REVIEW

Morphological identification of neuron types in the rat hippocampus

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Abstract

The cerebral cortex ensures an optimal interaction of mammals, including humans, with their environment, by encoding, storing and combining information about the surrounding world and the internal milieu. Probably the simplest and the most popular region for studying the cortical network is the hippocampal CA1 area, because it has the least heterogeneous neuronal population, the somata and dendrites of principal neurons (pyramidal cells) are arranged into well defined layers and the extrinsic and intrinsic inputs are segregated. The relatively homogeneous pyramidal cell population is supported by a very heterogeneous GABAergic interneuron population, which provides not only general inhibition, but also regulates the precise timing of pyramidal cell activity. Interneurons usually innervate distinct domains of the surface of their target cell. The strategic placement of inhibitory synapses, indicate that GABAergic interneurons belonging to different classes serve distinct functions in the hippocampal network. Neuron types are usually defined according to various morphological, molecular and physiological features. Under typical experimental conditions only some of these parameters are available, therefore an important scientific question is: which partial measures are sufficient for correct recognition of a class of cell. By immunohistochemistry it is possible to stain all neurochemically identical neurons in a given brain region, therefore it is the most widely used method for identifying neuron classes. This review presents the neuron types identified so far in the area CA1 of the rat hippocampus with special emphasis on the immunocytochemical characterization of these cells.

Keywords: hippocampus, interneurons, classification, immunocytochemistry.

The cerebral cortex ensures an optimal interaction of mammals, including humans, with their environment, by encoding, storing and combining information about the surrounding world and the internal milieu.

Although the neuronal and synaptic organization of the cerebral cortex is extremely complex, some salient features can be described by a basic cortical circuit, which is the triad of: (i) a subpopulation of principal neurons providing the main output of the brain region, (ii) the main excitatory input which activates these cells, and (iii) the local circuit neurons (interneurons) which regulate the interactions between the inputs and outputs of principal cells. Principal cells usually have spiny dendrites and release excitatory amino acid neurotransmitters (in most cases glutamate). Local circuit neurons commonly have smooth or sparsely spiny dendrites and release the inhibitory neurotransmitter: γ-aminobutyric acid (GABA). This very simplified cortical circuit is complemented by a sparse, but neurochemically very heterogeneous nonthalamic subcortical input.

In the neocortex it is difficult to recognize this basic cortical circuit, because the same basic pattern is superimposed in several layers in each column, the layers are richly interconnected, and the local recurrent axon collaterals overlap in space with the external afferents. Probably the simplest and the most popular region for studying the cortical network is the hippocampal CA1 area, because it has the least heterogeneous neuronal population, the somata and dendrites of principal neurons (pyramidal cells) are arranged into well defined layers and the extrinsic and intrinsic inputs are segregated.

Along with the adjacent cortical structures of the parahippocampal gyrus, the hippocampus is critically involved in learning and in memory formation for facts and events (explicit or declarative memory). Beyond its physiological role, the hippocampal region is of a particular interest for scientist and clinicians because of its high seizure susceptibility and its possible role in Alzheimer disease and schizophrenia.

In order to understand the functional architecture of any neural network, the component neuron types and their connections must be known. Principal cells generally form a relatively homogeneous population as opposed to interneurons, which are highly heterogeneous regarding their morphological, molecular and electrophysiological features. Neuron types can be defined on the basis of: (i) brain area and cell domain-specific distribution of input and output synapses, (ii) expression of proteins involved in cell signaling, (iii) intrinsic membrane properties reflecting the expression

of ion channel proteins, and (iv) temporal distribution of firing *in vivo* [1, 2]. In most cases only some of these parameters are available in a given experiment, therefore intense research is carried out to establish which partial measures are sufficient for correct recognition of a class of cell.

From the above criteria, only the second can be determined directly experimentally by immunocyto-chemistry for the whole population in a certain brain region. The other three are available only for individual neurons (by intracellular electrophysiological recording and/or labeling) or small clusters of neighboring neurons.

In many pathological cases, and animal models of these pathological states (e.g. epileptogenesis, neuro-degenerative disorders, etc.) we are interested in the modification of a neuronal subpopulation as a whole. By immunohistochemistry it is possible to stain all neurochemically identical neurons in a given brain region. Therefore, this review will present the neuron types identified so far in the area CA1 of the rat hippocampus with special emphasis on the immuno-cytochemical characterization of these cells.

It is estimated that a typical CA1 pyramidal neuron receives approximately 30000 excitatory and 1700 inhibitory inputs [3]. The dendritic tree of pyramidal cells is covered by spines on which asymmetrical, excitatory synapses are found. The apical dendrite (usually one or two, giving rise to many small oblique branches in stratum radiatum) and the basal dendrites in stratum oriens receive glutamatergic input mainly from the hippocampal CA3 area, local axon collaterals and the amygdala. The apical dendritic tuft receives excitatory input mainly from the entorhinal cortex and thalamus. Beside the cell body and axon initial segment, which have solely GABAergic input, all dendrites receive local inhibitory input from interneurons (Figure 1).
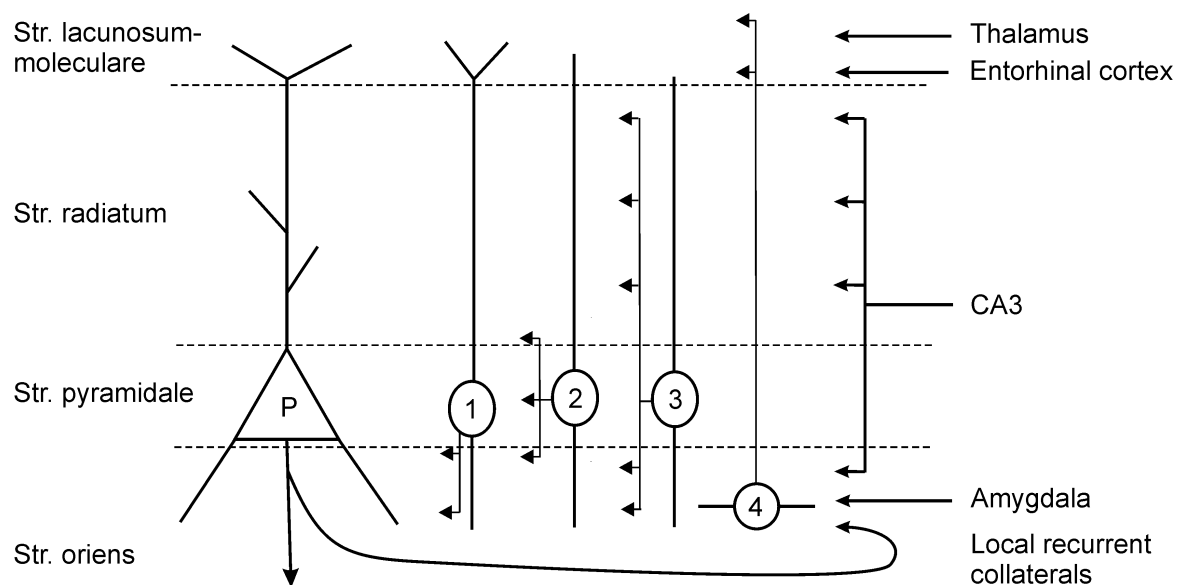

Figure 1 – *Innervation of pyramidal cells in the hippocampal CA1 area. Only the four best characterized interneurons are shown. The most important glutamatergic inputs and their laminar alignment is indicated in the right. The interneuron dendrites are drawn with thick lines, the axons with thin lines. The main synaptic termination regions are indicated by arrows. P: Pyramidal cell, 1: Axo-axonic cell, 2: Basket cell, 3: Bistratified cell, 4: O-LM cell.*

Pyramidal neurons can be divided in several functional domains. The dendrites receive most of the cell's excitatory and inhibitory synaptic inputs and locally integrate them. The cell body integrates inputs from the dendrites and receives only GABAergic synapses whereas the axon initial segment is the most likely site of action potential initiation and also receives only GABAergic input. The axon has no synapses and transmits the output of the neuron to its targets without further modification.

In most publications, **pyramidal cells** are presented as a single population, but there may be at least three subgroups. There are two types of pyramidal cells in the stratum pyramidale: (i) which are weakly immuno-positive to calbindin and are found in the compact layer close to stratum radiatum, and (ii) which are immuno-negative for calbindin, are located towards stratum oriens, are more sparsely encountered and are larger then cells from the previous group [4]. The third subtype of pyramidal cells is located in stratum radiatum (initially denominated radiatum giant cells) [5]. Knowledge regarding the functional significance of the differences between pyramidal cell subtypes is very limited.

The relatively homogeneous pyramidal cell population is supported by a very heterogeneous GABAergic interneuron population, which provides not only general inhibition, but also regulates the precise timing of pyramidal cell activity. The laminar segregation of afferent fibers and the compartmentalized structure of pyramidal neurons make possible for these cells to perform spatially segregated information processing at the same time. Interneurons usually innervate distinct domains of the surface of their target

cell. The strategic placement of inhibitory synapses, indicate that GABAergic interneurons serve distinct functions in the hippocampal network. In the hippocampus, there is a large diversity of distinct types of interneurons. Currently more than 20 types innervating pyramidal cells and/or other interneurons are described [6, 1, 7, 8].

The **axo-axonic cells** provide GABAergic synapses exclusively to the axon initial segments of up to 1200 pyramidal cells [9]. The synaptic boutons contacting a given postsynaptic cell are lined up in most cases on a single presynaptic branch, producing a characteristic axon terminal field which inspired some authors to call these neurons as chandelier cells [10, 11]. The dendrites extend into str. oriens and str. radiatum, forming a more extensive tuft in str. lacunosum-moleculare than the dendrites of basket and bistratified cells [12]. Immunocytochemically, the axo-axonic cells are parvalbumine (PV) positive, this calcium binding protein can be visualized in the soma, dendrites and axon terminals. This expression pattern can be found in some basket cells too.

Basket cells provide GABAergic synapses to the cell body and proximal dendrites of pyramidal cells and other basket cells. The axon terminals may be restricted to the pyramidal layer or they may extend to variable degree to str. radiatum and/or oriens. The larger the penetration into the layers above and below str. pyramidale, the proportion of output synapses on dendrites is higher [13]. Basket cell dendrites are present in str. oriens and radiatum. The radial dendrites enter str. lacunosum-moleculare but rarely branch. There are at least three subgroups of basket cells. One subtype of basket cells is immunopositive for PV, but immunonegative for cholecystokinin (CCK), the other two subtypes are conversely PV$^-$ and CCK$^+$. Besides targeting the pyramidal cells, the PV$^+$ basket cells contact predominantly other PV$^+$ basket cells, whereas CCK$^+$ cell contact preferentially other CCK$^+$ cells. The interconnected PV$^+$ basket cells are capable to generate high frequency synchronous activity. The CCK$^+$ basket cell population can be further subdivided into a vasoactive intestinal polypeptide (VIP) immunopositive, vesicular glutamate transporter 3 (VGLUT3) immunonegative group and a VGLUT3$^+$, VIP$^-$ group. The expression of VIP and VGLUT3 was found to be mutually exclusive in CCK$^+$ interneurons [14], suggesting that there are two types of CCK+ basket cells, but it can not be excluded that the expression of VIP and VGLUT3 is dynamically regulated in time, and in fact there are not two groups, but two states of one cell type [1].

CCK$^+$ basket cells express high levels of presynaptic cannabinoid type 1 receptor (CB1) that mediate short-term depression of GABA release following depolarization of postsynaptic cells [15]. Endocannabinoid-mediated retrograde synaptic signaling is a key regulator of GABA release at synapses formed by these basket cells.

The **bistratified cells** have an axonal arbor distributed in str. radiatum and oriens that matches the Schaffer collateral, commissural input pathway. The axon terminals usually avoid the str. pyramidale, therefore these cells act on dendritic domains. The bistratified cell's dendrites are found in str. oriens and radiatum but the radial dendrites usually do not enter str. lacunosum-moleculare, sometimes they even bend back at the radiatum/lacunosum-moleculare border. Immunocytochemically bistratified cells are PV$^+$, somatostatin$^+$, neuropeptide Y (NPY)$^+$, GABA$_A$ receptor α1 subunit$^+$ and CCK$^-$. High intensity labeling of GABA$_A$ receptor α1 subunit outlines the soma and dendrites of these cells.

O-LM cells have their soma and horizontal dendrites in str. oriens. The axon is distributed in str. lacunosum-moleculare, innervating distal dendrites of pyramidal cells and other interneurons. They express somatostatin and PV, but the calcium binding PV is expressed at lower level then in the previous three-neuron types. O-LM cells have high level of mGluR1α in the extrasynaptic membrane [16]. Other interneurons also express mGluR1α but at markedly lower level [17]. Another metabotropic receptor, mGluR7 can be found in the presynaptic terminals, but this receptor is also present in terminals innervating other cell types. However, the high level of both mGluRs is unique feature of O-LM cells.

Ivy cells and neurogliaform cells have very dense axonal arbor. The fine axons of ivy cells innervate mostly basal and oblique pyramidal cell dendrites, co-aligned with CA3 input [18]. Ivy cells represent the most numerous interneuron class described so far in the hippocampal CA1 area. Neurogliaform cells innervate the apical tuft of pyramidal cells co-aligned with the entorhinal input, the axons often cross the fissure into the dentate gyrus, therefore ivy and neurogliaform cells have spatially complementary axon termination field. In both cell types, the neuronal isoform of nitric oxide synthase (nNOS) is expressed. In this brain area they are a major source of nitric oxide, by which they modulate excitability at a slower timescale and more diffusely than do other interneurons. nNOS strongly colocalizes with neuropeptide Y in both ivy cells [18, 19] and a subset of neurogliaform cells. Ivy cells and neurogliaform cells are both derived from medial ganglionic eminence progenitors, but there is a subset of neurogliaform cells which arises from caudal ganglionic eminence progenitors and does not express nNOS [20]. Ivy cells express also high levels of GABA$_A$ receptor α1 subunit [18], but neither PV nor CCK expression can be detected [21].

The **Schaffer collateral associated cells** have their cell body in str. radiatum up to the border with str. lacunosum-moleculare. They innervate pyramidal cell dendrites in str. radiatum and to a lesser extent in str. oriens in conjunction with the Schaffer collateral/commisural pathways [22], They also innervate interneurons including perforant path associated cells and

other Schaffer collateral associated cells [23]. The dendrites are in str. radiatum but can enter all layers. These cells are immunopositive for CCK and calbindin and are immunonegative for somatostatin and NPY. The **apical dendrite targeting cell** is a morphologically similar cell type, but which innervate preferentially the main apical shaft of pyramidal cells instead of oblique and basal dendrites [24].

The **perforant path associated cells** have their soma in str. lacunosum-moleculare or str. radiatum. Their axons are centered to str. lacunosum-moleculare, associated with the entorhinal input to CA1 area. Some of these cells have also axons spreading into the dentate gyrus and str. radiatum, others give rise to branches to subiculum and presubiculum [1]. At least part of the perforant path associated cells express CCK and pre-synaptic CB1 cannabinoid receptors. Inhibitory outputs from perforant path associated and Schaffer collateral associated cells onto their postsynaptic pyramidal cells or interneurons are tonically reduced by activation of CB1 receptors [23]. By this mechanism the postsynaptic target cells, which release endocannabinoids, may influence the presynaptic GABA release. However, depolarization-induced suppression of inhibition is significantly less effective at dendritic compared with perisomatic synapses [25].

There are non-principal cells, which have wide-spread axon branches outside the CA1 area too. In this category can be included the **back-projection cell**, innervating the CA1 and CA3 area and the dentate hilus. **Hippocampo-septal cells** are located in str. oriens where they have horizontal dendrites. They project to the septum and to other areas of the hippocampal formation where they innervate mainly GABAergic neurons. Local axons in str. radiatum, pyramidale and oriens target preferentially other interneurons [26]. These cells are calbindin$^+$ and somatostatin$^+$, some of them express mGulR1α at lower level than O-LM cells [1]. The medial septum provides rhythmic drive to the hippocampus, and hippocampo-septal feedback synchronizes septal pacemaker units [27]. **Trilaminar cells** have a dense axon arbor in str. radiatum, pyramidale and oriens. The soma is located in str. oriens

and also the long horizontal dendrites are located in this layer [28]. Just a few trilaminar cells were identified so far. By *in vivo* recording and labeling, a trilaminar cell appeared as mGluR8-decorated and strongly muscarinic receptor (M2)-positive. This cell had a large projection from the CA1 area to the subiculum and a preferential innervation of interneurons in the CA1 area in addition to pyramidal cell somata and dendrites [29].

There are at least three types of **interneuron specific (IS) cells**, targeting preferentially other interneurons. IS-I cells are calretinin positive and their axon innervates mainly calbindin$^+$ and other calretinin$^+$ cells. IS-II cells predominantly innervate CCK/VIP positive basket cells whereas IS-III cells mainly innervate O-LM cells [30].

During *in vivo* network activity the different classes of interneurons have distinct firing patterns, therefore provide differential GABAergic input to distinct domains of pyramidal cells. Presumably, cellular diversity serves the temporal organization of cortical functions in the coordination of the activity of different subcellular domains of a single neuron as well as neuronal populations [21]. The best characterized interneuron types are the basket cells (a PubMed search in November 2010 with the keyword "basket cell" gave 1589 results), axo-axonic cells (250 PubMed hits), bistratified cells (199 PubMed hits) and O-LM cells (37 hits). The O-LM cells have their cell body in str. oriens, but the first three have their somata in str. pyramidale. How can we identify these cell populations based only on immunohistochemical data? All three types are PV$^+$ (the CCK$^+$ basket cells usually have their cell body outside the pyramidal layer) (Figure 2). Systematic investigation of their molecular expression profile demonstrated that even these three cell types can be reliably distinguished [31]. Somatostatin immuno-reactivity was found only in bistratified cells (Figure 3). Axo-axonic cells express significantly lower level of GABA$_A$ receptor α1 subunit in their dendritic membrane than do basket and bistratified cells. Using these molecular markers it was possible to estimate that in CA1 area of the hippocampus basket cells represent 60%, bistratified 25% and axo-axonic cells 15% of the PV-containing cells in str. pyramidale [31].
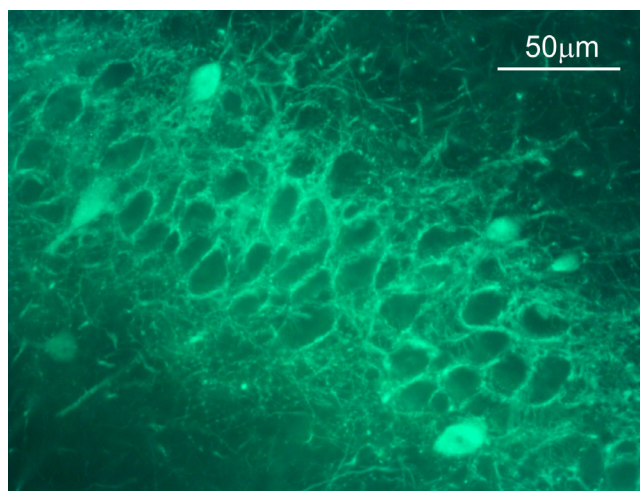

Figure 2 – Immunoreactivity for parvalbumin (PV) in area CA1 of the hippocampus (primary antibody: monoclonal mouse anti-PV, secondary antibody: FITC labeled donkey anti-mouse). The somata of PV$^+$ interneurons are located in str. pyramidale. PV$^+$ perisomatic axon branches form nest like twine around the PV immunonegative pyramidal cell bodies.
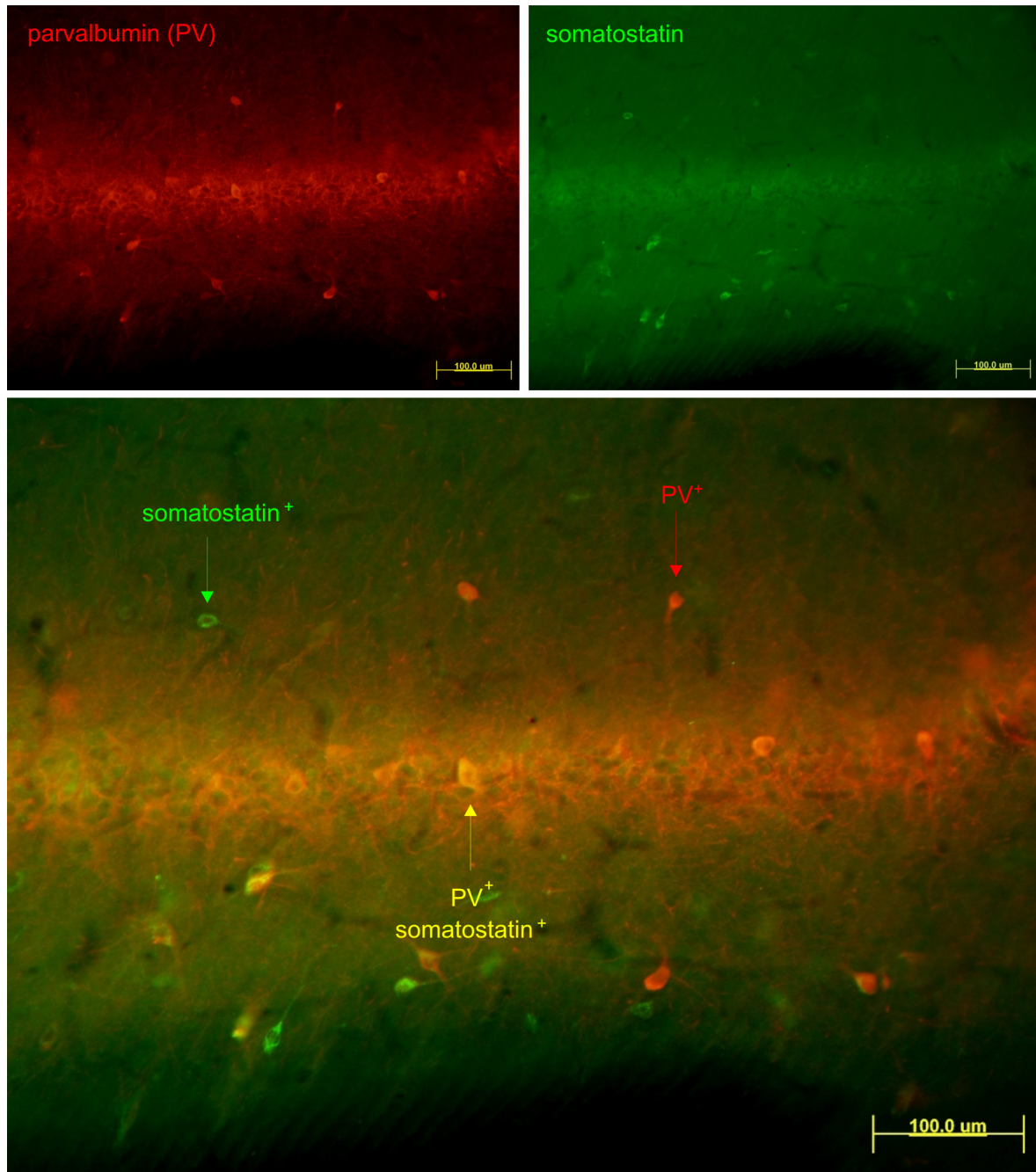
Figure 3 – *Double immunofluorescence micrographs testing the expression of parvalbumin (PV) and somatostatin (SOM) in hippocampal area CA1 (primary antibodies: monoclonal mouse anti-PV and polyclonal rabbit anti-SOM; secondary antibodies: Cy3 labeled donkey anti-mouse and Alexa488 labeled donkey anti-rabbit). Upper panels: images captured using filters for Cy3 (PV) and Alexa488 (SOM); lower panel: merged and enlarged visualization of the same micrographs. Cells stained in green (one indicated by green arrow) are immunopositive only for SOM, red staining indicates PV+ cells. Double-labeled cells appear in yellow (one indicated by yellow arrow). These neurons in str. pyramidale are putative bistratified cells, which provide dendritic inhibition.*

Interneuron types with somata outside the pyramidal layer can be identified based on their position compared to the layers of the hippocampus and their neuro-chemical profile. Although such an identification of these cell types is possible, relatively little is known about their *in vivo* electrophysiological properties, because due to the sparse arrangement of these neurons, only a small number of cells from any class were fully investigated. In consequence, the functional importance of these neuron types is less well understood and further research is required.

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