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Zonal distribution of perforant path cells in layer III of the entorhinal area projecting to CA1 and subiculum in the rat

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ABSTRACT

The distribution pattern of the cells that give rise to perforant path projections, including direct entorhino-CA1 and entorhino-subicular projections, was investigated in layer III of the medial and lateral entorhinal areas in the rat using retrograde labeling with horseradish peroxidase conjugated to wheat germ agglutinin and cholera toxin B subunit. Using two-dimensional unfolded maps of the entire hippocampal and parahippocampal fields, we found that cells projecting to a certain septotemporal level of CA1 and the subiculum were distributed in a band-like zone extending across the medial and lateral entorhinal areas. The transverse axis of these zones was disposed parallel to the rhinal fissure and their longitudinal axis was perpendicular to the boundary between the medial and lateral entorhinal areas. Projections to the septal CA1 originated from the zone near the rhinal fissure, whereas those to the temporal CA1 originated from the zone far from the rhinal fissure. Each zone in both the medial and lateral entorhinal areas involved many neurons projecting to a wide proximodistal range of CA1 and the subiculum. These results suggest that the entorhino-CA1 and entorhino-subicular perforant path projections are generally organized in a band-like zonal fashion with a gradient, rather than a point-to-point topographic arrangement.

1. Introduction

The entorhinal area (EA) is thought to play a crucial role in the neural circuitry underlying spatial representation and navigation, as various types of cells that participate in spatial recognition or directional orientation have been discovered in the rat EA (Witter and Moser, 2006). A physiological study by Sargolini et al. (2006) indicated that many grid cells, head-direction cells, and conjunctive cells (i.e., cells with conjunctive grid and head-direction properties) are distributed in the rat medial entorhinal area (MEA), and that a large proportion of conjunctive cells is located in layer III of MEA. Previous anatomical studies (e.g., Steward and Scoville, 1976) revealed that layer III of both MEA and the lateral entorhinal area (LEA) includes cells that give rise to the monosynaptic input pathway to CA1 and the subiculum (Sub). These EA layer III–CA1/Sub projections are important components of the perforant path, together with EA layer II–hippocampal projection (trisynaptic input pathway). Recently, a study using transgenic mice indicated that EA layer III–CA1 projection is more essential for temporal association memory than EA layer II–hippocampal projection (Suh et al., 2011).

Both EA layer III–CA1 and EA layer III–Sub projections are organized along proximodistal (i.e., close to or far from CA3; see Honda and Ishizuka, 2004) and septotemporal (i.e., dorsoventral) gradients. For instance, in the rat, MEA projects to the proximal part of CA1 and the distal part of Sub, whereas LEA projects to the distal CA1 and proximal Sub (Steward, 1976; Witter, 1993, 2006). These topographical differences in connectivity may hint at differences in the functional properties of the cells involved in spatial representation and navigation. Indeed, Henriksen et al. (2010) reported that spatial representation is organized along the transverse (proximodistal) axis of CA1 in the rat.

The question arises as to whether spatial and directional information issued from each part of the entorhinal area is provided to CA1 and Sub strictly in a point-to-point topographic manner. Is the innervation from MEA limited to the proximal (near CA3) part of CA1 and the distal (far from CA1) part of Sub, and is the innervation from LEA limited to the distal CA1 and proximal Sub?

Abbreviations: AB, angular bundle; CA, cornu ammonis; cc, corpus callosum; DG, dentate gyrus; EA, entorhinal area; hf, hippocampal fissure; IS, injection site; ld, lamina dissecans; LEA, lateral entorhinal area; MEA, medial entorhinal area; ParS, parasubiculum; PreS, presubiculum; PRh, perirhinal cortex; rf, rhinal fissure; RSG, retrosplenial granular cortex; Sub, subiculum.

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In order to answer these questions, the distribution of the cells of origin of EA layer III–CA1/Sub projections should be analyzed in the entire range of both MEA and LEA using retrograde-, as well as anterograde-labeling methods. However, in most previous reports, an anterograde axonal tracer was injected into several regions of the entorhinal area and the distribution of the labeled terminals was analyzed in restricted regions. Witter (2006) described the terminal fields of EA–CA1 and EA–Sub projections using unfolded representations. However, the distribution pattern of CA1 or Sub projection cells in layer III of the entire EA has not yet been reported. One of the possible obstacles to more thorough observation is that the hippocampal and parahippocampal areas form a distinctly curved shape.

The goal of the present study was to investigate the distribution pattern of perforant path cells in layer III along the entire extent of both MEA and LEA. Using the retrograde-labeling method, we studied the distribution pattern of EA–CA1 and EA–Sub projection cells in layer III of EA in sections cut perpendicular to the "extended" hippocampi (Ishizuka et al., 1990; Ishizuka, 2001; Honda and Ishizuka, 2004; Honda et al., 2008), and reconstructed the distribution in two-dimensional unfolded maps of the entire hippocampal and parahippocampal fields.

2. Materials and methods

The experimental procedures were approved by the Animal Care and Use Committee of Tokyo Women's Medical University, and all conformed to the guidelines for the care and use of laboratory animals (NIH). We used 18 adult male Wistar rats (290–320 g, Clea Japan Inc., Tokyo, Japan) and every effort was made to minimize the number of animals used. Of these rats, 14 were used for retrograde labeling with horseradish peroxidase–conjugated wheat germ agglutinin (WGA–HRP), and 4 for retrograde labeling with cholera toxin B subunit (CTB).

2.1. Surgery and injections

The rats were initially anesthetized with isoflurane (Forane; Abbott Laboratories, North Chicago, IL, USA), and a surgical level of anesthesia was maintained by intramuscular injection of a mixture of ketamine (60 mg/kg body weight, Ketalar 50, Parke-Davis, Ann Arbor, MI, USA) and xylazine (20 mg/kg body weight, Selaktar, Bayer, Leverkusen, Germany). Each animal was placed in a stereotaxic frame, and a hole was drilled in the skull at coordinates derived from the atlas of Paxinos and Watson (1986) (Table 1). Then, a glass micropipette (outer diameter, 30–50 μm) filled with 4–8% WGA–HRP (Toyobo, Osaka, Japan) in phosphate buffer (0.1 M, pH 7.4) was lowered through the hole with manipulator guidance. The tracer solution (10–40 nl) was injected by pressure using a 1-μl Hamilton syringe that was inserted into each glass capillary tube (Table 1). A solution of 1% CTB (List Biological Laboratories, Inc., Campbell, CA, USA) dissolved in phosphate buffer (PB; 0.1 M, pH 7.4) was also injected iontophoretically in separate animals using a 2-mm glass capillary tube with a filament inside (tip outer diameter, approximately 30 μm). A positive current of 5.0 μA was delivered for 20–30 min using a high-voltage current source (Midgard™, Stoelting, Wood Dale, IL, USA) at a rate of 7 s on/7 s off. All injections were performed unilaterally (left side).

2.2. Fixation and cutting

After a survival period of 48 h (WGA–HRP injections) or 7 days (CTB injections), rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (80 mg/kg body weight, Nembutal, Abbott Laboratories), and perfused transcardially with physiological saline followed by a fixative solution. We used a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in PB in both WGA–HRP and CTB experiments. After the brains were removed, we made the "extended" hippocampal formation in order to facilitate analysis of the laminar and topographical pattern of retrogradely labeled cells. Procedures were performed as described in detail elsewhere (Ishizuka et al., 1990; Ishizuka, 2001; Honda and Ishizuka, 2004; Honda et al., 2008). In brief, cerebral hemispheres were dissected free from the diencephalon by transecting the corpus callosum, internal capsule, and basal part of the forebrain. To reduce the natural concavity of the hippocampal formation and parahippocampal areas, the dissected hemispheres were gently "flattened" in a fixative between two filter papers. After postfixation for 6–8 h, the blocks were stored in 20% glycerin solution in PB overnight for cryoprotection. Transverse sections of the flattened hemisphere, perpendicular to the "extended" septotemporal (longitudinal) axis of the hippocampal formation, were cut at a thickness of 50 μm using a freezing microtome.

2.3. HRP histochemistry

Sections were collected in order in ice-cold PB (0.1 M, pH 6.0). For detection of HRP activity, the modified TMB-AHM (tetramethyl benzidine-ammonium heptamolybdate) method (Olucha et al., 1985) was used. The sections were incubated in a solution of 0.25% AHM, 0.2% TMB (Dotite TMBZ, Dojin Chemical, Tokyo, Japan), and 0.008% H_2O_2 in PB for 2 h at room temperature. After being washed in PB, the sections were divided into two series of alternating sections and mounted onto gelatin-coated glass slides. One of the series was counterstained with neutral red, while the other was left unstained for dark-field microscopy. The specimens were

Table 1
Summary of tracer injection locations and volumes.

Injection site	Case no.	Tracer (concentration)	Volume in nl	Coordinates		
				AP (from Bregma)	LM	DV
Septal, proximal CA1	H1	WGA–HRP (6%)	40	−2.8	1.0	3.1
Septal, distal CA1	H3	WGA–HRP (6%)	40	−3.6	1.4	2.7
mid-septotemporal, proximal CA1	S3	WGA–HRP (4%)	40	−5.5	4.7	4.28
mid-septotemporal, mid-proximodistal CA1	S75	WGA–HRP (4%)	20	−5.2	4.0	3.1
Temporal, proximal CA1	H4	WGA–HRP (6%)	40	−5.4	5.4	7.6
Temporal, distal CA1	S76	WGA–HRP (4%)	30	−6.0	4.8	3.86
Septal, distal Sub	H2	WGA–HRP (6%)	40	−5.6	1.9	3.1
mid-septotemporal, mid-proximodistal Sub	S73	WGA–HRP (4%)	40	−6.8	3.8	3.6
mid-septotemporal, proximal Sub	S17	WGA–HRP (8%)	10	−7.4	4.0	5.32
mid-septotemporal, distal Sub	S74	WGA–HRP (4%)	40	−6.8	3.8	3.6
Temporal, mid-proximal	CH2	CTB (1%)	[a]	−7.2	5.2	4.6
Temporal, distal	S18	WGA–HRP (8%)	10	−7.29	4.0	5.32

[a] CTB was injected iontophoretically as described in text (exact volume unknown).