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MORPHOMETRIC CHARACTERISTICS OF THE NEURONS OF THE HUMAN SUBICULUM PROPER

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Abstract - The human subiculum is a significant part of the hippocampal formation positioned between the hippocampus proper and the entorhinal and other cortices. It plays an important role in spatial navigation, memory processing and control of the response to stress. The aim of our study was identification of the morphometric characteristics of the neurons of the human subiculum proper: the maximum length and width of cell body and total dendritic length and volume of cell body. Comparing the measured parameters of different types of subicular neurons (bipolar, multipolar, pyramidal neurons with triangular-shaped soma and neurons with oval-shaped soma), we can conclude that bipolar neurons have the lowest values of the measured parameters: the maximum length of their cell body is 14.1 ± 0.2 μm, the maximum width is 13.9 ± 0.5 μm, and total dendritic length is 14597 ± 3.1 μm. The lowest volume value was observed in bipolar neurons; the polymorphic layer is 1152.99 ± 662.69 μm^3. The pyramidal neurons of the pyramidal layer have the highest value for the maximal length of the cell body (44.43 ± 7.94 μm), maximum width (23.64 ± 1.89 μm), total dendritic length (1830 ± 466.3 μm) and volume (11768.65±4004.9 μm^3) These characteristics of the pyramidal neurons indicate their importance, because the axons of these neurons make up the greatest part of the fornix, along with the axons of neurons of the CA1 hippocampal field.

Key words: Neurons, human subiculum, hippocampal formation, dendrites, morphometry

INTRODUCTION

The human hippocampal formation is a complex structure which consists of the hippocampus proper (fields CA1, CA2, and CA3), gyrus dentatus, the subicular complex and entorhinal cortex (Insauti and Amaral, 2004). The subiculum proper is a part of the subicular complex that includes the presubiculum and parasubiculum (Amaral and Insauti, 1990; Amaral and Witter, 1995; O'Mara et al., 2001). It is one of the links of the trisynaptic circuit, is a major target of CA1 projections and represents the principal outflow of the hippocampal formation. The subiculum in turn projects to the hippocampus, providing massive, topographically organized innervation to the limbic cortex, lateral septum, bed nucleus of the stria terminalis, nucleus accumbens, hypothalamus and preoptic area (Naber and Witter, 1998; Swanson et al., 1978; Tamamaki and Nojyo, 1990). In contrast to the hippocampus, which is the most studied part of the hippocampal formation, few scientific articles concerning the anatomy and function subiculum have been published (Commins et al., 1998; Commins et al, 2002; Anderson and O'Mara, 2003).

The subiculum proper plays a significant role in memory processes, the consolidation of memory and learning (O'Mara et al, 2009). Its role had been described in the processing of spatial information and in spatial memory (Morris et al, 1990), as indicated by lesion studies (Schenk and Morris, 1985; Taube et al, 1992; Sharp and Green, 1994). The dorsal subiculum is relatively more related to space and memory, and the ventral subiculum is related to stress, anxiety and reward. The hypothalamo-pituitary-adrenocortical (HPA) stress response is vital for the survival and well-being of all mammals. Some of the products of HPA activation are glucocorticoid hormones. In the brain, glucocorticoids modulate the memory of aversive events and alter emotionality and mood. In stressful situations and the stress response, the ventral part of the subiculum proper (Herman and Mueller, 2006) inhibits the HPA axis trans-synaptically through GABAergic neurons that give projections directly to the paraventricular nucleus or hypothalamic autonomous control system. However, the role of the subiculum in the integration of stress response is still unclear. The results of some studies indicate that it could exert an excitatory effect on the HPA axis under certain circumstances (Herman and Mueller, 2006; Jacobson and Sapolsky, 1991; Herman and Cullinan, 1997).

Some changes are perceived in the subiculum proper in pathophysiological states such as epilepsy (Arellano et al., 2004, Dreier and Heinemann, 1991; Cohen et al. ,2002), schizophrenia (Weinberger, 1999; Steven, 2000; Shulman and Tibbo, 2005) and Alzheimer's disease (Van Hoesen and Damasio, 1987; Falke et al., 2003).

For a better understanding of the function of the subiculum proper, it is necessary to determine the details of not only the morphological, but also the morphometric characteristics of its neurons.

MATERIALS AND METHODS

The current study included 10 human brains (20 hemispheres), of both gender, 20 to 70 years old. The brains had no discernible pathological changes and no neuropsychiatric diseases. After fixation in 10% pufferized solution of formaldehyde that lasted at least three months, the brains were subjected to the Golgi method of staining.

Blocks of tissue of dimensions of 2x2x1 cm were used for the Golgi method. After fixation in formaldehyde, the blocks of tissue were treated with 2.5% $KCrO_5$ at 37°C in the dark, with frequent changes of solution during 2 to 4 days. The block of tissue was rinsed in a 2.5% solution of $AgNO_3$. The next step was impregnation of the block in a 2.5% solution of $AgNO_3$ during 4 days in the dark at room temperature. After transferring through a series of alcohols with rising concentrations (60%, 100%), the block of tissue was shaped in paraffin. The block was cut on a microtome into 80 to 100 μm-thick samples. Deparaffinization of a sample was performed on micro slides, and the tissue was covered with DPX and cover glasses. The Golgi method is highly selective and impregnates only a small proportion of the neurons. The neurons and fibers were extracted from the preparations and reproduced in camera lucida (Reichert-Jung, Polyvar) drawings. The total number of Golgi impregnation neurons chosen from the human subiculum was 100. Photographs, made with an Olympus C-35 AP-4, of the selected neuronal types were also taken under different magnifications. The drawings of the neuronal types were first recorded by scanning, subsequently digitalized and measured.

The classification of neurons was performed according to the following criteria: a) shape of the cell bodies; b) dendritic organization-position, number, length and branching patterns, and c) density of the spines covering the dendrites.

The maximum length (Dmax) and width (Dmin) of the cell body and total dendritic length (TDL) and volume (V) of the cell body were measured for each neuron, using a Zeiss Axiovision 3.0.6. Comparison of the neurons was based on the types of neurons, following these parameters, in different layers of the human subiculum proper.

Table 1. Morphometric analysis of neurons of molecular layer of subiculum proper.

Types of neurons	Dmax ± SD (μm)	Dmin ± SD (μm)	TDL ± SD (μm)	V ± SD (μm^3)
Bipolar neurons	14.1 ± 0.2	13.9 ± 0.5	145.97 ± 3.1	1426 ± 1.3
Multipolar neurons	29.33 ± 1.13	15.71 ± 2.26	1202.01 ±215.4	3850.56 ± 0.2

Dmax (the maximum length of cell body), Dmin (the maximum width of cell body), TDL (total dendritic length) and V (volume of cell)

Table 2. Morphometric analysis of neurons of pyramidal layer of subiculum proper.

Types of neurons	Dmax±SD (μm)	Dmin±SD (μm)	TDL±SD (μm)	V±SD (μm^3)
pyramidal neurons	44.43 ± 7.94	23.64 ± 1.89	1830 ± 466.3	11768.65 ± 4004.9
neurons with triangular-shaped soma	30.27 ± 6.82	20.32 ± 5.43	1275.41 ± 134.9	7514.19 ± 156.9
neurons with oval-shaped soma	29.35 ± 4.97	22.75 ± 2.67	1294.19 ± 377.36	8009.57 ± 234.7
multipolar neurons	33.99 ± 5.14	22.62 ± 3.64	1248.92 ± 344.91	7693.87 ± 148.9
bipolar neurons	24.36 ± 0.01	14.21 ± 1.06	803.36 ± 203.6	2583.2 ± 25.4

Dmax (the maximum length of cell body), Dmin (the maximum width of cell body), TDL (total dendritic length) and V (volume of cell)

Table 3. Morphometric analysis of neurons of polymorphic layer of subiculum proper.

Types of neurons	Dmax±SD (μm)	Dmin±SD (μm)	TDL±SD (μm)	V±SD (μm^3)
Bipolar neurons	33.61 ± 10.4	20.76 ± 4.35	1152.99 ± 662.69	1152.99 ± 662.69
Multipolar neurons	26.54 ± 0.35	20.84 ± 0.46	815.65 ± 44.9	6031.5 ± 231

Dmax (the maximum length of cell body), Dmin (the maximum width of cell body), TDL (total dendritic length) and V (volume of cell)

RESULTS

The human subiculum proper is the laminar structure represented by three archicortical layers. The sparse neurons, classified into two types as bipolar and multipolar neurons respectively, were found in the molecular layer of the subiculum proper. Morphometric parameters of the neurons are shown in Table 1.

The presence of the pyramidal layer of the human subiculum proper is clearly visible deeper from the pial surface and the molecular layer. The neurons of this layer were classified into two main types: type I – pyramidal cells and type II – nonpyramidal cells, interneurons which were divided in four subtypes of neurons: IIa – bipolar subtype; IIb – multipolar subtype; IIc – neurons with triangular-shaped soma; IId – neurons with oval-shaped soma (microphotographs 1, 2, 3 and 4). The pyramidal cells were the predominant cells in this layer. These neurons have the typical pyramidal shape of soma and thick apical and many thinner basal dendrites. The nonpyramidal cells, interneurons, represent a morphologically heterogeneous type of neurons, situated among the pyramidal neurons in all portions of this layer (Table 2).

The polymorphic layer is the deepest layer of subiculum proper. We defined two types of neurons (Table 3) in this layer: multipolar and bipolar.

DISCUSSION

The subiculum is a very important output structure

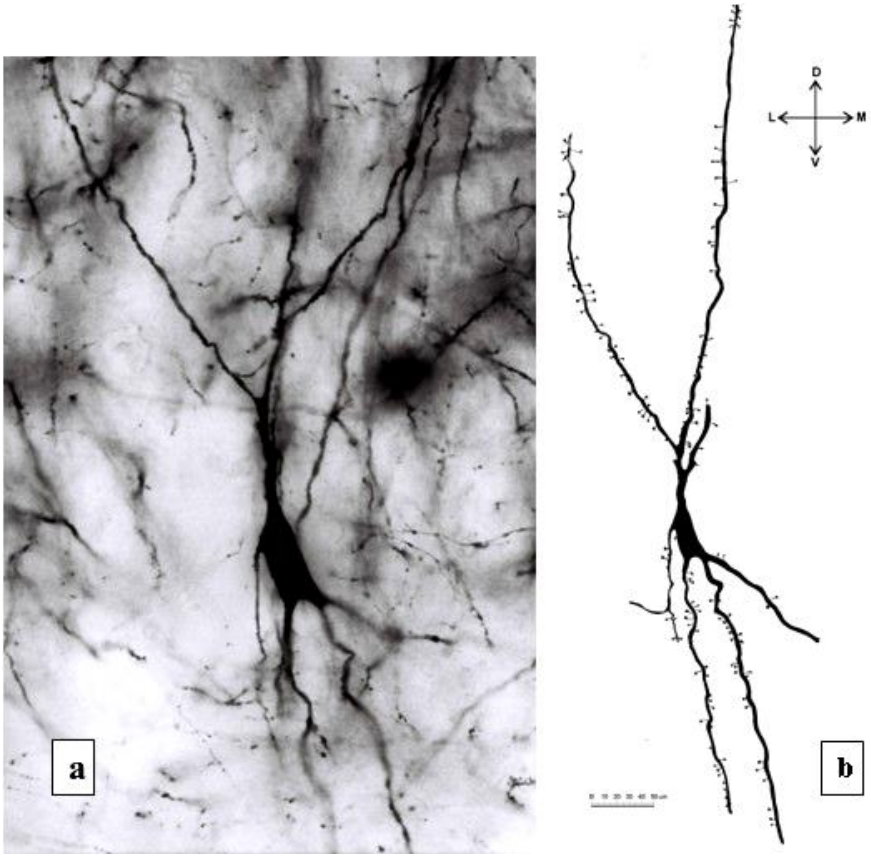

Fig. 1. (a) Microphotograph of Golgi impregnated pyramidal neuron in pyramidal layer, magnification x 400. (b) Drawing of Golgi impregnated pyramidal neuron in pyramidal layer

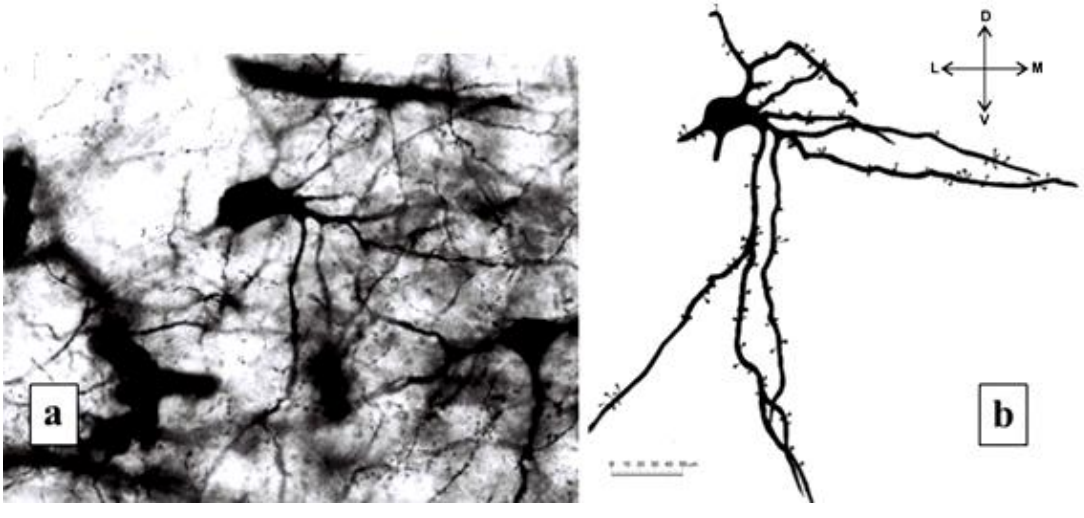

Fig. 2. (a) Microphotograph of neuron with oval-shaped soma in pyramidal layer, magnification x 400. (b) Drawing of Golgi impregnated neuron with oval-shaped soma in pyramidal layer.

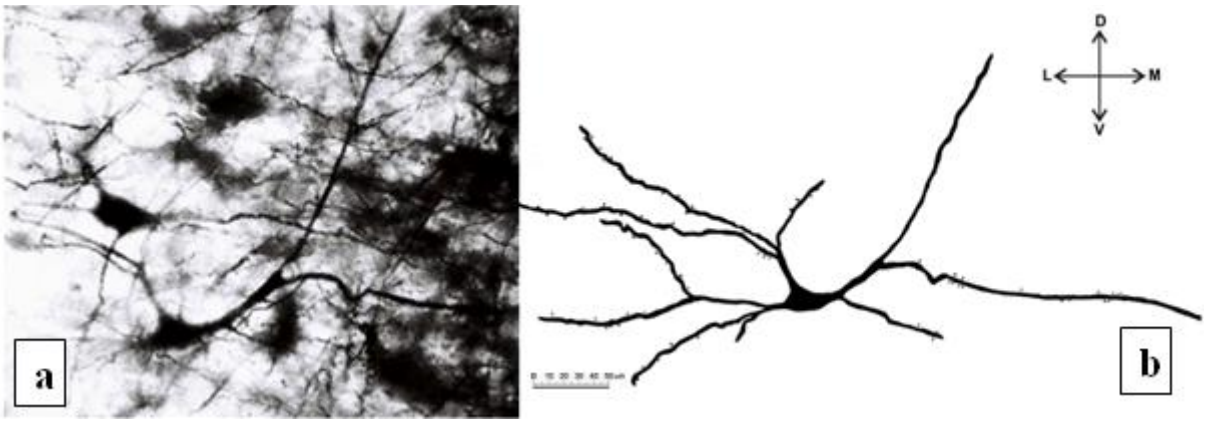

Fig. 3. (a) Microphotograph of Golgi impregnated triangular-shaped neuron in pyramidal layer, magnification x 400. (b) Drawing of Golgi impregnated triangular-shaped neuron in pyramidal layer

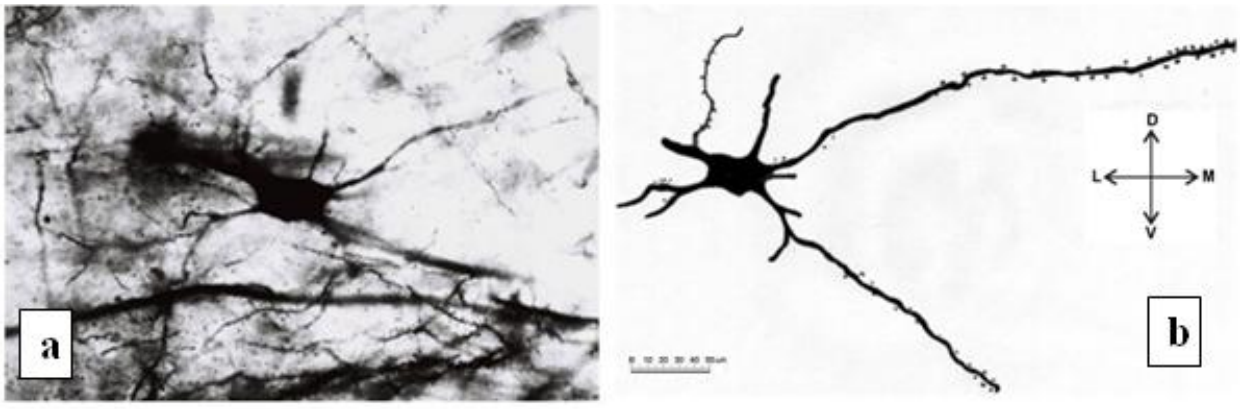

Fig. 4. (a) Microphotograph of Golgi impregnated multipolar neuron in pyramidal layer, magnification x 400. (b) Drawing of Golgi impregnated multipolar neuron in pyramidal layer

of the whole hippocampal formation. There are only a few studies of its anatomy, physiology and function as the interface between the hippocampal complex and cerebral cortex; there are no data about the cellular morphology and morphometrics of the human subiculum proper (O'Mara, 2005).

The human subiculum proper is a three-layer archicortex, in which the superficial layer is a molecular layer that is continuous with the stratum lacunosum-moleculare and radiatum of the hippocampal CA1 field. The bipolar neurons of the molecular layer have the lowest values of measured parameters: Dmax 14.1 ± 0.2 μm, Dmin 13.9 ± 0.5 μm and TDL 145.97 ± 3.1 μm. A wide pyramidal cell layer with large pyramidal neurons is deeper, and the deepest is the polymorphic layer. The principal cell layer of the subiculum is populated with pyramidal neurons: these are consistent in their shape and size and extend their apical dendrites into the molecular layer and their basal dendrites into deeper portions of the pyramidal cell layer (Insauti and Amaral, 2004; Amaral and Insauti, 1990; Amaral and Witter, 1995; O'Mara et al., 2001). The pyramidal neurons were described as large neurons by Amaral and Insauti (1990), which we confirmed in our study. Comparing the measured parameters, we can conclude that the pyramidal neurons of the pyramidal layer have the highest value: Dmax (44.43 ± 7.94 μm), Dmin (23.64 ± 1.89 μm), TDL (1830 ± 466.3 μm) and V (11768.65 ± 4004.9 μm^3). Taking into account that the axons of the pyramidal

neurons of the pyramidal layer make up the greatest part of the fornix postcommissuralis (Amaral and Insauti, 1990), these values can be expected. Among the pyramidal cells, there are many smaller neurons; these are considered the interneurons of the subiculum (Amaral and Insauti, 1990; Swanson et al, 1987). The bipolar neurons of the polymorphic layer have the lowest value of the volume layer ($1152.99 \pm 662.69\ \mu m^3$).

The dendritic and axonal characteristics of rat subicular neurons were described in electrophysiological studies. However, the morphological characteristics of neurons of human and animal subiculum have not yet been sufficiently investigated (Vulovic et al., 2010). It is particularly noticeable that there is almost no information about the morphological characteristics of human and animal subicular neurons. For this reason, we could not compare our findings with the research of other scientists.

The results of this study, in which we have described the morphometric characteristics of neurons of the human subiculum proper, are just a part of the missing information. Future study of the human subiculum proper will provide further information which will improve our understanding of the complex functions of the subiculum proper.

REFERENCES

Amaral, D. G. and *R. Insauti* (1990). Hippocampal formation, In: *The human nervous system*, (Ed. G. Paxinos), 711–755. Academic Press, New York.

Amaral, D. G., and *M. P. Witter* (1995). Hippocampal formation. In: *The rat nervous system*, 2 (Ed. G. Paxinos), 344-393. Academic Press, New York

Anderson M. I. and *S. M. O'Mara* (2003). Analysis of recordings of single-unit firing and population activity in the dorsal subiculum of unrestrained, freely moving rats. *J. Neurophysiol.* **90**(2), 655-65.

Arellano, J. I., Munoz, A., Ballesteros-Yanez, I., Sola, R. G., and *J. De Felipe* (2004). Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus. *Brain*, **127**, 45–64.

Commins, S., Gigg, J., Anderson, M. and *S. M. O'Mara* (1998). The projection from hippocampal area CA1 to the subiculum sustains long-term potentiation. *NeuroReport,* **9**, 847–850.

Commins, S., Aggleton, J. P. and *S. M. O'Mara* (2002). Physiological evidence for a possible projection from dorsal subiculum to hippocampal area CA1. *Exp. Brain Res.* **146**(2), 155-160.

Cohen, I., Vincent, N., Stephane, C., Michel, B. and *M. Richard* (2002). On the Origin of interictal activity in human temporal lobe epilepsy in vitro. *Science*, **298**, 1418-1421.

Dreier, J. P. and *U. Heinemann* (1991). Regional and time dependent variations of low Mg2 induced epileptiform activity in rat temporal cortex slices. *Exp. Brain Res.* **87**, 581–596.

Falke, E., Jonathan, N., Mitchell, T. W., Bennett, D. A., Trojanowski, J. Q., and *E. A. Steven* (2003). Subicular dendritic arborization in correlates with neurofibrillary tangle density Alzheimer's disease. *American Journal of Pathology*, **163** (4), 1615-1621.

Herman, J. P. and *W. E. Cullinan* (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in Neuroscience*, **20**, 78-84.

Herman, J. P. and *N. K. Mueller* (2006). Role of the ventral subiculum in stress integration. *Behavioural Brain Research*, **174**, 215–224.

Insausti, R. and *D. G. Amaral* (2004). Hippocampal Formation. In: *The human nervous system*, 2 (Ed. G. Paxinos), 871-913. Academic Press, New York.

Jacobson, L. and R. *M. Sapolsky* (1991). The role of the hippocampus in feedback regulation of the hypothalamo-pituitary-adrenocortical axis. *Endocr. Rev.* **12**, 118-134.

Naber, P. A. and *M. P. Witter* (1998). Subicular efferents are organized mostly as parallel projections: a double-labeling, retrograde-tracing study in the rat. *J. Comp. Neurol.* **393**, 284–297.

Morris, R. G. M., Schenk, F., Tweedie, F. and *L. E. Jarrard* (1990). Ibotenate lesions of the hippocampus and/or subiculum: dissociating components of allocentric spatial learning. *Eur. J. Neurosci.* **2**, 1016–1028.

O'Mara, S .M., Comins, S., Anderson, M., and *J. Gigg* (2001). The subiculum: a review of form, physiology and function. *Progr. Nerobiol.* **64**, 129–55.

O'Mara S. **(**2005). The subiculum: what it does, what it might do, and what neuroanatomy has yet to tell us. *J. Anat*. **207**, 271–282.

O'Mara, S. M., Sanchez-Vives, M. V., Brotons-Mas, J. R. and *E O'Hare*. (2009). Roles for the subiculum in spatial information processing, memory, motivation and the temporal control of behaviour. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **33**(5), 782-790.

Sharp, P. E. and *C. Green* (1994). Spatial correlates of eating patterns of single cells in the subiculum of the freely moving rat. *J. Neurosci.* **14**, 2339-2356.

Schenk, F. and *R. G. M. Morris* (1985). Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Exp. Brain Res*. **58**, 11-28.

Shulman, Y. and *P. Tibbo* (2005). GABA-ergic deficits in schizophrenia: evidence and implications. *Reviews UAHSJ***, 2**, 23-27.

Steven, E. A. (2000). Cellular and molecular neuropathology of the parahippocampal region in schizophrenia. *Annals of the New York Academy of Sciences*. **911**, 275-292.

Swanson, L. W., Wyss, J. M. and *W. M. Cowan* (1978). An autoradiographic study of the organization of intrahippocampal association pathways in the rat. *J. Comp. Neurol.* **181**, 681–716.

Swanson, L. W., Kohler, C. and *A. Bjorklund* (1987). The limbic region. I. The septohippocampal system. In: *Handbook of Chemical Neuroanatomy*, *Vol. 5, Integrated Systems of the CNS, Part I* (Eds. T. Hökfelt, A. Björklund and L.W. Swanson), 125-227. Elsevier, Amsterdam

Tamamaki, N. and *Y. Nojyo* (1990). Disposition of slab-like modules formed by axon branches originating from single CA1 pyramidal cell neurons in the rat hippocampus. *J. Comp. Neurol*. **291**, 509-519.

Taube, J. S., Kesslak, J. P. and *C. W. Cotman* (1992). Lesions of the rat post-subiculum impair performance on spatial tasks. *Behav. Neural Biol*. **57**, 131-143.

Van Hoesen, G. W. and *A. R. Damasio* (1987). Neural correlates of cognitive impairment in Alzheimer s disease. In: *Handbook of Physiology* (Ed: F. Plum), 871-898. Williams & Wilkins, Baltimore.

Vulović, M., Živanovic-Mačužić, I., Sazdanović, P., Jeremić, D. and *J. Toševski* (2010). Morphology of neurons of human subiculum proper. *Med. Pregl*. **LXIII** (5-6), 356-360.

Weinberger, R. D. (1999). Cell biology of the hippocampal formation in schizophrenia. *Society of Biological Psychiatry*, **45**, 395-402.