Neuroscience 337 (2016) 242–266

IMMUNOHISTOCHEMICAL INVESTIGATION OF THE INTERNAL STRUCTURE OF THE MOUSE SUBICULUM

YOSHIHISA ISHIHARA AND TAKAICHI FUKUDA *

Department of Anatomy and Neurobiology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-8556, Japan

Abstract—The subiculum is the output component of the hippocampal formation and holds a key position in the neural circuitry of memory. Previous studies have demonstrated the subiculum's connectivity to other brain areas in detail; however, little is known regarding its internal structure. We investigated the cytoarchitecture of the temporal and mid-septotemporal parts of the subiculum using immunohistochemistry. The border between the CA1 region and subiculum was determined by both cytoarchitecture and zinc transporter 3 (ZnT3)-immunoreactivity (IR), whereas the border between the subiculum and presubiculum (PreS) was partially indicated by glutamate receptor 1 (GluR1)-IR. The subiculum was divided into proximal and distal subfields based on cytoarchitecture and immunohistochemistry for calbindin (CB), nitric oxide synthase (NOS) and Purkinje cell protein 4 (PCP4). The proximal subiculum (defined here as subiculum 2) was composed of five layers: the molecular layer (layer 1), the medium-sized pyramidal cell layer (layer 2) that contained NOS- and PCP4-positive neurons, the large pyramidal cell layer (layer 3) characterized by the accumulation of ZnT3- (more proximally) and vesicular glutamate transporter 2-positive (more distally) boutons, layer 4 containing polymorphic cells, and the deepest layer 5 composed of PCP4-positive cells with long apical dendrites that reached layer 1. The distal subiculum (subiculum 1) consisting of smaller neurons did not show these features. Quantitative analyses of the size and numerical density of somata substantiated this delineation. Both the proximal–distal division and five-layered structure in the subiculum 2 were confirmed throughout the temporal two-thirds of the subiculum. These findings will provide a new structural basis for hippocampal investigations. **© 2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: subiculum, immunohistochemistry, ZnT3, VGluT2, NOS, PCP4.

INTRODUCTION

The hippocampal formation plays a crucial role in learning and memory. This formation consists of three components, including the dentate gyrus, Ammon's horn and subiculum, and information is primarily transferred in this sequence. Therefore, the subiculum is the output region of the hippocampal formation (Rosene and Van Hoesen, 1977; Witter and Groenewegen, 1990; O'Reilly et al., 2013), which receives major intrahippocampal afferents from the CA1 region of Ammon's horn and sends efferents to many cortical and subcortical regions. The subiculum also establishes reciprocal connections with its targets (O'Mara et al., 2001; Witter and Amaral, 2004; Ding, 2013). Thus, the subiculum holds a key position in the neural circuitry of memory formation. Previous studies have established the details of the afferent and efferent connections of the subiculum, and the pronounced topographical relationships between the source and target areas of these connections have been demonstrated (Tamamaki and Nojyo, 1990; Witter and Groenewegen, 1990; Canteras and Swanson, 1992; Ishizuka, 2001: Witter, 2006; O'Reilly et al., 2013; Honda and Ishizuka, 2015).

However, the internal structure of the subiculum has only been partially understood, and many fundamental issues remain to be examined, including the exact boundaries between the subiculum and neighboring regions, the presence or absence of divisions inside the subiculum, the number of laminations in principal cell layers, and the potential differences along its septotemporal (dorsoventral) and proximodistal axes. This is in sharp contrast compared with the Ammon's horn and dentate gyrus, in which both the morphological and physiological properties have been investigated in substantial detail (Frotscher et al., 1988; Freund and Buzsáki, 1996; Johnston and Amaral, 2004; Witter and Amaral, 2004; Witter, 2012).

The greater part of the subiculum is located between the presubiculum (PreS) and the hippocampal CA1 region, whereas its septal end adjoins the retrosplenial

*Corresponding author. Address: Department of Anatomy and Neurobiology, Graduate School of Medical Sciences, Kumamoto University, Honjo 1-1-1, Chuo-ku, Kumamoto 860-8556, Japan.
E-mail address: tfukuda@kumamoto-u.ac.jp (T. Fukuda).

Abbreviations: AChE, acetylcholine esterase; BLA, basolateral nucleus of amygdala; BMA, basomedial nucleus of amygdala; BSA, bovine serum albumin; CB, calbindin; CLSM, confocal laser scanning light microscopy; DAB, diaminobenzidine chloride; GAD, glutamic acid decarboxylase; GluR1, glutamate receptor 1; IB, intrinsically bursting; IR, immunoreactivity; NADPH-d, nicotinamide adenine dinucleotide phosphate diaphorase; NeuN, neuronal nuclei; NOS, nitric oxide synthase; PaS, parasubiculum; PB, phosphate buffer; PBS, phosphate-buffered saline; PCP4, Purkinje cell protein 4; PreS, presubiculum; RS, regular spiking; Sub, subiculum; VGluT1, vesicular glutamate transporter 1; VGluT2, vesicular glutamate transporter 2; VTA, ventral tegmental area; ZnT3, zinc transporter 3.

http://dx.doi.org/10.1016/j.neuroscience.2016.09.027
0306-4522/© 2016 IBRO. Published by Elsevier Ltd. All rights reserved.

granular cortex instead of the PreS. The borders between these regions and the subiculum are rather difficult to define, particularly in the temporal hippocampus. For example, the objective boundary between the PreS and subiculum can be determined only in their superficial layers based on the differences in both the cytoarchitecture (Ramón y Cajal, 1911; Blackstad, 1956; Slomianka and Geneser, 1991a,b; Witter and Amaral, 2004; Witter, 2012; Ding, 2013) and histochemistry for acetylcholine esterase (AChE) (Slomianka and Geneser, 1991a,b; Fujise et al., 1995; Ding, 2013). In contrast, the deep cell layers of both the PreS and subiculum appear to form a continuum, and AChE histochemistry does not visualize the clear border in this location. The other border between the CA1 region and subiculum is also unclear at the temporal level because the cell layer of the temporal CA1 gradually widens toward the subiculum rather than exhibiting an abrupt widening as demonstrated in the septal hippocampus.

The potential partition of the subiculum into two divisions, the proximal and distal subiculum, has also been problematic. Ramón y Cajal (1911) regarded the subiculum as one homogeneous region, whereas Lorente de Nó (1934) divided the subiculum into two components: the subiculum and prosubiculum. The "subiculum" defined by Lorente de Nó may comprise the distal region of the subiculum near the PreS, and the "prosubiculum" may comprise the proximal region of the subiculum close to the CA1 region. Since then, it has long been debated whether the "prosubiculum" exists, and a conclusion has not been reached. One reason for a lack of consensus may be the confusion about the usage of "prosubiculum"; Lorente de Nó (1934) defined the area based on the detailed observations of its differential cytoarchitecture, whereas this term has sometimes been used to indicate narrow transitional zone between the subiculum and the CA1 region (Rosene and Van Hoesen, 1987), which, at least in rodents, appears to be different from the region identified by Lorente de Nó.

The aim of this study is to investigate the internal structure of the subiculum. We used immunohistochemistry for various substances and acquired objective chemical markers to delineate several subfields and layers within the subiculum. The septotemporal differences in the newly identified structures are also demonstrated. The present results will provide a morphological basis for not only the investigation of connectivity between the subiculum and other brain regions but also physiological investigations in the subiculum and other related areas that are responsible for memory formation.

EXPERIMENTAL PROCEDURES

Tissue preparation

All experiments and animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996), and all protocols were approved by the Institutional Animal Care and Use Committee at Kumamoto University. All efforts were made to minimize the number of animals used and their suffering.

Twenty-five male C57BL/6J mice (21–25 g, 7–8 weeks old) were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.). The mice were perfused via the ascending aorta with phosphate-buffered saline (PBS, pH 7.4), followed by 50 ml of 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer (PB, pH 7.4) at room temperature. The brains were removed from the skull and stored overnight in 4% paraformaldehyde in 0.1 M PB at 4 °C. The next day, the fixative was replaced by PBS that contained sodium azide.

Immunohistochemistry

Serial 40-μm-thick sections were cut in a horizontal (n = 19 animals) or sagittal (n = 6 animals) direction using a vibrating microtome (TTK-3000, Dosaka, Kyoto, Japan) from the brain block that contained the entire hippocampal formation. Following cryo-protection in 25% sucrose in PBS, the sections placed on aluminum foil were rapidly frozen in the vapor of liquid N_2, rapidly thawed in 25% sucrose in PBS, and subsequently processed for immunohistochemistry, as previously described (Fukuda and Kosaka, 2000; Tajima and Fukuda, 2013). Briefly, for the immunoperoxidase staining, the sections were incubated with 1% bovine serum albumin (BSA; Sigma–Aldrich, St. Louis, MO, USA) and 0.3% Triton-X in PBS overnight, with a primary antibody listed in Table 1 diluted in 1% BSA–0.3% Triton-X in PBS for 7 days at 20 °C, with a biotinylated secondary antibody listed in Table 2 overnight, and with a mixture of avidin DH and biotinyl HRP (Vectastain ABC Standard Kit, Vector Laboratories, Burlingame, CA, USA) in PBS-0.3% Triton-X for 3 h at 20 °C. The sections were stained using diaminobenzidine chloride (DAB) as a chromogen, dried on glass slides, treated with 0.4% OsO_4 in PB for 10 min to enhance the staining intensity, and embedded in Multi Mount 480 (Matsunami Glass Ind., Kishiwada, Japan). A long incubation period with the primary antibodies was essential to improve the permeation of the antibodies into the deep part of the 40-μm-thick sections (Fukuda et al., 1998; Fukuda and Kosaka, 2000).

For the triple fluorescent immunohistochemistry, the sections were incubated with 1% BSA and 0.3% Triton-X in PBS overnight, with a mixture of mouse anti-calbindin (CB) (1:5000, Swant, Marly, Switzerland), rabbit anti-zinc transporter 3 (ZnT3) (1:2500, Synaptic Systems, Goettingen, Germany) and guinea-pig anti-vesicular glutamate transporter 2 (VGluT2) (1:1000, Frontier Institute, Ishikari, Japan) antibodies diluted in 1% BSA and 0.3%Triton-X in PBS for 7 days at 20 °C, with biotinylated donkey anti-mouse IgG (1:500, Jackson ImmunoResearch, West Grove, PA, USA) overnight, and with a mixture of streptavidin-Alexa 647 (1:1000, Jackson ImmunoResearch), Alexa 488-conjugated donkey anti-rabbit IgG (1:200, Millipore) and Cy3-conjugated donkey anti-guinea pig IgG (1:200, Millipore) overnight. Several other sets of double or triple immunostaining were performed by combining the primary and secondary antibodies listed in Tables 1 and

Download English Version:

https://daneshyari.com/en/article/6270729

Download Persian Version:

https://daneshyari.com/article/6270729

Daneshyari.com