1974; Steward and Scoville, 1976; Ruth et al., 1982; 1988, Tamamaki and Nojyo, 1993; Deller, 1998; Dolorfo and Amaral, 1998b), and a distinct projection to the contralateral dentate gyrus is present. Further, comparable to findings in the rat by Deller (1998) and Deller et al. (1996) we noticed a small projection from the entorhinal cortex that terminates outside the "normal" perforant path area (i.e., outside of the outer two-thirds of the molecular layer of the dentate gyrus). These EC terminals are present near (above and below) and within the granule cell layer.

Comparable to most other species, in the mouse the projection from the entorhinal cortex to CA3 and CA1 terminates in stratum lacunosum-moleculare (Stanfield et al., 1979). In CA1 the axons from LEA terminate near the subiculum (i.e., in CA1c), whereas the MEA terminals are found near CA3, i.e., in CA1a and in CA1b. In the rat the terminals from the entorhinal cortex in CA3 and CA1 are present in stratum lacunosum-moleculare. In the mouse and the rat these projections are similarly radially organized (Steward, 1976; Wyss, 1981; Witter et al., 1989a). In the mouse the projection from the entorhinal cortex to CA3 and CA1 originates predominantly from neurons in layer III, however a small number of neurons in the deeper layers of the entorhinal cortex contributes to this projection. In contrast to the rat, in the mouse the layer II neurons of the entorhinal cortex do not seem to project to CA3.

In the mouse, the projection from the entorhinal cortex to the subiculum originates predominantly from neurons in layer III, however a small number of neurons in the deeper layers of the entorhinal cortex contributes to this projection. In the rat the innervation of the subiculum similarly derives from neurons in layer III of both LEA and MEA, but predominantly from lateral parts of these areas (Witter et al., 1989a).

The rat, the rabbit, and the cat show a distinct projection from the entorhinal cortex to the contralateral dentate gyrus, which is, however, limited to the septal part of the dentate gyrus (Goldowitz et al., 1975; Hjorth-Simonsen and Zimmer, 1975; Steward and Scoville, 1976; Wyss, 1981; Witter and Groenewegen, 1984), in contrast, in the mouse the projection to the contralateral dentate gyrus is extremely modest and limited to the septal tip of the dentate gyrus only.

In the rat the EC-DG organization has been studied most extensively (Hjorth-Simonsen, 1972; Steward, 1976; Wyss, 1981; Ruth et al., 1982, 1988; Pohle and Ott, 1984; Dolorfo and Amaral, 1988; Amaral and Witter, 1989). Together, these studies have demonstrated that the EC-DG projection in the rat is topographically organized such that, lateral parts of the entorhinal cortex principally terminate in the septal part of the dentate gyrus, whereas medial parts of the entorhinal cortex primarily terminate in the temporal part of the dentate gyrus. The mouse demonstrates a similar organization, i.e., lateral EC-to-septal DG and medial EC-to-temporal DG, of this projection. However, it should be noted that these lateral and medial zones in the entorhinal cortex cut across the cytoarchitectonically defined borders of LEA and MEA. This arrangement of the medial and lateral zones in the entorhinal cortex that project, respectively, to temporal and septal parts of the hippocampus is similar to what has previously been described in the rat by Dolorfo and Amaral (1988) and cat

by Witter and Groenewegen (1984).

Several studies have indicated that functional differences exist between dorsal and ventral parts of the hippocampus in the rat, these are related to differences in inputs between medial and lateral areas of the entorhinal cortex (e.g., Witter et al., 1989a; Moser and Moser, 1998). Lateral parts of entorhinal cortex predominantly receives inputs from cortical multimodal sensory areas, whereas medial parts of the entorhinal cortex receive inputs that are more related to visceral information. The existence of a relatively similar topographical organization of the projection from the entorhinal cortex to the hippocampal formation in the mouse (compared to the rat) suggests that a similar functional difference likely exists in the mouse.

6.2.2. *Differences in the organization of the projection from entorhinal cortex to the hippocampal formation between the mouse and the rat*

Most of the differences between mice and rats in the projection from the entorhinal cortex to the hippocampus were outlined above (6.2.1), In short, in the rat the projection from the entorhinal cortex to the contralateral dentate gyrus is primarily confined to the septal part of the dentate gyrus, this contralateral projection is practically nonexistent in mice. However, mice, similar to rats and cats, have a relatively large projection from the entorhinal cortex to contralateral CA3, CA1 and subiculum.

In rats, the layer II cells of the EC have been shown to project to the dentate gyrus, similarly, in mice the projection from the entorhinal cortex to the dentate gyrus originates predominantly from layer II neurons, and only a very small number of neurons in deeper layers of the entorhinal cortex contribute to this projection.

Comparable to most other species, in mice, the projection from the entorhinal cortex to CA1 and to the subiculum originates predominantly from neurons in layer III, however a very small number of neurons in the deeper layers of the entorhinal cortex also contributes to this projection. In the mouse, CA3 is predominantly innervated by the neurons from layer III of the entorhinal cortex, in contrast, in rats the projection from the entorhinal cortex to CA3 originates predominantly from layer II neurons.

The projection of the entorhinal cortex to the hippocampal formation is organized topographically. Injections into the lateral part of LEA label axons and terminals in the dorsal, septal third of the hippocampal formation. In contrast, an injection into the more medial part of LEA gives rise to labeled axons and terminals in the intermediate (septotemporal axis) part of the hippocampal formation, and injections into the medial part of

LEA label terminal fields in the ventral, temporal part of the hippocampal formation. Thus, injections that are situated increasingly more medial in LEA give rise to terminal fields in the hippocampal formation that are located more and more ventrally (temporal). A similar organization of the hippocampal terminal fields is found following MEA injections. In summary, neurons that are in the lateral part of the entorhinal cortex (from both medial and lateral entorhinal areas) project to the dorsal third (or septal pole) of the hippocampal formation, whereas the projection to the ventral third of the hippocampal formation originates from neurons that are located in medial parts of the entorhinal cortex. Similar to the mouse, the rat EC-DG projection is topographically organized such that lateral parts of the entorhinal cortex terminate in the septal part of the dentate gyrus, whereas medial parts of the entorhinal cortex predominantly terminate in the temporal part of the dentate gyrus.

Our data demonstrate that in comparison to rats, the mouse, in general, has a similar organization of the EC to hippocampal formation projection. The primary species difference is the lack of an appreciable contralateral projection from EC to DG in the mouse. However, mice have a more extensive projection to the contralateral entorhinal cortex, thus communication with the other hemisphere takes place at a different level of information processing. Further, the mouse EC to DG projections have a smaller septotemporal extent than in the rat, indicating stronger functional differences between dorsal and ventral hippocampus in the mouse. This reiterates the need to carefully characterize the connections in a species/strain prior to comparative studies within that species/strain.

6.2.3. *Differences in lesion-induced hippocampal plasticity between mice and rats*

This study demonstrates that the process of neuronal reorganization in adult female mice (C57BL/6J) is very similar to that shown by adult female rats (Wistar). However, the process of neuronal regeneration shows differences in the response to denervation.

The staining pattern for synaptophysin in the dentate gyrus molecular layer shows some differences between the sham (i.e., normal) mice and rats. In our material, the inner one-third of the molecular layer of the dentate gyrus is stained more densely in the mouse than in the rat (e.g., Cabalka, 1992; White et al., 2001), however, others have shown a staining pattern for synaptophysin in the molecular layer of the dentate gyrus of the rat that is more similar to our mouse pattern (e.g., Masliah et al., 1991). Some of these variations in the staining pattern for synaptophysin in the mouse are probably attributable to the use of different primary and secondary antibodies (unpublished observations, Masliah et al., 1991; Cabalka, 1992). The use of different rat and mouse strains probably further contributes to the

observed differences (unpublished observations). However, we have analyzed the data such (we compared the non-lesioned side with the lesioned side) that differences in staining density, that are a relatively common problem in immunohistochemical techniques, and that are present in our material between batches stained at different times, are circumvented (e.g., Calhoun, 1996).

The results show that there are no differences between rats and mice in the upregulation of the presynaptically expressed protein synaptophysin (Südhof, 1987) in the dentate gyrus molecular layer following entorhinal cortex lesions. Both species display an increased staining density, which suggests sprouting and/or an increased number of presynaptic elements four weeks following unilateral entorhinal cortex lesions. These experiments also demonstrate a clear relation between the position of the lesion in the entorhinal cortex and the band of increased staining in the molecular layer of the dentate gyrus. The lesions of the lateral part of the lateral entorhinal cortex (that we use in this study) result in changes in staining density only in the lateral perforant path terminal field in the dorsal, septal hippocampus (Wyss, 1981; Dolorfo and Amaral, 1998; Van Groen et al., 2001). It should be noted that in the mouse there are no significant changes in the density of synaptophysin staining in other parts of the lateral entorhinal cortex terminal field in the hippocampus (i.e., in areas CA3 and CA1). In contrast, the rat does show an increased density of staining for synaptophysin in stratum lacunosum-moleculare, but this layer is more densely innervated by the lateral entorhinal cortex in the rat than in the mouse (Wyss, 1981; Van Groen et al., 2001).

It can be argued that due to shrinkage of the molecular layer of the dentate gyrus there is an increase in the density of staining for synaptophysin (e.g., Wagner et al., 1983). However, in contrast to earlier studies in the *rat* (e.g., Van Groen et al., 2001) the molecular layer of the dentate gyrus of the *mouse* does not show shrinkage following unilateral entorhinal cortex lesions. It should be noted that Stone et al. (1998) actually have demonstrated an *increase* in the size of the outer molecular layer of the dentate gyrus of the ovariectomized female mouse with estrogen replacement therapy, two weeks following perforant path transection. In contrast, our data from the rat agree with earlier findings in the rat demonstrating shrinkage of the molecular layer of the dentate gyrus following entorhinal cortex lesions (Steward, 1991). Similar to our data, Steward and Vinsant (1983) have shown a decrease in the size of the molecular layer to about 75 % of the contralateral molecular layer at 12 days post-lesion. Further, this decrease in width has been shown by Diekmann et al. (1996) to be accompanied by significant shortening in the size of the dendritic tree of the

dentate granule cells, measurable even 60 and 270 days following entorhinal cortex lesions. They demonstrated that the denervated dendrites do not reach the hippocampal fissure any more, but terminate in the middle molecular layer of the dentate gyrus. In the mouse the shortening of the apical dendrites of the granule cells of the dentate gyrus does not seem to occur (unpublished observations). It should be noted that our measurements of the width of the molecular layer (and outer molecular layer) of the dentate gyrus correspond to earlier published data (Steward and Vinsant, 1983; Steward, 1991; Deller and Frotscher, 1997; Stone et al., 1998).

The analysis of the sprouting in the denervated zone of the dentate gyrus following entorhinal cortex lesions is, however, not only related to sprouting of surviving entorhinal cortex neurons. In the rat several fiber systems have been shown to participate in the reinnervation of the dentate granule cell dendrites following entorhinal cortex lesions. In the rat, the normally sparse, crossed entorhinal-dentate pathway proliferates within the denervated zone (e.g., Steward et al., 1974; Steward and Vinsant, 1983). However, since the mouse does not have an appreciable contralateral projection from the entorhinal cortex to the hippocampus (Van Groen et al., 2002), the sprouting in the mouse most likely derives from the axons of the non-lesioned neurons of the ipsilateral entorhinal cortex (Steward and Vinsant, 1978). Further, in the rat it has been demonstrated that following entorhinal cortex lesions there is an upregulation in the density of the cholinergic fiber system innervating the dentate gyrus molecular layer (Lynch et al., 1972; Nadler et al., 1977; Forster et al., 1997), the alterations in the AChE staining density that we have seen in this study are probably related to changes in this fiber system (Slomianka and Geneser, 1993). Thus, the possibility of concomitant sprouting of cholinergic septal axons can therefore not be excluded, since an increase in staining density in AChE in the molecular layer of the dentate gyrus is present in our AChE stained material, similar to the descriptions in the rat. It should be noted that the synaptophysin staining does not allow to distinguish between types of presynaptic terminals (Calhoun et al., 1996). In the rat it has also been shown that there is an expansion of the terminal field of the commissural/association system into the zone formerly occupied by the entorhinal axons and terminals following complete entorhinal cortex lesions in young animals (Lynch et al., 1976; Staübli et al., 1984). We have not been able to replicate this finding, we do not see any significant increase in the width of the commissural/associational system of the dentate gyrus (the inner part of the molecular layer). Thus, this increase in the size of the associational system does not seem to happen following partial entorhinal cortex lesions in either the adult, female mouse or rat. However, it should be noted that a recent study using

apoE transgenic mice has demonstrated an increase in the size of the inner molecular layer following an unilateral entorhinal cortex lesion with ibotenic acid (White et al., 2001).

Together, the data from these experiments suggest that a real change in the number of presynaptic terminals (i.e., the density of innervation) in the molecular layer of the dentate gyrus is present in the mice following entorhinal cortex lesions, whereas in our data from the rat this is less clear (see above; Steward, 1991; Kelly and Steward, 1998). However, many electron microscopic studies in the rat have demonstrated that regeneration is present in the molecular layer of the dentate gyrus following entorhinal cortex lesions (e.g., Matthews et al., 1976b; Steward and Vinsant, 1983). Taken together, this suggests that the rat and the mouse show a similar reinnervation of the dentate gyrus molecular layer following lesions of its main input, the entorhinal cortex, but the response to denervation is different between these two species. In the rat the denervated molecular layer shrinks in size (and the dendritic tree shrinks), whereas this does not occur in the mouse.

6.2.4. *Effects of the presence of the mutated human presenilin 1 gene (M146L; hps1*) on lesion-induced sprouting in the hippocampus of the mouse*

Recent studies have shown that PS1 could play a role in brain plasticity (Selkoe, 2000). Mice expressing a human *ps1* gene containing an AD mutation display increased hippocampal long-term potentiation compared to mice expressing the normal gene (Parent et al., 1999). It has been shown that PS1 is induced in surviving neurons in the hippocampus following transient ischemia (Tanimukai et al., 1998), suggesting that PS1 expression might be related to protection or recovery of damaged neurons in vivo. Further, recently it has been demonstrated that in the rat following entorhinal cortex lesions there is an upregulation in both APP and PS1 mRNAs in the hippocampus (Ramirez et al., 2001). In contrast to our findings, it has been suggested that Alzheimer's disease linked *ps1* mutations impair neurite outgrowth in cell culture studies (Dowjat et al., 1999). But, recently, another study demonstrated that *ps1* mutations deregulate neurite growth, such that in differentiated neurons the *ps1* mutation promotes a marked increase in total neurite length (Pigino et al., 2001). Similarly our data demonstrate that the presence of a familial Alzheimer's disease mutation in the *ps1* gene increases the sprouting response following lesions in transgenic mice, but it should be noted that the "normal" human *ps1* gene has the same effect. The enhanced sprouting can, therefore, be the result of the higher levels of PS1 protein present in these animals (Czech et al., 1997), although most PS1 protein that is present in these animals is human PS1 (Czech et al., 1997; Leutner et al., 2000). In our mice the level of transgenic

human PS1 slightly exceeds the level of endogenous murine PS1 that is present in normal mice (Leutner et al., 2000), therefore the improved sprouting response could be caused by the increased amount of PS1 protein. However, high levels of the PS1 protein have been shown to decrease plasticity in hippocampal neurons (Guo et al., 1999), making this explanation less likely. Further, the level of full-length PS1 is tightly regulated; PS1 is mostly present as the cleaved C- and N-terminal fragments (Czech et al., 2000). On the other hand, the enhanced sprouting is most likely directly related to the functioning of PS1, and not to its effects on Aβ processing since both the "normal" and the mutated human gene have a similar effect. However, an increased or decreased level of (or change in) APP processing with an effect on sprouting cannot be completely ruled out (Seabrook and Resahl, 1999; Czech et al., 2000). In a recent report (De Strooper et al., 1998) it was demonstrated that in transgenic mice that lack the *ps1* gene a decreased secretion of Aβ is present in primary neurons cultured from these mice, despite the normal level of the full length APP or the amyloidogenic COOH-terminal fragment. This argues for the notion that PS1 is an important factor for the proteolytic processing of APP at the COOH-terminus of APP by γ-secretase (De Strooper et al., 1998). A recent paper has confirmed these results by demonstrating that cells that have both *ps1* and *ps2* knocked-out do not secrete any Aβ (Zhang et al., 2000). Moreover, very recently it has been demonstrated that PS1 is either the gamma secretase itself and/or closely associated with the gamma secretase complex (Esler et al., 2000; Herreman et al., 2000; Li et al., 2000a,b). Together, these data indicate that PS1 can directly influence plasticity (Mattson and Guo, 1997; Parent et al., 1999) or can indirectly modulate plasticity through its actions on APP processing (Czech et al., 2000) and thus change brain plasticity (Mattson, 1997; Selkoe, 2000).

These results demonstrate that the presence of either the normal human *ps1* gene or the mutated human *ps1** gene in these transgenic mice, compared to the mouse *ps1* gene, enhances plasticity of the transgenic mouse brain following unilateral entorhinal cortex lesions.

6.2.5. *Effect of estrogen on axonal sprouting in the mouse hippocampal formation*

These studies demonstrate that in mice, following ovariectomy, the axonal sprouting response of the hippocampal formation to lesions of its main cortical input, the entorhinal cortex, is substantially reduced. Estrogen supplementation (ES), both at a moderate dose and at a high dose, restores the sprouting response to the level of the non-ovariectomized, the normal mouse.

Following ovariectomy, with its concomitant reduction in estrogen levels, the uterine weights of the mice are very much reduced, similar to findings in other studies (e.g., Ryan and Schwartz, 1980; Stone et al., 1998). In contrast, the uterine weights are increased following ES compared to normal mice, as expected, since the moderate dose pellets of estradiol (M-ES) result in blood levels of estrogen slightly above the normal, diestrous levels of estrogen (i.e., 75 -100 pg/ml and 20 - 40 pg/ml, respectively; Bronson and Desjardins, 1974; Ryan and Schwartz, 1980). Earlier studies have shown that there is a relation between estrogen levels and uterine weight (Bronson and Desjardins, 1974; Ryan and Schwartz, 1980; Stone et al., 1998). Similarly, following H-ES the uterine weights are significantly increased compared to M-ES, since the implantation of the high dose pellets of estradiol results in blood levels of estrogen somewhat above the normal, proestrous levels of estrogen (i.e., from around 250 pg/ml to 300 - 400 pg/ml, respectively (Bronson and Desjardins, 1974; Ryan and Schwartz, 1980). We have not measured the blood levels of estrogen in our animals in this study, since in a separate study we have measured these levels (Rissanen et al., 1999) and they have been shown to be similar to the above levels. Further, the amount of blood necessary (approximately 0.25 ml) for measurement of the blood estrogen levels could have interfered with the proper perfusion fixation of the animals which is essential for the semi-quantitative immunohistochemistry.

Ovariectomy with the ensuing low estrogen levels can affect the size of the ibotenic acid lesion, and thus the amount of the sprouting response. However, no difference in the size of the ibotenic acid lesion in the entorhinal cortex is present in the OE group compared to any of the other groups.

We have only measured changes in the synaptophysin (and AChE) staining density in the dorsal, septal part of the hippocampus, since the axons arising from (the lesioned) lateral parts of the entorhinal cortex predominantly terminate in this part of the hippocampus (Turner et al., 1998; Van Groen et al., 2001). Following the lateral entorhinal area lesions there is an increase in the density of staining for synaptophysin in the outer one-third of the dentate gyrus molecular layer ipsilateral to the lesion, however since shrinkage of this layer would have affected the synaptophysin staining density measurements (e.g., Wagner et al., 1983), we measured the width of the dentate gyrus molecular layer. None of the groups of mice showed any change in the width of the dentate gyrus molecular layer or in the width of stratum lacunosum-moleculare+stratum radiatum of CA1. In contrast, it should be noted that Stone et al. (1998) showed an increase in the width of the outer molecular layer in estrogen-treated wild-type mice following perforant path transection.

Degenerating terminals are being removed from the first day of the lesion by activated microglia (Gall et al., 1979), microglia becomes activated within 24 h postlesion and is maximally activated by 3 days postlesion (Jensen et al., 1994). It has been demonstrated that microglia express estrogen receptors (Mor et al., 1999) and that estrogen has anti-inflammatory effects on microglia (Mor et al., 1999; Bruce-Keller et al., 2000). Thus the lack of estrogen could have interfered with the functioning of microglial cells and therefore with the clearance of debris, and this would have hindered regeneration. However, in an earlier study we have examined the activity of microglia and astrocytes, and there were no differences between normal and OE animals two and four weeks after entorhinal cortex lesions (Kadish et al., 2001).

The later, "regeneration" phase (which starts approximately one week after the lesion) is characterized by reinnervation of the denervated zone, i.e., the sprouting of new axons and the formation of new terminals and synapses in the molecular layer of the dentate gyrus (Matthews et al., 1976b; Steward et al., 1990). In our previous studies we have shown that there are no differences in the upregulation of two presynaptically expressed proteins (i.e., GAP-43 and synaptophysin) following entorhinal cortex lesions, suggesting that there is an apparent increase in the staining of presynaptic elements four weeks following ibotenic acid lesions of the entorhinal cortex (Kadish et al., 2001). Several electron microscopic studies in the rat have demonstrated that regeneration of synapses is present in the molecular layer of the dentate gyrus following entorhinal cortex lesions (e.g., Matthews et al., 1976b; Steward and Vinsant, 1978). Electrophysiological studies have indicated that the growth of new synapses is accompanied by the re-establishment of the entorhinal evoked field potentials in the dentate gyrus (West et al., 1975; Steward et al., 1983). Further, Steward (1982) and Ramirez (1997) have reviewed the functional significance of the entorhinal cortex lesion-induced plasticity in the hippocampus, they have demonstrated that the time-course of the ingrowth of entorhinal cortex axons corresponds with the improvement in behavior and electrophysiological properties.

Several fiber systems have been shown to participate in the reinnervation of the dentate granule cells following entorhinal cortex lesions in rats. Thus, the analysis of the effects of entorhinal cortex lesions on sprouting in the denervated zone of the dentate gyrus is complex since several fiber systems could sprout into the denervated part of the molecular layer of the dentate gyrus. In the mouse the crossed entorhinal-hippocampal pathway is practically non-existent (Van Groen et al., 2001), and therefore not likely to contribute significantly to the sprouting response. Further, Lynch et al., (1972) and Zimmer (1973) have

shown in young rats that there is an expansion of the terminal field of the commissural/association system into the zone formerly occupied by the entorhinal axons and terminals following complete entorhinal cortex lesions. West (1984) and Deller and Frotscher (1997) have demonstrated a similar expansion of the commissural fiber system following entorhinal cortex lesions in the adult rat. However, no change in the size of the layer occupied by this system is present in our animals. In contrast, White et al. (2001) have shown an expansion of the inner molecular layer in human apoE transgenic mice. Likely these differences are (partly) caused by the partial entorhinal cortex lesions (with degeneration of the lateral perforant path only) in this study compared to complete entorhinal cortex lesions in the other studies.

It has been demonstrated that the AChE containing septohippocampal pathway proliferates within the denervated zone (e.g., Lynch et al., 1972; Nadler et al., 1977a,b). Nonetheless, no change in choline acetyltransferase (ChAT) staining is present following the entorhinal cortex lesions in the mouse (Kadish et al., 1999). Likewise, the change in density of staining for AChE is not related to an actual increase in axon number (Calhoun and Mouton, 2001; preliminary studies). High power analysis of the zone of upregulated AChE staining shows a higher density of staining per axon, and clumps of AChE associated with axons, together this indicates a higher expression of AChE per axon. Besides, it should be noted that not all entorhinal cortex lesions result in significant changes in AChE staining, whereas all lesions result in significant changes in synaptophysin staining. Further, Henderson et al. (1998) have demonstrated in the rat an apparent increase in the density of ChAT containing terminals following unilateral entorhinal cortex lesion. However, they concluded that this apparent increase in staining density could entirely be accounted for by the tissue shrinkage. In contrast, it should be noted that Nyakas et al. (1988) demonstrated an increased innervation from the septum to the hippocampus following unilateral entorhinal cortex lesions in the rat. Together, this may indicate that possibly some of the non-cholinergic (i.e., GABAergic) septal axons possibly sprout following entorhinal cortex lesions. But it should be noted that these axons mainly terminate on interneurons and are relatively sparse.

Ovariectomy per se also influences the cholinergic system, Luine (1985) has shown a significant decrease in ChAT staining in the hippocampus two weeks following ovariectomy in the mouse, but Gibbs et al. (1994) have demonstrated that four weeks following an ovariectomy the ChAT levels in the hippocampus have returned back to normal. Therefore we assume that the ovariectomy did not negatively affect our measurements of the changes in

ChAT staining density (Kadish et al., 1999). We do, however, show a significant increase in the density of staining for AChE following the entorhinal cortex lesion in the mouse, indicating that this enzyme is upregulated in the hippocampus. This would also concur with the suggestion that AChE has a function in regulating plasticity as such (Small, 1989).

The sprouting response in the dentate gyrus following entorhinal cortex lesions is dramatically reduced in ovariectomized animals, suggesting that a lack of estrogen leads to a reduction in plasticity. This hypothesis is corroborated by the results of the ES mouse groups, since the estrogen supplementation in the ovariectomized mice enhanced the regeneration response of the brain to lesions to a level similar to that of normal, non-ovariectomized mice.

Many studies have suggested that estradiol is a trophic hormone in the brain during fetal development (e.g., Beyer, 1999). In recent studies it has been demonstrated that the adult brain remains highly plastic and hormone-regulated (e.g., Garcia-Segura et al., 1994; Desmond and Levy, 1997), and estradiol influences this plasticity by acting as an important trophic and protective factor throughout the life span, even in the human brain (Garcia-Estrada et al., 1993; Garcia-Segura et al., 1994; McEwen and Alves, 1999). Estradiol's neurotrophic effects are many: induction of neurite outgrowth, dendritic spines and synaptogenesis, influence on long term potentiation and excitability, and enhancement of gene expression (e.g., Garcia -Segura et al. 1994; Desmond and Levy, 1997; McEwen and Alves, 1999). Our findings that estradiol maintains the plasticity of neurons suggests that neurons may experience resistance to injury and/or facilitated regeneration from injury. It has been shown that sex differences are present in the reaction of the rat brain to injury (Milner and Loy, 1980; Morse et al., 1986), and that estrogen supplementation modulates axonal sprouting and/or the regeneration of lesioned neuronal processes (Morse et al., 1992).

Estradiol may exert its trophic and protective effects by acting via classic genomic mechanisms (i.e., through the estrogen receptor) on a variety of genes including the neurotrophins and their receptors, cell death proteins and/or structural proteins that allow maintenance of synapses and neurite outgrowth (e.g., McEwen and Alves, 1999). There are two known estrogen receptors, ER-α and ER-β, and the colocalization of estrogen receptors, neurotrophins, and their cognate receptors suggests potential interactions between estrogen and neurotrophins (e.g., Gibbs et al., 1994). Further, estradiol may exert direct protective effects by modifying blood flow (Mendelsohn, 2000). These effects are both short-term, i.e., direct modulation of vascular smooth muscle cell relaxation, and long-term, i.e., through estrogen receptor-mediated changes in gene and protein expression (Mendelsohn, 2000). Another pathway through which estrogen could exert its effects is by means of the non-

genomic membrane receptors and their signaling pathways (such as cAMP and IP_3 [Beyer, 1999; McEwen and Alves, 1999]).

In summary, our data demonstrate that low levels of estrogen lead to a reduction in the sprouting response in the dentate gyrus following entorhinal cortex lesions, i.e., low estrogen levels lead to a reduction in brain plasticity. Numerous studies from humans and animal models have suggested that estrogen may be beneficial in preserving cognitive function (e.g., Desmond and Levy, 1997; Henderson et al., 2000), further, in postmenopausal women estrogen has been shown to help improve specific aspects of cognitive function (Fillit et al., 1986; Brinton et al., 2000; Henderson et al., 2000). The ability of gonadal steroids to alleviate neurological symptoms has been addressed in human studies (Fillit et al., 1986). Further, recently it has been also reported that estradiol may be beneficial in neurodegenerative diseases such as AD (Brinton et al., 2000; Henderson et al., 2000). Together, these data may provide a better comprehension of the clinical observations of improved cognitive function and decreased neurodegeneration in women that receive hormone replacement therapy.

CONCLUSIONS

The goals of this thesis were to study and describe the projections from the entorhinal cortex to the hippocampal formation in mice, and report the differences in these projections between mice and rats. Further, we report the differences in hippocampal sprouting following entorhinal cortex ablation between mice and rats. In addition, our aim was to evaluate the effects of a *ps1* mutation on lesion-induced synaptic plasticity in the hippocampus of mice and to study the effects of different estrogen levels on this lesion-induced synaptic plasticity. From the results of these studies we can draw the following conclusions:

1) the detailed study of the projections from the entorhinal cortex to the hippocampal formation in mice demonstrated while in comparison to the primate, cat, and rat, the mouse has, in general, a similar organization of the entorhinal cortex to hippocampal formation projections, species differences are present in these connections. The three primary differences between mice and rats in the projections from the entorhinal cortex to the hippocampal formation are: 1) the lack of an appreciable contralateral projection from EC to DG, 2) layer III of the EC projecting to CA3 (in the rat it is layer II), and 3) a more limited septotemporal extent of the labeling in the hippocampus in the mouse compared to the rat. This therefore reiterates the need to carefully characterize (anatomically and physiologically) the connections in a species prior to extrapolating from the results of studies in other (similar looking) species.

2) the description of the similarities and differences between mice and rats in lesion-induced sprouting in the hippocampus. showed that differences between rats and mice exist in the reaction of the hippocampus to deafferentation of its main input, the entorhinal cortex. The primary difference between rats and mice in the reaction of the hippocampus to entorhinal cortex lesion is shrinkage of the denervated area in the rat, but not in the mouse. Further, in general, together these studies show, that, despite the relatively common assumption, mice are <u>not</u> small rats.

3) the results from EC-lesion experiments in single transgenic mice demonstrate that the presence of either the normal human *ps1 gene* or the mutated human *ps1** gene in these mice, compared to the mouse *ps1* gene, enhances neuronal plasticity in the brain following unilateral ECL. Together, these data indicate that PS1 can directly influence plasticity or indirectly modulate plasticity through its actions on APP processing, and thus change the response of the brain to injury.

4) Our studies on EC-lesions and the effects of ovariectomy, demonstrate that low levels of estrogen lead to a reduction in the sprouting response in the dentate gyrus following entorhinal cortex lesions, i.e., low estrogen levels lead to a reduction in brain plasticity. These data may provide a better comprehension of the clinical observations of improved cognitive function and decreased neurodegeneration in women that receive hormone replacement therapy.

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APPENDIX: ORIGINAL PUBLICATIONS (I-V)

I

The entorhinal cortex of the mouse: Organization of the projection to the hippocampal formation

Van GroenT, Miettinen P, Kadish I

Hippocampus 2002; 12: In Press

II

Species differences in the projections from the entorhinal cortex to the hippocampus

Van Groen T, Kadish I, Wyss JM

Brain Res Bull 2002;57:553-556

III

Transgenic mice expressing the human presenilin gene demonstrate enhanced hippocampal reorganization following entorhinal cortex lesions

Kadish I, Pradier L, van Groen T

Brain Res Bull 2002;57:587-594

IV

Differences in lesion-induced hippocampal plasticity between mice and rats

Kadish I, van Groen T

Manuscript

V

Low levels of estrogen significantly diminish axonal sprouting following entorhinal cortex lesions in the mouse

Kadish I, van Groen T

J Neurosci 2002: In Press