Consequences of sequences: Studies on convergent and divergent elements of neocortical inhibitory microcircuits

Ph.D. thesis

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INTRODUCTION AND AIMS

The human cerebral cortex, the most elaborated living structure, is responsible for higher order brain functions such as perception, cognition and consciousness. It contains an extraordinary number of neurons, the basic units of the brain. The cerebral cortex has two major types of neurons: principal or pyramidal cells and local interneurons. Pyramidal cells use the excitatory amino acid glutamate as neurotransmitter and send axons to neighbouring and distal cortical and subcortical areas. They provide about 80 % of neurons in the neocortex. Inhibition in cortical circuits is mediated by local interneurons releasing the neurotransmitter γ-aminobutyric acid (GABA). These interneurons exhibit more diverse morphological and physiological characteristics than excitatory neurons. Cortical neurons are connected to each other and with subcortical areas by a vast number of synapses (~10^{12}). These chemical synapses transform all-or-none action potentials of the presynaptic axon into graded responses in postsynaptic neurons. The incoming signals are processed in the dendrites interacting with specific active and passive properties and output is generated in the axonal region allowing neurons to spatially separate different stages of computation. Each neurons provide coincident inputs to their postsynaptic follower when elicit action potentials and the properties of these connections depend both on pre- and postsynaptic cells. Each neuron receive thousand of synaptic inputs originated form other neurons. These inputs are integrated by the neurons and determine their output generation. Therefore, relations of cortical neurons are spatially organized by divergent and convergent connections. The activity of cortical neurons is temporally organized and oscillations express temporal relation between neurons. Different cortical rhythms can be linked to particular behavioural patterns. In general, firing of single neurons is related to the rhythm of the cortical networks, however they fire usually at a lower frequency. The firing of distinct types of neurons is coupled to stereotyped phases of these oscillations. However, the issues that how single neurons contribute in these spatiotemporally organized networks, and what is the impact of single inputs in the behaviour of single cortical cells, is less understood.

Single inhibitory inputs are able to generate rhythmic activity by interacting with intrinsic conductances and excitatory inputs in their postsynaptic target cells. Activation of individual GABAergic cells are sufficient to synchronize the firing of their postsynaptic cells providing a temporal reference which could be followed by hundreds of neurons. However, GABAergic cells subdivide the surface of their target neurons. Most experiments addressing synchronization either did not examine the location of the inputs or were focused on perisomatic mechanisms. Thus, our first aim was to investigate the postsynaptic effects of dendritically terminating GABAergic interneurons in somatosensory cortex. We compared the effects of two distinct population of interneurons targeting the dendritic or perisomatic region of the postsynaptic cells at behaviuorally relevant frequencies.

Electrical synapses play a role in neuronal synchrony and gap junctional coupling can promote synchronous activity in connections of cortical interneurons. The precise spatiotemporal cooperation of gap junctional coupling with GABAergic synapses between basket cells further enhances populational coherence. Next we investigate how this cooperation appears between dendritic inhibitory synapses and electrical coupling formed by regular spiking non-pyramidal cells (RSNPC).

There are two major types of postsynaptic inhibition in the cerebral cortex. Extracellular stimulation of afferent cortical fibers elicits biphasic inhibitory postsynaptic potentials (IPSPs) in cortical cells. The early phase is due to the activation of $GABA_A$ receptors resulting in a Cl^- conductance, the late phase is mediated by K^+ channels linked to $GABA_B$ receptors through G-proteins. Although dual recordings revealed several classes of interneurons evoking fast, $GABA_A$ receptor mediated responses in the postsynaptic cells, it is not clear whether distinct groups of inhibitory cells are responsible for activating $GABA_A$ and $GABA_B$ receptors. Repetitive firing of interneurons and/or cooperation of several interneurons is thought to be necessary for the activation of $GABA_B$ receptors possibly by producing extracellular accumulation of GABA to levels sufficient to activate extrasynaptic receptors. To find the origin of neocortical slow inhibition we investigated the kinetics, pharmacology and postsynaptic

localization of inhibitory inputs evoked by distinct type of interneurons in pyramidal cells.

Nerve cells integrate thousands of convergent postsynaptic potentials and the rules of input summation are crucial in determining neuronal output properties. The rules of synaptic summation are thought to depend on the dendritic geometry of the postsynaptic cell, on a variety of synaptic and voltage dependent conductances distributed heterogeneously over the dendritic tree and on the relative position and timing of inputs. To test the effect of synapse location on the integration of inputs, we have identified the sources, effect, and subcellular location of local cortical afferents converging onto simultaneously recorded neocortical neurons . The summation of two glutamatergic or GABAergic inputs, evoked by interneurons with specific target preference, was tested as a function of the relative location of synapses and the relative timing of inputs.

Activation of voltage gated conductances, such as hyperpolarization activated cation channel (Ih) can influence the integration of dendritic inputs. The weight of individual dendritic inhibitory synapses might be less than that of perisomatic synapses due to the passive properties of the dendrites and the preferential dendritic expression of some voltage-dependent conductances. Such mechanism would counteract the relatively higher number of synapses targeting the dendrites as compared to perisomatic inputs, equalizing the inhibitory effectiveness of perisomatic and dendritic channels of information. Therefore, we compared the summation of two inhibitory inputs regarding their location on the postsynaptic pyramidal cells and the contribution of Ih channel to these integrations.

Finally, we addresses a fundamental aspect of cortical information processing: how subthreshold interaction of inputs would be manifested in the firing of postsynaptic neurons? We have chosen a connection, the inputs from pyramidal neurons to fast spiking interneurons, which has been proposed to participate in the fine tuning of temporal characteristics of cortical microcircuits. We investigated the mechanisms by means of individual and simultaneous effects

of two inputs are manifested in the output of postsynaptic neurons and how these mechanisms promote the reading of the temporal sequences of excitatory inputs.

METHODS

Young (P17-30) Wistar rats were anaesthetized with halothane, and following decapitation coronal and sagital slices (300-320 μm thick) were prepared from their somatosensory cortex. During the recordings the oxigenated extracellular solution composed of (in mM) 130 NaCl, 3.5 KCl, 1 NaH_2PO_4, 24 $NaHCO_3$, 3 $CaCl_2$, 1.5 $MgSO_4$, 10 D(+)-glucose at 34-35 ˚C. Micropipettes (5-7 MΩ) were filled with (in mM) 126 K-gluconate, 4 KCl, 4 ATP-Mg, 0.3 GTP-NA_2, 10 HEPES, 10 kreatin-phosphate and 8 biocytin (pH 7.25; 300 mOsm). Somatic whole-cell current-clamp recordings were obtained from concomitantly recorded pairs, triplets and quadruplets of interneurons and pyramidal cells visualized in layers II/III by infrared differential interference contrast videomicroscopy. The firing patterns of the neurons were recorded at -60 mV with 800 ms square pulses, starting from -100 pA and increasing by 20 pA steps. The recorded cells were identified by their firing patterns, synaptic connections and morphological properties.

Presynaptic cells were stimulated with brief (2 ms) pulses to reliably evoke action potential when investigating synaptic connections. During subthreshold paradigms, the membrane potential of postsynaptic cells were usually held at -51 ± 4 mV. For suprathreshold trials, postsynaptic cells were depolarized with constant current injections above the threshold to elicit firing. During convergent experiments, presynaptic cells were stimulated in cycles containing single presynaptic cell activations and synchronous and asynchronous dual presynaptic activation. For synchronous presynaptic activation, action potentials were timed to synchronize maximal unitary postsynaptic current amplitudes measured prior to the experiments testing convergence. Voltage clamp recordings were excluded

from analysis if postsynaptic series resistance was higher than 25 MΩ. Traces were excluded from the analysis, if spontaneous PSPs occurred 20 ms before or 100 ms after the activation of identified responses. The biocytin filled cells were visualized with the avidin-biotin-horseradish peroxidase method and light microscopically reconstructed in three-dimension. Light microscopically detected presumed synapses were checked on the ultrathin sections in electron microscope.

RESULTS AND DISCUSSION

Input and frequency specific entrainment of postsynaptic firing by IPSPs of perisomatic or dendritic origin

The recorded fast-spiking cells innervated the soma and proximal dendrites of the postsynaptic neurons (20 ± 16 μm from the soma, including synapses on the soma); therefore, we identify these cells as basket cells. In contrast, more distal dendrites were innervated by bitufted cell synapses, which were on average 65 ± 25 μm from the soma. Perisomatic and dendritic GABAergic inputs provided by fast spiking and bitufted interneurons respectively, entrain the timing of postsynaptic spikes differentially in both pyramidal cells and interneurons when activated at beta and gamma frequencies. Entrainment of pyramidal as well as regular spiking non-pyramidal cells was input site and IPSP frequency dependent. Gamma frequency input from fast spiking cells entrained pyramidal cells on the positive phase of an intrinsic cellular theta oscillation, whereas input from bitufted cells was most effective in gamma frequency entrainment on the negative phase of the theta oscillation. The discharge of regular spiking interneurons was phased at gamma frequency by dendritic input from bitufted cells, but not by perisomatic input from fast spiking cells. Action potentials in fast spiking GABAergic neurons were phased at gamma frequency by both other fast spiking and bitufted cells, regardless of whether the presynaptic GABAergic input was at gamma or beta frequency.

Our results provide evidence that both perisomatic and dendritic GABAergic inputs are capable of entraining several types of postsynaptic neuron in cortical networks at behaviourally relevant frequency ranges. Convergence and divergence of the connections, some identified here for the first time, increase the complexity as well as the computational power of network operations. The interaction of cell type-specific intrinsic properties and location-selective GABAergic inputs could result in a spatiotemporally regulated synchronization and gating of cortical spike propagation in the network.

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

Regular spiking non-pyramidal cells (RSNPCs) were identified based on their physiological and anatomical properties. 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 %). Dendritic GABAergic (75 ± 18 μm from the soma) interactions between RSNPCs phased postsynaptic activity at beta frequency (19 Hz), but were ineffective in phasing at gamma rhythm (37 Hz). Electrical interactions of RSNPCs were transmitted via 2-8 gap junctions between dendritic shafts and/or spines (59 ± 21 μm from the soma). Elicited at beta and gamma frequencies, gap junctional potentials timed postsynaptic spikes with a phase lag, 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.

Electrically and synaptically coupled networks of dendrite targeting RSNPCs could provide a pathway for rhythmic activity spatially segregated from network of interneurons with perisomatic postsynaptic target preference.

Identified sources and targets of slow inhibition in the neocortex

GABAergic neurogliaform cells showed late spiking firing pattern and possessed compact axonal and dendritic arborization in the neocortex. Neurogliaform cells

predominantly innervated dendritic spines (71 %) and shafts (29 %) of the postsynaptic cells. Postsynaptic potentials in pyramidal neurons elicited by neurogliaform cells showed slower ($p < 0.001$, Mann-Whitney test) 10-90 % rise times (23.4 ± 9.8 ms, n = 54) when compared to IPSPs due to basket (5.8 ± 2.0 ms, n = 19) or bitufted cell (6.5 ± 1.7 ms, n = 15) activation. The decay of neurogliaform to pyramid IPSPs could not be fitted with single or double exponential functions. We thus measured the half-width of IPSPs for statistical comparison and found that neurogliaform to pyramid IPSPs were significantly longer ($p < 0.001$; 183.9 ± 82.5 ms, 61.3 ± 16.3 ms, and 58.9 ± 17.9 ms for neurogliaform, basket and bitufted to pyramid connections, respectively). Neurogliaform to pyramid IPSPs were composed of two components. The early component is due to chloride-permeable, bicuculline and gabazine sensitive $GABA_A$ receptors. The delayed component requires the activation of CGP35348 sensitive $GABA_B$ receptors and it is driven by potassium ions.

Our results provide evidence that neurogliaform cells, in contrast to other GABA-releasing cells, elicit combined $GABA_A$ and $GABA_B$ receptor mediated responses with single action potentials.

Cell type and subcellular position dependent summation of unitary postsynaptic potentials in neocortical neurons

We simultaneously recorded from three neocortical neurons to investigate the effects of subcellular position of two convergent inputs on the response summation in the common postsynaptic cell. The sources, and subcellular location of local cortical inputs were identified using correlated light- and electron microscopy. When scattered over the somatodendritic surface, combination of two coincident excitatory or inhibitory synaptic potentials summed linearly in layer 2/3 pyramidal cells as well as in GABAergic interneurons. Slightly sublinear summation with connection specific kinetics was observed when convergent inputs targeted closely placed sites on the postsynaptic cell. The degree of linearity of summation also depended on the type of connection, the relative timing of inputs. Similar moderate sublinear summation were observed during the

interaction of neighboring inhibitory inputs in the axon initial segment of the postsynaptic cell. Although, this cellular compartment has a relatively small volume, and in this case a considerable portion of all afferents converging to the same domain is simultaneouly active.

These results suggest that, when few inputs are active, linear or moderately sublinear summation dominates the integration of inputs maintaining the importance of individual inputs. However, compartment and connection specific nonlinear interactions between synapses located close to each other could increase the computational power of individual neurons in a cell type specific manner.

Contribution of Ih in summation of dendritic IPSPs

We found previously that blockade of Ih channels shifts the summation of two perisomatic IPSPs towards sublinearity. Our results show that two dendritic IPSPs often sum sublinearly, however the synapses arriving from the different presynaptic cells are not close to each other. The Ih blocker, ZD 7288, have diverse effect on the summation of IPSPs where at least one of them located on the dendritic region of the postsynaptic cell. In some experiments ZD 7288 increased the sublinearity similarly to the summation of two perisomatic IPSPs. But in the majority of cases ZD 7288 shifted the summation towards linear particularly if both presynaptic inhibitory cells targeted the dendritic region. Sublinear summation caused by Ih could be observed only when one or both IPSPs targeted the dendritic domain.

These results show that Ih channels are actively involved in location dependent modulation of IPSP summation. Therfore, they could regulate the weighting of the impact of dendritic and perisomatic inhibitions.

Readout of spatial and temporal input summation by single neocortical interneurons

We have chosen the pyramidal to fast spiking cell connection to investigate the issue that how subthreshold interaction of inputs would be manifested in the firing of postsynaptic neurons. First, the postsynaptic membrane potential dependence of

subthreshold input summation was measured. We found that the degree of slight non-linearity was depended on postsynaptic membrane potential and timing of EPSPs. Suprathreshold interaction of the same inputs, however, was highly nonlinear that is limited to a relatively narrow time window. Simultaneous activation of two EPSPs elicited action potentials with decreased temporal jitter and shorter latency by shifting the peaks of postsynaptic spike distributions towards presynaptic spikes by 0.29 ± 0.09 ms. The temporal distribution of postsynaptic firing also indicates the relative temporal position of subthreshold EPSPs preceding postsynaptic spikes and enabling FS cells to generate different readouts for various sequences of asynchronous inputs. The latency of postsynaptic action potentials was dependent on the interval between presynaptic spikes. When the two EPSPs were separated by 2 ms, the peak of postsynaptic spike distributions were shifted towards the presynaptic spike with 0.4 ± 0.24 ms. We could not detect similar differences when the interval between presynaptic activation was 5 ms. However, when the two inputs were separated by 10 ms, dual presynaptic activation shifted postsynaptic spike distributions away from the presynaptic spike with 0.75 ± 0.28 ms relative to the response to synchronous presynaptic activation. In contrast to synchronous activation, none of tested preceding EPSPs could increase the precision of spike timing of follower EPSPs relative to single activation. We found that differential availability of sodium channel during EPSPs could antagonize the effects of temporal summation and decrease the spike triggering efficacy of a follower EPSP in a sequence relative to the same, but solitary EPSP.

Our results provide evidence that the interaction of spatial and temporal summation rules with differential voltage-gated channel activation could be responsible for timing dependent input processing in single fast-spiking cell. This scenario also allows each single excitatory input to dinamically influence the postsynaptic firing behavior. Transformation of linear subthreshold summation rules to nonlinear and dynamic spike transmission reflects the sequence and synchrony of inputs and could enhance temporal precision of information flow in cortical networks.

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