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# The alvear pathway of the rat hippocampus

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**Abstract.** Neurons of the entorhinal cortex project to the hippocampus proper and dentate gyrus. This projection is called the "perforant pathway" because it perforates the subiculum; current usage applies this term to all entorhino-hippocampal fibers. However, entorhinal fibers also reach Ammon's horn via the alveus ("alvear pathway"), an alternative route first described by Cajal. The anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHAL) was used in order to analyze the contribution of this pathway to the temporo-ammonic projection. In the temporal portion of the rat hippocampus, most of the entorhinal fibers reach Ammon's horn after perforating the subiculum (classical perforant pathway). At more septal levels, the number of entorhinal fibers that take the alvear pathway increases; in the septal portion of the hippocampal formation, most of the entorhinal fibers to hippocampal subfield CA1 reach this subfield via the alveus. These fibers make sharp right-angle turns in the alveus, perforate the pyramidal cell layer, and finally terminate in the stratum lacunosum-moleculare. The crossed temporo-ammonic fibers reach their termination area in the stratum lacunosum-moleculare of CA1 almost exclusively via the alveus. These data indicate that the alveus is a major route by which entorhinal fibers reach their targets in CA1.

**Key words:** *Phaseolus vulgaris* leucoagglutinin – Anterograde tracing – Entorhinal cortex – Crossed temporo-ammonic pathway – Crossed temporo-dentate pathway – Rat (Sprague Dawley)

## Introduction

The entorhinal cortex (EC) collects all neocortical information to the hippocampus, integrates this input, and finally distributes the neocortical signals to the hippocampus in a precise topographical fashion: Neurons of layer II project to the outer molecular layer of the dentate gyrus and stratum lacunosum-moleculare of hippocampal subfield CA3 (Tamamaki and Nojyo 1993), neurons of layer III project to the stratum lacunosum-moleculare of hippocampal subfield CA1 (Steward and Scoville 1976; for review, see Witter 1993), and neurons located in the deeper layers of the EC project to the inner molecular layer, granule cell layer, and hilus of the temporal portion of the dentate gyrus (Deller et al. 1996b). Cajal (1911) was the first to describe the trajectory of entorhinal fibers to the hippocampus and named this projection the "perforant pathway", because he was intrigued by the observation that entorhinal fibers have to perforate the subiculum in order to reach their target cells in the hippocampus. He also noticed a second pathway of entorhinal fibers that reach their target cells via the alveus, the "temporo-ammonic alvear pathway". However, it has recently been suggested that the term "perforant pathway" should be used for all entorhinal fibers to the hippocampus, regardless of fiber type and layer or area of origin (Witter 1989; Amaral and Witter 1995), and that the "alvear pathway" should be discounted in the rat because it consists of only a few or "aberrant" fibers (Witter et al. 1989). Such a restriction would be misleading, however, if the alvear pathway contributed considerably to the entorhinal innervation of the hippocampus. Moreover, standard techniques such as anterograde degeneration (e.g., Brodal 1981), retrograde tracer injections (e.g., Brodal 1981), and electrical stimulation (e.g., Leung 1979; Klapstein and Colmers 1993; Buhl et al. 1994; Leung et al. 1995; Schmitz et al. 1995) also affect fibers of passage, and, therefore, a precise knowledge of the trajectory of the entorhino-hippocampal fiber tracts is essential for a better understanding of the organization of this projection. We have used the anterograde tracer

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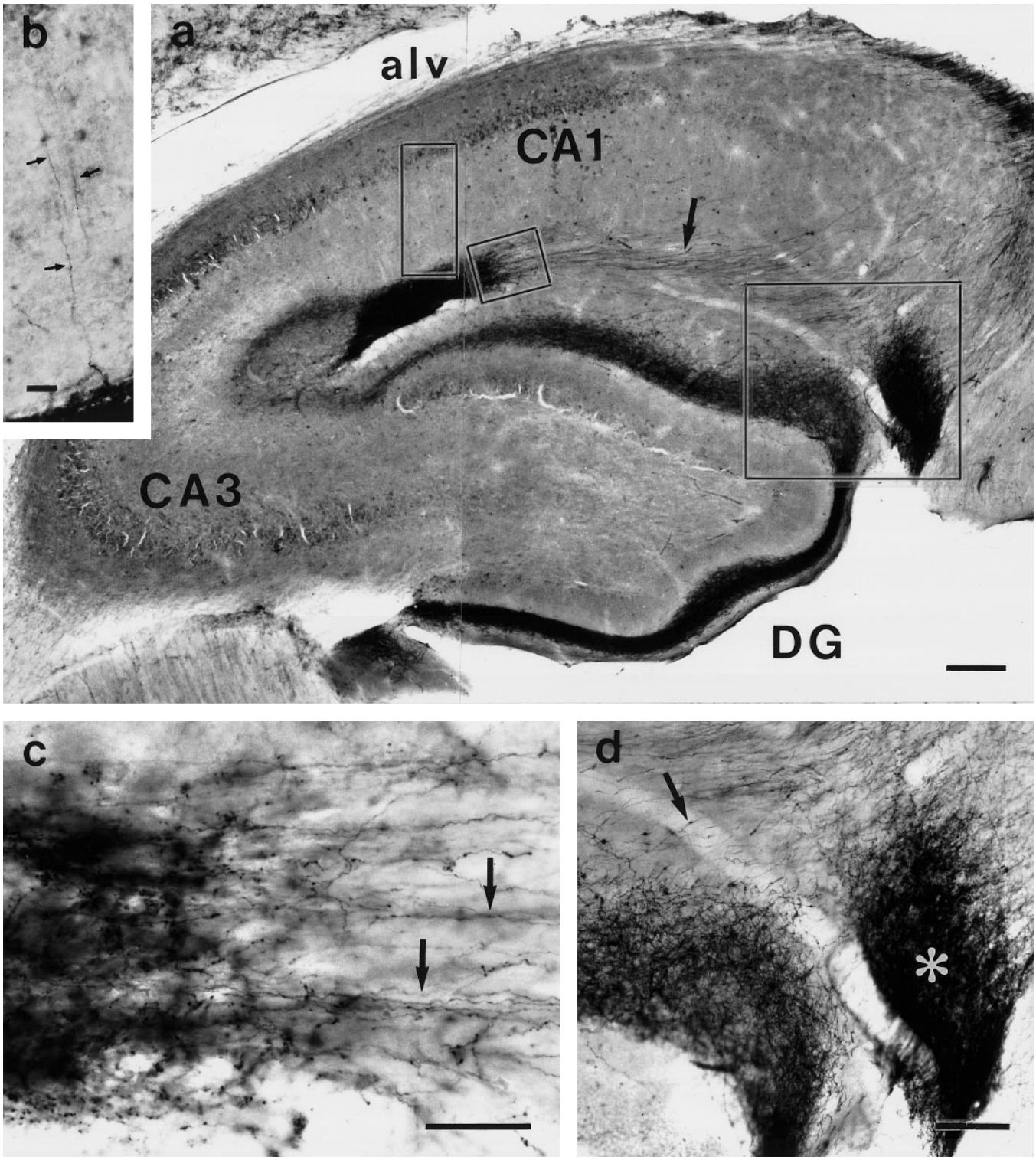



**Fig. 1a–d.** Entorhino-hippocampal fibers at a temporo-occipital level of the hippocampal formation. **a** Horizontal section through the hippocampus after a PHAL injection into the ipsilateral EC. The temporo-dentate and temporo-ammonic projections are labeled with the tracer. Note that most of the temporo-ammonic fibers reach Ammon's horn after perforating the subiculum (*bold arrow*). These fibers traverse the stratum lacunosum-moleculare before entering their termination area (*middle rectangle*; higher magnification shown in **c**). Only a few fibers reach CA1 via the alveus (*left rectangle*; higher magnification shown in **b**). The temporo-dentate fibers perforate the subiculum and cross the hippocampal fissure or turn to more septal levels (*right rectangle*; higher magnification shown in **d**). *alv*, Alveus; *DG*, dentate gyrus. **b** Higher magnification of the *left rectangle* in **a**. *Arrows* indicate weakly stained entorhinal fibers that reach CA1 via the alveus. **c** Higher magnification of the *middle rectangle* in **a**. *Arrows* indicate entorhinal fibers in the stratum lacunosum-moleculare. The terminal field of these entorhinal fibers can be seen *left*. **d** Higher magnification of the *right rectangle* in **a**. Temporo-dentate fibers cross the hippocampal fissure and enter the outer molecular layer of the dentate gyrus (*arrow*). Other entorhinal fibers form a tight fiber bundle and turn toward more septally located portions of the hippocampal formation (*asterisk*). *Bars:* 200 µm in **a**; 35 µm in **b**; 40 µm in **c**; 100 µm in **d**

*Phaseolus vulgaris* leucoagglutinin (PHAL), which labels individual fibers and their collaterals, to analyze the trajectory of entorhinal fibers in the alveus and to map their contribution to the temporo-ammonic projection at different septo-temporal levels of the hippocampal formation.

## Materials and methods

Male and female Sprague-Dawley rats (*n*=16; 250–350 g; Harlan Winkelmann, Borchen, Germany) housed under standard laboratory conditions were used. Surgical procedures were performed under deep anesthesia (Nembutal at a dose of 50 mg/kg body weight) in agreement with German legislation on the use of laboratory animals. A solution of 2.5% PHAL (Vector Laboratories, Burlingame, Calif.) in 10 mM phosphate buffer (PB), pH 7.8, was iontophoretically delivered (Gerfen and Sawchenko 1984) into the medial or lateral EC at different vertical levels (coordinates from Bregma: medial: AP -8,5; L 3,8–4,5; V 5,8–6,8; lateral: AP -8,6; L 5,0–6,0; V 5,8–6,8; Paxinos and Watson 1986) by using a glass micropipette (tip diameter 15–30 µm, 5 µA positive current applied every other 5 s for 20–30 min). The animals were allowed to survive for 10 days following the injection of the anterograde tracer. After this time, the rats were deeply anesthetized with Nembutal and transcardially perfused with a fixative containing 4% paraformaldehyde, 0.08% glutaraldehyde, 15% picric acid in 0.1 M PB (pH 7.4). Brains were removed and postfixed for 2 h in glutaraldehyde-free fixative. Sections (100 µm thick) were cut with a Vibratome and washed in PB.

Immunocytochemistry was used to visualize the PHAL-containing axons. Free-floating sections were incubated at 4°C in biotinylated goat anti-PHAL (1:400), 1% normal horse serum, 0.1% $NaN_3$, 0.5% Triton X-100 in 0.1 M PB for 2 days. After the sections had been rinsed in PB, they were incubated in avidin-biotin-peroxidase complex (ABC-Elite, Vector Labs.) for 3 h. Following subsequent washes, the sections were immersed for 5–10 min in a nickel/diaminobenzidine solution (0.05% 3,3′diaminobenzidine, 0.02% nickel ammonium chloride, 0.024% cobalt chloride, 0.001% $H_2O_2$) to give a dark-blue reaction product. Sections were then placed on gelatin-coated slides, dehydrated in ethanol, mounted in Eukitt and investigated under the light microscope. The trajectory of PHAL-labeled entorhinal fibers could be followed from the PHAL injection site to the target area by means of serial sections taken from the hippocampus (see also Deller et al. 1996b).

In order to show the relative contribution of the alvear path to the entorhinal projection to CA1 more clearly, the entorhinal projection to CA1 as visualized in single sections was drawn with the aid of a camera lucida. These drawings represent planar projections of all PHAL-labeled entorhinal fibers within a single 100-µm-thick section. In contrast, the photomicrographs of the same 100-µm-thick sections show only one plane of focus, and, therefore, fewer PHAL-labeled entorhinal fibers. Camera lucida drawings were made from single sections at different septo-temporal levels.

## Results

### *Injection sites*

All animals received a single PHAL injection into the EC. The injection sites were found in the medial and lateral entorhinal areas at different septo-temporal levels and usually covered several cell layers. Injection sites were 100–800 µm in diameter. An increased immunocytochemical background staining could be observed for an additional 100–500 µm beyond the central injection site. Injection sites were 500–800 µm in their longitudinal extent (Deller et al. 1996a, b).

### *The perforant pathway to the dentate gyrus and CA3*

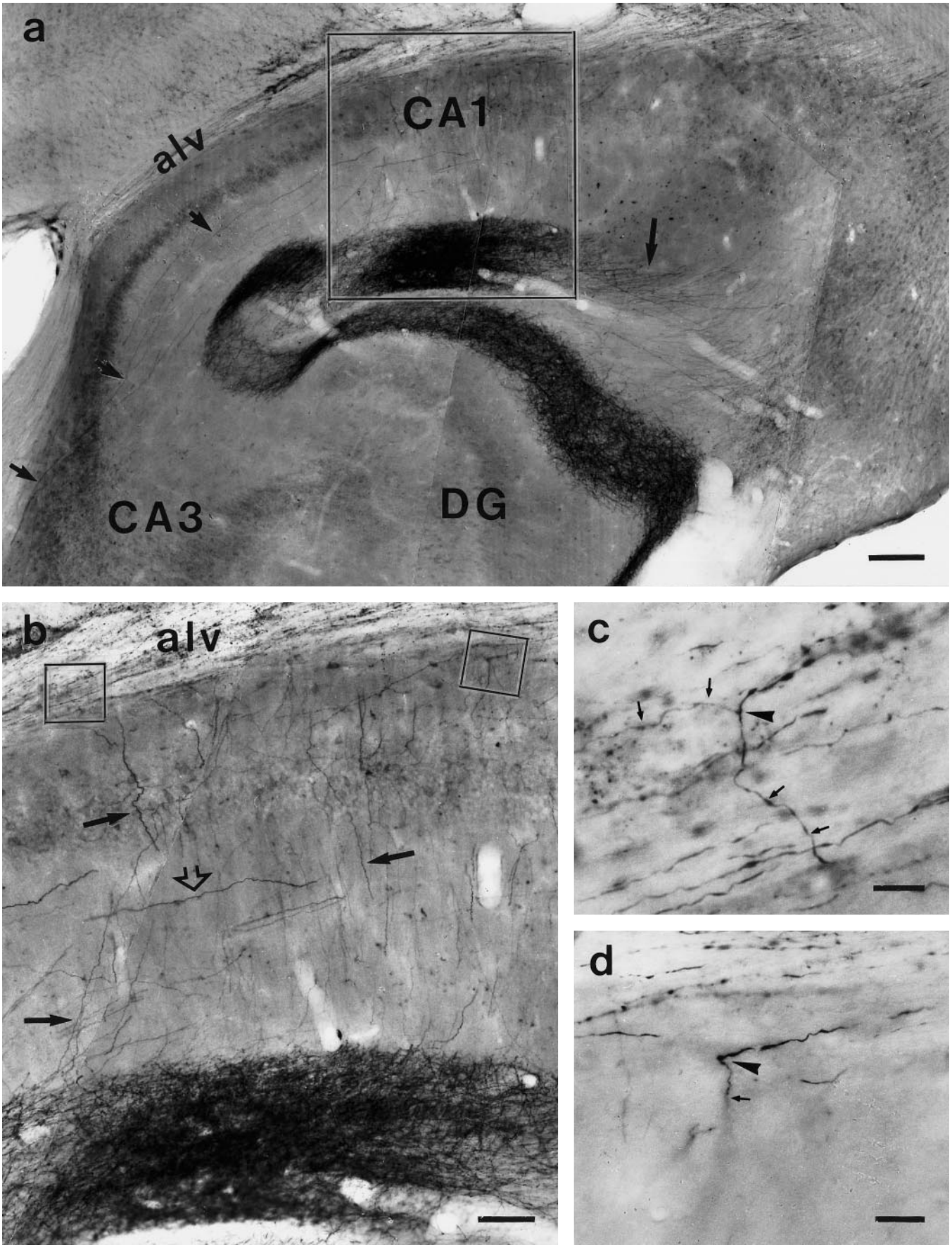
As shown in detail earlier (e.g., Steward 1976; Wyss 1981; Deller et al. 1996a, b; for review, see Amaral and Witter 1995), the entorhino-dentate projection from layer II of the EC terminates exclusively in the outer two thirds of the molecular layer. This projection was heavily labeled after PHAL injections into the EC and showed the septo-temporal topography typical of the perforant pathway (Steward 1976; Wyss 1981; Amaral and Witter 1995; Deller et al. 1996b). Two trajectories of single entorhino-dentate fibers could be observed at temporal levels of the hippocampal formation. One group of fibers traversed the hippocampal fissure (Fig. 1a, d) and participated in the formation of the dense entorhinal fiber plexus in the outer molecular layer of the dentate gyrus. A second group of fibers participated in the formation of a dense fiber bundle located opposite the crest of the dentate gyrus in layer I of the presubiculum (Fig. 1a, d). These fibers continued in a rostro-dorsal direction and terminated in more septally located portions of the dentate gyrus.

The entorhinal projection to the ipsilateral CA3 area was also labeled (Figs. 1a, 2a, 3a). This projection arose from the same layer II entorhinal neurons as the entorhinal projection to the dentate gyrus (Tamamaki and Nojyo 1993), and, in agreement with these authors, entorhinal fibers to CA3 followed a similar route.

### *The perforant pathway is the main trajectory of entorhinal fibers to CA1 at temporal levels*

The entorhinal projection to CA1 was labeled at temporal levels of the hippocampal formation. Two trajectories of the entorhinal fibers to CA1 could be observed: One

**Fig. 2a–d.** Entorhino-hippocampal fibers at an intermediate septo-temporal level. **a** Horizontal section through the hippocampus after a PHAL injection into the ipsilateral EC. The corresponding contralateral hippocampus is shown in Fig. 4. The temporo-dentate and temporo-ammonic projections are labeled. Numerous fibers reach Ammon's horn after perforating the subiculum (*long bold arrow*), but an almost equal number arrives via the alveus (*rectangle*; higher magnification shown in **b**). Note that some PHAL-labeled entorhinal fibers follow a trajectory parallel to the pyramidal cell layer in the stratum radiatum. These fibers perforate CA3 and enter the fimbria (*short bold arrows*). *alv*, Alveus; *DG*, dentate gyrus. **b** Higher magnification of the *rectangle* in **a**. Entorhinal fibers reach CA1 via the alveus (*alv*). These fibers make sharp right-angle turns in the alveus (*rectangles*), traverse the pyramidal cell layer, and finally enter the stratum lacunosum-moleculare (*bold arrows*). *Left* and *right rectangles* are shown at higher magnification in **c** and **d**, respectively. An entorhinal fiber in the stratum radiatum that follows a trajectory parallel to the pyramidal cell layer is indicated (*open arrow*). **c** Higher magnification of the *left rectangle* in **b**. Note that the PHAL-labeled entorhinal fiber in the alveus comes to a branching point (*arrowhead*) where two collaterals arise (*arrows*). One collateral makes a right-angle turn toward CA1, whereas the other collateral continues in the alveus. **d** Higher magnification of the *right rectangle* in **b**. The labeled axon makes a sharp right-angle turn in the alveus (*arrowhead*) and continues toward CA1 (*arrow*). *Bars:* 200 µm in **a**; 75 µm in **b**; 10 µm in **c**, **d**


a
alv
CA1
CA3
DG
b
alv
c
d

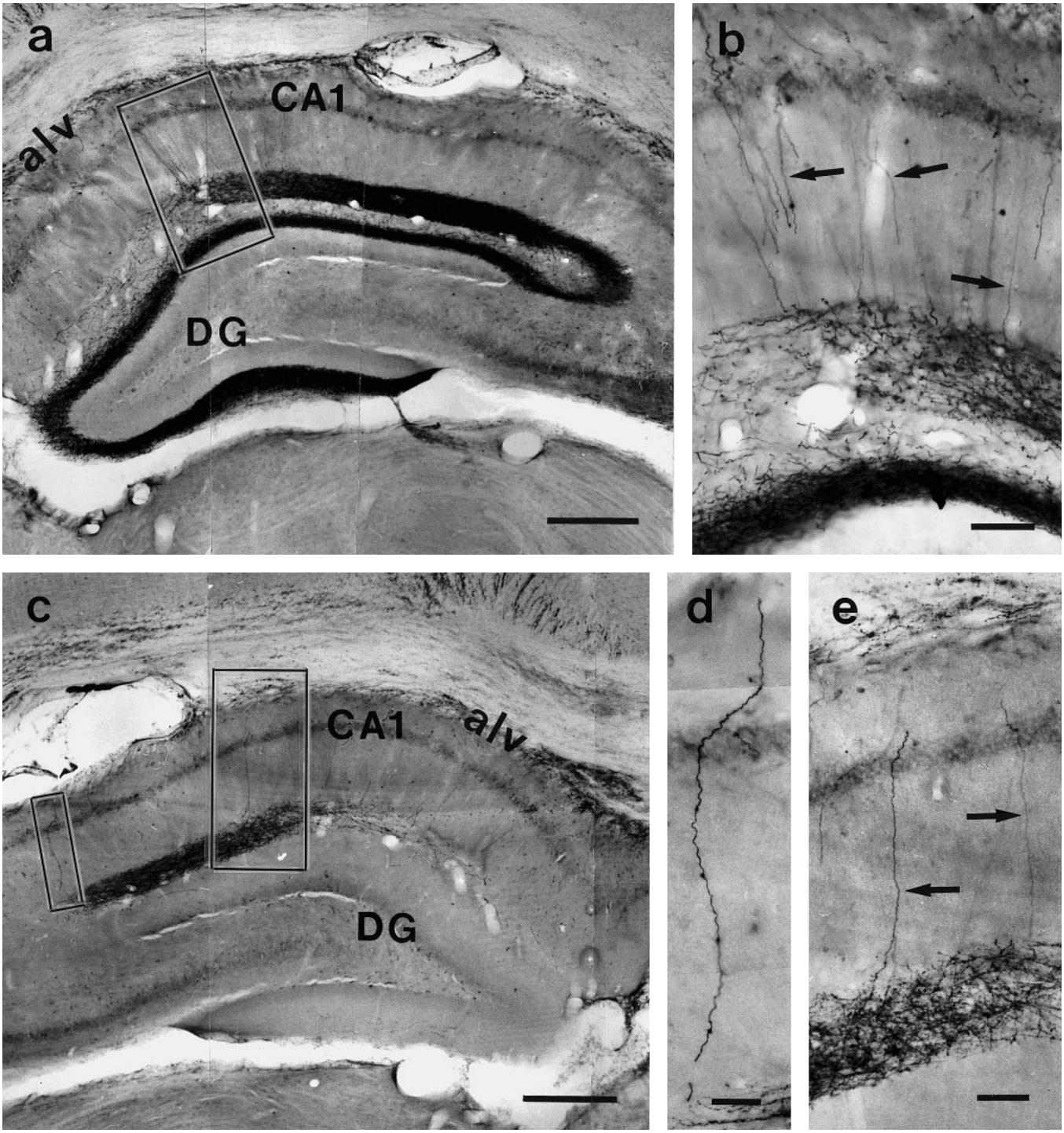
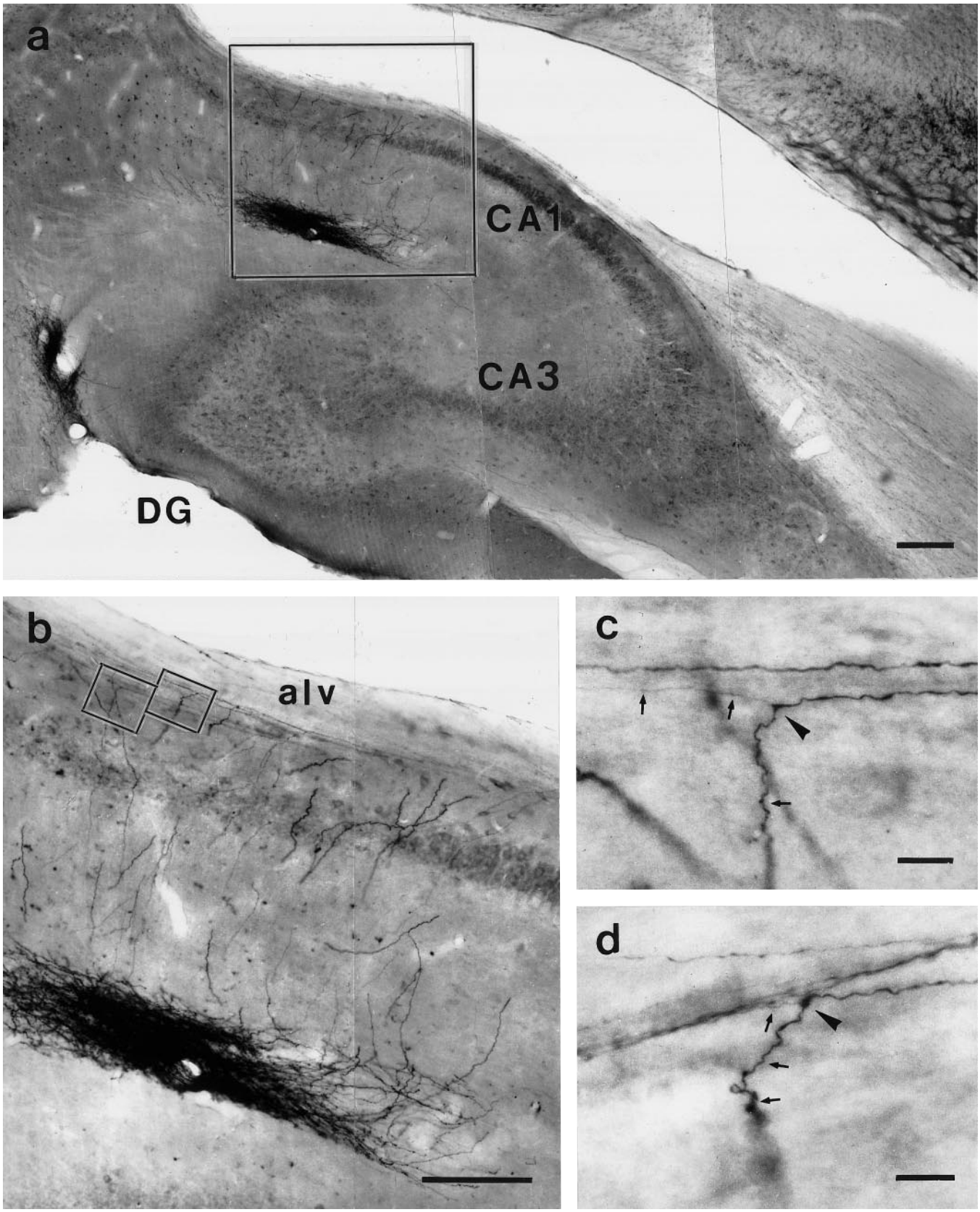



**Fig. 3a–e.** Entorhino-hippocampal fibers to the ipsi- and contralateral hippocampus at a septal level. **a** Frontal section through the hippocampus after a PHAL injection into the ipsilateral EC. The corresponding contralateral hippocampus is shown in **c**. The temporo-hippocampal projections are labeled with the tracer. Most of the fibers reach the stratum lacunosum-moleculare via the alveus (*rectangle*; at higher magnification in a serial section in **b**). *alv*, Alveus; *DG*, dentate gyrus. **b** Serial section of the hippocampus and higher magnification of the *rectangle* shown in **a**. *Arrows* indicate fibers that reach the stratum lacunosum-moleculare via the alveus. **c** Contralateral hippocampus to **a**. The crossed temporo-hippocampal projections are labeled. The temporo-dentate projection is weak compared with the temporo-ammonic projection. The entorhinal fibers reach the stratum lacunosum-moleculare via the alveus (*rectangles*). Higher magnification of the *left* and *right rectangles* shown in **d** and **e**, respectively. **d** Higher magnification of the *left rectangle* in **c**. Crossed temporo-ammonic fibers can be followed from the alveus through the CA1 pyramidal cell layer to the stratum lacunosum-moleculare. **e** Higher magnification of the *right rectangle* in **c**. Crossed temporo-ammonic fibers reach the stratum lacunosum-moleculare from the alveus (*arrows*). *Bars:* 500 µm in **a**, **c**; 100 µm in **b**, **e**; 50 µm in **d**


a
CA1
CA3
DG
b
alv
c
d

group of fibers followed a trajectory similar to that of the entorhino-dentate fibers. These fibers could be followed from the injection site to more septal levels of the hippocampal formation in serial sections of the hippocampus. They were located laterally to the entorhino-dentate fiber bundle, perforated the subiculum, and entered the stratum lacunosum-moleculare of CA1 (Figs. 1a, c, 6b). These fibers continued within this layer until they reached their topographically corresponding termination field (Amaral and Witter 1995; Tamamaki and Nojyo 1995). A second group of fibers left the injection site, entered the angular bundle, and continued in a rostro-dorsal direction to more septally located portions of the hippocampal formation. However, at this temporal level of the hippocampal formation, only a few entorhinal fibers could be followed into the alveus. These fibers continued in the alveus until they were located opposite the termination field of the perforating fibers, traversed the strata oriens, pyramidale, and radiatum, and, finally, terminated in the same field as the perforating entorhinal fibers (Figs. 1b, 6b).

### *The alvear pathway is a major trajectory of entorhinal fibers to CA1 at septal levels*

At a more septal level (intermediate level), a much larger number of entorhinal fibers could be observed that reached their target zone in Ammon's horn via the alveus (Figs. 2a, b; 5b, c). These fibers continued in the alveus until they were located opposite the termination field of the perforating fibers, made right-angle turns (Fig. 2c, d), and traversed the CA1 layers until they finally terminated in the same field as the perforating entorhinal fibers (Fig. 2a, b). Interestingly, a small number of PHAL-labeled entorhinal fibers was also observed in the stratum radiatum. These fibers followed a trajectory parallel to the pyramidal cell layer of CA1 and CA3 (Fig. 2a, b), before traversing the CA3 pyramidal cell layer and entering the fimbria. Moreover, the entorhinal fibers in the alveus frequently branched with one collateral turning toward the entorhinal termination zone in CA1 and the other collateral continuing in the alveus toward the fimbria (Figs. 2c, 4c, d).

At a hippocampal level close to the septal pole, the alvear path seemed even more prominent (Fig. 3a): Numerous entorhinal fibers reached the stratum lacunosum-moleculare via the alveus (Figs. 3b, 5a), but no perforating fibers could be seen at this level of the hippocampal formation.

### *The alvear pathway is the main route for crossed entorhinal fibers to CA1*

In contrast to the weak crossed entorhino-dentate and crossed entorhino-CA3 projections (Zimmer and Hjorth-Simonsen 1975; Steward 1976, 1980; Wyss 1981; Deller et al. 1996a), the crossed entorhinal projection to CA1 was almost comparable in density to the entorhino-ammonic projection on the injection side (Steward 1976; Wyss 1981; Deller et al. 1996a; Figs. 3c, d, e, 4, 5a, 6a). These entorhinal fibers reached their termination zone via the alveus. Again, entorhinal fibers made right-angle turns (Fig. 4c, d), traversed the layers of CA1 (Fig. 3c, d, e, 4b), and terminated in the stratum lacunosum-moleculare. Many crossed entorhino-ammonic fibers branched (Fig. 4c, d). One collateral turned toward the stratum lacunosum-moleculare; the other collateral continued in the alveus.

## Discussion

In the present study, anterograde tracing with PHAL was used to analyze the contribution of the alvear pathway to the entorhino-ammonic projection. Our data indicate that the alvear pathway is a major route of entorhinal fibers to the CA1 region in the septal portion of the hippocampus and the main route of entorhinal fibers to the contralateral CA1 region.

### *The alvear pathway of the rat hippocampus*

Entorhinal fibers in the alveus were first mentioned by Cajal (1911) who described a projection of entorhinal fibers to the subiculum and named these fibers the "temporo-ammonic alvear path". Later, Lorente de Nó (1934) demonstrated numerous entorhinal fibers in the alveus using the Golgi technique. He distinguished between a "deep" commissural system of the entorhinal cortex and a "superficially" located fiber system to the prosubiculum and to CA1a. Results obtained by autoradiography and silver staining after EC lesions led Steward (1976) to suggest that the entorhinal fibers present in the alveus only represented the most laterally originating component of a more extensive projection system to both the subiculum and the CA1 region, and that they did not represent a separate pathway. The alvear path is also mentioned in the autoradiographic study of Wyss (1981) who suggested that the entorhinal fibers in the alveus represent the commissural projection system of the EC, with

**Fig. 4a–d.** Entorhino-hippocampal fibers to the contralateral hippocampus at an intermediate septo-temporal level. **a** Horizontal section through the hippocampus after a PHAL injection into the contralateral EC. The corresponding ipsilateral hippocampus is shown in Fig. 2. The crossed temporo-hippocampal projections are labeled with the tracer. The crossed temporo-dentate projection is weak compared with the temporo-ammonic projection to CA1. Most of the fibers reach CA1 via the alveus (*rectangle*; higher magnification shown in **b**). *DG*, Dentate gyrus. **b** Higher magnification of the *rectangle* in **a**. The entorhinal fibers reach the stratum lacunosum-moleculare via the alveus (*alv*). These fibers make sharp right-angle turns in the alveus (*rectangles*), traverse the pyramidal cell layer, and finally enter the stratum lacunosum-moleculare. *Left* and *right rectangles* shown at higher magnification in **c** and **d**, respectively. **c, d** Higher magnification of the *left* and *right rectangles* in **b**. A PHAL-labeled entorhinal fiber in the alveus branches (*arrowhead*) into two collaterals (*arrows*). One collateral makes a sharp turn toward the stratum lacunosum-moleculare. The other collateral continues within the alveus. *Bars:* 200 μm in **a**; 150 μm in **b**; 10 μm in **c**, **d**

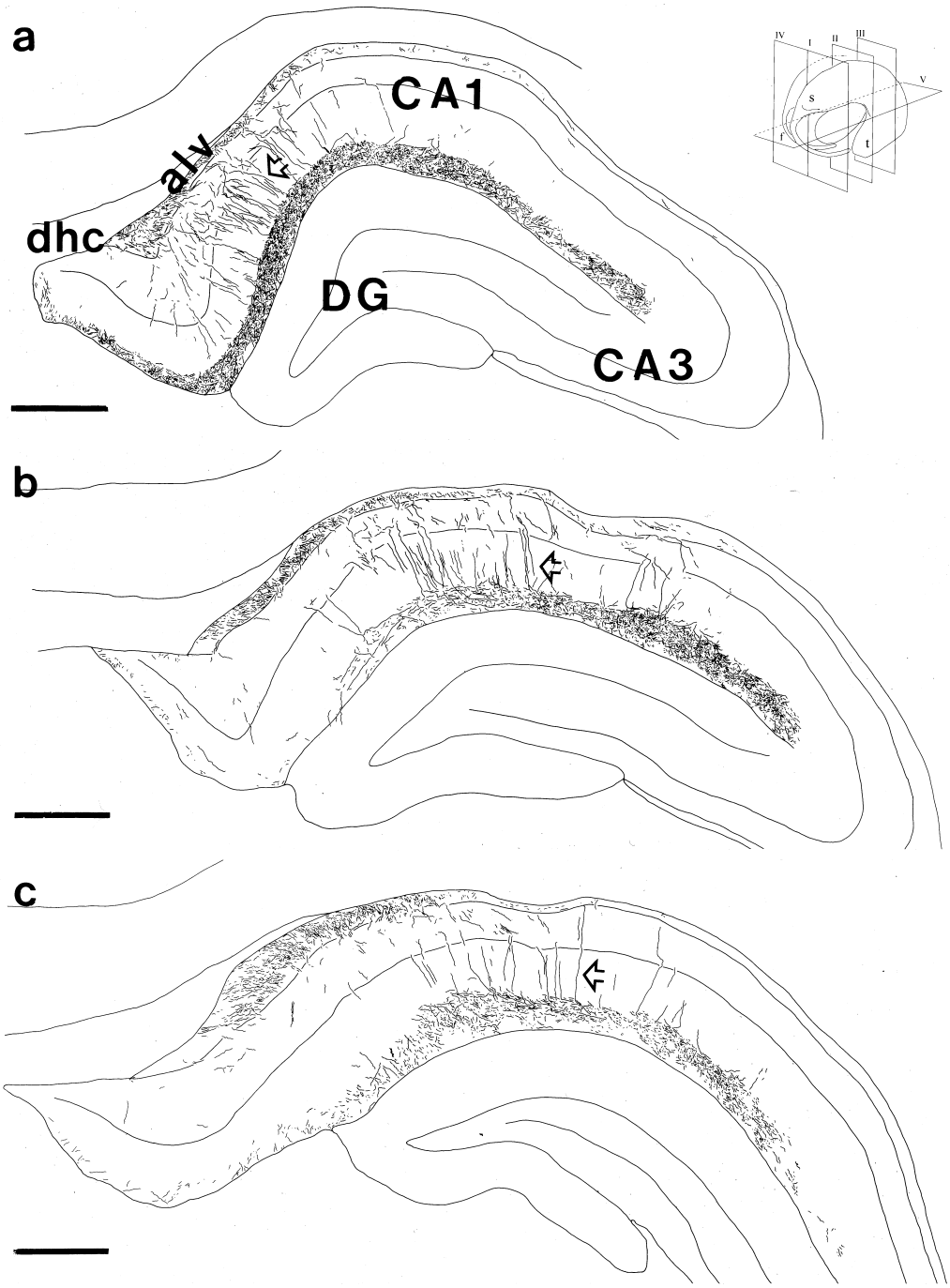



**Fig. 5a–c.** Camera lucida drawings of the ipsilateral temporoammonic pathway to CA1 at different coronal levels (coordinates given from Bregma). **a** Coronal section of the hippocampus (*plane I* in the *inset*) located close to the septal pole of the hippocampal formation (AP -3.6 mm). Note the large number of entorhinal fibers that reach the stratum lacunosum-moleculare of CA1 via the alveus (*open arrow*). *alv*, Alveus; *DG*, dentate gyrus; *dhc*, dorsal hippocampal commissure. *Inset:* Planes of sectioning of the hippocampi illustrated in Figs. 5, 6. *f*, Fornix; *s*, septal pole of the hippocampus; *t*, temporal pole of the hippocampus. **b** Coronal section of the hippocampus (*plane II* in *inset*; AP -4 mm). The *open arrow* indicates fibers that reach the stratum lacunosum-moleculare via the alveus. **c** Coronal section of the hippocampus (*plane III* in *inset*; AP 1–5 mm). The *open arrow* indicates fibers that reach the stratum lacunosum-moleculare via the alveus. *Bars:* 400 μm

only some superficial axons terminating in CA1a and the adjacent parts of the subiculum. More recent studies by Witter and co-workers (for review, see Witter et al. 1989), using the sensitive anterograde tracer PHAL, have demonstrated entorhinal fibers that reach the stratum lacunosum-moleculare of CA1 via the alveus. However, these workers have suggested that, in the rat, this alvear pathway consists of aberrant fibers that should be discounted. In line with this interpretation of the alvear pathway, it has been proposed (Witter 1989; Amaral and Witter 1995) that all entorhino-hippocampal fibers should be referred to as the "perforant pathway" in order to simplify anatomical terminology. The same authors mention a small fascicle of entorhinal fibers that is present in the alveus of the monkey (Witter and Amaral 1991) and that shows anatomical similarities to the alvear pathway described in the present study. Our data, however, show that the alvear pathway is present in the rat and demonstrate numerous entorhinal fibers reaching the ipsi- and contralateral CA1 region of the hippocampus via the alveus.

### *The importance of the alvear pathway for the entorhinal projection to CA1*

The relative contribution of alvear pathway fibers to the ipsilateral entorhinal projection to CA1 is difficult to estimate. Perforating entorhinal fibers to CA1 clearly dom-

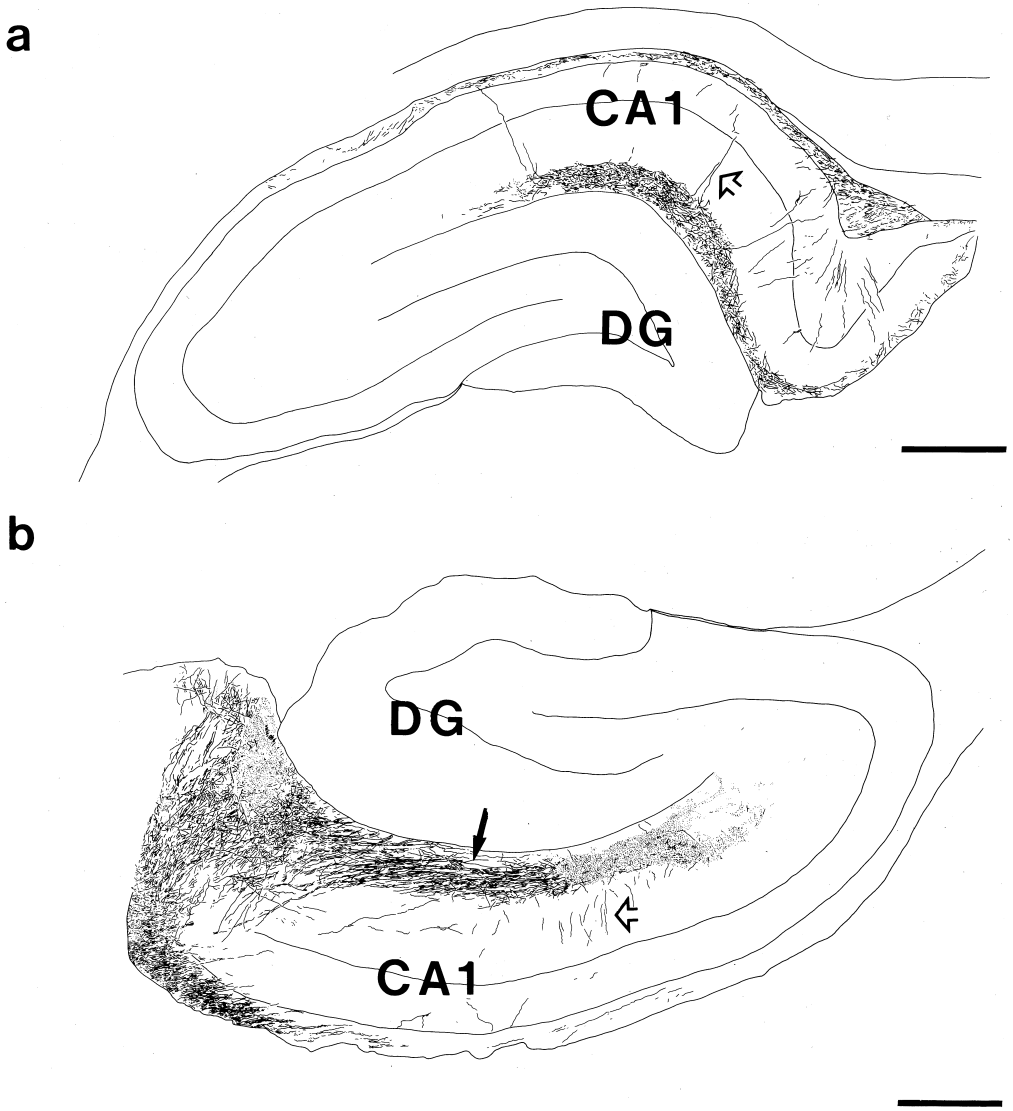



**Fig. 6a, b.** Camera lucida drawing of the temporo-ammonic pathway to CA1 (coordinates given from Bregma). **a** Coronal section of the septal (dorsal) hippocampus contralateral to the injection side (plane IV of *inset* in Fig. 5a; AP -3.6 mm). Numerous PHAL-labeled crossed temporo-ammonic fibers reach the stratum lacunosum-moleculare via the alveus (*open arrow*). **b** Horizontal section of the hippocampus ipsilateral to the injection side (plane V of *inset* in Fig. 5a; V -4.3 mm), same section as in Fig. 1a. At this level, entorhinal fibers reach CA1 after perforating the subiculum. They continue in the stratum lacunosum-moleculare (*bold arrow*) until they reach their termination zone. Only a few fibers pass through the alveus and ascend to the stratum lacunosum-moleculare of CA1 (*open arrow*). *DG*, Dentate gyrus. *Bars:* 400 µm

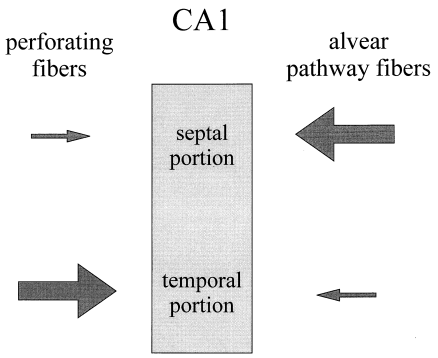



**Fig. 7.** Fiber tracts from the entorhinal cortex to CA1. In the septal portion of the hippocampal formation (*top*), most of the entorhinal fibers to CA1 reach the stratum lacunosum-moleculare via the alveus. In the temporal portion of the hippocampal formation (*bottom*), most of the entorhinal fibers reach CA1 after perforating the subiculum

inate at temporal levels of the hippocampus. Since these fibers may also have a septo-temporal spread within the stratum lacunosum-moleculare (for review, see Amaral and Witter 1995), perforating fibers could reach the septal portion of CA1 by following a course within the stratum lacunosum-moleculare in a longitudinal (temporo-septal) direction. In contrast, the high specificity of the entorhino-CA1 projection described recently (Tamamaki and Nojyo 1995) suggests that the septo-temporal spread of the entorhinal projection to CA1 is limited and that entorhinal fibers to the septal portion of CA1 are mostly derived from alvear pathway fibers (Fig. 7). Unfortunately, little is known about the septo-temporal spread of a single perforating entorhinal fiber to CA1; therefore, a contribution of the perforating fibers to the septal portion of CA1 cannot be excluded (Fig. 7).

### *Bilaterally projecting entorhinal fibers in the alvear pathway*

The number of fibers that reach CA1 via the alvear pathway increases toward septal levels of the hippocampal

formation (Figs. 5, 6). This septo-temporal distribution is similar to the septo-temporal topography of the crossed entorhino-hippocampal projections (Steward 1976; Wyss 1981). Interestingly, it has been suggested that entorhinal fibers in the alveus belong to the crossed and commissural projections of the EC (Wyss 1981), i.e., projections that terminate in the contralateral hippocampus (Steward 1976; Wyss 1981; Deller et al. 1996b) and contralateral EC (Köhler 1986, 1988; Adelmann et al. 1996). Since many entorhinal fibers branch in the alveus and show one collateral to the ipsilateral stratum lacunosum-moleculare of CA1, and a second collateral that continues in the alveus toward the fimbria, the findings of Wyss (1981) may only need to be slightly extended: Entorhinal fibers in the alveus also appear to have an ipsilateral collateral to CA1. Thus, entorhinal fibers that project to the ipsilateral and to the contralateral CA1 area could be those that make up the alvear pathway.

*Cells of origin of the alvear path fibers*

Neurons in the entorhinal cell layer III have been identified as the cells of origin of the entorhinal projection to the ipsilateral and contralateral CA1 area of the hippocampus (Steward and Scoville 1976). However, since no retrograde double-labeling experiments have been performed, it remains unclear whether the same neuron projects both ipsilaterally and contralaterally to CA1 or whether two different subpopulations of neurons are involved. The branching of entorhinal axons in the alveus observed in the present study provides evidence for a bilateral innervation of the CA1 area by single layer III neurons of the EC. One collateral reaches the ipsilateral CA1 area, the other collateral crosses the midline and innervates the contralateral CA1 region. Such a bilateral innervation of the hippocampus is also known for the associational and commissural projection of the dentate gyrus (Blackstad 1956; Zimmer 1971; Gottlieb and Cowan 1973; Swanson et al. 1978; Laurberg 1979; Laurberg and Sorensen 1981; Deller et al. 1995, 1996c). In this commissural system, single hilar neurons have ipsilateral and commissural collaterals (Berger et al. 1980). Moreover, it has recently been shown that commissural and associational axons also terminate in corresponding layers of the dentate gyrus (Deller et al. 1996c), thereby innervating similar target regions and, possibly, target cells. It remains to be seen whether single layer III entorhinal neurons that contribute to the ipsilateral and crossed entorhinal projection to CA1 show a similar bilateral specificity.

*Target specificity of entorhinal fibers to CA1*

Entorhinal fibers in the alveus branch opposite their target area (Figs. 5, 6). This observation is noteworthy, since layer III neurons of the EC project only to a limited portion of the CA1 subfield that corresponds to a specific cortical column of the EC (Witter 1993; Tamamaki and Nojyo 1995). Thus, precise guiding cues have to be postulated as being present during development. First, there need to be signals inducing axon collateral formation and, secondly, the correct target pyramidal cells have to be recognized. Such information might be present bilaterally, thereby enabling the crossed collateral to find its corresponding target in the contralateral hippocampus. Interestingly, similar branching processes (right angles) can be seen in other parts of the central nervous system, such as the corticospinal tract. Axons of this system branch in the pons with one collateral turning at a right angle toward the pontine nuclei and the other collateral continuing into the spinal cord (O'Leary and Terashima 1988; Heffner et al. 1990; for review, see O'Leary et al. 1991). Thus, it remains to be shown which cues facilitate the pathfinding of the alvear axons in the hippocampus; cellular elements, such as guidepost cells (Palka et al. 1992), target cell derived chemoattractants (O'Leary et al. 1991), or factors present in the extracellular matrix (Goodman and Shatz 1993) are suggested.

*Electrophysiological characteristics of the alvear pathway*

In electrophysiological experiments, alvear stimulations have frequently been used to analyze the functional characteristics of hippocampal neurons in vivo and in slice preparations of the hippocampus (e.g., Leung 1979; Klapstein and Colmers 1993; Buhl et al. 1994; Leung et al. 1995; Schmitz et al. 1995). In particular, the CA1 region has been analyzed by using alvear stimulation, and the electrophysiological effects observed on CA1 pyramidal neurons are generally interpreted as antidromic stimulation of CA1 axon collaterals (e.g., Leung 1979; Klapstein and Colmers 1993). Although alvear pathway fibers may be involved when stimulating the alveus (e.g., Leung 1979), such an effect has been neglected, mainly because its anatomical basis has previously been unclear (Leung 1979). Moreover, alvear stimulation may yield different results in hippocampal slices depending on the septo-temporal level of the hippocampus from which the slices are made. The alvear fibers may indeed be discounted if the slices are taken from temporo-occipital levels but should be taken into account whenever slices are taken from septal levels of the hippocampus.

*Conclusions*

The alvear pathway appears to be a major trajectory of entorhinal fibers to the septal portion of the ipsilateral and contralateral CA1 region of the hippocampus. Entorhinal neurons of layer III that project to the ipsilateral and to the contralateral CA1 area are putative cells of origin. Specific guiding cues in the alveus are required during development in order to enable these fibers to reach their specific target area.

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## References


Adelmann G, Deller T, Frotscher M (1996) Organization of identified fiber tracts in the rat fimbria-fornix: an anterograde tracing and electron microscopic study. Anat Embryol 193:481–493

Amaral DG, Witter MP (1995) Hippocampal formation. In: Paxinos G (ed) The rat nervous system, 2nd edn. Academic Press, San Diego New York Boston London Sydney Tokyo Toronto, pp 443–493

Berger TW, Semple-Rowland S, Basset J (1980) Hippocampal polymorph neurons are the cells of origin for ipsilateral association and commissural afferents to the dentate gyrus. Brain Res 215:329–336

Blackstad TW (1956) Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. J Comp Neurol 105:417–537

Brodal A (1981) Neurological anatomy in relation to clinical medicine. I. Introduction, methods, correlations. Oxford University Press, Oxford New York, pp 3–46

Buhl EH, Han ZS, Lorinczi Z, Stezhka VV, Karnup SV, Somogyi P (1994) Physiological properties of anatomically identified axo-axonic cells in the rat hippocampus. J Neurophysiol 71:1289–1307

Cajal SR (1911) Histologie du système nerveux de l'homme et des vertébrés. CSIC, Madrid

Deller T, Nitsch R, Frotscher M (1995) *Phaseolus vulgaris* leucoagglutinin (PHAL) tracing of commissural fibers to the rat fascia dentata: evidence for a previously unknown commissural projection to the outer molecular layer. J Comp Neurol 352: 55–68

Deller T, Frotscher M, Nitsch R (1996a) Sprouting of crossed entorhino-dentate fibers after a unilateral entorhinal lesion: anterograde tracing of fiber reorganization with *Phaseolus vulgaris*-leucoagglutinin (PHAL). J Comp Neurol 365:42–55

Deller T, Martinez A, Nitsch R, Frotscher M (1996b) A novel entorhinal projection to the rat dentate gyrus: direct innervation of proximal dendrites and cell bodies of granule cells and GABAergic neurons. J Neurosci 16:3322–3333

Deller T, Nitsch R, Frotscher M (1996c) Heterogeneity of the commissural projection to the rat dentate gyrus: a *Phaseolus vulgaris* leucoagglutinin tracing study. Neuroscience (in press)

Gerfen CR, Sawchenko PE (1984) An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an axonally transported plant lectin, *Phaseolus vulgaris* leucoagglutinin. Brain Res 290:219–238

Goodman CS, Shatz CJ (1993) Developmental mechanisms that generate precise patterns of neuronal connectivity. Cell 72/Neuron 10 (Suppl):77–98

Gottlieb DI, Cowan WM (1973) Autoradiographic studies of the commissural and ipsilateral association connections of the hippocampus and dentate gyrus of the rat. I. The commissural connections. J Comp Neurol 149:393–422

Heffner CD, Lumsden AG, O'Leary DD (1990) Target control of collateral extension and directional axon growth in the mammalian brain. Science 247:217–220

Klapstein GJ, Colmers WF (1993) On the sites of presynaptic inhibition by neuropeptide Y in the rat hippocampus in vitro. Hippocampus 3:103–111

Köhler C (1986) Intrinsic connections of the retrohippocampal region in the rat brain. II. The medial entorhinal area. J Comp Neurol 246:149–169

Köhler C (1988) Intrinsic connections of the retrohippocampal region in the rat brain. III. The lateral entorhinal area. J Comp Neurol 271:208–228

Laurberg S (1979) Commissural and intrinsic connections of the rat hippocampus. J Comp Neurol 184:685–708

Laurberg S, Sorensen KE (1981) Associational and commissural collaterals of neurons in the hippocampal formation (hilus fasciae dentatae and subfield CA3). Brain Res 212:287–300

Leung LS (1979) Potentials evoked by alvear tract in hippocampal CA1 region of rats. I. Topographical projection, component analysis, and correlation with unit activities. J Neurophysiol 42:1557–1570

Leung LS, Roth L, Canning KJ (1995) Entorhinal inputs to hippocampal CA1 and dentate gyrus in the rat: a current-source-density study. J Neurophysiol 73:2392–2403

Lorente de Nó R (1934) Studies on the structure of the cerebral cortex. II Continuation of the study of the ammonic system. J Psychol Neurol 46:113–177

O'Leary DD, Terashima T (1988) Cortical axons branch to multiple subcortical targets by interstitial axon budding: implications for target recognition and "waiting periods". Neuron 1:901–910

O'Leary DD, Heffner CD, Kutka L, Lopez-Mascaraque L, Missias A (1991) A target-derived chemoattractant controls the development of the corticopontine projection by a novel mechanism of axon targeting. Development (Suppl) 2:123–130

Palka J, Whitlock KE, Murray MA (1992) Guidepost cells. Curr Opin Neurobiol 2:48–54

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn, Academic Press, Sidney Orlando San Diego New York Austin London Montreal Tokyo Toronto

Schmitz D, Zhang CL, Chatterjee SS, Heinemann U (1995) Effects of methysticin on three different models of seizure-like events studied in rat hippocampal and entorhinal cortex slices. Arch Pharmacol 351:348–355

Steward O (1976) Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. J Comp Neurol 167:285–314

Steward O (1980) Trajectory of contralateral entorhinal axons which reinnervate the fascia dentata of the rat following ipsilateral entorhinal lesions. Brain Res 183:277–289

Steward O, Scoville SA (1976) Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. J Comp Neurol 169:347–370

Swanson LW, Wyss JM, Cowan WM (1978) An autoradiographic study of the organization of intrahippocampal association pathways in the rat. J Comp Neurol 181:681–716

Tamamaki N, Nojyo Y (1993) Projection of the entorhinal layer II neurons in the rat as revealed by intracellular pressure-injection of neurobiotin. Hippocampus 3:471–480

Tamamaki N, Nojyo Y (1995) Preservation of the topography in the connections between the subiculum, field CA1, and the entorhinal cortex in rats. J Comp Neurol 353:379–390

Witter MP (1989) Connectivity of the rat hippocampus. In: Chan-Palay V, Köhler C (eds) The hippocampus – new vistas. Liss, New York, pp 53–69

Witter MP (1993) Organization of the entorhinal-hippocampal system: a review of current anatomical data. Hippocampus 3 (Suppl):33–44

Witter MP, Amaral DG (1991) Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. J Comp Neurol 307:437–459

Witter MP, Groenewegen HJ, Lopes da Silva FH, Lohman AHM (1989) Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. Prog Neurobiol 33:161–253

Wyss JM (1981) An autoradiographic study of the efferent connections of the entorhinal cortex in the rat. J Comp Neurol 199:495–512

Zimmer J (1971) Ipsilateral afferents to the commissural zone of the fascia dentata, demonstrated in decommissurated rats by silver impregnation. J Comp Neurol 142:393–416

Zimmer J, Hjorth-Simonsen A (1975) Crossed pathways from the entorhinal area to the fascia dentata. II. Provokable in rats. J Comp Neurol 161:71–102