GENERAL REVIEW

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Hippocampus – Why is it studied so frequently?

Hipokampus – zašto se toliko proučava?

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Introduction

From the very beginnings of brain research, the hippocampus has been the focus of attention for anatomists. Nowadays, its complexity and clinical importance have attracted the interest of a vast number of researchers of different profiles.

Work on hippocampal tissue facilitated some important neurophysiological discoveries: identification of excitatory and inhibitory synapses, transmitters and receptors, discovery of long-term potentiation and long-term depression, role of oscillations in neuronal networks, underlying mechanisms of epileptogenesis and of memory disorders [1].

The Neuron Theory and the hippocampus

When Theodor Schwann and Matthias Schleiden (1839) proclaimed that a cell is a basic functional unit of all living things, they did not believe this applicable to the nervous system [2]. That was going to change after Camillo Golgi in 1873 introduced the black visualisation of neurons by silver impregnation. Suddenly, nerve cell and the full extent of its "protoplasmic processes", later named "dendrites" by Wilhelm His (1889) and "axons" by Albert von Kölliker (1896), became highly visible in sharp contrast[3]. Wilhelm von Waldeyer-Hartz, who was aware of the findings of His and August Forel on individuality of the nerve cell function, after being introduced to Golgi and Santiago Ramon y Cajal images of the hippocampal nerve cells, understood the nature of the organization of the nervous system, coined the name "neuron" and promoted the Neuron Theory in 1891 [4–6].

On the hippocampus nomenclature

According to the *Terminologia Anatomica* (1998), hippocampus is the name for practically the entire protrusion on the medial wall of the temporal horn of the lateral ventricle. The name hippocampus to this structure was given by the Bolognese anatomist Giulio Cesare Aranzio-Arantius in 1564 which crossectional appearance resembled a seahorse [1] to him. Winslow in 1732, used the term *cornu arietis* (ram`s horn) for the appearance of the hippocampal section, which De Garengeot in 1742 turned to *cornu Ammonis* after the Egyptian god Ammon who was depicted with a human body and the head of a ram with the horn[7]. The hippocampus proper is the more commonly used name for Ammon's horn. The name *cornu Ammonis* survived as the acronim *CA* for the subdivisions of the hippocampus proprius as it was proposed by Lorente de No [8] in 1934 and is still in use.

On the hippocampal anatomy

Anatomists are equivocal about the structures involved within hippocampus, yet, it is generally accepted that the term hippocampus by itself is not quite adequate. Various criteria have been used to define the hippocampus, such as: the anatomical findings, the cell types and connectivity, the number of cortical layers, but most frequently the combination of listed, related to authors.

The contemporary terms in use are the hippocampal region [9–11], the hippocampal system [12], the hippocampus with joined structures [13] or the hippocampal formation [14, 15] and all of them include further subdivisions. Macroanatomically, hippocampus appears as bilaminar, with the lamina of the gyrus dentatus rolled up inside the lamina of the hippocampus proprius.

Laminar organization and cytoarchitectonic characteristics

We consider as useful the division of the hippocampus into the three layered and the six layered areas. The hippo-

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campus proper with its four subfields: CA1, CA2, CA3 and CA4 [8], dentate gyrus and the subiculum are the simplest part of the cortex [16] sharing the characteristic three-layered appearance of the so-called allocortex [13]. The six layered appearance that characterizes the neocortex[17] consists of presubiculum, parasubiculum and entorhinal cortex [14] (Figure 1), comprising perirhinal and postrhinal cortices.

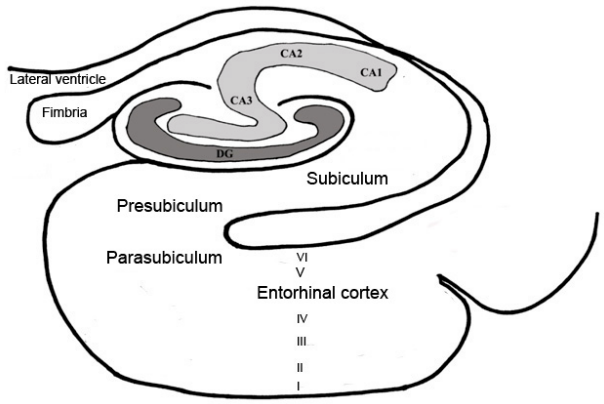

Fig. 1 – Structures of the human hippocampal formation.
DG – dentate gyrus;
CA – cornu ammonis.

There are two major groups of neurons within the hippocampal formation: the principal cells (Figure 2), which are the main source for extrahippocampal connections, and a range of interneurons, which are mainly local-circuit neurons [18].

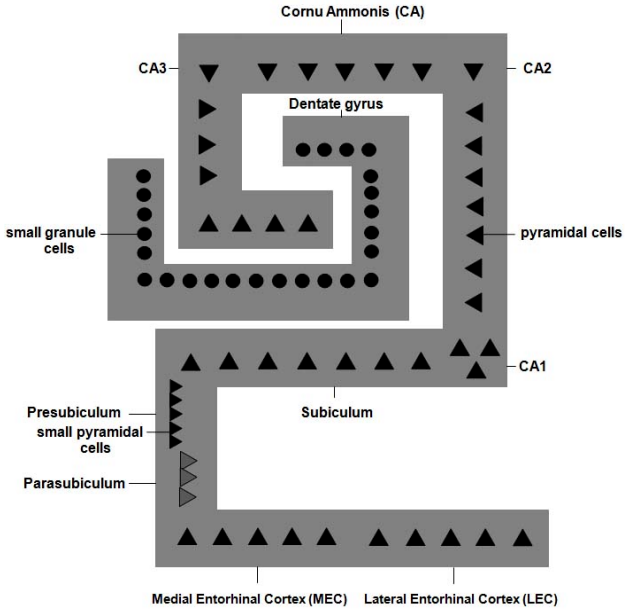

Fig. 2 – Principal cells within the hippocampal formation.

The three layered division

Dentate gyrus

Dentate gyrus has three layers: the molecular, the granular and the polymorphic cell layer. The first one of the three, the molecular layer is continuous with that of the hippocampus proprius. It lies closest to the hippocampal fissure, and was considered cell free for exception few interneurons [13]. Recently, six interneuron types and two GABA negative types were differentiated within dentate molecular layer in the rabbit hippocampus [19]. The principal, granular layer, contains small granule cells with axons which form the mossy fiber pathway in the overlaying molecular layer. The granule cell is the only cell type that gives axons to innervate the CA3 region of the hippocampus proprius. The contact of the dentate mossy fibers with the spines of pyramidal cells occurs in the stratum lucidum of CA3 region [20, 21]. The axons of the dentate gyrus granule cells (mossy fibers) are excitatory glutamatergic but also contain GABA and the opiate peptide dynorphin [22, 23]. Beside the granule cells, in the granular layer are also excitatory mossy cells and inhibitory interneurons. Mossy cells have extensive ramification reminding to moss, and their axons innervate contralateral dentate gyrus. There are also various interneurons which connections remain within the gyrus dentatus [24]. The third dentate gyrus layer is the polymorphic cell layer [14] (also called the hilus [25]).

Cornu ammonis (hippocampus proprius)

The hippocampus proprius has three layers: stratum oriens, stratum pyramidale, and the molecular zone [13]. The well-defined pyramidal cell layer formed by excitatory pyramidal neurons is the principal layer of the hippocampus proper. It consists of large pyramidal cells tightly packed in CA1, and less tightly packed in CA2 and CA3 fields. The rest of the tissue consists of axons and dendrites and various types of interneurons which inhibit the pyramidal cells. CA1 field continues from the subiculum. CA2 is a narrow zone, between CA1 and CA3 field and CA3 continues into the hilus of the dentate gyrus. The hilar part in rats, and not in humans, is defined by Lorente de No [8] as CA4. In primates, the hilus is dominated by pyramidal cells and the assignment of these neurons to a specific hippocampal subfield remains controversial. All pyramidal neurons within the human dentate hilus can be designated as the CA3 hilar neurons [26].

Further, hippocampal three layers could be subdivided into six sublayers [13]. Starting under the ependyma of the ventricular surface these sublayers are: the alveus, the stratum oriens, the stratum pyramidale, the stratum lucidum, the stratum radiatum and the stratum lacunoso-moleculare. The alveus contains powerful efferent axons of the hippocampal and subicular pyramidal neurons which are passing toward fimbria and fornix and also afferent fibers mainly from the septum [13]. The stratum oriens, a layer between the alveus and the pyramidal cell bodies, contains basal dendrites of pyramidal cells, inhibitory basket interneurons and commissural fibers from the contralateral hippocampus, as well as afferents from the septum. These contralateral hippocampal connections are better developed in rodents than in primates. The stratum lucidum is exclusively interposed between the pyramidal cell bodies and the stratum radiatum, and can be seen in the CA3 field as a narrow cell-free zone occupied by the mossy fibers from the dentate granule cells which have contact with the proximal dendrites of pyramidal cells in the

field CA3. The stratum lucidum is not as prominent in humans as it is in primates. The stratum radiatum contains apical dendrites of pyramidal cells which interconnect with Schaffer colaterals (fibers from CA3 to CA1), fibers from septal nuclei, and commissural fibers. The stratum lacunosum is a thin layer containing Schaffer colaterals and perforant fibers from upper layers of entorhinal cortex. The stratum lacunoso-moleculare contains perforant fibers originating from entorhinal cortex which form synapses on the distal apical dendrites of pyramidal cells [11, 17, 27].

Subiculum

The border between CA1 and subiculum is characterised by the abrupt discontinuation of pyramidal cell layers of the CA1 which is replaced by a wide molecular layer of subiculum. The principal cell layer of the subiculum contains large pyramidal neurons among which smaller interneurons are present. At the border with presubiculum a significant decrease in the size of pyramidal cells is visible [10].

The six layered division

Presubiculum

The presubiculum should be considered an anatomical transition zone between the three-layered and six-layered areas because its six layers are not so well defined [28]. The most characteristic cells of the presubiculum are densely packed small, darkly Nissl-stained pyramidal cells located in the external cell layer [14].

Parasubiculum

The cells of the parasubiculum are densely packed, lightly stained pyramidal neurons which, by the use of distinctive staining, Timm sulfide silver method [29], differentiate the parasubiculum from both, presubiculum and entorhinal cortex.

Entorhinal cortex

The cells of the entorhinal cortex serve as the main interface between the hippocampus and other parts of the brain. There are four cellular (II, III, V and VI) and two acellular or plexiform (I and IV) layers. Layer II contains stellate cells grouped in clusters which caudally tend to merge. In layer III pyramidal cells are predominant. Pyramidal cells in layer V vary from grouped large, darkly stained neurons to rather loose arranged smaller pyramidal cells altogether with polymorphic cells. Cells in layer VI are heterogenous in size and shape. Columnar organization at caudal levels is present in layers V and VI [14]. The entorhinal cortex is the most heavily damaged in Alzheimer's disease and is the site of early onset of the disease [30].

The neuronal connections of the hippocampus

The quality of some nerve fiber staining can be augmented by fiber degeneration. However, several hippocampal fiber systems contain thinner axons as compared to other parts of the central nervous system [31]. This, probably, was the reason why the first attempts of Marchi and Algeri in 1886 only stained the thicker degenerating fibers [1]. Improvement was achieved by applying silver impregnation methods to degenerating hippocampal fibers [32] altogether with the electron microscopy [33] and the fiber tracing methods based on intra-axonal transport of injected radioactive amino acids [34].

Intrinsic hippocampal connections

Gathered data on intrinsic hippocampal neuronal connections showed that each part of hippocampal formation gives fibers to neighboring regions but does not always get reciprocal connections [35]. That is not the case with neocortical areas which are generally reciprocally interconnected [36]. Unidirectional intrahippocampal connections could be shown by the so-called "trisynaptic circuit" (Figure 3) [37].

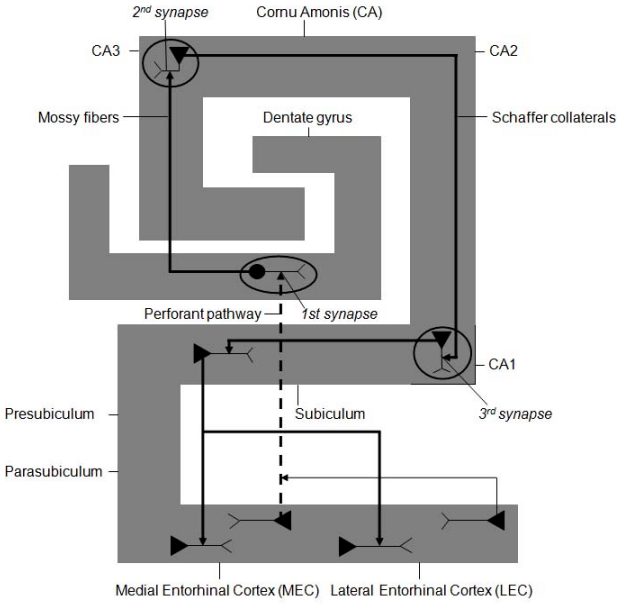

Fig. 3 – Mostly unidirectional intrahippocampal "trisynaptic circuit".

The excitatory glutamatergic [37] "trisynaptic circuit" arises in layer II of the entorhinal cortex [38], its axons perforate the subiculum forming the "perforant path". The majority of the perforant path fibers reach the molecular layer of the gyrus dentatus where they have synapse (the first synapse of the trisynaptic circuit) with the dendrites of the granular cells [39]. The gyrus dentatus does not project back to the entorhinal cortex. Similarly, the axons of the dentate gyrus granule cells called "mossy fibers" have synapse (the second synapse of the "trisynaptic circuit") with the apical dendrites of CA3 pyramidal cells and do not project back to the granule cells. The CA3 pyramidal cells axons emit the so-called "Schaffer collaterals" [1] to have synapse (the third synapse of the trisynaptic circuit) with the apical dendrites of CA1 pyramidal cells which also do not project back to the CA3 pyramidal cells. The same unidirectionality principle for intrahippocampal connections was confirmed for the CA1-subiculum and CA1-entorhinal cortex connections. The subiculum, further, sends axons to the presubiculum, parasubiculum and into the deep layers of the entorhinal cortex.

The CA1 and the subiculum with the projections to the entorhinal cortex are closing the circuit from the entorhinal cortex *via* the dentate gyrus, the hippocampus proprius, and back to the entorhinal cortex [1, 13, 14]**.** Thus, it could be that to some extent this entorhinal cortex-entorhinal cortex loop compensates the unidirectional type of neuronal connections between the hippocampal structures. Duvernoy [13] introduced the name "polysynaptic pathway", based on the findings that the subiculum also takes part in the intrinsic hippocampal circuitry. The polysynaptic pathway thus, is the chain of at least four synapses connecting the entorhinal area (presubiculum, parasubiculum and entorhinal cortex), the gyrus dentatus, the cornu ammonis fields, and the subiculum. It is accepted that there is also the direct intrahippocampal pathway originating from layer III of the entorhinal cortex and projecting directly to the CA1 pyramidal neurons by a different pathway from that of the perforant path [40].

Extrinsic hippocampal connections

The hippocampal formation is connected with numerous subcortical and cortical structures (Figure 4) [15].

from the amygdala. The afferents from the septal nuclei mainly release acetylcholine having a modulatory excitatory effect on the hippocampal pyramidal cells. Adrenergic and serotoninergic afferents from the locus ceruleus and the raphe nuclei have modulatory effects on the hippocampal long term potentiation which is related to the attention and motivation that both influence learning and memory functions [16].

Outputs (efferents)

The hippocampal formation efferents reach subcortical, as well as cortical areas. There are two main outways from where hippocampal efferent fibers leave the hippocampal region: rostral, toward subcortical and caudal, and toward cortical areas [17].

Rostral fibers, raising mainly from the subiculum [41] and adjacent areas, gather and through the fimbria of fornix reach the mammillary bodies, continuing *via* the mamillothalamic tract into the anterior thalamic nucleus, and further to the cingulate cortex. Some of fibers reach the nucleus accumbens, the ventromedial thalamus and the amygdala indicating that the hippocampus is also connected with neuronal groups related to emotions and motivation [15].

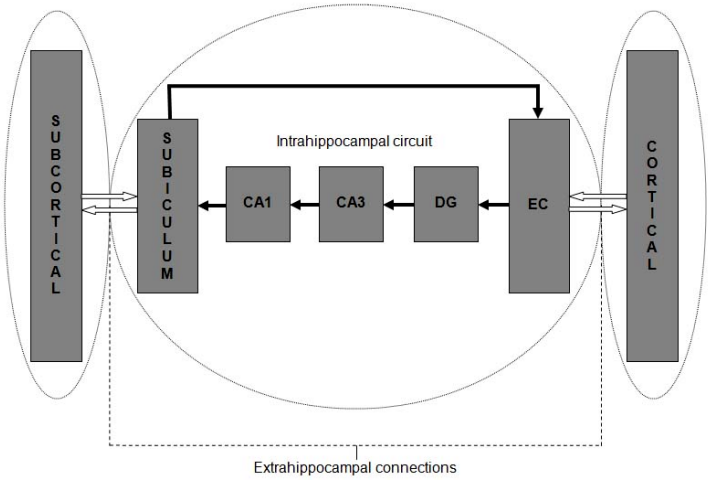

Fig. 4 – Intrahippocampal and extrahippocampal connections.
EC – entorhinal cortex; DG – dentate gyrus; CA – cornu ammonis; ← Intrahippocampal circuit

Inputs (afferents)

The main afferents to the hippocampal formation enter *via* the entorhinal area [15]. The neocortical inputs to the entorhinal cortex of the rat comprise two groups: those that terminate in the superficial layers (I–III) and those that terminate in the deep layers (IV–VI) [14].

The entorhinal area recevies the majority of the afferents from the parahippocampal gyrus and from the perirhinal cortex which receive information from association neocortical centers (the visual, auditory and somatosensory) and from the polysensory association areas which integrate various sensory data. Inputs are coming also from the cingulate gyrus, the insula and the prefrontal cortex. Important inputs to the hippocampal formation are from the septal nuclei, from the brainstem monoaminergic neurons (locus ceruleus and raphe nuclei), from the hypothalamus, from the thalamic nuclei and

Caudally, three parallel efferent pathways are reaching primarily the temporal and frontal association areas. One direct and one indirect bands, originate from the entorhinal cortex and project to the temporal lobe, medial and orbital parts of the prefrontal cortex and to the polysensory areas in the superior temporal gyrus. Indirect projections coming from the entorhinal cortex *via* parahippocampal gyrus and the perirhinal cortex terminate in the same areas. The third pathway reaches these areas directly from the CA1 and the subiculum [15].

The neurotransmitters of the hippocampus

Glutamate has been stressed as the main neurotransmitter in the mammal brain [42, 43]. Excitatory neurotransmitters have been localized in principal neurons of the neocortex [44]. On the other side, GABA plays the major inhibitory role in the brain [45, 46] mainly localized in interneurons [24].

The granule and pyramidal cells of the hippocampus are excitatory glutamatergic, whereas the interneurons are GABAergic [47]. In comparison with glutamate and GABA, other neurotransmitters are present at far fewer synapses in the hippocampal region [46, 48]. In particular, the hippocampus proprius and gyrus dentatus contain neurons producing substance P, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), somatostatin, corticotropin-releasing factor (CRF), and neuropeptide Y [13], which all are involved in local inhibitory or excitatory circuits [49–52]. Enkephalin and glutamate containing hippocampal afferent fibers arise from the adjacent entorhinal cortex [53]. The gyrus dentatus granular neurons also may produce enkephalins and dynorphins [54].

On the human hippocampus functions

Various theories based on anatomical and clinical correlations have been proposed for the human hippocampus role, such as roles in olfactory function, emotions, attention and memory [1, 55]. Furthermore, many functions of the hippocampus have been recognized and based on the addressed clinical impairment.

The hippocampus and emotional behavior

At present, emotional behavior is mostly attributed to the amygdala whose central nucleus is believed to modulate the autonomic reactions produced by emotions and whose basolateral nucleus projections to the dorsomedial thalamic nucleus and further to the prefrontal cortex are thought to regulate an individual`s behavior. The hippocampal involvement in emotional behavior remained related to pain; the polysynaptic pathway gives fibers to the anterior cingulate cortex where end the spinoreticulothalamic pathways involved in the perception of some aspects of pain [13].

The hippocampus and memory

Bekhterev (1900) was the first who pointed out the importance of the subicular complex in memory processes, when he studied the damage of the temporal lobe [1].

The case of a patient is famous who in 1950 underwent bilateral resection of the hippocampus and surrounding areas in order to control his otherwise intractable grand mal epilepsy. After the surgery, the patient could recall memories from early life but could not acquire long-term memory for new facts or events (anterograde amnesia). Subsequent detailed neuropsychological assessment of that patient revealed severity of memory impairment and furthermore an array of cognitive and mnemonic functions unaffected by bilateral lesion [56].

It is believed that the hippocampus (in particular CA3 region) is the site where new declarative (episodic, explicit or declarative memory that can be described in words [57]) events and facts (short-term memories) are processed and encoded into memory trace and as such transferred to the other parts of the brain where they turn into long-term memories [58, 59]. An isolated damage of the hippocampal formation usually does not abolish the recall of older memories, although it prevents the learning of new material [15]. There is a general acquired agreement that the hippocampus is not involved in the memory process of acquiring motor (procedural, implicit or non-declarative memory) skills and procedures [1, 57].

There are two types of the hippocampal electroencephalographic (EEG) activities registered in animals. While exploring their environment theta 5 Hz–10 Hz frequencies were recorded. In a period of quiet wakefulness sharp-wave activity of large amplitude replaced theta frequencies. It was speculated that during theta EEG pattern the hippocampus is acquiring a new information while during sharp-wave (quiet) and slow-wave sleep activities the hippocampus is transfering this information elsewhere in the brain [25].

Duvernoy [13] considers that the hippocampus is implicated in all aspects of the declarative memory, i.e., the semantic memory, which involves memory of facts and concepts, the episodic memory, which permits conscious recollection of events and the relations between them, and the spatial memory, which involves spatial location recognition.

Based on the huge amount of data, Stark [60] elaborated four conclusions related to the human hippocampus involvement in memory along with the adjacent cortical structures in the parahippocampal gyrus: the hippocampus is critically involved in memory for facts and events, this involvement is time limited, the hippocampus is not involved in immediate or working memory process and is not involved in implicit or non-declarative long-term memory process, the hippocampus is not involved in non-mnemonic aspects of cognition including spatial processing.

Certain differences in opinion related to the role of the hippocampus in spatial location recognition, i.e. spatial processing could be better understood having in mind the case of second patient. Unlike the first patient (above described), the second one has what is likely complete loss of the hippocampal region [59], thus he could retrieve spatial information and navigate in a spatial environment learned long before the onset of his amnesia but apparently has not learned any spatial information about his environment after the onset of his amnesia. His performance dropped from 83% correct in his childhood environment to 0% correct in his current environment on the navigation tasks. With complete hippocampal loss, spatial processing therefore appears normal despite a complete inability to acquire new spatial information [61, 62].

The hippocampal involvement in stress

The findings that the density of mineralocorticoid receptors in the hippocampus is the highest in the brain [63, 64] linked the hippocampus with stress, having in mind that increased corticosterone is released from the adrenals during stressful situations [65, 66]. Chronically elevated levels of cortisol induce atrophy of the hippocampus in Cushing`s disease [67]. Certain volume decrease also was found in the hippocampus, putamen and caudate nucleus in posttraumatic stress disorder patients [68].

Plasticity of hippocampal neurons

Plasticity represents the capacity of cell to change in response to various stimuli or injury. In relation to neuronal cells it is related to the well-known synaptic activity as well to anatomical changes. Different laboratories identified axonal sprouting and synaptogenesis in the hippocampus after lesions

of the fimbria. Synaptogenesis was manifested in new multiple synaptic buttons appearance at dendritic spines [69, 70].

Further efforts were directed to explore regenerative capacity of transplanted neurons to give axons toward neuronal target cells of recipient tissue. It was shown that transplanted adrenergic and cholinergic neurons could extend axons and reinervate the hippocampus with followed restoration of maze learning ability [71, 72]. Further, the transplanted hippocampal tissue showed electrophysiological properties typical of hippocampal neurons when grafted into the cerebellum [73].

Conclusion

There is no simple answer to the question why hippocampus is studied so frequently. It attracted early investigators by its distinct, relatively simple and well-structured form as compared to other parts of pallium. However, certain anatomical and neurobiological features of the hippocampus such as: single cell layer and strictly laminated inputs, predominantly unidirectional connections toward different cortical regions, numerous contacts with target neuronal dendrites, highly plastic synapses, tissue suitable for transplantation studies, neurons that can be successfully grown in culture, acute or cultured slices surviving for prolonged periods *in vitro* are of contemporary interest especially having in mind its proven role in memory process.

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