Dendritic information processing: placement and effect of GABAergic synapses, gap junctions, and voltage-gated ion channels on cortical neurons

Ph.D. thesis

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Introduction and aims

Nerve cells of the cerebral cortex possess an astonishingly complex dendritic tree on which they receive thousands of synaptic inputs. The integrative operations of neurons are determined by the passive properties of dendrites, complexity of dendritic morphology together with the distribution of active conductances on the dendritic tree. Synaptic inputs arriving to distinct parts of the dendritic tree therefore can have different effects on the neuronal output and/or the dendritic excitability depending on their location. Despite the thorough investigation of excitatory synaptic integration, very little is known about that of inhibitory synapses, although they constitute every fifth synapse in the cortex.

GABAergic inhibitory cells release GABA as neurotransmitter. GABA is indispensable for the maintenance of normal cortical excitatory operations, and has a critical role in timing sub-, and suprathreshold neuronal activity. Two types of postsynaptic receptors mediate GABA evoked responses. $GABA_A$ receptors are ionotropic Cl^--channels, whereas metabotropic $GABA_B$ receptors open K^+-channels via G-protein activation. GABAergic cells target specific subcellular compartments of postsynaptic cells. For example, basket cells innervate perisomatic regions, axo-axonic cells terminate specifically on axon initial segments, whereas dendrite targeting cells in visual cortex cells and double bouquet cells innervate preferably dendritic shafts and spines respectively. Perisomatic inhibitory synapses can regulate the local generation of sodium action potentials. Dendritic inhibitory synapses can interact with voltage-gated ion channels, shunt excitatory synapses distal to the soma and backpropagation of Na^+-dependent action potentials, control synaptic plasticity, and regulate dendritic Ca^{2+} spikes. In spite of the existence of GABAergic cells innervating dendritic regions, their distinct, cell type specific impact on the excitability and / or output of postsynaptic cells is not well understood.

Thus, our first aim was to classify dendritically terminating GABAergic interneurons in somatosensory cortex. We also investigated whether the effect of different dendrite targeting interneurons is related to the subcellular position of their synapses on the postsynaptic cell.

We performed whole cell recordings with biocytin filling from synaptically coupled pairs of presynaptic interneurons and postsynaptic pyramidal cells and interneurons. Whole-cell patch-clamp recording from cell pairs provides a useful tool for the physiological identification of a presynaptic neuron evoking a distinct response in the postsynaptic cell. The effect of the presynaptic cell on the sub-, and suprathreshold activity of the postsynaptic cell can be also investigated, that was followed by the visualization and anatomical classification of pre-, and postsynaptic cells. Combination of this

method with the electron microscopic identification of the sites of synaptic interactions is suitable for investigating the cell type dependent effect of presynaptic GABAergic neurons.

Several studies suggested that dendritic inhibition is mediated by $GABA_B$ receptors. Experiments provided evidence only for pure dendritic $GABA_A$ responses evoked by single cells. There is no evidence for $GABA_B$ receptor mediated responses evoked by single cell either in hippocampus or in neocortex. To address this question we examined the connections between neocortical interneurons and pyramidal cells. We analyzed their postsynaptic response in pyramidal cells and determined the number and spatial distribution of the sites of interactions.

Cortical GABAergic mechanisms have been implicated in synchronizing population activity at behaviorally relevant frequencies. Gamma band EEG activity have been observed in the neocortex in vivo associated with a number of cognitive processes, such as perception or attentional mechanisms and beta rhythms at 20 Hz are related to voluntarily controlled sensorimotor actions. Electrical synapses play a role in neuronal synchrony and gap junctional coupling can promote synchronous activity in connections of cortical interneurons. Perisomatically terminating GABAergic inputs are effective in timing postsynaptic action potentials, and in our earlier study, we revealed that it is dependent on the type of connection. Basket cells synchronize each other via gap junctions combined with neighboring GABAergic synapses. Previous experiments either did not examine the location of the inputs or were focused on perisomatic mechanisms. In our second experiment, we investigated the efficiency of dendritic inputs in timing somatic action potentials.

In addition to their exact subcellular location, the functional impact of inhibitory synapses is highly dependent on the distribution of voltage gated-ion channels they interact with. Dendrites of nerve cells express numerous types of voltage-gated ion channels, but little is known about their cell surface distribution. Several experiments mapped voltage-gated channels on the axo-somato-dendritic surface of nerve cells using patch-clamp recordings. This technique is extremely useful as it reveals the location of functional channels. However, small subcellular compartments remain inaccessible with this approach and a differential current density does not necessarily mean distinct densities of channels. In our third experiment, we applied EM immunogold localizations of HCN1 to reveal the rules of its cell surface distribution. HCN1 is one of the four known subunits (HCN1-4) of the hyperpolarization-activated and cyclic-nucleotide-gated nonselective cation channels. The homo- or heteromeric assemblies of these subunits are mainly responsible for the functional diversities of H-current (I_h). Hyperpolarization of postsynaptic membrane potential caused by IPSPs can activate HCN channels, therefore

revealing the exact subcellular distribution of these channels could further increase our knowledge on the impact of dendritic inhibitory synapses.

Materials and methods

Slices were obtained from Wistar rats (P19-35) and transferred to a recording chamber, where slices were continuously perfused with oxygenated artificial cerebrospinal fluid (ACSF). Whole-cell patch clamp recordings were carried out from concomitantly recorded pairs, triplets or quadruplets of layers 1-4 putative interneurons and/or pyramidal cells. Synaptic connections were monitored online, while presynaptic cells were stimulated to fire with brief suprathreshold pulses. Depolarizing current pulses employed during recording resulted in an adequate filling of neurons by biocytin. Slices were fixed, cryoprotected, freeze-thawed in liquid nitrogen, and embedded in gelatin blocks. 300 µm thick slices embedded in gelatin blocks were resectioned at 70 µm thickness. Cells filled with biocytin were visualized with the avidin-biotin-horseradish peroxidase method. 3'3-diaminobenzidine tetrahydrochloride (0.05 %) was used as chromogen and 0.01% H_2O_2 as oxidant. Sections for electron microscopy were postfixed with 0.5-1% OsO_4, contrasted in 1% uranyl acetate, dehydrated in graded alcohol series and embedded on slides into epoxy resin.

Three-dimensional light microscopic reconstructions of recovered cells were carried out using Neurolucida system with 100x objective from the 70 µm thick serial sections representing the entire slice. Dendrogram constructions and synaptic distance measurements were aided by Neuroexplorer software. The entire somatodendritic surface of recorded cells was tested for close appositions with filled axons or filled dendrites, each of which was traced back to the parent soma. Light microscopically detected presumed synapses were checked on the ultrathin sections in electron microscope.

Axon rich areas, including all layers covered by the axonal field, were cut out from sections on the slide and re-embedded for ultrathin sectioning. 70 nm serial sections were scanned under electron microscope and biocytin filled axon profiles were followed until they established synaptic connections with unlabelled postsynaptic profiles. Since all profiles were followed and the plane of the section randomly cuts through axonal branches, the above procedure ensured a random sample of postsynaptic targets. Tracing of serial sections were also used for the identification of postsynaptic target.

<u>Pre-embedding immunocytochemistry of HCN1</u>

Sixteen adult (P35-67) male Wistar rats were deeply anaesthetized and were

perfused through the aorta. The brains were removed and blocks were cut out from the forebrain and 60 µm thick horizontal vibratome sections were prepared. After blocking, sections were incubated in polyclonal primary antibody raised in rabbit or guinea pig against different epitopes of HCN1. Sections for immunoperoxidase reactions were incubated in biotinylated GAR or GAGp IgGs, followed by incubation in ABC. The enzyme reaction was revealed by DAB. Sections for immunogold reaction were incubated in GAR and GAGp IgGs coupled to 0.8 nm gold particles and silver enhanced. Sections for electron microscopy were postfixed with 0.5-1% OsO_4, contrasted in 1% uranyl acetate, dehydrated in graded alcohol series and embedded into epoxy resin.

We have chosen subicular pyramidal cells to quantitatively evaluate the relative densities of HCN1 in distinct subcellular compartments, because the strongest immunolabeling was observed in the subiculum, allowing the achievement of the highest signal to noise ratio. Electron micrographs were taken within the same ultrathin section from randomly selected fields in stratum moleculare representing the distal part of apical dendrites and in deep subicular layers representing somata, proximal dendrites and spines. We calculated the nonspecific immunoparticle density over pyramidal cell nuclei. The immunoparticle density was calculated in particle / effective membrane area (in particle / μm^2) over all plasma membrane compartments and in particle / area (in particle / μm^2) for somatic, dendritic and spine cytoplasm. The immunoparticle densities were statistically compared to the nonspecific labeling density with the paired t-test.

Results and discussion

1. Identified sources and targets of slow inhibition in the neocortex

Neurogliaform cells (NGFCs) were identified based on late spiking firing pattern and their compact axonal and dendritic morphology. Random electron microscopic sampling of postsynaptic targets showed that NGFCs predominantly innervated dendritic spines (71 %) and shafts (29 %). Three dimensional light microscopic mapping of NGFC to pyramid connections confirmed the results of random electron microscopic analysis of targets postsynaptic to NGFCs and predicted synapses on dendritic spines and shafts of pyramidal cells at distances 62 ± 28 µm from the somata. Full electron microscopic analysis of all light microscopically mapped synapses was performed on a randomly selected pair and revealed one synapse on a dendritic spine neck, three on spine heads and one on a dendritic shaft 63 ± 27 µm (25 - 92 µm) from the soma.

IPSPs in pyramidal neurons elicited by NGFCs showed slower 10 - 90 % rise

times (23.4 ± 9.87 ms) and decay when compared to IPSPs due to basket (5.8 ± 2.0 ms) or bitufted cell (6.5 ± 1.7 ms) activation. Pharmacological analysis of NGFC to pyramid interactions revealed that these IPSPs were composed of two components. The early component could be blocked by a $GABA_A$ receptor antagonist, bicuculline, the late component could be blocked by further addition of the $GABA_B$ receptor antagonist CGP35348. Excitatory synapses innervate dendritic spines, therefore neurogliaform cells can locally inhibit EPSPs, thus effectively control dendritic excitability. This influence of neurogliaform cells is further enhanced by their ability to place their synapses on the origin of spine neck, where a more powerful control of EPSPs has been proposed as compared to spine heads. NGFCs could evoke postsynaptic responses only at very low frequencies, suggesting a physiological role tuned for sparse temporal operation and for metabotropic receptors activating an array of biochemical pathways

Our results provide evidence that slow, $GABA_B$ receptor-mediated IPSPs arrive from unitary sources in cortical networks. We identify the first cell type, NGFCs, which consistently recruit postsynaptic $GABA_B$ receptors in addition to $GABA_A$ channels.

2. β and γ frequency synchronization by dendritic GABAergic synapses and gap junctions in a network of cortical interneurons

Regular spiking nonpyramidal cells (RSNPCs) were identified based on their physiological and anatomical properties. The dendrites of RSNPCs originated from the two poles of their elongated somata and were sparsely spiny. The axons formed a dense cloud around the dendritic tree and sent a loose bundle of radial branches spanning all layers of the cortex. Electron microscopic analysis of unlabeled postsynaptic targets taken from layers 2-5 showed that RSNPCs innervated dendritic spines (53 ± 12 %) and shafts (45 ± 10 %) and occasionally somata (2 ± 4 %). Detailed analysis of serial sections revealed that only 44 ± 21 % of postsynaptic targets identified as dendritic spines received asymmetrical synapses.

We identified GABAergic, electrical and combined GABAergic and electrical connections between RSNPCs. GABAergic interactions were mediated by 4 ± 2 axo-dendritic synapses at a mean distance of 63 ± 28 µm from the somata and phased postsynaptic activity at beta frequency, but were ineffective in phasing at gamma rhythm. Electrical interactions of RSNPCs were transmitted via 2-8 gap junctions between dendritic shafts and/or spines at a mean distance of 77 ± 34 µm from the somata. Gap junctional

potentials timed postsynaptic spikes with a phase lag at beta and gamma frequencies, however strong electrical coupling could synchronize pre- and postsynaptic activity. Combined unitary GABAergic and gap junctional connections of moderate strength produced beta and gamma frequency synchronization of the coupled RSNPCs. Gap junctions as well as chemical synapses were located in the dendritic domain of the postsynaptic cells at a mean distance of 59 ± 21 and 75 ± 18 μm from the somata, respectively.

Our results provide evidence that dendritic GABAergic and gap junctional mechanisms effectively transmit suprathreshold information in a population of interneurons at behaviourally relevant frequencies. A coherent network of GABAergic cells targeting the dendrites could provide a pathway for rhythmic activity spatially segregated from perisomatic mechanisms of synchronization.

3. Polarized and compartment-dependent distribution of the hyperpolarization-activated channel HCN1 in pyramidal cell dendrites

The regional and cellular distribution of HCN1 immunostaining as revealed with the two antibodies was practically identical. Light microscopy revealed in the neocortex strongly immunopositive apical dendrites of layer V pyramidal cells. The labeling intensity of the apical dendrites appeared at the border of layers III and IV, and increased toward layer I, dendritic tufts showing the strongest labeling. In the hippocampal formation, the most intense immunolabeling for HCN1 was detected in the subiculum, followed by the CA1, CA3 areas, and the dentate gyrus showed the least intense labeling. In particular, the stratum moleculare of the subiculum was the strongest subregion of the entire hippocampal formation, followed by the stratum lacunosum-moleculare of the CA1 area containing the distal parts of apical dendrites of pyramidal cells. Intense labeling of the plasma membrane of the apical dendrites of layer V, CA1 and subicular pyramidal cells was observed at high magnifications.

Electron microscopic immunogold analysis demonstrated that the majority of immunoparticles for HCN1 are attached to plasma membranes of distal apical dendrites and spines in layer V, CA1 and subicular pyramidal cells. We evaluated quantitative comparison of HCN1 content of proximal and distal subcellular compartments in subicular pyramidal cells, since the strongest labeling was found here.

Pyramidal cell axon initial segments, small diameter axons, and axon terminals have an undetectable level of HCN1, whereas somata, proximal and distal dendrites express significant amounts of HCN1 (domain-dependence). To determine the density differences between distinct proximal and distal plasma membrane compartments of

pyramidal cells, we compared immunoparticle densities in those plasma membrane compartments, which had significant amounts of HCN1 labeling. The density of immunoreactive HCN1 was ~60-times higher in distal dendritic plasma membranes than in somatic membranes. The ~16-times difference between proximal and distal dendrites was not the consequence of differences in the diameter of the dendrites, as we have selected small diameter proximal dendrites and found their density very similar to the large diameter proximal apical dendrites. These results demonstrate a distance-dependent increase in the surface density of HCN1 on the somato-dendritic domains of subicular pyramidal cells. However, when the density of immunoreactive HCN1 was compared in the stratum moleculare, we found a ~ 4 -times higher value in distal dendritic shafts than in spines. This later finding establishes that not only the distance from the soma, but the actual subcellular domain also plays an important role in determining the surface density of HCN1.

Our results reveal the complexity in the cell surface distribution of voltage-gated ion-channels, and predict its role in increasing the computational power of single neurons via subcellular domain and input specific mechanisms.

Publications

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