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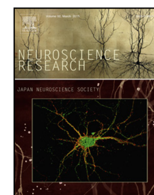

Topographic distribution of cortical projection cells in the rat subiculum

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ABSTRACT

The topographic distribution of subicular pyramidal cells, which give rise to projections to the entorhinal cortex, presubiculum, parasubiculum, and the retrosplenial granular cortex, was investigated in the rat using retrograde labeling with wheat germ agglutinin-horseradish peroxidase. Using two-dimensional unfolded maps of the entire hippocampal and parahippocampal fields, we found that the cells originating the projections to the above cortical areas were consistently observed throughout the entire septotemporal extent of the subiculum. In the transverse plane, most of the cortical projection cells were vertically located in the middle region of the subicular pyramidal cell layer. The cells giving rise to the projections to the lateral entorhinal cortex were predominantly located in the most proximal (near CA1), superficial region. Few cortical projection cells were located in the deepest (adjacent to the angular bundle) region. The distribution of cortical projection cells showed an oblique tri-laminar pattern, which was similar to the previously reported laminar pattern of subcortical projection cells in the subiculum. These results suggest that cortical projection cells in middle and superficial regions of the subiculum may correspond to layer V of the isocortex and cells in the deepest region corresponding to layer VI.

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1. Introduction

Memory signals processed in the internal circuit of the hippocampal formation are propagated to the subcortical and cortical structures. These efferent systems of the hippocampus originate primarily from the subiculum (Swanson and Cowan, 1977; Sørensen and Shipley, 1979; Sørensen, 1980). We previously investigated the descending systems of the subiculum, and reported that cells giving rise to projections to the nucleus accumbens, the medial mammillary body, and the anteroventral thalamic nucleus are arranged in a laminar fashion in the subicular pyramidal cell layer (Ishizuka, 2001). In addition, the subiculum gives rise to cortical projections to the entorhinal cortex, presubiculum, parasubiculum, retrosplenial granular cortex, perirhinal cortex, postrhinal cortex, and the anterior cingulate cortex (guinea pig: Sørensen and Shipley, 1979; Sørensen, 1980, rat: Köhler, 1985; van Groen and Wyss, 1990; Kloosterman et al., 2003; Jay and Witter, 1991; Agster and Burwell, 2013, cat: van Groen et al., 1986, monkey: Amaral et al., 1984; Blatt and Rosene, 1998; Kobayashi and Amaral, 2003). However, it has not been elucidated how these cortical projection cells are organized throughout the subiculum. In the present study, a retrograde labeling technique was used to investigate the organization of subicular cells, which give rise to projections to the entorhinal cortex, presubiculum, parasubiculum, and retrosplenial granular cortex.

2. Materials and methods

The experimental procedures were approved by the Animal Care and Use Committees of both Tokyo Women's Medical University and Tokyo Metropolitan Institute of Medical Science. All procedures conformed to the guidelines for the care and use of laboratory animals (NIH). We used 22 adult male Wistar rats (270–320 g, Clea Japan Inc., Tokyo, Japan) and every effort was made to minimize the number of animals used. All animals were used for experiments involving retrograde labeling with wheat germ agglutinin – horseradish peroxidase (WGA-HRP). Injections were made into the retrosplenial granular cortex (RSG), presubiculum (Pre),

Abbreviations: AB, angular bundle; CA, cornu ammonis; cc, corpus callosum; DG, dentate gyrus; EC, entorhinal cortex; hf, hippocampal fissure; IS, injection site; LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex; OCC, occipital cortex; Par, parasubiculum; Pre, presubiculum; PRh, perirhinal cortex; rf, rhinal fissure; RSG, retrosplenial granular cortex; RSA, retrosplenial agranular cortex; Sub, subiculum.

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Table 1
Summary of tracer injection locations and volumes.

Injection site			Case no.	% conc. of WGA-HRP	Volume in nl	Coordinates		
Area	Septotemporal localization	Proximodistal localization				AP (from Bregma)	LM	DV
RSG	Septal	Distal	S63	4	20	−2.3	0.51	2.41
RSG	Mid	Proximal	S65	4	10	−4.8	0.3	1.5
RSG	Temporal	Distal	S43	4	20	−7.3	1.4	2.2
RSG	Temporal	Mid-to-distal	S66	4	10	−6.3	1.0	2.5
Pre	Septal	Mid-to-distal	S33	8	70	−7.3	2.9	3.0
RSG Pre	Temporal Septal	Proximal Mid-to-distal	S34	8	77	−7.6	2.4	3.0
Pre	Mid	Mid-to-proximal	S31	8	62	−7.3	3.2	3.4
Pre	Mid	Mid	S29	8	30	−7.3	3.2	3.4
Pre Par	Mid Septal	Distal Proximal	S61	4	25	−8.3	3.1	3.2
Pre Par	Mid-to-temporal Temporal	Proximal-to-distal	H19	4	30	−6.8	3.8	7.1
MEC Par	Septal Septal	Proximal-to-distal Mid-to-distal	H8	4	40	−8.72	3.5	4.2
MEC	Mid-to-septal	Mid-to-distal	S45	4	60	−8.4	4.2	5.6
Par MEC	Mid Septal	Proximal-to-distal Proximal	H41	4	20	−7.64	4.1	5.32
MEC	Temporal	Mid-to-distal	H31	4	20	−8.2	5.4	6.38
MEC Sub	Temporal Temporal	Mid-to-proximal Mid-to-distal	S46	4	60	−7.8	5.0	7.6
LEC	Septal	Mid-to-distal	H34	4	20	−8.0	6.2	6.6
LEC	Mid	Mid-to-distal	S48	4	20	−7.4	5.8	7.6
LEC	Temporal	Mid-to-distal	H36	4	20	−5.2	6.8	7.8
LEC	Septal	Mid	LEA3	4	20	−7.04	5.4	7.4

parasubiculum (Par), medial entorhinal cortex (MEC), and lateral entorhinal cortex (LEC) at various sites along the septotemporal and proximodistal axes. Of these, 19 cases are described in Table 1. In all cases, many subicular neurons were retrogradely labeled on the same side of the injection, with only a few observed in the contralateral side. Thus, in the present study, the analysis was focused on the ipsilateral side of the injection.

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). A glass micropipette (outer diameter, 30–50 μm) filled with 4–8% WGA-HRP (Toyobo, Osaka, Japan) in 0.1 M phosphate buffer (PB), pH 7.4, was lowered through the hole with manipulator guidance. The tracer solution (10–93 nl) was injected under pressure using a 1 μl Hamilton syringe that was inserted into the glass capillary tube (Table 1).

2.2. Fixation and cutting

After a survival period of 48 h, 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 containing 4% paraformaldehyde and 0.1% glutaraldehyde in PB. After the brains were removed, we configured the tissue into the “extended” hippocampal formation in order to facilitate analysis of the laminar and topographical patterns 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 the basal region 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 hemispheres, 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

Ordered sections were collected 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% H2O2 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 dehydrated in a graded series of alcohols, cleared with xylene, and coverslipped.

2.4. Data analysis

The locations of the retrogradely labeled cells, the sites of tracer injections, and the borders of cortical areas and layers were mapped using a computer-assisted microscope system (Neurolucida™, MicroBrightField, Inc., Colchester, VT, USA). In addition, two-dimensional unfolded maps were prepared, as previously described (Honda and Ishizuka, 2004), to aid in the

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