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LAMINAR ORGANIZATION OF HUMAN SUBICULUM PROPER

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LAMINARNA ORGANIZACIJA SUBICULUM PROPER ČOVEKA

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SAŽETAK

Ljudski subiculum proper je laminarna struktura koja predstavlja troslojni arhikorteks smešten između arhikorteksa hippocampus proper-a i šestoslojnog entorinalnog korteksa. Subiculum proper predstavlja mesto izlaska aksona iz cele hipokampalne formacije. Nasuprot hippocampusu koji je intenzivno proučavan postoji veoma malo informacija o ljudskom subiculum proper-u.
Cilj našeg istraživanja bio je identifikacija morfoloških karakteristika njegovih lamina upotrebom Golgi i Nissl tehnika bojenja. Istraživanje je izvršeno na 10 ljudskih mozgova (20 hemisfera), oba pola.
Naši rezultati pokazuju postojanje tri lamine ljudsog subiculum proper-a. Molekularni sloj nalazi se najpovršnije, ka pialnoj površini. U dubljim delovima ovog sloja, prema piramidalnom sloju, ukazali smo na prisustvo sporadič nih neurona netipičnih za ovaj sloj. Središni sloj, piramidalni, sastavljen je od piramidalnih neurona. Ovaj sloj je jasno podeljen na dve sublamine, spoljašnju i unutrašnju. Aksoni svih piramidalnih ćelija usmereni su ka molekularnom sloju. Polimorfni sloj je najdublji i u njemu se nalaze različiti tipovi neurona.
Buduća istraživanja morfoloških karakteristika neurona ljudskog subiculum proper-a, daće veliki doprinos razumevanju njegove značajne funkcije, a posebno uloge u memoričkim procesima.
Ključne reči: subiculum, hippocampus, Golgi

ABSTRACT

Human subiculum proper is the laminar structure represented by a three archicortical layers, that is placed between archicortex of hippocampus proper and a six-layers entorhinal cortex. Subiculum proper is the origin for the axons of the whole hippocampal formation. The informations about human subiculum proper are quite limited, in contrast to hippocampus which was intensively studied. The purpose of our research was identification of morphological particularity of its laminae, by using Golgi and Nissle method of staining. The research was performed on 10 brains (20 hemispheres) of both gender. In our investigation we found three layers of human subiculum proper. The superficial, molecular layer is directed to pial region. In deeper parts of this layer, toward the pyramidal layer, we pointed to sporadical presence of neurons which are considered as untypical for the superficial lamina. Middle layer is pyramidal, composed of pyramidal neurons. This layer is clearly divided into external and internal sublamina, and the axons of all pyramidal cells are directed to molecular layer. The polymorphic layer is the deepest one, and there are different types of neurons in it.
Further examinations of human subiculum proper that are related to morphological characteristics of neurons, would give the great contribution to the clarification of its important functions, connected with distribution of axons of hippocampal formation toward the other parts of brain, and to assumptive part in memory processes.
Key words: subiculum, hippocampus, Golgi

INTRODUCTION

Hippocampal formation is limbic structure that consists of: hippocampus proper (fields CA1, CA2 and CA3), gyrus dentatus, entorhinal cortex, and subicular complex (1). Subicular complex comprises several fields whose number and names varied in course of time, but there is general agreement that the subicular complex consists of three fields: subiculum proper, presubiculum and parasubiculum (1-3). Anatomical, physiological and functional characteristics of hippocampus proper and gyrus dentatus are intensively examined (1, 4-7). In contrast to that, subiculum proper is less explored structure, although it is the main place where the axons come out of the whole hippocampal formation (1, 2, 8).
The studies of subiculum proper showed that it is archicortex, composed of three layers, in which the superficial layer is molecular layer which is continuous with stratum lacunosum-moleculare and radiatum of the CA1 field (3). A wide pyramidal cell layer with large pyramidal neurons is deeper, and the deepest is polymorphic layer. Braak divided pyramidal layer into two sublaminae: external sublamina, near the molecular layer, and internal sublamina (9). According to Braak`s pigmentoarchitectonic examinations, there is accumulation of lipofuscin in the proximal portion of apical dendrites of pyramidal cells of the external layer. The main layer of cells of subiculum proper, the pyramidal layer, is composed of large pyramidal neurons that are equal in shape and size, with apical dendrites directed to the molecular layer, while basal dendrites are directed to the deep parts of pyramidal layer. Pyramidal cells of this layer of subiculum proper are less densely packed than those that are seen in CA1 field, and among them are smaller neurons – interneurons (2, 10).
Cognitive brain functions, memory, learning and their disorders are subject of interest of the scientists from

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the ancient times. Behterev (1900) was the first who pointed out the importance of subicular complex in memory processes, when he studied disorders that were caused by the damage of temporal lobe (11). Today it is considered that the hippocampal formation and its component, subiculum proper are the place of consolidation of memory, transferring the short-term memory into long-term, permanent memory (12). Beside its part in the process of learning and remembering, subiculum proper influences behaviour of an individual in stressful situations. Mechanism of the response of the organism to the stress goes through hypothalamus-pituitary-adrenal gland (HPA) axis. Many limbic structures have the influence on HPA axis. The amigdala and nucleus of the stria terminalis have excitatory effect. Hippocampal formation has inhibitory influence on HPA axis, as ventral part of subiculum proper inhibits HPA axis transsynaptically through GABA-ergic neurons that gives projections directly to paraventricular nucleus or hypothalamic autonomous control system (13, 14).

Some changes are discerned in subiculum proper in different pathological conditions as: Alzheimer's disease (15-17), temporal epilepsy (18, 19) and schizophrenia (20, 21). The studies of subiculum proper were done on brains of rats and monkeys in the past (3). The intention was paid to examination of neuron function in the region of CA1-subiculum proper of rat (3, 22). The studies of human subiculum proper are scarce.

Hippocampal formation has laminar organization (23, 24). Subiculum proper together with hipocampal fields CA1, CA2, CA3 and gyrus dentatus is a three-layer archicortex in cytoarchitectonic manner, while presubiculum, parasubiculum and entorhinal cortex are consisted of six layers. According to the great functional importance of subiculum proper, the purpose of our examination was discerning of potentially morphological particularity of its laminae, according to more explored other parts of hippocampal formation, first of all hippocampus.

MATERIAL AND METHODS

The examination of laminar organization of human subiculum proper was performed on 10 human brains (20 hemispheres), of both gender, 25 to 75 years old. The brains were with no discernible pathological changes and without neuropsychiatric anamnesis. After fixation in 10% puferised solution of formaldehyde that lasted at least three months, brains were subjected to Golgi and Nissl method of staining. The blocks of tissue dimensions of 2x2x1 cm were used for Golgi method. After fixation in formaldehyde blocks of tissue were treated with 2.5% KcrO5 at 37 C temperature in dark, with frequent changes of solution during 2 to 4 days. The block of tissue was rinsed in 2.5% solution of AgNO3. The next step was impregnation of the block in 2.5% solution of ANO3 during 4 days in dark at room temperature. After transferring through series of alcohol with the rising concentration (60%-100%), block of tissue was shaped in paraffin. The block was cut into samples 80 to 100 μm on microtome. Deparaffinisation of a sample was performed on micro slides, and the tissue was covered by DPX and cover glasses. For Nissl method paraffin blocks were cut into tissue samples 8–10 μm wide. After that preparations were deparaffinised and led to the solution of the lowest concentration by successive changing of the solution. Those preparations were stained in 1% of water solution of Cresyl violet for 60 min. After rinsing and differentiation by acetate acid and covering by DPX, preparations were covered by cover glass. Luminous microscopy of the preparations was executed on luminous microscope Leica DMLB.

RESULTS

The laminar organization of subiculum proper that is composed of three layers: molecular, pyramidal and polymorphic, is clearly discernible on the preparations of human brain stained by Nissl method. Morphological characteristics of neurons of those layers are decribed by observerving the preparations of human subiculum proper that are stained by Golgi method. Molecular layer of subiculum proper is placed superficially in relation to pyramidal and polymorphic layer. It is placed toward pial area, i.e. hippocampal fissure (Figure 1).

Figure 1. Layers of subiculum proper, Nissl method (x200).

In this layer, on Nissle stained preparations, there are sparse smaller bipolar and larger multipolar neurons (beside granular cells of glia), that do not have the characteristics of deeper placed pyramidal layer. Those sparse neurons of molecular layer are specially discernible in Golgi preparations from our series. They are located only in the deeper parts of this layer, toward pyramidal layer, while they are not discernible in the other parts and according to their morphological characteristics they do not belong to pyramidal neurons. The fibres that penetrate from adjacent pyramidal layer are visible in molecular layer (Figure 2).

The middle layer is pyramidal. It is named after pyramidal cells, that are the most numerous in this layer. In Golgi stained human preparations, pyramidal cells with its morphological characteristics are clearly visible (Figure 3).

Figure 2. Molecular layer of human subiculum proper, Golgi method (x200).

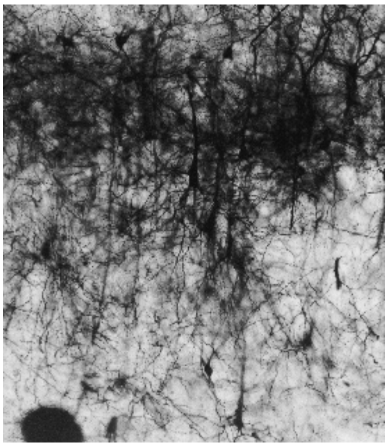

Figure 3. Pyramidal layer of human subiculum proper, Golgi method (x200).

Apical dendrites of all pyramidal neurons are directed to molecular layer. In this layer neurons that are among pyramidal neurons are visible, but they are not of the same morphological characteristics as those. This layer was more intensively coloured in Nissle too (Figure 1), that conditionally can be used as comparative criterion of this layer in relation to the other. There are two visible laminae in pyramidal layer in Nissle stained preparations: external and internal. External lamina contains pyramidal cells that are larger than the eponymous in internal lamina (Figure 4).

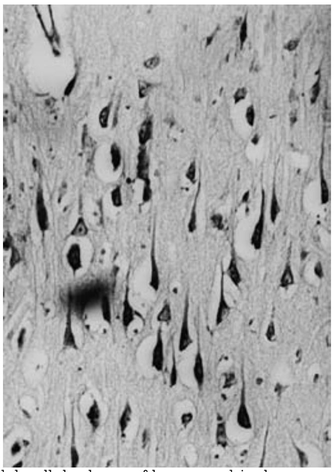

Figure 4. Pyramidal cellular layer of human subiculum propr, Nissl method (x 200).

The deepest layer of subiculum proper is polymorpic layer. The different types of neurons are present in this layer (Figure 5).

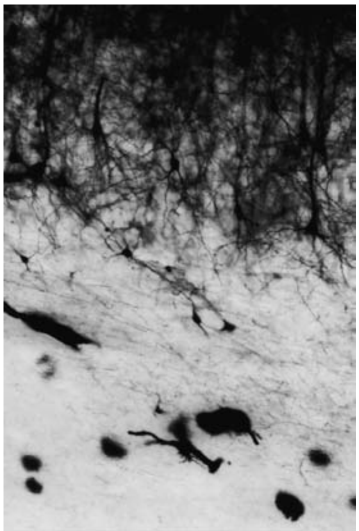

Figure 5. Polymorphic layer of human subiculum proper, Golgi method (x200).

DISCUSSION

There is a significant disproportion in literature in number of research articles devoted to such functionaly important morphological complex as hippocampal formation. It is very interesting that one part of hippocampal formation, hippocampus proper, is probably the most studied part of brain, while on the other side, there is incomparably less data about subiculum, that is functionally unbreakable connection with hippocampus proper.

Intensive studying of hippocamppus gave the enormous contribution to majority of the general processes in the brain, that are not typical only for pyramidal cells CA1 field of hippocampus, on which the studies were most often performed. Lately, some authors who were led by the fact that complete comprehension of function of hippocampus is not possible without comprehension of its executive part, paid more attention to this region. The aim of our study was to further investigate this brain region. Using Golgi method of staining the preparations we got by the series of frontal section of human hippocampal formation, we found three layers of subiculum proper: molecular, pyramidal and polymorphic. Eponymous layers were described by Amaral and Insauti (1), and O'Mara (3). This three-layer structure is very similar according to its substance to hippocampus proper, i.e. primeval archicortex. Observing the molecular layer that is superficially located, toward the pial region, it can be concluded that it is the continuation of the eponymous layer of hippocampal field CA1. Amaral and Insauti (1) and O'Mara (3) describing the molecular layer of subiculum proper indicated that it is the continuation of strata lacunosum-moleculare and radiatum of the CA1 field of the hippocampus.

Specially interesting findings on our preparations are concerned with the molecular layer of subiculum. Traditionally molecular layer is the nearest to pia and there are no neurons in it. It is specific by the presence of the fibres that come from the other layers of this structure. In our preparations stained by Golgi method, neurons in the molecular layer of subiculum proper are clearly visible.

Going deeper from the pial region, after molecular layer we come to the pyramidal layer of cells in which there are pyramidal neurons. This layer is the most important part of subiculum proper. Describing the pyramidal layer in Nissle stained preparation of the brain of rat, Braak (9) discerned two laminae: external and internal. Our examination on Nissle stained preparations of human brain indicates that there are two layers or laminae in the pyramidal layer, external lamina, in which pyramidal cells with larger bodies are located, and internal lamina. The pyramidal cells of the internal lamina that is closer to the polymorphic layer are with smaller bodies in comparison to the eponymous cells of external lamina. These results are similar to the Braak`s findings (9). Observing the pyramidal layer of cells we can say that it's the most important and the most numerous cells are pyramidal cells. As Shane O'Mara described this layer in the different animal species, we can agree with his conclusion that the apical dendrites of pyramidal cells are directed toward the molecular layer, while basal dendrites are directed to the deeper parts of the pyramidal layer. Among the pyramidal neurons of pyramidal layer, there are neurons that are diferent from the typical pyramidal neurons. In the experiments performed on the rats, Swanson (10), Amaral and Witer (2) named these neurons interneurons. These authors clasiffied all neurons that are smaller than pyramidal neurons as interneurons. We did not group these neurons because we thought that we should know the morpological characteristics of these neurons that are still unknown.

The characteristics of polymorphic layer of cells are still unknown both in animals and in humans. Our results indicate that in this layer there are different types of neurons, as was indicated by Amaral and Insauti (1). It is obvious that in this layer there are no cells that are similar according to morphological characteristics to the pyramidal cells.

Studying those morphological characteristics of subiculum proper that are still unknown, we would get more informations that are necessary for understanding the complex memory functions in which the bigger importance of subiculum proper is approved. Memory function disorder is of great clinical importance since psychiatric disorders are common and increasingly frequent.

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