Interactions between the entorhinal cortex and hippocampal formation
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Chapter 2

Topographical and laminar organization of subicular projections to the parahippocampal region of the rat

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ABSTRACT

In this study, we analyzed in detail the topographical organization of the subiculo-parahippocampal projection in the rat. The anterograde tracers Phaseolus vulgaris leucoagglutinin-L (PHAL) and biotinylated dextran amine (BDA) were injected into the subiculum at different septo-temporal and transverse levels. Deep layers of the ento-, peri- and postrhinal cortices are the main recipients of subicular projections, but in all cases we noted that a small fraction of the projections also terminates in the superficial layers II and III. Analysis of the fiber patterns in the parahippocampal region revealed a topographical organization, depending on the location of the cells of origin along both the transverse and septo-temporal axis of the subiculum. Projections originating from subicular cells close to CA1, i.e. proximal part of subiculum, terminate exclusively in the lateral entorhinal cortex (LEA) and in the perirhinal cortex. In contrast, projections from cells closer to the subiculum-presubiculum border, i.e. distal part of subiculum, terminate in the medial entorhinal cortex (MEA) and in the postrhinal cortex. In addition, cells in septal portions of the subiculum project to a lateral band of entorhinal cortex parallel to the rhinal sulcus and to peri- or postrhinal cortices, whereas cells in more temporal portions project to more medial parts of the EC.

These results indicate that subicular projections to the parahippocampal region precisely reciprocate the known inputs from this region to the hippocampal formation. We thus suggest that the reciprocal connectivity between the subiculum and the parahippocampal region is organized as parallel pathways which serve to segregate information flow and thus maintain the identity of processed information. Although this parallel organization is comparable to that of the CA1-parahippocampal projections, differences exist with respect to their degree of collateralization.
INTRODUCTION

Numerous studies employing behavioral, anatomical and physiological techniques have indicated that the medial temporal lobe or hippocampal system plays an important role in normal memory function (Eichenbaum, 1999, Squire and Zola-Morgan, 1991, Suzuki and Eichenbaum, 2000). This system comprises the hippocampal formation (dentate gyrus, CA sub-fields and subiculum) and the adjacent parahippocampal region (in the rodent, the main constituents are the ento-, peri- and postrhinal cortices) (Scharfman et al., 2000). In order to understand the specific contribution of these areas to memory processes, detailed knowledge of their connectivity is indispensable.

It has become clear that the entorhinal cortex forms an important portal for cortical information entering the hippocampal formation (Amaral, 1993, Leung et al., 1995, Steward and Scoville, 1976, Witter et al., 1992). Efferent fibers originating from the superficial layers of the entorhinal cortex provide the main cortical input to the hippocampal formation. Entorhinal layer II input to the dentate gyrus has been extensively studied, both anatomically and physiologically (Dolorfo and Amaral, 1998a, Leung et al., 1995, Tamamaki, 1997). In addition, the entorhinal cortex also projects, through layer III, directly to the other sub-fields of the hippocampal formation (CA1 and subiculum) (Desmond et al., 1994, Empson and Heinemann, 1995, Leung et al., 1995, Paré and Llinas, 1995, Steward, 1976, Yeckel and Berger, 1995). Alternatively, cortical information may also be relayed to area CA1 and the subiculum through peri- and postrhinal projections (Canning et al., 2000, Kosel et al., 1983, McIntyre et al., 1996, Naber et al., 1999, Naber et al., 2001b).

After processing of the cortical inputs, the hippocampal formation returns its output to various brain areas. Area CA1 and the subiculum represent the main output structures of the hippocampal formation, since their projections terminate in various cortical and sub-cortical regions, like the medial prefrontal cortex, retrosplenial cortex, parahippocampal region, septal complex, the mammillary nuclei, the thalamus and the amygdala (Gaykema et al., 1991, Köhler, 1985, Ptkänen et al., 2000, Swanson and Cowan, 1977, Tamamaki and Nojyo, 1995, van Groen and Wyss, 1990, Witter et al., 1989, Witter et al., 1990, Wyss and Van Groen, 1992). Hippocampal output to the neocortex is mainly mediated by the entorhinal cortex (Burwell and Amaral, 1998a), although additional output routes make use of the perirhinal and postrhinal cortices, which also receive direct output from CA1 and subiculum (Deacon et al., 1983, Swanson and Cowan, 1977).

In our previous studies on the organization of the parahippocampal-hippocampal connections, we have demonstrated a characteristic topographical organization of the projections from the ento-, peri- and postrhinal cortices to the hippocampal formation (Naber et al., 1999, Naber et al., 2000, Witter, 1993). On the other hand, little is known about the topographical organization of the return projections from subiculum to the parahippocampal region. The organization of this projection is especially relevant for our electrophysiological studies on
FIGURE 2.1 Overview of the location of anterograde tracer injections in the subiculum as observed in coronal sections (A; left to right = anterior to posterior) and schematically represented on an unfolded map of the hippocampal formation (B). All cases described in the text are shown. In A, only the center of the injection is drawn, but in some cases injections spread to adjacent septal and temporal levels as well (see B). Note that the injections covered the full extent of the septo-temporal axis of the subiculum and included proximal and distal subiculum. The inset in A shows a drawing at mid-sagittal level, in which vertical lines indicate the location of the coronal sections on the rostro-caudal axis. The unfolded map was adapted from Swanson (1998). The locations of the injections in the unfolded map were determined with the help of coronal isolines that are part of the map as used in Swanson (1998). The shaded area represents subiculum. For abbreviations, see list. Scale bar = 1 mm.
entorhinal-hippocampal re-entrant circuits, which heavily rely on the exact positioning of stimulation and recording electrodes. Therefore, the studies reported in the present paper have been designed to investigate the organization of the projections of subicular neurons to the parahippocampal region in the rat. In particular, we were interested in determining whether projections from different portions of subiculum are overlapping or segregated in peri-, post- and entorhinal cortices and whether these projections display the same topographical features as the input pathways from the parahippocampal region to subiculum.

MATERIALS & METHODS

In total 40 female Wistar rats (weight 200-220 g: Harlan Centraal Proefdierbedrijf, Zeist, The Netherlands) were used in this study. Female rats were chosen for this study, since all our previous studies were carried out in female animals as well. Other studies addressing hippocampal-parahippocampal connectivity generally use male rats, but no apparent gender differences in connectivity have been reported (Burwell et al., 1995, Burwell and Amaral, 1998a, b, Naber et al., 1997, Naber et al., 1999, Naber et al., 2001a). All animals were fed ad libitum and housed in cages with enriched litter. All experimental procedures were according to the guidelines of the ethical committee of animal experimentation Vrije Universiteit.

Surgery and tracer injection

Animals were anesthetized with a mixture of ketamine and xylazine, (4 parts of a 1% solution of Ketaset (ketamine; Aesco, Boxtel, The Netherlands), and 3 parts of a 2% solution of Rompun (xylazine; Bayer, Brussels, Belgium); total dose: 1 ml/kg body weight. Glass micropipettes (CG-150F-15 Clark, Reading, UK) with a tip diameter of 10-15 μm were filled with either a 5% solution of biotinylated dextran amine MW 10.000 (BDA, Molecular Probes Inc., Eugene, OR) in 0.01 M NaH$_2$PO$_4$/Na$_2$HPO$_4$ buffer, pH 7.3 or with a 2.5% Phaseolus vulgaris leucoagglutinin-L solution (PHAL, Vector, Burlingame, CA) in 0.1 M NaH$_2$PO$_4$/Na$_2$HPO$_4$ (phosphate) buffer, pH 7.4. These two tracers can be used interchangeably, since no differences have been reported (Wouterlood and Jorritsma-Byham, 1993).

After rats were mounted in a stereotaxic frame, small holes were made in the skull and the pipettes were lowered into desired areas. Coordinates were selected such that the total of injections covered both the extent of the septotemporal and the transverse axes of the subiculum (see fig. 2.1), using stereotaxic coordinates derived from Paxinos and Watson (Swanson, 1998). BDA was injected by applying a small positive pulsed DC current onto the micropipette (6.5 μA, 7 s on, 7 s off) for 10 min., whereas PHAL was delivered by applying small positive 7.5 μA DC currents (7 s on, 7 s off) for 10 min.
FIGURE 2.2 A: Schematic three-dimensional view of the rat brain showing the location of the hippocampal formation and its septotemporal axis. B,C: Photomicrographs (digital montage) of a Nissl-stained coronal section of the septal (B) and temporal (C) subiculum. Indicated are the borders with surrounding areas, the molecular and pyramidal layer of the subiculum and the proximo-distal axis of the subiculum. For abbreviations, see list. Scale bars = 1 mm.
After one week of survival, the animals were deeply anesthetized with sodium pentobarbital (Nembutal i.p., 60 mg/kg body weight, Ceva, Paris, France) and rapidly transcardially perfused with a small volume of physiological saline (0.9% NaCl), followed by 500 ml of a solution of 4% freshly depolymerized paraformaldehyde (Merck, Darmstadt, Germany), 0.05% glutaraldehyde (Merck), and 0.25% saturated picric acid (Merck) in 0.1 M phosphate buffer.

Histochemistry

After removal from the skull, the brains were stored for 24 hr in the perfusion fixative. Following three rinses in phosphate buffer, brains were infiltrated with 20% glycerin (Merck) and 2% dimethyl sulfoxide (Merck) in phosphate buffer. After sufficient infiltration, the brains were frozen with 30% sucrose in phosphate buffer onto the stage of a freezing microtome and 40 μm thick sections were cut in the coronal plane. Sections were collected in phosphate buffer. Subsequently, sections were rinsed three times in 0.05 M Tris/HCl (Merck) supplemented with 0.15 M NaCl, pH 7.4 (TBS), which was followed by three rinses in TBS containing 0.5% Triton-X-100 (Merck) (TBS-T). Next, sections were stained for the presence of either BDA or PHAL.

Sections from brains injected with BDA were incubated overnight at 4 °C in avidin-biotin-peroxidase complex (Vector, Burlingame, CA) in TBS-T and rinsed in 0.05 M Tris/HCl buffer, pH 7.6. Sections from brains injected with PHAL were incubated for 48 hr at 4 °C in goat-anti-PHAL (Vector), diluted 1:1000 in TBS-T. After several rinses in TBS-T, these sections were incubated for 18 hr at room temperature in donkey-anti-goat IgG (Nordic, Tilburg, The Netherlands), diluted 1:100 in TBS-T, followed by an incubation in rabbit-peroxidase-anti-peroxidase (Nordic), diluted 1:200 in TBS-T for 4 hr at room temperature. Finally, both BDA and PHA-L were visualized by incubating the sections in diaminobenzidine (DAB) substrate: 5 mg 3,3’ diaminobenzidine-tetrahydrochloride (Sigma, St. Louis, MO) and 3.3 μl of 30% H₂O₂ in 10 ml Tris/HCl, pH 7.6. The staining reaction was monitored by viewing the sections at regular time intervals and as soon as nonspecific background staining became visible the reaction was terminated by several rinses in Tris/HCl.

Several series of sections of each experimental case were mounted on microscope slides from a 0.1% solution of gelatin (Oxoid, Basingstoke, UK) in Tris/HCl pH 7.6, air-dried, and at least one series was subsequently counterstained in a 0.2% aqueous solution of cresyl violet (Merck). Next, sections were dehydrated through an ascending series of alcohol and through two rinses of xylene and finally coverslipped with Entellan (Merck).

Analysis of the topography of subiculo-parahippocampal projections

Series of coronal sections comprising the entire rostro-caudal extent of the parahippocampal region were studied with the use of a light microscope equipped with a camera lucida. The contours of sections, the injection sites, and the corresponding labeling in
FIGURE 2.3 A: Schematic drawing of the rat brain showing the position of the perirhinal, postrhinal, and entorhinal cortices in relation to the rhinal sulcus. Arrow indicates approximate viewing angle of image in B. (Adapted from Burwell et al., 1995). B: Ventro-caudo lateral view of the parahippocampal region. Peri- and postrhinal cortices (light grey), lateral entorhinal area (medium grey) and medial entorhinal area (dark grey) are shown, as well as their subdivisions. (Adapted from Insausti et al., 1997). C: Photomicrographs (digital montages) of Nissl-stained coronal sections, illustrating the laminar organization and subdivisions of the parahippocampal region. The most caudal section (bottom right) demonstrates that the postrhinal cortex is not always straightforward to recognize in coronal sections. Borders demarcating PER and POR, as well as the subdivisions of EC are indicated. LEA comprises DLE, DIE, VIE and AE; MEA comprises ME and CE. The layers are indicated by roman numerals. The lamina dissecans is drawn as a dashed line. For abbreviations, see list. Scale bar = 1 mm.
the peri-, post- and entorhinal cortices were drawn. The exact location of both the injection site in the subiculum and the corresponding labeling in the peri-, post- and entorhinal cortices was determined by projecting the contours and cell layers of the sections, as revealed by cresyl violet staining, onto the drawings. In each case labeling shows two different features: either fibers are branched and covered with many varicosities (inset fig. 2.4A,C) or fibers are more or less smooth with no branching. The first type is considered to represent terminal fibers (van Haeften et al., 1995), whereas the smooth fibers are considered to be passing fibers. To facilitate comparisons of projection patterns of different experiments all data were subsequently mapped onto a series of standard coronal sections (figs. 2.5-8).

In order to visualize the specific topographical pattern of termination of the subicular projections, 3-dimensional topographical reconstructions were made of the exact location of the subicular plexus in the parahippocampal region. For this, the contours of all sections containing the peri-, post- and entorhinal cortices were drawn with the use of a microscope equipped with a camera lucida and the specific location of the terminal field in that section was transposed onto the contour of the section. Next, all contours were mapped with a graphics tablet (Scriptel, Columbus, OH) with the use of reconstruction software (PC3D, Jandel Scientific Products, CA), and transformed into a 3-dimensional representation.

Photomicrographs were acquired using the MCID® system (ImagingResearch Inc., St. Catharines, Ontario, Canada). Final preparation of the figures, including contrast enhancement, were done using Corel PhotoPaint (Corel Corporation, Ottawa, Ontario, Canada).

RESULTS

Nomenclature

Subiculum

The subiculum, like the other subfields of the hippocampal formation, appears as an elongated structure with its long axis extending in a C-shaped fashion from the septal nuclei, rostrally and dorsally, to the temporal region caudally and ventrally. The long axis of the subiculum is therefore referred to as the septotemporal axis (fig. 2.2A). Perpendicularly oriented to the septotemporal axis of the subiculum is the transverse axis, on which a proximal (close to CA1) and a distal (close to presubiculum) pole can be distinguished (fig. 2.2B,C).

We adhere to the cytoarchitectonic description of the subiculum as used by Köhler (1985), Swanson et al. (1987), Witter et al. (1990), and Amaral and Witter (1995). In summary, the subiculum consists of two layers. Starting superficially (i.e. close to the hippocampal fissure), these layers include a molecular layer and a deep thick layer of large pyramidal neurons (fig. 2.2B,C).
Subicular-parahippocampal projections

C

D

(figure 2.4)
FIGURE 2.5 The distribution of labeled fibers as observed in coronal sections (top-left to bottom-right = anterior to posterior) after anterograde tracer injection in septal proximal subiculum (filled circle, exp. 85392). The core of the labeled plexus is located in perirhinal cortex and adjacent lateral entorhinal area. Labeled fibers outside the parahippocampal region are omitted for clarity. Arrows indicate area borders. For abbreviations, see list.

FIGURE 2.4 Photomicrographs (digital montages) to illustrate the dense fiber plexus in layer V of the entorhinal, perirhinal and postrhinal cortices as seen at low magnification (left). Areas enclosed by rectangles are presented at higher magnification on the right, showing that terminal fibers are also present in layer II, III and VI. A: Projections from septal proximal subiculum to perirhinal cortex and LEA (case #85392). Higher magnification figure on the right shows labeling of varicose, terminating fibers in both perirhinal and entorhinal cortex (area DLE), which is further illustrated in the enlarged cutout. B: Projections from temporal intermediate subiculum to LEA and MEA (case #88384). Higher magnification figure on the right shows clear terminal labeling in layers V and deep III of areas VIE and DIE of LEA. C: Projections from septal distal subiculum to postrhinal cortex (exp. 89261). Higher magnification figure on the right shows labeled fibers in deep layers, as well as in deep layer III of the postrhinal cortex. Enlarged cutout illustrates the varicose nature of the labeled fibers, indicating that they are terminating fibers. D: Projections from temporal distal subiculum to MEA (exp. 88562). Higher magnification figure on the right shows that terminal labeling is predominantly present in layer V of area ME of MEA. In all panels the layers are indicated by roman numerals. Borders of perirhinal and postrhinal cortices and entorhinal subdivisions are demarcated and indicated by arrows at the cortical surface. The locations of the borders are based on adjacent Nissl-stained sections or dark-field images. Abbreviations: d, dorsal; v, ventral; l, lateral; m, medial. For other abbreviations, see list. Scale bars = 1 mm for low magnification figures, 0.4 mm for higher magnification figures.
We use the description of the layers of the entorhinal cortex originally introduced by Lorente de Nó (1933) (see also Amaral and Witter (1995) and Insausti et al. (1997)). Accordingly, we consider the entorhinal cortex to consist of six layers, which are grouped into superficial layers (I-III) and deep layers (V-VI), separated by the cell-free lamina dissecans (layer IV) (fig. 2.3C).

For a topographical description of the location of the subicular terminal fields in the entorhinal cortex, we adhere to the division of the entorhinal cortex as proposed by Insausti et al. (1997). Based on differences in cyto-architecture, six sub-fields are distinguished (fig. 2.3B,C): a caudal entorhinal field (CE), which makes up the dorsal part of the caudal pole of the hemisphere; a medial entorhinal field (ME), occupying the most ventro-medial part of the entorhinal cortex; a ventral intermediate entorhinal field (VIE), which is located caudo-medially; an amygdalo-entorhinal transitional field (AE), located between VIE and the amygdalo-hippocampal transitional area; a dorsal intermediate entorhinal field (DIE), which forms the ventro-lateral part of the entorhinal cortex; and a dorsal lateral entorhinal field (DLE), which is located adjacent to the rhinal sulcus. The first two sub-fields (CE and ME) together are commonly referred to as the medial entorhinal area (MEA), and the other four

Entorhinal cortex

FIGURE 2.6 The distribution of labeled fibers as observed in coronal sections after anterograde tracer injection in temporal proximal subiculum (filled circle, exp. 86087). The core of the plexus is located in the ventro-medial part of lateral entorhinal area. Labeled fibers outside the parahippocampal region are omitted for clarity. For abbreviations, see list.
sub-fields (VIE, AE, DIE, DLE) are referred to as the lateral entorhinal area (LEA) (e.g. see fig. 2.3B). This latter, simpler, nomenclature will be used for the summary of the projections as well as for the discussion.

Peri- and postrhinal cortices

Based on cytoarchitectural and connectional criteria, the strip of cortex, which borders the entorhinal cortex dorsally over its entire rostrocaudal extent, is parcelled into two different regions. The more rostrally located region is named the perirhinal cortex, whereas the caudal region is named the postrhinal cortex (fig. 2.3A-C). A detailed description of both areas can be found elsewhere (Burwell et al., 1995, Burwell, 2001, Naber et al., 1997).

Description of tracing experiments

Since large injections of anterograde tracer in the subiculum will not reveal the delicate topography of projections, injections have been deliberately made such to cover a small area and to obtain optimal transport of the tracer. In a selection of rats (n=20), the injection sites appeared to be restricted to a relatively small portion of the transverse axis of

FIGURE 2.7 The distribution of labeled fibers as observed in coronal sections after anterograde tracer injection in septal distal subiculum (filled circle, exp. 86292). The core of the plexus is located in postrhinal cortex and adjacent medial entorhinal area. Labeled fibers outside the parahippocampal region are omitted for clarity. For abbreviations, see list.

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Subicular-parahippocampal projections

FIGURE 2.8 The distribution of labeled fibers as observed in coronal sections after anterograde tracer injection in temporal distal subiculum (filled circle, exp. 89350). The core of the plexus is located in the ventral part of medial entorhinal area. Labeled fibers outside the parahippocampal region are omitted for clarity. For abbreviations, see list.

Laminar distribution of subicular fibers in the parahippocampal region

Entorhinal cortex. Our findings confirm earlier observations by Köhler (1985) that subiculo-entorhinal projections are strictly ipsilateral. In addition, we observed that projections originating from injections at distinct locations on the transverse and septo-temporal axes of the subiculum did not differ in laminar terminal distribution in the entorhinal cortex. In all cases, it appeared that the labeled fibers always terminated in a dense plexus located deep to the lamina dissecans (fig. 2.4A-D). Fiber density was especially high in the superficial part of layer V (layer Va) where the fibers formed many varicosities, most likely representing terminal boutons (see inset fig. 2.4A,B; cf. van Haefen et al., 1995). A smaller fraction of the fibers terminated in a significantly less dense fashion in layer Vb or VI, or
crossed the lamina dissecans to end in superficial layers I-III. Although between cases the relative density of labeled fibers in superficial layers, compared to the plexus in layer V, could vary, there was no systematic relation with the injection site in the subiculum.

Peri- and postrhinal cortex. Projections to peri- and postrhinal cortices were found to originate mainly from septal parts of the subiculum (see below). Labeled fibers were predominantly observed ipsilateral to the injection site, but occasionally a much less dense projection to the contralateral side was found. Subicular fibers predominantly terminated in the deeper layers (layers V-VI) of both peri- and postrhinal cortex (fig. 2.4A,C). Few fibers were observed in layers I-III.

**Topography of subicular projections**

The overall topographical organization of the subiculum efferents to the parahippocampal region will be described on the basis of four tracer injections in proximal and distal subiculum at septal and temporal levels. All other cases adhere to the overall pattern and are summarized in the following description and in figure 2.9.

**Proximal subiculum**

A representative example of the fiber pattern in entorhinal cortex resulting from a tracer injection into the proximal part of the septal subiculum is shown in figure 2.5 (exp. 85392, see also fig. 2.4A). The injection site, involving the molecular and pyramidal cell layer of proximal subiculum, abutted field CA1 and extended slightly into the intermediate part of subiculum and the adjacent part of field CA1.

Generally, most labeling was present in perirhinal cortex and in the entorhinal areas comprising LEA. At rostral levels, a very dense plexus of labeled fibers and varicosities was observed in the perirhinal cortex (fig. 2.4A). The plexus extended into the deep layers of the rostral-most part of area DLE of the entorhinal cortex and the dorsal part of the adjacent piriform cortex. At more caudal levels, the plexus was still present in perirhinal cortex, whereas the density and size of the labeled plexus in DLE increased. Labeling in DIE was present, but at a lower density than in DLE. At very caudal levels, the highest density of labeled fibers was found in DLE. At these levels, a less dense fiber plexus was found in the dorsal parts of areas DIE and VIE. No labeled projections were observed in the areas, which comprise the medial entorhinal cortex or postrhinal cortex, except for a few passing fibers.

Other tracer injections in septal proximal subiculum analyzed in detail using camera lucida drawings and 3D reconstruction (e.g. exp. 95278 and 87052L) showed considerable overlap with injection 85392. These cases showed a similar distribution of labeled varicose fibers in perirhinal cortex and areas DLE and DIE of the entorhinal cortex. In case 87052L it appeared that the core of the plexus extended towards more ventral parts of area DIE (see also fig. 2.9).
An injection in the proximal part of the temporal subiculum (exp. 86087, fig. 2.6), extending slightly into the intermediate part of the temporal subiculum, involved neurons in all layers. The labeled fiber plexus was mainly found in the central parts of the entorhinal cortex, predominantly in those areas, which comprise LEA, and appeared to be rather diffuse. Rostrally, a low-density plexus was observed in DLE. The highest density of labeled fibers was found in the ventral parts of area DIE and most of area VIE. A few isolated fibers were observed in areas AE, CE and ME and the perirhinal cortex, but no labeling was present in the postrhinal cortex. This pattern was similar to that observed in another experiment with an injection in temporal proximal subiculum (exp. 86013, see fig. 2.9).

Distal subiculum

The injection in experiment 86292 (fig. 2.7) was restricted to the distal part of the septal subiculum and labeled neurons in both the molecular and pyramidal cell layer. A densely labeled plexus was observed in all layers of the dorsal presubiculum, and the deep layers of the parasubiculum, together forming the core of the plexus. At more caudal levels, high density labeling was also found in the postrhinal cortex and in the dorsal part of entorhinal area CE. A less dense labeling was observed in the more ventral parts of area CE and the dorsal part of area ME. Thus, labeling was confined to those entorhinal areas that constitute MEA, and no labeled fibers were found in any of the areas, which constitute the lateral entorhinal area, or in the perirhinal cortex. In experiment 87052R the injection was also located in septal distal subiculum, but more temporal than case 86292. In this case the densest labeling was found in area CE, but the plexus also extended into the ventral part of the postrhinal cortex and into the dorsal part of area ME. In one experiment (89261) the injection appeared to be positioned extremely septal in distal subiculum. The resulting fiber plexus covered the whole postrhinal cortex, a few fibers were also found in the adjacent perirhinal cortex, but the plexus did not extend into the entorhinal cortex (see figs. 2.4C and 2.9).

The labeled plexus resulting from an injection in distal parts of the temporal subiculum (exp. 89350) is shown in figure 2.8. The injection included all layers and was not completely restricted to the distal part of the subiculum but extended slightly into the more intermediate parts. The corresponding plexus of labeled fibers was found in the more caudal portions of the entorhinal cortex, predominantly MEA. When compared with the injections in distal septal subiculum, dense labeling was not found in dorsal CE, but in the more ventral parts of MEA, i.e. area ME. At more caudal levels, the plexus extended, in a less dense fashion, also dorsally into the ventral part of area CE. At even more caudal levels, the plexus covered a large extent of area CE. Only a few fibers were observed in areas DIE, AE, and DLE and peri- and postrhinal cortices. A very similar pattern of labeled fibers was also found with another injection in temporal distal subiculum (exp. 88562; see figs. 2.4D and 2.9).
(figure 2.9)
Summary of topography

From the four tracing studies described here in detail, the concept emerges that the subiculo-parahippocampal projection is organized according to two distinctive topographical principles. The other tracing experiments do support this conclusion as summarized in figure 2.9.

One principle is related to the location of the neurons of origin along the transverse axis of the subiculum. Fibers originating in proximal (i.e. close to area CA1) parts of the subiculum preferentially target those parts of the entorhinal cortex which constitute the lateral entorhinal area (LEA: areas DLE, DIE, AE, VIE) and the adjacent perirhinal cortex (exp. 85392, 95278, 87052L, 86087, 86013; left column fig. 2.9). On the other hand, projections arising from distal parts of the subiculum (i.e. close to presubiculum) are restricted to those areas of the entorhinal cortex which are delineated as the medial entorhinal area (MEA: areas ME and CE) and the postrhinal cortex (exp. 89261, 86292, 87052R, 88562, 89350; right column fig. 2.9). Injections intermediate to the proximal and distal poles of subiculum gave rise to labeling in both peri- and postrhinal cortex and in LEA and MEA (exp. 90200R, 86188, 88384; middle column fig. 2.9).

The second topographical principle of subicular projections is related to a septal-to-temporal origin. Regardless of their location on the transverse axis, neurons in septal parts of the subiculum project to the lateral band of the entorhinal cortex, more or less parallel to the rhinal sulcus (including parts of both LEA and MEA) (i.e. exp. 89261, 86292, 85392, 95278, 87052L, 87052R). Neurons located in temporal subiculum give rise to projections to more medial portions of LEA and MEA (exp. 86013, 89350, 86087, 88562). In contrast to the rather rigid transverse topography, this septo-temporal topography is more gradually organized. When moving the injection site along the longitudinal axis of the subiculum (top to bottom in fig. 2.9), starting septally, a gradual shift of the terminal plexus from dorso-lateral to medio-ventral parts the entorhinal cortex occurs. Subicular input to the perirhinal and postrhinal cortices arises mainly from the septal part of the subiculum although some labeled fibers were present in perirhinal cortex in some cases after temporal subicular injections (e.g. fig. 2.6).

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**FIGURE 2.9** Summary of all selected cases demonstrating the topography of subiculum projections to perirhinal, postrhinal and entorhinal cortices. The locations of terminal plexi are indicated in schematic three-dimensional representations of the parahippocampal region for injections in proximal (left column), intermediate (middle column) and distal (right column) subiculum at different septo-temporal levels (top to bottom). The position of each scheme within the figure is related to the location of the corresponding injection site in subiculum according to the arrows. The area that has the highest density of labeled fibers (core) is indicated in dark gray, whereas the extent of the total plexus is indicated in light gray. Inset shows the position of the different entorhinal subdivisions and peri- and postrhinal cortices on a three-dimensional representation of the rat brain (see fig. 3). For abbreviations, see list.
DiscusSion

To the best of our knowledge, this study is the first to describe in detail the organization of subicular projections to the perirhinal, postrhinal and entorhinal cortices in the rat. With the use of anterograde tracing it was demonstrated that subicular projections to the entorhinal, perirhinal and postrhinal cortices follow a topographical pattern, related to both the transverse and septo-temporal axes of the subiculum. These results corroborate and extend previous studies on subicular-entorhinal projections and demonstrate for the first time a clear topographical projection from subiculum to peri- and postrhinal cortices.

Topographical and laminar organization

In the rat, only a few studies have reported on the topographical organization of the projections from subiculum to the parahippocampal region. Our results led us to identify two organizational principles of the subiculo-rhinal pathway (fig. 2.10). The first one, regarding the subiculo-entorhinal pathway, is that the septo-temporal axis of the subiculum corresponds to a lateral-to-medial gradient of termination in both LEA and MEA (fig. 2.10A). In contrast to the entorhinal cortex, peri- and postrhinal cortices do not receive projections from the full longitudinal extent of subiculum. Thus subicular projections to peri- and postrhinal cortices arise mainly from subicular neurons toward the septal end of the longitudinal axis and not from the temporal end of subiculum, as has also been reported by Deacon et al. (1983).

A second organizational principle of the subiculo-parahippocampal projection is related to the transverse locations of the subicular cells of origin (fig. 2.10B). Tamamaki and Nojyo (1995) observed that cells in proximal and distal parts (with respect to CA1) of the dorsal subiculum project to LEA and MEA, respectively. The present results confirmed this topographical relation and, in addition, extend previous conclusions by showing that this topography applies to all septo-temporal levels of the subiculum. A new finding here is that peri- and postrhinal cortices are not targeted by projections from neurons at all levels on the transverse axis of subiculum. The proximal part of subiculum projects to perirhinal cortex, whereas the distal part of subiculum projects to postrhinal cortex. Based on the data presented here it is clear that projections from neurons in the proximal and distal poles of the subiculum do not overlap in the entorhinal cortex or peri-/postrhinal cortices. We found that injections in intermediate subiculum gave rise to a labeled plexus around the borders between peri- and postrhinal cortices and between LEA and MEA. This suggests that cells with a central position in subiculum (i.e. intermediate subiculum), in contrast to cells in the proximal and distal poles, have terminals in both LEA/perirhinal cortex and MEA/postrhinal cortex. However, our data cannot exclude the alternative of a strict separation between a proximal group and a distal group of cells, which exclusively project to LEA/perirhinal cortex or MEA/postrhinal cortex respectively.
Subicular-parahippocampal projections

A

septal

temporal

B

FIGURE 2.10 A: Summary of the topography of subicular projections to the parahippocampal region, indicating the relationship between the septo-temporal origin in the subiculum with a lateral-to-medial termination in the parahippocampal region. Septal subiculum projects to PER, POR, and adjacent lateral portions of both LEA and MEA. Temporal subiculum projects to medial portions of LEA and MEA, but not to PER or POR. B: Summary of the organization of subicular projections to the parahippocampal region, indicating that proximal subiculum (i.e. close to CA1) projects to PER and LEA and distal subiculum (i.e. close to presubiculum) projects to POR and MEA.

Subicular neurons preferentially target layer Va, just deep to the lamina dissecans of the entorhinal cortex. This laminar distribution of subicular projection confirms the results of previous studies (Köhler, 1985, van Haeften et al., 1995). In addition we have shown that a small but appreciable part of the projection extends into the superficial layers I-III and deeper layers Vb and VI. Furthermore, we have found that the laminar organization is similar in all subdivisions of the entorhinal cortex. Regarding the subicular output to the peri- and postrhinal cortices, we have shown that fibers mainly reach deep layers. Only few fibers target the superficial layers I-III of peri- and postrhinal cortices.

Species comparison

Similar to rodents, the entorhinal cortex in cat, monkey and human can be subdivided into LEA and MEA, although further subdivisions of these areas may differ between species.
Previous anatomical studies have indicated the existence of projection fibers from 
the subiculum to the entorhinal cortices (rat: Beckstead, 1978; Deacon et al., 1983; Köhler, 
1985; Swanson and Cowan, 1977; Tamamaki and Nojyo, 1995; guinea pig: Sorensen and 
Ino et al., 2001, Rosene and Van Hoesen, 1977, Saunders and Rosene, 1988, van Groen et al., 
1986). In most studies this projection has been shown to terminate mainly in deep layers of 
the entorhinal cortex, although a smaller projection to superficial layers has also been 
reported. Generally, the topographies reported in this study for subiculo-entorhinal projections 
in the rat are comparable to those described for other species.

With respect to subiculum projections to the perirhinal cortex, it has been reported that 
in primates the perirhinal cortex receives strong input from the prosubiculum (Blatt and 
Rosene, 1998). Since the prosubiculum as defined by Blatt and Rosene is comparable to the 
proximal part of the subiculum of the rat (Amaral and Witter, 1995), there does not seem to be 
an obvious species difference. In contrast, with respect to the subiculum projections to the 
parahippocampal cortex (area TF and/or TH) in primates, which is thought to be homologue 
to the postrhinal cortex in the rat (Burwell et al., 1995), differences are apparent. According 
to Blatt and Rosene (1998), area TH receives its input from the subiculum, which would be the 
distal part of the subiculum in the rat. In contrast, the origin of the subiculum projection to 
area TF overlaps with that of the origin of the subiculo-perirhinal projections. It thus appears 
that with respect to the origin of subicular projections, area TF is more similar to the 
perirhinal cortex, whereas area TH might be the true homologue of the postrhinal cortex of 
the rat.

**Functional implications**

The entorhinal cortex is an important relay station in the communication between the 
hippocampal formation and the neocortex. Previous studies showed that projections from the 
parahippocampal region to the hippocampal formation are topographically organized. 
Anatomical studies have indicated that in the entorhinal cortex of the rat three lateral-to-
medial bands, more or less parallel to the rhinal sulcus, can be recognized that project to 
different septo-temporal levels of the hippocampal formation (Dolorfo and Amaral, 1998a). 
Similar observations have been reported for the entorhinal to dentate gyrus projections in the 
monkey (Witter and Amaral, 1991). In addition, inputs to subiculum from LEA and MEA 
subdivisions of the entorhinal cortex are segregated along the transverse axis (Witter, 1993). 
Similarly, peri- and postrhinal cortices also project to distinct portions along the transverse 
axis of the subiculum, but much more restricted than the entorhinal cortex does (Naber et al., 
1999, Naber et al., 2001b).

Our present findings clearly show that the subiculo-parahippocampal projections 
adhere, with a remarkable precision, to the transverse and, albeit to a lesser extent, the septo
temporal topography of parahippocampal projections to the subiculum. Such an interaction between the subiculum and the parahippocampal region by means of a set of parallel channels, may serve to segregate information flows and thus maintain identity of the information during processing. Interestingly, the organization of the projections from entorhinal cortex to peri- and postrhinal cortices fits very well into this picture of parallel output channels (Burwell and Amaral, 1998b). Thus, perirhinal cortex is targeted by LEA and both areas receive direct input from the proximal part of subiculum. In contrast, postrhinal cortex is targeted mainly by MEA, and both these areas receive direct input from the distal part of subiculum. Apparently, peri- and postrhinal cortices receive two versions of subicular output: the initial, direct output and after modification in the entorhinal cortex. This modification may consist of integration between subicular output channels, since these channels are likely to overlap in the entorhinal cortex and since there are extensive associational connections within the entorhinal cortex (Dolorfo and Amaral, 1998b). The existence of parallel hippocampal output pathways through the parahippocampal region may be analogue to the parallel input pathways that we recognized in a previous paper (Naber et al., 2000).

Hippocampal output does not only originate in subiculum, but also in CA1. Tamamaki and Nojyo (1995) argued that CA1 represents the major hippocampal output structure, since the number of labeled neurons in CA1 was larger than in subiculum after retrograde tracer injection in entorhinal cortex. Our tracing studies in subiculum, however, revealed a massive subicular output to ento-, peri- and postrhinal cortices, indicating that the subiculum is a major hippocampal output structure as well. Although the number of subicular cells projecting to the entorhinal cortex may be smaller compared to CA1, it is possible that subicular cells possess a more extensive axonal collateralization within the entorhinal cortex.

This appears to be opposite to the degree of collateralization of CA1 and subiculum among target areas. Thus, the output of single CA1 neurons is not specific for a single target structure (Naber and Witter, 1998), nor is it specific for the ipsilateral side (van Groen and Wyss, 1990), but within the entorhinal cortex CA1 neurons may project rather focally. In contrast, single subicular neurons may rather specifically project to only the entorhinal cortex on the ipsilateral side (Köhler, 1985, Naber and Witter, 1998; this study), but they may collateralize extensively within the entorhinal cortex.

The question remains what the implication is of having two hippocampal output pathways, which distribute in essence to the same target regions, but show a strikingly different connectional organization. It is to be expected that relevant data pertinent to this question will come from studies in which firing characteristics of CA1 and subicular neurons are measured and compared during different tasks and behaviors (Barnes et al., 1990, Hampson et al., 2000, Sharp, 1999). In this context it appears relevant that the responses in subiculum after CA1 stimulation are decreased by acetylcholine (Hasselmo and Cekic, 1995), dopamine (Behr et al., 2000) and serotonin (Behr et al., 1997, Boeijinga and Boddeke, 1996).
This suggests that, depending on the activity states of these modulatory systems, CA1 and subiculum may either act together in distributing processed output or act independently. Clearly, more studies are needed to reveal the exact contribution of both CA1 and subiculum to hippocampal output.