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

Ph.D. thesis

János Szabadics

Supervisor:
Gábor Tamás, Ph.D.
Department of Comparative Physiology, University of Szeged, Szeged

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Szeged

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1. GENERAL INTRODUCTION

Brain functions are carried out by densely connected networks of neurons. One of the recent aims of neuroscience research is to understand the contributions and impacts of single neurons in the information flow in these networks. During my Ph.D. studies I focused on two aspects of information processing in neocortical circuits: divergence of signaling in separate inhibitory networks and temporal and spatial convergence of two inputs on single cells.

Wiring of the cortex

The human brain contains an extraordinary number (approximately $28 \cdot 10^9$) of neurons, the basic units of the brain, and approximately the same number of glial cells. The human cerebral cortex, the most elaborated living structure, is responsible for higher order brain functions such as perception, cognition and consciousness. Despite the complexity, fundamental cortical structure and basic cells types appear similar from individual to individual within a species, and species to species within mammals. Mammalian neocortices show a uniform horizontal and vertical organization: they form six anatomical layers and functional columns. Ramon y Cajal noticed over a century ago (1893) that neurons found in any cortical area are far from being uniform regarding their morphology and connectivity. This heterogeneity suggests that they interact with each other in a complex and diverse manner. Neurons have two morphologically different processes: axons and dendrites. Dendrites receive and locally process inputs then transmit incoming signals towards the cell body. Output impulses are generated in relatively proximal regions of axons and propagate to the axon terminals to activate synapses. Cortical neurons are connected to each other and to subcortical areas by a vast number of synapses ($\sim 10^{12}$). These chemical synapses transform all-or-none action potentials of the presynaptic axon into graded responses in postsynaptic neurons. Cortical neurons, therefore, integrate and process signals arriving from lower brain areas and other cortical structures within the framework of their connections. The complexity of cortical operations, therefore, depends both on the specialized characteristics of individual neurons and also on their precisely organized connections.

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 and can be found in all cortical layers except layer I. 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. Anatomical studies revealed that interneurons target distinct subcellular domains on principal neurons and distinct populations of interneurons selectively target the axon, the perisomatic region and distal dendrites (Freund and Buzsaki, 1996; Somogyi et al., 1998). Basket cells innervate the perisomatic region, chandelier or axo-axonic cells (Somogyi et al., 1983) target exclusively the axon initial segment of pyramidal cells (Somogyi, 1977; Somogyi et al., 1982) and dendrites receive GABAergic inputs from various populations of interneurons. Double bouquet cells innervate distal dendritic shafts and more frequently dendritic spines (Tamas et al., 1997) and dendrite targeting cells (Tamas et al., 1997) primarily target dendritic shafts. Excitatory synapses arriving from pyramidal cells are located on specialized neuronal compartments called dendritic spines (Peters et al., 1991). Thousands of spines decorate the dendrites of pyramidal neurons. Most of them consist of a short or long spine neck and the spine head located at the end. Spines are the smallest biophysical and biochemical compartments in the dendritic tree (Shepherd, 1990; Shepherd, 1996) forming basic units of synaptic integration (Yuste and Denk, 1995). Interestingly, the dendrites belonging to the majority of GABAergic interneuron lack spines and receive excitatory synapses on dendritic shafts.

The synaptic connectivity of the neocortex is extremely complex. Each neuron receives 5,000–60,000 synapses (Cragg, 1967; DeFelipe and Farinas, 1992), 70 % of which originate from other cortical areas (Gruner et al., 1974; Ahmed et al., 1994; Gulyas et al., 1999; Megias et al., 2001; Martin, 2002). Approximately 20 % of synapses are GABAergic (Mountcastle, 1997; Somogyi et al., 1998). Neurotransmitters activate receptors composed from various subunits in the postsynaptic membranes. These subunits determine the different effects, kinetics, pharmacological sensitivity of receptors and are expressed in cell-type specific and subcellular domain selective patterns (Hollmann and Heinemann, 1994; Barnard et al., 1998).

Integration in single neurons

Neurons fire action potentials and the primary site of action potential generation is the proximal portion of the axon (Colbert and Pan, 2002). Action potentials or spikes can be produced also in the dendritic region (Stuart and Sakmann, 1994), but the axonal threshold is the lowest for generating of action potentials. Synaptic inputs are located primarily on the dendritic tree and partly on soma and axon initial segment of neurons. The output generating

properties of single cells are determined by their passive membrane properties and active conductances. Inputs are integrated according to the input resistance and membrane time constant of the postsynaptic cell, and the output is also determined by the current-voltage relationship of sodium channels. According to cable theory, postsynaptic potentials are filtered as they spread along the dendrites (Rall and Shepherd, 1968) and the impact of individual synapses decreases with increasing distances from the soma (Rall, 1977; Spruston et al., 1994). However, voltage-gated ion channels expressed in the dendrites of neurons could promote backpropagation of action potentials and shift the initiation zone to the dendrites from the axon (Stuart et al., 1997). Currents carried by dendritic voltage-gated channels are important in shaping the subthreshold integrative properties of neurons (Lipowsky et al., 1996; Hoffman et al., 1997; Fricker and Miles, 2000). For instance, inward currents can provide extra ion flux to replace the excitatory synaptic currents lost by dendritic filtering. Dendritic ion channels can affect the temporal summation of trains of inputs (Magee, 1999; Williams and Stuart, 2000) by shaping the decay phase of PSPs (Magee and Cook, 2000). Spike generating capability of single cells can reflect the preceding activity by differential states of sodium channel activation (Azouz and Gray, 2000). Slowly activating potassium channels could lead to decreasing firing frequencies during sustained activation (Lien et al., 2002) and transient activation of potassium currents lead to sustained high frequency spike trains without frequency adaptations by facilitating the recovery from inactivation of sodium channels (Erisir et al., 1999). Taken together, 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.

The synaptic connections between cortical neurons show short-term modulations reflecting the frequency of presynaptic activity (Thomson and West, 1993). Cortical pyramidal cells can evoke either depressing or facilitating EPSPs depending on the type of postsynaptic neurons (Reyes et al., 1998; Scanziani et al., 1998). These postsynaptic target cell-specific dynamic behaviours are regulated by postsynaptic receptor desensitization (Rozov et al., 2001a), presynaptic calcium accumulation (Dittman et al., 2000; Rozov et al., 2001b) and persistent metabotropic receptor activations (Losonczy et al., 2003). When inhibitory cells discharge repetitively, the efficacy of their synapses changes showing depression or facilitation only, or combined facilitating-depressing kinetics. Both presynaptic and postsynaptic neurons are involved in generating the type of temporal dynamics shown by cortical GABAergic synapses (Gupta et al., 2000).

Theoretical analysis of spatial input summation (i.e. addition of events arriving simultaneously onto separate regions of the dendritic tree) began by assuming that passive cables serve as reasonable models of dendrites (Jack et al., 1975; Segev et al., 1995). According to cable theory, electrotonically isolated inputs sum linearly, whereas closely located inputs produce an attenuated response as a consequence of reduction in the ionic driving force or a decrease in dendritic input resistance leading to shunting of synaptic currents (Jack et al., 1975; Segev et al., 1995). However, as discussed above, dendritic membranes are not passive (Hoffman et al., 1997; Lorincz et al., 2002), they contain voltage dependent conductances, which could selectively amplify distal inputs or subserve local nonlinear operations (Koch et al., 1983; Mel, 1993). Recent results show that independent nonlinear computation occurs in branches of structurally and functionally complex dendritic trees. Dendrites perform multi-layer analysis on the spatial contexts of inputs. Thin dendritic branches serve as the first layer, their output is passed to parent dendrites (second layer) and finally to the action potential initiation zone (third layer). Each layer follows differential non-linear rules for integration (Poirazi et al., 2003a, b; Polsky et al., 2004).

Information flow in neuronal networks

Activity-dependent inhibition, interacting with excitatory inputs, has been functionally divided into two different types: feedforward and feedback inhibition. Feedforward inhibition is provided by divergent excitatory inputs that activate inhibitory neurons converging on the targets of excitation (Buzsaki, 1984). Feedforward inhibition arrives at the postsynaptic neuron with a short delay after excitation, and thus shortens the duration of excitation. Accordingly, spikes triggered by excitation occur in a limited time window, thus the temporal precision of postsynaptic output is increased (Pouille and Scanziani, 2001). Feedback inhibition is triggered by recurrent excitatory collaterals to local inhibitory neurons, thus temporally limiting the output leaving the local circuit (Andersen et al., 1963).

Cortical circuits are composed of huge numbers of basically similar neurons (Mountcastle, 1997) and single presynaptic spikes were suggested to have limited effect on postsynaptic cells due to the relatively small number of synapses which transmit information with restricted efficacy (Komatsu et al., 1988; Mason et al., 1991; Nicoll and Blakemore, 1993; Thomson and West, 1993). However, the activity of cortical neurons is temporally and spatially organized in humans and other mammals leading to signals which were first recorded on the electroencephalogram (Berger, 1929). Characteristic types of neural population dynamics occur in behaving animals. Neuronal networks in the mammalian

forebrain show oscillations in several bands covering frequencies from approximately 0.05 Hz to 500 Hz (Buzsaki and Draguhn, 2004). Different cortical rhythms can be linked to particular behavioural patterns: theta oscillations (4-12 Hz) were recorded in the hippocampal formation of mammals and humans during sensory-motor integration (Buzsaki, 2002; Caplan et al., 2003) and in neocortex during memory tasks (Caplan et al., 2001); beta oscillations (15-25 Hz) are related to simple motor tasks (Salmelin et al., 1995; Caplan et al., 2003); gamma oscillations (40-80 Hz) occur during processes related to perception, REM sleep and working memory (Bragin et al., 1995; Fries et al., 2001b; Howard et al., 2003) and 200 Hz sharp-wave ripples can be observed during quiet sleep, consummatory behaviour and immobility (Buzsaki, 1986; Siapas and Wilson, 1998). In general, firing of single neurons is related to the rhythm of the cortical networks, however they fire usually at a lower frequency (Fisahn et al., 1998; Fries et al., 2001a). The classical view considers cortical neurons as integrate-and-fire devices. This allows the temporal summation of synaptic events over temporally extended intervals and the model implies that inputs contribute to output generation regardless of their timing. Information is carried by the rate at which neurons discharge action potentials. This view is supported by in vivo observations showing that the spikes of single pyramidal cells from the motor cortex can evoke whisker movements independent from the ongoing local activity in the cortex. The evoked movement depends on the number and frequency of initiated action potential trains (Brecht et al., 2004). The alternative concept on the operation of single neurons in the network is the so-called coincidence detection theory, stating that only synchronous signals contribute effectively to output generation, therefore the number of relevant inputs is small compared to the total number of inputs arriving to a neuron. This model emphasizes the critical role of input timing and synchronous oscillatory patterns in cortical information flow (Konig et al., 1996; Stevens and Zador, 1998; Salinas and Sejnowski, 2001). Coincidence detection could be a particularly effective way of enhancing response saliency. Supralinear boosting of input summation by synchronous activity could effectively enhance transmission without raising discharge rates. Thus, synchronization can express unambiguous relations among neurons because it selectively amplifies the impact of synchronous responses (Singer and Gray, 1995).

The membrane potential of single cortical neurons is fluctuating between two states (up and down states) in vivo (Steriade et al., 1993b; Lampl et al., 1999). Elevated firing rates in up state follow sensory stimulation (Anderson et al., 2000) and provide a background for persistent activity, a network state that might contribute to working memory (Goldman-Rakic, 1995; Durstewitz et al., 2000; Wang, 2001). However, up and down states can also be

generated intracortically (Steriade et al., 1993b) and reflect the operational state of the circuit and could play a role in signal propagation and grouping. According to the synfire chain theory, synchronous activity of a group of neurons, as defined by their divergent and convergent connections, propagates in a feedforward manner through the network in precise spatiotemporal sequences (Abeles, 1991). Modelling and experimental results show the existence of stereotyped firing sequencies (Prut et al., 1998; Ikegaya et al., 2004) and the ability of cortical networks to propagate such sequencies at high temporal fidelity (Diesmann et al., 1999; Reyes, 2003). Recent results show that such sequences can be assembled during synchronous, long lasting depolarization of the membrane potential (up state) in neocortical networks (Cossart et al., 2003).

Although mechanisms linking oscillating brain states and cellular mechanisms are not clear, recent studies emphasize the importance of GABAergic processes originating from local networks. In vivo recordings show that oscillatory activity in the neocortex is associated with rhythmic IPSPs (Steriade et al., 1993c; Sik et al., 1995; Ylinen et al., 1995). The rhythmic activity of inhibitory networks was proposed to have a crucial role in generating gamma activity in the cortex (Whittington et al., 1995). 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 (Cobb et al., 1995). In hippocampal slices, pharmacologically evoked 40 Hz oscillations have been shown to depend on the interaction of neuronal excitatory network and inhibition mechanisms (Fisahn et al., 1998). Recent results provide another mechanism through which inhibitory cell discharges might be synchronized (Galarreta and Hestrin, 1999; Gibson et al., 1999; Beierlein et al., 2000; Tamas et al., 2000). Gap junctions exist when membranes of two cells are connected by proteins of the connexin family (Venance et al., 2000). The precise spatiotemporal cooperation of gap junctional coupling with GABAergic synapses between basket cells further enhances populational coherence (Tamas et al., 2000). More recently it was found that firing of distinct types of hippocampal interneurons is coupled to stereotyped phases of oscillations (Klausberger et al., 2003; Hajos et al., 2004; Klausberger et al., 2004) suggesting that distinct GABAergic pathways might be involved in the execution of particular tasks of spatiotemporal signal processing.

2. AIMS

This thesis studies aspects of synchronous and sequential events of divergent and convergent neocortical networks. The main questions of our experiments were the following:

1. How differentially placed inhibitory inputs entrain distinct populations of postsynaptic cells at β and γ frequencies?
2. How dendritic electrical coupling and dendritic inhibitory inputs time postsynaptic action potentials in the β and γ frequency ranges?
3. What is the origin of slow inhibitory postsynaptic potentials in the neocortical network?
4. How spatiotemporal properties of inputs influence the arithmetics of subthreshold summation?
5. How subthreshold rules of unitary input summation would be translated to suprathreshold postsynaptic activity?

Contributions

In chapters 4.1 and 4.2, correlated light- and electron microscopy was performed by Andrea Lőrincz and Gábor Tamás; in chapter 4.3 by Andrea Lőrincz, Anna Simon and Gábor Tamás; in chapters 4.4, 4.5 and 4.7 by Gábor Tamás. I carried out the majority of electrophysiological recordings and data analysis shown in chapters 4.1, 4.2, 4.3, 4.4 and 4.5, and all recordings and analysis presented in chapter 4.6 and 4.7.

3. MATERIAL AND METHODS

Electrophysiology. 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. Slices were cut with Leica VT1000S or Microm HM650 vibratome in cold (3-6 ˚C) solution containing of (in mM) 85 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCl_2$, 4 $MgSO_4$, 25 D(+)-glucose, 75 sucrose and saturated with 95 % O_2 and 5 % CO_2. After 1 hour incubation at 30 ˚C, the solution was gradually replaced with a room temperature solution composed of (in mM) 130 NaCl, 3.5 KCl, 1 NaH_2PO_4, 24 $NaHCO_3$, 1 $CaCl_2$, 3 $MgSO_4$, 10 D(+)-glucose. The solution used during recordings differed only in that it contained 3 mM $CaCl_2$ and 1.5 mM $MgSO_4$. 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 at ~36˚C from concomitantly recorded triplets and quadruplets of interneurons and pyramidal cells visualized in layers 2/3 by infrared differential interference contrast videomicroscopy (Zeiss Axioskop microscope, Hamamatsu CCD camera, Luigs & Neumann Infrapatch set-up and two HEKA EPC 9/double patch-clamp amplifiers). Signals were filtered at 8 kHz, digitized at 16 kHz and analyzed with PULSE software (HEKA).

The passive membrane parameters and firing patterns of the recorded cells were investigated at -60 mV with 800 ms square pulses, starting from -100 pA in 20 pA steps. The inward resistances of the cells were determined in the first trial at the maximum of the voltage response. The membrane time constant were measured by fitting single exponential to the onset of the voltage response to hyperpolarizing square current pulse. Presynaptic cells were stimulated with brief (2 ms) pulses to reliably evoke action potential when investigating synaptic connections. The short-term dynamics of a given synapses were measured with 60 ms interval paired pulse protocols. Recording of synaptic connection trials were delivered at >5s intervals, to minimize intertrial variability, except in chapter 4.3 (>60s). During subthreshold paradigms, the membrane potential of postsynaptic cells were usually held at -51 ± 4 mV to separate the chloride driven IPSPs (reversal potential at -72 mV) and sodium or calcium driven EPSPs. Unless specified, traces shown are averages of 30-200 episodes. The amplitude of postsynaptic events was defined as the difference between the peak amplitude and the baseline value measured prior to the PSP onset. All traces were offseted to align their baselines for the period from -20 to 0 ms prior to the onset of current injections into the presynaptic neuron. Data for analysis were used only from epochs in which the postsynaptic

response remained stationary, i.e. the mean amplitude of 10 consecutive events remained within ±10 % of the mean of the first 10 events of the epoch. Statistica for Windows software package was used for statistical analysis (StatSoft, Tulsa, Oklahoma). The significance level for all comparisons was set at $p < 0.05$.

For phasing trials (Chapters 4.1 and 4.2), postsynaptic cells were depolarized with constant current injections above threshold so as to elicit firing at 6.9 ± 3.0 Hz, without significant differences between types of connections and presynaptic paradigms. The postsynaptic spikes were collected from 50-100 consecutive trials. The timing of the postsynaptic action potentials was measured at their peaks. First, postsynaptic firing probabilities were measured at low timing resolution in each experiment. In these protocols width of the bins are corresponded to the interval of presynaptic action potentials. During the presynaptic stimulation the presynaptic spikes determined the onset of a given bin and prior or after the stimulation the bins are determined by the first or last presynaptic action potentials. Postsynaptic firing probability was normalized to allow comparison of pairs of a particular type of connection (Fig. 1.2D). Friedman's nonparametric repeated measures test was applied to determine significant changes of average firing probability during individual cycles relative to the average of control; consecutive cycles, which were consistently similar to or different from control in all cases of a given type of connection, were grouped. These groups of bins were then compared to one another to validate the grouping relative to control. When all cycles were different from control, bins during presynaptic activity were compared to one another and were grouped accordingly. When no consistent trends were obvious, we split the train of cycles into two groups containing cycles 1-5 and 6-11 at 37 Hz, and 1-3 and 4-6 at 19 Hz. For higher timing resolution probability plots the timing of postsynaptic action potentials were compared to preceding presynaptic spike in each cycles. These timing values were binned at 3.375 ms (37 Hz) and 6.5 ms (19 Hz) within each cycle. The measured distributions were averaged in the corresponding groups where the amounts of postsynaptic spike were similar (Fig. 1.2E). Controls were collected prior to the onset of the presynaptic spike train using identical cycle duration, and data obtained during presynaptic activation were normalized to control. Friedman's test was used to determine differences relative to control and between bins of different latency in individual presynaptic stimulation cycles. Each bin was compared to the control, then bins were grouped and groups were again compared; if all bins were different from control, we searched for tendencies comparing consecutive bins and/or comparing the bin containing maximal and minimal average firing probability with the

rest of bins. Data are given as mean ± s.e.; differences were accepted as significant at $p < 0.05$.

In experiments, where convergent inputs were studied (Chapter 4.4 - 4.7), the 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. Unless specified, traces shown are averages of 30-60 consecutive episodes. Traces were excluded from the analysis, if spontaneous PSPs occurred 20 ms before or 100 ms after the activation of identified responses; this process resulted in the elimination of <10 % of events in a particular paradigm. The difference between the algebraic sums of single input responses and the recorded summed response was calculated during postsynaptic responses and expressed as a percentage of the maximal amplitude of the calculated response at the given time point. The resulting waveform is used as a measure of the degree of linearity over time.

When investigating the effects of convergent unitary EPSPs on the postsynaptic firing, the onset of EPSPs were synchronized by systematically shifting presynaptic action potentials in order to compensate for submillisecond latency differences in experiments with synchronous presynaptic activation. All sweeps were included in the analysis regardless of the occurrence of postsynaptic spikes prior to the activation of presynaptic cells. High resolution distributions of postsynaptic spikes were calculated by Gaussian convolution (2ms) as described by Szűcs (Szucs, 1998). In voltage-clamp experiments with currents applications similar to EPSP waveforms, signals were sampled at 100 kHz (Chapter 4.7). In these experiments series resistances were between 16 and 24 MΩ. Leak currents were measured by applying 10 alternating polarity, tenth amplitude pulses during each trial and subtracted automatically by the acquisition software. Current and voltage traces were recorded in parallel to follow the quality of voltage clamp. Only that traces were used for analysis where the leak and series resistance were at least in 25 protocol cycles unchanged.

The Ih channel blocker, ZD7288 (40 μM), the AMPA receptor antagonist, NBQX disodium salt (10 μM), the NMDA receptor antagonist, D-AP-5 (20 μM), the $GABA_B$ receptor antagonist, CGP35348 (60 μM) and the $GABA_A$ receptor antagonist, (-)-bicuculline-methiodide (10 μM) and SR-95531/gabazine (20 μM) were applied extracellularly. Drugs were dissolved according to the instructions of suppliers and perfused through the recording

chamber similarly to the control solution. The sodium channel blocker QX314 (5 mM) were added to the intracellular solution.

Histology. I have not contributed to the anatomical investigation of recorded cells. Visualization of biocytin was carried out by Éva Tóth, Andrea Lőrincz and Gábor Tamás, light microscopic reconstructions were performed by Andrea Lőrincz, Gábor Tamás, Éva Tóth, Anna Simon and Gergely Komlósi and the correlated electron microscopy by Gábor Tamás and Andrea Lőrincz. Therefore I refer to the original publications for the detailed description of these methods (Tamas et al., 2000; Szabadics et al., 2001). Briefly, three-dimensional light microscopic reconstructions were carried out using Neurolucida (MicroBrightfield) with a 100x objective. Distances between dendritic contact sites of different sources were measured along the dendrites and, when applicable, the somatic distances linking the root of the dendrites were added. For calculating the mean distance between the identified or light microscopically predicted synaptic sites of two afferents to the same postsynaptic cell, the distances between each synaptic site of one afferent from each synaptic site of the other one were averaged.

4. RESULTS

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

Summary

Correlated activity of cortical neurons underlies cognitive processes. Networks of several distinct classes of GABAergic interneuron are capable of synchronizing cortical neurons at behaviourally relevant frequencies. Here we show that perisomatic and dendritic GABAergic inputs provided by two classes of GABAergic cells, fast spiking and bitufted interneurons respectively, entrain the timing of postsynaptic spikes differentially in both pyramidal cells and interneurons 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. The interaction of cell type specific intrinsic properties and location selective GABAergic inputs could result in a spatio-temporally regulated synchronization and gating of cortical spike propagation in the network.

Introduction

Neurons of the cerebral cortex communicate by complex temporal and spatial dynamics, but how signals of different frequencies are routed and assessed by cortical networks remains to be explained in terms of specific connections. Electroencephalograms indicate synchronous cortical population activity with dominant frequency components corresponding to particular behaviours (Barlow, 1993; Niedermeyer and Lopes da Silva, 1993). Slow oscillations around 1-4 Hz are associated with sleep, and rhythms in the beta and gamma frequency bands (15-70 Hz) are typical in the awake states (Steriade et al., 1993a). Moreover, gamma rhythms are associated with a number of cognitive processes, such as perception and attentional

mechanisms (Lisman and Idiart, 1995; Mainen and Sejnowski, 1995; Singer and Gray, 1995; Jefferys et al., 1996). Fast network oscillations were proposed to gate the flow of neural information (Salinas and Sejnowski, 2001) and to establish dynamic temporal correlations between spatially distributed neurons, possibly contributing to higher order sensory representations (Singer and Gray, 1995).

Cortical networks have a variety of intrinsic mechanisms that could contribute to synchronous activity (Ritz and Sejnowski, 1997), and GABA-mediated processes appear prominent in governing rhythmogenesis (Lytton and Sejnowski, 1991; Buzsaki and Chrobak, 1995; Cobb et al., 1995; Jefferys et al., 1996; Csicsvari et al., 1999; Bartos et al., 2001; Hirsch et al., 2003). Synchronous, high frequency firing of putative interneurons has been recorded *in vivo* (Steriade et al., 1998; Swadlow et al., 1998; Csicsvari et al., 1999), and both synaptic and gap junctional coupling can promote synchronous activity in connections of cortical interneurons (Galarreta and Hestrin, 1999; Gibson et al., 1999; Koos and Tepper, 1999; Beierlein et al., 2000; Tamas et al., 2000; Venance et al., 2000; Bartos et al., 2001). Inhibitory postsynaptic potentials (IPSPs) elicited by some interneuron classes were shown to be highly effective in entraining the firing of postsynaptic neurons (Lytton and Sejnowski, 1991; Cobb et al., 1995; Bush and Sejnowski, 1996; Jefferys et al., 1996; Bartos et al., 2001). Different types of GABAergic cells subdivide the surface of their target neurons (Somogyi et al., 1998), and both perisomatically and dendritically terminating populations of interneurons can be synchronized within the same cell class via precise spatiotemporal cooperation of gap junctional coupling with GABAergic synapses (Tamas et al., 2000; Szabadics et al., 2001). Dendritically and perisomatically terminating cortical interneurons are recruited at characteristic temporal domains during cortical network operations (Klausberger et al., 2003; Hajos et al., 2004; Klausberger et al., 2004). Cortical GABAergic responses exhibit a wide range of dynamic characteristics, which are determined by both pre- and postsynaptic factors (Gupta et al., 2000). Computational models and experiments suggested that the time scale of rhythmic cortical activity could be determined by intrinsic subthreshold membrane potential oscillations and by the decay constant of GABAergic inhibitory synaptic potentials onto the postsynaptic cells (Cobb et al., 1995; Traub et al., 1996; Hausser and Clark, 1997; Stuart, 1999; Bartos et al., 2001; Tiesinga et al., 2001). Here we explore how IPSPs evoked by different classes of cortical interneurons entrain postsynaptic firing in distinct types of target cell and how pyramidal cells recruit firing in these interneurons.

Results

In order to identify the cellular properties underlying the flow of rhythmic activity in the cortex, we have recorded pairs and triplets of interneurons and/or principal cells coupled by GABAergic connections in layers 2-3 of the somatosensory cortex. Following the physiological classification of cell types and their interactions, we analyzed the spatial distribution of synapses mediating the interactions. Having identified distinct cell populations and stereotyped arrangement of synapses on the target cells, next we tested some factors which might influence postsynaptic firing behaviour under the influence of rhythmically arriving IPSPs, focusing primarily on the cell type dependence of entrainment and only to a small extent on the underlying mechanisms.

Identification of cell types

According to previously established electrophysiological and anatomical classification of cell types, we have focused our study on the output of fast spiking (FS) and bitufted (Bt) cells innervating FS, Bt, regular spiking non-pyramidal (rs) and pyramidal neurons (McCormick et al., 1985; Cauli et al., 1997; Kawaguchi and Kubota, 1997; Reyes et al., 1998) (Fig. 1A). In agreement with earlier studies, input resistance and membrane time constant were, on average, 211 ± 36 MΩ and 26 ± 6 ms in pyramidal cells (n = 23), 112 ± 28 MΩ and 7 ± 1 ms in FS (n = 44) cells, 282 ± 54 MΩ and 26 ± 7 ms in Bt (n = 40) and 306 ± 94 MΩ and 23 ± 11 ms in rs (n = 16) neurons. Similarly to previously determined properties of EPSPs targeting cortical interneurons (Reyes et al., 1998; Szabadics et al., 2001), unitary input from neighbouring pyramidal cells showed paired pulse depression on FS (n = 8, to 58 ± 248 %) and rs (n = 26, 49 and 56 %) cells and paired pulse facilitation on Bt (n = 9, 232 ± 108 %) cells at a paired pulse interval of 60 ms. Based on intrinsic membrane and firing characteristics and the facilitatory EPSPs received, the Bt cell class of this study is similar to that of bitufted cells defined by Reyes et al. (1998) and to the regular spiking nonpyramidal-somatostatin cell type in (Kawaguchi and Kubota, 1997; Cauli et al., 2000) and to the low threshold spiking cells characterized earlier (Gibson et al., 1999).

Spatial arrangement of the input from FS and Bt interneurons on the target cells

One of the emerging principles of cortical circuitry is the spatial specificity of GABAergic cells (Szentagothai and Arbib, 1974; Kawaguchi and Kubota, 1997; Somogyi et al., 1998). We searched for potential differences in the spatial arrangements of connections between

GABAergic cells and from GABAergic cells to pyramidal cells. We assessed the number and position of synapses mediating the interactions by correlated light and electron microscopy (Fig. 1.1B-G). From each class of connection tested for postsynaptic spike timing, five cell pairs were selected for light microscopic mapping of connectivity; the selection was based on the relative completeness of the presynaptic axonal and postsynaptic dendritic trees from the classes in which we had more than five examples. Of these, full electron microscopic evaluation of predicted synapses was performed in 11 pairs, including all classes of

connections. In addition, in the rest of the selected pairs, somata (n = 16) postsynaptic to FS cells were completely examined in serial ultrathin sections to avoid the light microscopic underestimation of the number of synapses targeting the cell body. Both FS and Bt cells innervated postsynaptic neurons through similar numbers of synapses (5 ± 2 and 3 ± 2, respectively). However, the subcellular positions of synapses formed by FS and Bt cells were significantly different ($p \leq 0.02$; Mann-Whitney U-test), regardless of the type of target neuron. The FS 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 Bt cell synapses, which were on average 65 ± 25 μm from the soma.

Connection dependent efficacy of single IPSPs in phasing postsynaptic action potentials

Given the spatial separation of GABAergic inputs on the surface of postsynaptic cells in our sample, we tested whether a solitary IPSP could reset the phase of rhythmic firing evoked by depolarization in interneurons, following reports that in pyramidal cells rhythmic firing was phase locked by a single IPSP (Cobb et al., 1995; Miles et al., 1996; Stuart, 1999; Gupta et al., 2000). We recorded single IPSPs in FS cells evoked by different types of presynaptic cells (FS, n = 3; Bt, n = 6) on the perisomatic and dendritic domain, but having similar decay time constants recorded at the soma (20 ± 10 ms and 17 ± 10 ms, respectively). Solitary IPSPs elicited either by FS cells or Bt cells could phase lock the firing of the postsynaptic FS cells to the IPSP at frequencies of 27 ± 6 Hz for up to three successive cycles (Fig. 1.2A). We have also found that a single IPSP elicited perisomatically by FS (n = 4) or dendritically by Bt (n = 5) cells phase locked postsynaptic pyramidal cell firing at theta frequency for two to three consecutive cycles (not shown).

The kinetics of IPSPs differ between cell types and may also differ between distinct inputs to the same cell. The duration of somatically detected IPSPs may also constrain rhythmic firing. We searched for kinetically distinct IPSPs targeting the same postsynaptic cell type, and found them reproducibly in one of the interneuron types in our sample, the rs cell (Fig. 1.2B). Interestingly, IPSPs elicited dendritically by Bt cells had faster rise and decay characteristics than those evoked by FS cells around the soma. Averaged IPSPs elicited by single presynaptic spikes of FS (n = 5) or Bt (n = 5) cells in rs cells had 10-90% rise times of 15.2 ± 8.5 ms and 4.4 ± 0.5 ms, respectively ($p \leq 0.05$, Mann-Whitney U-test) and decay times of 88 ± 29 ms and 27 ± 9 ms ($p \leq 0.001$). The firing of postsynaptic rs cells was decreased for 62 ± 11 ms by FS cells, and for 31 ± 7 ms by Bt cells ($p \leq 0.01$; bin width, 13

ms). These results demonstrate that the duration of IPSPs contributes to the period of altered firing in the same type of cell. In rs cells, however, postsynaptic firing did not show significant periodicity before or after a single IPSP.

Timing of postsynaptic action potentials by differentially placed compound IPSPs

Because many interneurons fire repeatedly at high frequency in vivo (Steriade et al., 1993a; Swadlow et al., 1998; Csicsvari et al., 1999; Klausberger et al., 2003), we systematically measured the efficacy of GABAergic inputs at beta and gamma frequency on setting the phase of postsynaptic action potentials in three types of target neuron. The method of quantitative evaluation of action potential timing during different periods of rhythmic presynaptic GABAergic inputs is shown in fig. 1.2C-E in an example of a FS cell to FS cell connection. Presynaptic cells were stimulated with trains of action potentials at gamma (37 Hz) and beta (19 Hz) frequency, and compound unitary IPSPs were recorded postsynaptically. Then, postsynaptic cells were depolarized above threshold to characterize the effect of the same unitary IPSP series on influencing the timing of postsynaptic spikes. Cortical rhythmic activity at higher frequencies is often nested in slower ongoing oscillations (Wang, 2003; Ritz and Sejnowski, 1997; Buzsaki and Chrobak, 1995; McBain and Fisahn, 2001); therefore, when observed, we analyzed postsynaptic firing according to different phases of ongoing membrane behaviour as detected in separate subthreshold paradigms (see methods). In order to reduce the number of variables, comparisons were made between IPSPs having similar ($p > 0.05$) somatically recorded amplitudes. To test input specificity, IPSPs evoked by terminals in either the somatic/perisomatic, or the more distal dendritic domain of the postsynaptic target cells were compared. Electrical and combined electrical and GABAergic interactions between cells were not included in this paper because in depth analysis of all permutations would result in an exceedingly large and complex data set.

Input to FS cells

First we compared the effect of GABAergic input from FS and Bt cells on FS cells (Fig. 1.3A-D). Postsynaptic FS cells responded with unitary IPSPs of decremental amplitude to trains of action potentials elicited at beta and gamma frequency (19 and 37 Hz, respectively) in either of the two presynaptic cell types. The initial amplitudes of FS to FS and Bt to FS IPSPs were similar and decreased to approximately 28-49 % of the first response regardless of the source of the input. Presynaptic spike trains of Bt cells at beta and gamma frequencies moderately decreased the mean frequency of depolarization evoked postsynaptic firing in FS

cells to 85 ± 13 % and 80 ± 14 % of the control values, respectively; input from FS cells had a variable effect on the average rate of firing in FS cells. In two pairs the firing rate of the postsynaptic cell was unaffected; in three postsynaptic cells it was reduced. As a result of this variability, the average rate of firing in FS cells was not reduced significantly (Fig. 1.3A). Postsynaptic firing rate remained relatively stable during presynaptic cell activation when comparing the averages during individual cycles, showing that postsynaptic FS cells were not rhythmically entrained at frequencies lower than the applied test frequencies. However, postsynaptic FS cell firing was entrained within each cycle for the entire duration of presynaptic activation. After the preceding presynaptic spike, gamma frequency GABAergic inputs from both FS and Bt cells significantly decreased the probability of postsynaptic FS cell firing only for a limited period of time (Fig. 1.3A-B). Firing probability decreased in FS to FS cell connections in the second and third bins (3.4-10.1 ms; $p \leq 0.01$) and in Bt to FS cell connections from the second to fifth bins (3.4-16.8 ms, $p \leq 0.01$; Fig. 1.3A-B). During beta frequency presynaptic activation of either FS or Bt cells, postsynaptic firing probability returned to control levels following the preceding presynaptic spike after the second or third bins (13-19.5 ms; $p \leq 0.05$; Fig. 3C-D). Both 19 and 37 Hz frequency presynaptic activation suppressed FS cell firing for similar periods of time corresponding to the cycle length of gamma-band cortical oscillations. Thus, FS cells perform a transformation of beta frequency presynaptic input to gamma frequency rebound activation irrespective of the location of the presynaptic input on their somato-dendritic domain.

Input to rs cells

Postsynaptic rs cells responded differently to inputs from FS and Bt cells (Fig. 1.4). The summated amplitude of unitary IPSPs elicited by FS cells increased substantially in response to the first two presynaptic action potentials, then declined irrespective whether presynaptic firing was evoked at gamma or beta frequencies (Fig. 1.4A,C). On the other hand, repetitive activation of presynaptic Bt cells evoked IPSPs of relatively stable amplitude. The latter IPSPs had faster rise and decay kinetics, as mentioned previously.

Presynaptic activity of FS cells at gamma frequency phased action potentials at theta frequency in postsynaptic rs cells in parallel with a rebound depolarization developing in the rs cells following the first 3 IPSPs; postsynaptic firing was strongly suppressed during the first three presynaptic cycles ($p \leq 0.04$); then it returned close to control levels during the 4-10th cycles and was significantly reduced again during the 11th cycle ($p \leq 0.02$; Fig. 1.4A). Following the cessation of IPSPs there was strong rebound firing significantly exceeding the

control level ($p \leq 0.05$). Such theta frequency entrainment of rs cells was not apparent during FS cell input at 19 Hz, although postsynaptic firing was reduced during the first cycle ($p \leq 0.02$). The firing of rs cells was not entrained at gamma frequency by FS cells, as shown by irregular probability distributions of postsynaptic spikes relative to presynaptic cycles of firing (Fig 1.4A). At beta frequency, however, rs cells were synchronized to FS cell activity. Postsynaptic firing probability was higher than in the rest of the bins ($p \leq 0.04$) during the first cycle, and rs cells fired below control levels in the first five cycles during the rest of the presynaptic cycles ($p \leq 0.02$).

In contrast to FS cells, Bt cells effectively entrained rs cells at gamma and beta frequency throughout the entire duration of presynaptic activity, as shown by the highest postsynaptic firing probabilities around the end of the cycle and by groups of neighbouring bins significantly different from control values ($p \leq 0.05$; Fig. 1.4B,D bottom panels). The control firing rate of rs cells was decreased by Bt cells during the first five cycles at gamma ($p \leq 0.03$), and during the first cycle at beta frequency ($p \leq 0.02$; Fig. 1.4B,D middle panels). Low frequency postsynaptic rhythmicity was not detected by statistical comparison of firing probability of the bins in rs cells in response to Bt cell inputs (Fig. 1.4B,D).

Input to pyramidal cells

Pyramidal cells were differentially entrained by inputs from FS and Bt cells (Fig. 1.5), but unlike in rs cells, subthreshold responses were similar in amplitude and kinetics to both inputs. Unitary IPSPs showed an initial summation; then the amplitude of the compound response declined to a plateau at 74 ± 12 % and 69 ± 19 % of the summated amplitude at gamma and beta frequency, respectively, to both FS and Bt cells.

Presynaptic activity of FS and Bt cells at gamma frequency phased action potentials at theta frequency in tonically firing pyramidal cells. Postsynaptic firing was strongly suppressed during the first three presynaptic cycles in FS to pyramid connections ($p \leq 0.01$) then it returned to control levels during the 4-7th cycles and was significantly reduced again during the 8-11th cycles (Fig. 1.5A,B). In response to inputs from Bt cells, firing probability was decreased in pyramidal cells during the first two cycles ($p \leq 0.02$), was around control in the 3-7th cycles and dropped back to below control levels in the 8th 10th and 11th cycles ($p \leq 0.04$). Although firing probability was not significantly different from control in the 9th cycle ($p \leq 0.17$), there was a significant drop from the 7th to the 8th cycle ($p \leq 0.04$) and between the groups of 4-7th and 8-11th cycles ($p \leq 0.02$), therefore we grouped the 8-11th cycles. Following the last IPSP in the train, pyramidal cells generated rebound spikes. Presynaptic FS and Bt

cell activation at beta frequency reduced pyramidal firing during the first two cycles ($p \leq 0.03$); postsynaptic firing returned in the third cycle and remained similar to control until the end of presynaptic activity (Fig. 1.5C,D). Thus, gamma frequency IPSPs arriving to pyramidal neurons dendritically or perisomatically reset the phase of ongoing theta oscillations at the onset of postsynaptic response. Successive IPSPs within a train elicited at different frequencies become nested relative to the theta rhythm and their effect on postsynaptic firing can be tested on various phases of ongoing theta oscillations.

The phasing of pyramidal cell action potentials by FS and Bt cells at gamma frequency was dependent on the phases of the simultaneous theta rhythm in firing. The entrainment at gamma frequency by FS cells was apparent during the entire length of presynaptic activity. During the first three presynaptic cycles, when pyramidal cell firing was suppressed relative to control, postsynaptic firing probability showed a clear incease in the last bin relative to the rest of bins ($p \leq 0.004$; Fig 1.5A, blue). When pyramidal firing rate increased, phasing was clearly revealed by consecutive series of bins significantly different from control during the 4-7th ($p \leq 0.01$) and 8-11th ($p \leq 0.05$) cycle groups (Fig 1.5A, red and green). Comparing the amplitude of gamma frequency entrainment measured as differences of bins having maximal and minimal average firing probabilities showed significant differences between cycle groups representing positive (4-7th) and negative (1-3rd, 8-11th) phases of the ongoing theta rhythm. The amplitude of gamma frequency entrainment was about two times higher during the positive phase of the theta oscillation (4-7th cycles; 18.6 ± 8.6 %, $p \leq 0.03$) than during the 1-3rd and 8-11th cycles (7.6 ± 5.8 % and 9.7 ± 7.6 %). In contrast, entrainment of pyramidal cells by Bt cells was prominent during the first two and the 8-11th cycles, the negative phases of the theta oscillation. Consecutive series of bins were significantly different from control during the first two ($p \leq 0.05$) and 8-11th ($p \leq 0.05$) cycles (Fig 1.5B) and the amplitude of the gamma frequency entrainment was similar during these cycles. In response to beta frequency presynaptic spike trains in FS and Bt cells, postsynaptic firing dropped relative to control levels in the first two cycles ($p \leq 0.03$) then it returned close to control levels (Fig. 1.5C-D). Both FS and Bt cells entrained pyramidal neurons at beta frequency as demonstrated by differences in firing probability at the beginning and the end of the cycles ($p \leq 0.05$ and 0.03, respectively), but the degree of entrainment did not appear to change during the duration of presynaptic firing (Fig. 1.5C,D lower panels).

Input to Bt cells

We have searched for GABAergic responses in Bt neurons to FS (n = 24) and Bt (n = 32) cell activation, but we could only detect FS to Bt connections (n = 10, Fig. 1.6). These results support earlier data on the lack of chemical synapses between low threshold spiking cells which are likely to be equivalent to the Bt class of the present paper (Beierlein et al., 2000; Beierlein et al., 2003). Bitufted cells responded to gamma frequency input from FS cells with compound IPSPs of initially summating, then decrementing amplitude; the response to beta frequency input was slightly declining throughout. Postsynaptic firing was suppressed relative to control throughout presynaptic activity ($p \leq 0.05$). However, there was a significant increase in firing from the 4th to 5th cycle ($p \leq 0.01$), therefore we grouped cycles accordingly.

Entrainment of postsynaptic firing at gamma frequency was clearly defined by consecutive series of bins significantly different from control during the 5-11th cycles of presynaptic firing ($p \leq 0.03$), but the difference between the minimal and maximal bins was significant during the first four cycles as well ($p \leq 0.04$). Beta frequency presynaptic activation suppressed postsynaptic firing during the entire duration of input ($p \leq 0.05$). Postsynaptic firing showed significant increases from the first to second ($p \leq 0.01$) and from the third to fourth cycle ($p \leq 0.05$), therefore we grouped cycles accordingly. Phasing of Bt cells was detected during the entire duration of presynaptic FS cell activation. The 1st and 8th bins differed significantly from bins 2nd to 6th during the first presynaptic cycle ($p \leq 0.02$ and $p \leq 0.05$ respectively; Fig. 1.6B blue). The other bins with suppressed firing relative to control also showed effective beta frequency entrainment in the 2-3rd and 4-6th cycles ($p \leq 0.01$ for both cycle groups).

Discussion

Entrainment of postsynaptic firing by segregated GABAergic inputs

Our results provide evidence that perisomatic and dendritic GABAergic inputs are capable of entraining several types of postsynaptic neuron in cortical networks (Fig. 1.7). Entrainment of different non-pyramidal cells by GABAergic afferents might contribute to gamma rhythms, which also arise in mutually interconnected networks of interneurons in vitro (Whittington et al., 1995; Fisahn et al., 1998). Electrical coupling through gap junctions is prominent within populations of several classes of GABAergic neuron, including FS and Bt cells studied here, and can promote synchronous activity over a relatively wide range of frequencies (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000; Venance et al., 2000; Szabadics et al., 2001). Moreover, precise spatiotemporal cooperation between gap junctional potentials and GABAergic IPSPs is highly effective at synchronizing FS and rs cells at behaviourally relevant rhythms (Tamas et al., 2000; Szabadics et al., 2001). However, electrical interactions between different classes of interneurons are rare (Gibson et al., 1999; Venance et al., 2000), and only a few experimental studies addressing GABAergic connections between interneurons have tested oscillatory activity systematically (Hausser and Clark, 1997; Bartos et al., 2001).

Contribution of IPSP kinetics to postsynaptic spike timing

Our results show that the kinetics of IPSPs between different classes of interneurons are characteristic to particular connections (Gupta et al., 2000; Bartos et al., 2001), and in many cases resulted in an input dependent entrainment of postsynaptic activity. The similar

response of FS neurons to inputs from FS and Bt cells resulted in the transformation of beta frequency presynaptic input to a decreased firing probability for only about 20 ms, the duration of one gamma cycle. Therefore, FS cells seem to prefer gamma frequency operations (Pike et al., 2000; Fellous et al., 2001) and are likely to be involved in a frequency gating mechanism suppressing slower rhythms. The opposite effect could be observed in connections mediated by the relatively slow IPSPs from FS to rs neurons, which could not support high frequency entrainment. Paradoxically, IPSPs elicited on the dendritic domain of rs cells were faster than perisomatic IPSPs leading to the entrainment of postsynaptic firing in rs cells by presynaptic gamma and beta rhythms. Intrinsic, cell type specific mechanisms, counteracting filtering of dendritic IPSPs and resulting in faster decays than that of the somatic events, might include the local activation of voltage dependent conductances such as Ih, which might be preferentially expressed on distal dendrites of rs cells similar to pyramidal neurons (Magee, 1998). Differences in postsynaptic $GABA_A$ receptors may also contribute to input specific postsynaptic responses (Pawelzik et al., 1999; Thomson et al., 2000).

Our data support previous results showing that IPSP kinetics and short-term plasticity of unitary IPSPs are influenced both pre- and postsynaptically, and these characteristics are relatively homogeneous in a particular connection (Gupta et al., 2000; Bartos et al., 2001). Similar rules might apply to the entrainment of postsynaptic firing by GABAergic connections, as shown here. However, characteristics of IPSPs are not simply correlated with the efficacy of postsynaptic spike timing, indicating that intrinsic properties of the postsynaptic neurons interact effectively with GABAergic mechanisms in shaping suprathreshold activity. Compound IPSPs showing significant use dependent depression could lead to theta frequency entrainment in pyramidal and rs cells, but no detectable rhythmicity was detected at this frequency band in FS cells. In agreement with theoretical and experimental studies (Traub et al., 1996; Hausser and Clark, 1997; Magee, 1999; Bartos et al., 2001), the decay time constant of IPSPs appears to be an important factor for determining the duration of inhibition of firing, as the fastest decaying IPSPs in FS cells resulted in gamma frequency entrainment. However, relatively slower IPSPs were also effective at gamma frequency entrainment in another connection, the Bt to rs cell input. Furthermore, in pyramidal neurons, inputs from FS or Bt cells with similar somatically recorded IPSP kinetics, resulted in entrainment with different characteristics in phase–related nesting. Therefore, IPSP kinetics alone do not appear to determine the preferred frequency of entrainment.

Cell type specific interplay of GABAergic inputs and intrinsic properties in postsynaptic spike timing

We provide evidence that the time course of postsynaptic spiking probability is at least partly determined by the kinetics of the IPSP and, in addition, by intrinsic oscillatory properties of the postsynaptic cells. The preferential response frequency appears to be cell type specific, operating in the theta range in pyramidal cells and at low range gamma frequencies in FS cells, as suggested by experiments injecting sinusoidal currents into different cortical cell types (Pike et al., 2000; Fellous et al., 2001), and by the rhythmic phase locked firing after a single IPSP (Cobb et al., 1995; Magee, 1999). Cell type specificity of postsynaptic spike timing was further emphasized by reports showing that spike timing also depends on voltage gated conductances and synaptic background noise (Schreiber et al., 2004; Fellous et al., 2003).

Moreover, we show here that pyramidal cells can be entrained at frequency ranges above the intrinsic frequency by rhythmically nested GABAergic inputs. Whether similar mechanisms exist in different types of GABAergic neurons is not clear. Further studies will identify the biophysical parameters underlying the cell type dependent phasing efficacy of somatic versus dendritic GABAergic inputs. From our results, showing connection specific spike transmission, it is likely that additional factors determining postsynaptic entrainment are likely to include IPSP or IPSC amplitude, short-term plasticity of inputs and intrinsic properties of the postsynaptic membrane.

Signal combination by theta phase related nesting of spatially segregated inputs

The synchronized activity of pyramidal neurons is thought to be the basis for population signals recorded in the electroencephalogram (Barlow, 1993; Niedermeyer and Lopes da Silva, 1993). Perisomatically terminating basket and axo-axonic cells phase the activity of postsynaptic pyramidal cells at theta frequency range in the hippocampus in vitro (Cobb et al., 1995; Miles et al., 1996). The present results in the neocortex extend these observations. Firstly, dendritically arriving IPSPs can phase the discharge of pyramidal neurons, similar to rs cells reported earlier (Szabadics et al., 2001). Secondly, we identify two pathways effective in synchronizing pyramidal cells at gamma frequency operating via perisomatically and dendritically placed GABAergic synapses, respectively. Thirdly, the gamma frequency entrainment by the two inputs is most effective in two distinct phases of the simultaneously ongoing theta firing rhythm. The theta-phase related nesting of entrainment by differentially located inputs was only present in pyramidal neurons amongst the types of postsynaptic cells

tested so far. These findings suggest that the firing of pyramidal cells could be especially sensitive to encoding spatial and temporal characteristics of converging GABAergic pathways. Gamma frequency entrainment could promote such difference and it is possible that similar mechanisms could be involved in forming an oscillation dependent combination of signals during cognitive processes (Singer, 1999; Salinas and Sejnowski, 2001).

Connection specific frequency transformations in the cortical network

We show that the timing of postsynaptic firing in cortical GABAergic connections is highly variable, but also connection specific. The frequency of presynaptic firing may appear to have little influence (e.g. FS to rs), faithfully followed (Bt to rs), translated to a stereotyped rebound without (FS to FS and Bt to FS) and with nested intrinsic rhythms (FS and Bt to pyramid) by postsynaptic cells. In the same vein, FS cells were entrained similarly by perisomatic and dendritic IPSPs, but action potentials of rs and pyramidal cells were timed differentially by similar inputs. Regarding the functional significance of different connections, transformation of rhythmic action potential propagation in FS to FS and Bt to FS connections suggests a preferential role of FS cells in maintaining frequencies in the gamma band (Csicsvari et al., 1999; Pike et al., 2000; Bartos et al., 2001; Destexhe et al., 2001). In contrast, the difference in the efficacy of somatic and dendritic inputs in entraining rs and pyramidal cells might contribute to the preservation of specificity in the flow of information by gating particular channels at restricted frequencies. Differential theta-phase related entrainment of pyramidal cells by FS and Bt cells could provide a temporal segmentation for readout of multiple input signals (Roelfsema et al., 1997; Csicsvari et al., 1999). 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. Connection specific, spatio-temporally determined spike synchronization might provide a dynamic internal reference in cortical processing in several behaviourally relevant frequency ranges (Singer, 1999; Moore, 2004).

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

Summary

Distinct interneuron populations innervate perisomatic and dendritic regions of cortical cells. Perisomatically terminating GABAergic inputs are effective in timing postsynaptic action potentials, and basket cells synchronize each other via gap junctions combined with neighboring GABAergic synapses. The function of dendritic GABAergic synapses in cortical rhythmicity and their interaction with electrical synapses is not understood.

Using multiple whole cell recordings in layers 2-3 of rat somatosensory cortex combined with light and electron microscopic determination of sites of interaction, we studied the interactions between regular spiking nonpyramidal cells (RSNPCs). Random samples of unlabeled postsynaptic targets showed that RSNPCs placed GABAergic synapses onto dendritic spines (53±12 %) and shafts (45±10 %) and occasionally somata (2±4 %). GABAergic interactions between RSNPCs were mediated by 4 ± 2 axo-dendritic synapses 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. 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.

Our results provide evidence that dendritic GABAergic and/or gap junctional mechanisms effectively transmit suprathreshold information in a population of interneurons at behaviorally 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.

Introduction

Oscillatory activity in different frequency bands occurs in the EEG during various behavioral states in mammals including humans (Niedermeyer and Lopes da Silva, 1993). Particular cortical rhythms are clearly stimulus and task-specific (Buzsaki et al., 1983; Singer, 1993; Steriade et al., 1993a). 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 (Steriade et al., 1993b; Lisman and Idiart, 1995; Mainen and Sejnowski, 1995; Singer and Gray, 1995; Buzsaki, 1996; Jefferys et al., 1996; Steriade et al., 1996) and beta rhythms at 20 Hz are related to voluntarily controlled sensorimotor actions (Salmelin et al., 1995). Cortical GABAergic mechanisms have been implicated in governing population activity (Lytton and Sejnowski, 1991; Buzsaki and Chrobak, 1995; Cobb et al., 1995; Traub et al., 1996; Fisahn et al., 1998). Electrical synapses play a role in neuronal synchrony (Christie et al., 1989; Draguhn et al., 1998; Mann-Metzer and Yarom, 1999) and gap junctional coupling can promote synchronous activity in connections of cortical interneurons (Galarreta and Hestrin, 1999; Gibson et al., 1999; Koos and Tepper, 1999; Tamas et al., 2000; Venance et al., 2000). The precise spatiotemporal cooperation of gap junctional coupling with GABAergic synapses between basket cells further enhances populational coherence (Tamas et al., 2000).

GABAergic cells subdivide the surface of their target neurons (Somogyi et al., 1998), but most experiments addressing synchronization either did not examine the location of the inputs or were focused on perisomatic mechanisms (Cobb et al., 1995; Gupta et al., 2000; Tamas et al., 2000). Recent evidence suggests that a delicate balance of perisomatic and dendritic inhibition is essential in maintaining normal cortical rhythmogenesis since a deficit in dendritic inhibition could reduce seizure threshold, whereas enhanced somatic inhibition would prevent the continuous occurrence of epileptiform activity (Cossart et al., 2001). A particular subcellular domain of GABAergic and/or electrical communication might result in compartmental interaction of synaptic and voltage gated conductances, resulting in the domain specific processing of sub- and suprathreshold operations. In this work we identified a population of neocortical interneurons with dendritic target preference, which forms a network interacting via gap junctions, and GABAergic synapses. Neurons of this network are capable of engaging coherent activity and can be activated by local pyramidal cells at beta and gamma frequencies.

Results

Several hundred simultaneous dual, triple and quadruple recordings of neurons in layers 2-3 of rat somatosensory cortex provided 28 regular spiking nonpyramidal cell (RSNPC) to RSNPC connections and 12 pyramidal cell (PC) to RSNPC connections. RSNPCs were identified based on their physiological and anatomical properties (Cauli et al., 1997; Kawaguchi and Kubota, 1997; Cauli et al., 2000). Similar to RSNPCs identified earlier they responded to long (800 ms) depolarizing current pulses with a regular spiking firing pattern

showing first to second spike amplitude reduction of 23 ± 11 % and input resistance of 375 ± 117 MΩ (Fig. 2.1A). Local pyramidal cells elicited unitary EPSPs in RSNPCs with paired pulse depression (n = 12; amplitude of the first response: 1.24 ± 1.25 mV; paired pulse ratio: 56 ± 14 % (Porter et al., 1998). The dendrites of RSNPCs originated from the two poles of their elongated somata (Fig. 2.2C, 2.3B-C, 2.4C) and were sparsely spiny (Fig. 2.3E). The axons formed a dense cloud around the dendritic tree and sent a loose bundle of radial branches spanning all layers of the cortex (Fig. 2.3B). High order axonal branches of RSNPCs run radially and branched rectangularly (Fig. 2.2C, 2.4C). Electron microscopic random samples of unlabeled postsynaptic targets (n = 267) taken from layers 2-5 showed that RSNPCs (n = 10) innervated dendritic spines (53 ± 12 %) and shafts (45 ± 10 %) and occasionally somata (2 ± 4 %, Fig. 2.1B). Detailed analysis of serial sections revealed that only 44 ± 21 % of postsynaptic targets identified as dendritic spines received asymmetrical synapses.

Rhythmic activation of RSNPCs by local PCs was tested in six pairs at beta and gamma frequencies (19 and 37 Hz, respectively). Presynaptic PC firing at both frequencies resulted in use-dependent depression of postsynaptic unitary EPSPs in all RSNPCs (Fig. 2.1Ca). Presynaptic spike trains at beta rhythm increased the mean frequency of ongoing postsynaptic firing to 125 ± 14 % of the control value, respectively (from 4.2 ± 1.4 Hz to 5.3 ± 1.7 Hz; Fig. 2.1Ca, 2.5B). Beta frequency presynaptic activation entrained postsynaptic firing during the first three presynaptic cycles. During these cycles, postsynaptic firing probability was significantly higher in the first three bins (0 - 22.3 ms) after the preceding presynaptic spike than later (Fig. 2.1Cb, 2.5B). In parallel with the depression of unitary EPSPs, phasing effectiveness of PCs on RSNPC firing faded during the rest of presynaptic activity (Fig. 2.1Cb). Similar results were obtained at gamma frequency PC activation (Fig 2.5B). When driving the PCs at 37 Hz, the mean firing rate of postsynaptic RSNPCs was accelerated to 132 ± 26 % of the control and entrainment of postsynaptic firing was limited to the first two (n = 3 pairs) or three (n = 3 pairs) presynaptic cycles. During these cycles, postsynaptic firing probability was significantly higher in the second and third bins (7.4 - 22.3 ms) after the preceding presynaptic spike than during the rest of bins (Fig. 2.1Cb, 2.5B).

We identified GABAergic, electrical and combined GABAergic and electrical connections between RSNPCs. In pairs of RSNPCs connected by chemical synapses only (n = 12), light microscopic analysis of six fully visualized cell pairs indicated 4 ± 2 close appositions between presynaptic axons and postsynaptic dendrites at a mean distance of 63 ± 28 μm from the somata (Fig. 2.2C-D). All GABAergic connections were unidirectional

between RSNPCs in our sample. Measured at –51 ± 3 mV membrane potential, unitary IPSPs between RSNPCs were 0.54 ± 0.23 mV in amplitude (n = 12). Bicuculline completely abolished the responses (20 µM; n = 3). Repetitive presynaptic firing at 19 and 37 Hz resulted in the summation of postsynaptic unitary IPSPs followed by the stabilization of their amplitude ~ 198 ± 49 and 238 ± 57 % of the amplitude of averaged single events, respectively (n = 6; Fig. 2.2Aa, Ba). Presynaptic spike trains (19 Hz) decreased the mean frequency of spontaneous postsynaptic firing to 79 ± 18 % of the control value (from 4.6 ± 1.9 Hz to 3.6 ±

1.7 Hz). Postsynaptic firing was entrained for the entire duration of presynaptic activation. Postsynaptic firing probability was significantly smaller in the second and third bins (7.4 - 22.3 ms) after the preceding presynaptic spike than during the first, sixth and seventh bins (0 - 7.4 ms and 37.1 – 52 ms) in a cycle (Fig. 2.2Ab, 2.5C). Gamma frequency presynaptic activation (37 Hz) could decrease the mean postsynaptic discharge rate from 4.7 ± 1.7 Hz to 3.2 ± 1.3 Hz (69 ± 12 %) but was not effective in phasing postsynaptic action potential generation (Fig. 2.2B, 2.5C).

The second class of RSNPC to RSNPC connections was mediated by electrical synapses (n = 12). Light microscopic mapping in 5 fully recovered pairs detected 3 ± 3 close appositions (range, 2 - 8) exclusively between dendrites at a mean distance of 77 ± 34 μm from the somata (Fig. 2.3B-D). Electron microscopic analysis of the suspected coupling sites was carried out in one cell pair, leading to the identification of eight gap junctions in the connection at dendritic distances of 72 ± 16 μm (cell 1) and 63 ± 36 μm (cell 2) from the somata (Fig. 2.3C-F). Two gap junctions were established between dendritic spines, one between a spine and a dendritic shaft and four gap junctions linked dendritic shafts. Analysis of serial ultrathin sections showed in all four dendritic shaft to dendritic shaft cases that the junctional region was composed of the gap junction and an immediately adjacent patch of membranes running parallel for 0.3 - 0.9 μm at a rigid distance of 21 - 25 nm (Fig. 2.3F). The latter value is characteristic of desmosomes and/or synaptic clefts but the electron opaque reaction end product prevented further identification of junctional components.

All electrical connections between RSNPCs were reciprocal. Gap junctional potentials (GJPs) showed a relatively wide range in amplitude (0.07 - 2.42 mV; 0.62 ± 0.74 mV) at –50 ± 3 mV membrane potential and had an average duration of 19.6 ± 8.2 ms, as measured at half amplitude. They followed presynaptic action potentials with a delay of 0.40 ± 0.26 ms, measured as the period spanning the maximal rates of rise of the presynaptic action potential and the GJP, respectively. The average amplitude ratio (coupling coefficient) for GJPs and presynaptic potentials was 0.66 ± 0.83 % (range, 0.04 - 2.58 %) and 4.6 ± 3.1 % (range, 2.4 - 10.6 %) when eliciting action potentials and applying long current steps (200 pA, 300 ms duration) in the first neuron to elicit a response in the second neuron. Coupling strength was similar in both directions and did not show voltage dependence between –80 and –40 mV postsynaptic membrane potential (n = 4). Both amplitudes and kinetics of GJPs remained unchanged during repetitive presynaptic firing (Fig. 2.3Aa, Ga). The effect on unitary GJPs on postsynaptic suprathreshold activity was investigated at 19 and 37 Hz in 6 connections. For a given pair, timing of postsynaptic firing was similar at both frequencies tested and members

of a pair phased one another with similar efficacy regardless of the direction. In four out of six pairs, 19 Hz presynaptic activation accelerated the mean firing rate of postsynaptic RSNPCs to 120 ± 10 % of the control. Postsynaptic firing probability was significantly higher in the first two bins (0 – 14.9 ms) after the preceding presynaptic spike than during the last three bins (29.7 – 52 ms) of a cycle (Fig. 2.5D). GJPs arriving at gamma frequency increased ongoing postsynaptic firing and firing probability was significantly higher in the second bin (3.9 – 7.7 ms after the presynaptic spike) than in the rest of bins in a cycle (Fig 2.3G, 2.5D). In the two pairs with the highest coupling ratios and numbers of gap junctions, GJPs synchronized pre- and postsynaptic firing at beta and gamma frequencies with no apparent phase lag (bin width, 3.9 ms) and a relatively narrow temporal scatter of action potentials (Fig. 2.3A).

Six RSNPC to RSNPC connections were mediated by combined GJPs and IPSPs. The GABAergic component of all dual electrical and chemical connections was unidirectional. Light microscopic mapping of three fully recovered pairs revealed that 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. Detailed electron microscopic analysis of one pair confirmed such arrangement of connections and identified two gap junctions between dendritic shafts (one of them showing rigid widening of extracellular space adjacent to the gap junction) and three GABAergic synapses on dendrites (Fig. 2.4C-F). Postsynaptic responses were composed of GJPs followed by short-latency IPSPs of stable amplitude (Fig. 2.4A-B). Presynaptic spike trains at 19 and 37 Hz decreased the mean frequency of ongoing postsynaptic firing to 91 ± 11 % and 84 ± 8 % of control values, respectively (from 5.08 ± 1.19 Hz to 3.77 ± 1.13 Hz and from 5.08 ± 1.19 Hz to 3.77 ± 1.13 Hz). Following the onset of presynaptic spike trains postsynaptic firing was instantly synchronized at beta and gamma frequencies, with maximal postsynaptic action potential probability in the first bin (bin widths, 7.4 and 3.9 ms; Fig. 2.4Ab, Bb, 2.5E). Firing occurred synchronously in the coupled cells during the entire length of presynaptic activation.

Cluster analysis of postsynaptic firing probability in response to beta and gamma frequency presynaptic firing resulted in the clear delineation of controls, connections mediated by IPSPs and combined electrical and GABAergic coupling (Fig. 2.5F). Interactions via moderate gap junctional coupling and EPSPs clustered together (Fig. 2.5F) but the two electrical connections mediated by powerful coupling formed a separate group at both frequencies (not illustrated).

Discussion

We have identified a novel interneuronal network in the cortex interconnected by electrical and GABAergic synapses. RSNPCs form dendritic gap junctions with other RSNPCs and establish GABAergic synapses on the dendritic domain of postsynaptic cells. Electrical, GABAergic, or combined GABAergic and gap junctional signals targeted to the dendrites are capable of timing somatic action potential generation in the network of RSNPCs at behaviorally relevant frequencies.

Interneurons forming the network identified here are distinct of GABAergic cell classes known to form electrically coupled networks in the cortex (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000; Venance et al., 2000). The regular spiking firing pattern in combination with depressor unitary EPSPs demarcate RSNPCs both from fast spiking cells and from low threshold spiking/bitufted cells (Reyes et al., 1998; Gibson et al., 1999) and these electrophysiological parameters are similar to those of vasoactive intestinal polypeptide immunoreactive interneurons (Kawaguchi and Kubota, 1996; Cauli et al., 2000). In agreement with earlier results (Kawaguchi and Kubota, 1997; Tamás et al., 1997), we have found that RSNPCs place symmetrical synapses onto dendritic spines and shafts. These results would identify RSNPCs as double bouquet cells (Somogyi and Cowey, 1981; Tamás et al., 1997), but the finding that only less than half of postsynaptic targets identified as dendritic spines received asymmetrical synapses questions the origin of postsynaptic dendritic appendages. Although the synaptology of interneuronal spines are not known, the spines receiving excitatory synapses are most likely originate from pyramidal dendrites; the ones receiving GABAergic innervation only could belong to pyramidal cells as well as GABAergic interneurons. Double bouquet cells target other GABAergic neurons (Tamas et al., 1998) and there are known types of interneuron preferentially innervating other GABAergic cells in the rat (Gulyas et al., 1996; Meskenaite, 1997) and the distinction between these classes are not yet clear. Gap junctions between RSNPCs also connect dendritic shafts and spines and, moreover, GABAergic synapses and gap junctions are placed at similar distances from the

soma of RSNPCS. Therefore, similarly to the network of cortical basket cells (Tamas et al., 2000), chemical and electrical synapses target the same subcellular domain of RSNPCs equalizing the time required for postsynaptic signal propagation. Accurate spatial integration could be further promoted by juxtaposition of gap junctions, dendro-dendritic synapses and/or desmosomes found between smooth dendritic shafts in the primate motor cortex (Sloper and Powell, 1978) and between parvalbumin immunoreactive dendrites in the hippocampus (Fukuda and Kosaka, 2000). We found a rigid widening of the extracellular space next to the gap junctions in six out of five contacts between dendritic shafts of RSNPCs, but our method for the visualization of functionally coupled cells did not allow the differentiation of desmosomes and dendro-dendritic synaptic junctions.

We provide evidence that dendritically targeted GABAergic synapses are effective in timing somatic action potentials in the postsynaptic RSNPCs. Mechanisms underlying such phasing are not yet known. GABAergic cell types elicit IPSCs and IPSPs with remarkably different kinetics (Gupta et al., 2000), which could reflect distinct postsynaptic receptor properties (Draguhn et al., 1990; Mody et al., 1994). Currents activated by hyperpolarization (Ih) are present in RSNPCs, but not as prominent as in pyramidal cells and bitufted cells (our unpublished data and (Cauli et al., 2000). Therefore, activation and/or deinactivation of other voltage gated cation conductances and/or by passive dendritic properties could be the major factors in shaping the relatively fast decay of IPSPs. Dendritic GABAergic synapses between RSNPCs are able to phase postsynaptic cells in the beta frequency band, which is clearly faster than the theta frequency range of rebound activation detected in connections mediated by perisomatically placed GABAergic synapses on pyramidal cells (Cobb et al., 1995) as well as on interneurons (Tamas et al., 2000). The rebound activation might be even faster in vivo, when the input resistance of the cells is likely to be lower due to ongoing background synaptic activity. This could improve phasing between RSNPCs in the gamma frequency band. Theta and beta/gamma rhythms are linked to distinct behaviors (Buzsaki et al., 1983; Singer, 1993; Steriade et al., 1993a), and our results suggest that diverse populations of GABAergic cells might be differentially involved in cortical network operations during a particular activity.

Unitary EPSPs could initiate firing in RSNPCs with latencies slightly longer to what has been found in hippocampal interneurons (Fricker and Miles, 2000). The authors measured the timing of spikes elicited from just subthreshold membrane potentials, but we have tested the effectiveness of GJPs and EPSPs (and IPSPs and dual coupling) on spontaneous ongoing firing. The set of voltage gated conductances active at subthreshold membrane potentials and during spontaneous repetitive firing is likely to be different and might explain the

discrepancy. The amplitude of GJPs versus EPSPs might also influence the immediacy of spikes after the onset of the postsynaptic potentials by activating different amounts and or populations of voltage gated channels. In addition to spike triggering, suprathreshold activity of RSNPCs can be rhythmically timed with a phase lag by neighboring pyramidal cells in the beta and gamma frequency range.

Strong bidirectional electrical coupling could produce synchronization of RSNPCs, but on average, combined chemical and electrical unitary connections were most effective in synchronizing pre- and postsynaptic firing. In our sample, all GABAergic connections were unidirectional between RSNPCs including the GABAergic component of dual electrical and chemical connections. The implications of unidirectional GABAergic coupling in combination with reciprocal gap junctional connections are not clear. RSNPCs are embedded into a network interconnected by chemical, electrical and dual unitary connections and a particular postsynaptic cell is likely to receive convergent GJPs and IPSPs from some RSNPCs which might be precisely synchronized by dual coupling. This scenario suggests that dual connections would rule the operation of RSNPCs at the network level. Preferred rhythms of RSNPC population oscillations might be in the beta and probably gamma frequency band set by the timing of rebound activation following unitary IPSPs within the network. Coherent output of RSNPCs could provide a powerful dendritic pathway of rhythmic information processing spatially and temporally segregated from perisomatic mechanisms of synchronization. The cooperation of GABAergic synapses and gap junctions appears to be limited to a single population of interneurons (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000; Venance et al., 2000) therefore synchronization might be more prominent within populations than across different interneuron types. This might explain the effectiveness of soma and dendrite targeting interneurons in timing postsynaptic activity of pyramidal cells though a precisely synchronized, robust, but perisomatically and dendritically channeled GABAergic flow of information.

4.3 Identified Sources and Targets of Slow Inhibition in the Neocortex

Summary

There are two types of inhibitory postsynaptic potentials inhibition in the cerebral cortex. Fast inhibition is mediated by ionotropic $GABA_A$ receptors and slow inhibition is due to metabotropic $GABA_B$ receptors. Several neuron classes elicit inhibitory postsynaptic potentials through $GABA_A$ receptors, but possible distinct sources of slow inhibition remain unknown. We identified a class of GABAergic interneurons, the neurogliaform cells that in contrast to other GABA-releasing cells, elicited combined $GABA_A$ and $GABA_B$ receptor mediated responses with single action potentials and predominantly targeted dendritic spines of pyramidal neurons. Slow inhibition evoked by a distinct interneuron in spatially restricted postsynaptic compartments could locally and selectively modulate cortical excitability.

Introduction

Gamma aminobutyric acid (GABA) is the major inhibitory transmitter in the cerebral cortex (Krnjevic and Schwartz, 1967). 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 (Dutar and Nicoll, 1988; Mody et al., 1994; Misgeld et al., 1995; Barnard et al., 1998; Bowery et al., 2002). 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. GABAergic neurons terminate on separate subcellular domains of target cells, (Freund and Buzsaki, 1996; Somogyi et al., 1998) and several studies suggest that dendritic inhibition is mediated by $GABA_B$ receptors and possibly by a discrete group of interneurons (Lacaille and Schwartzkroin, 1988; Benardo, 1994) which can modulate dendritic excitability (Larkum et al., 1999). IPSPs with similar kinetics to $GABA_B$ receptor mediated responses are produced by interneurons possibly targeting the dendritic regions in the hippocampus (Lacaille and Schwartzkroin, 1988), but other experiments provide evidence for pure $GABA_A$ responses evoked on dendrites (Buhl et al., 1994a; Miles et al., 1996; Thomson et al., 1996; Gupta et al., 2000). Moreover, repetitive firing of interneurons and/or cooperation of several interneurons is thought to be necessary for the activation of $GABA_B$ receptors (Mody et al., 1994; Thomson et al., 1996; Kim et al., 1997; Thomson and Destexhe, 1999) possibly by producing

extracellular accumulation of GABA to levels sufficient to activate extrasynaptic receptors (Dutar and Nicoll, 1988; Isaacson et al., 1993; Destexhe and Sejnowski, 1995; Thomson et al., 1996; Kim et al., 1997; Thomson and Destexhe, 1999).

Results

Whole cell recordings with biocytin filling from synaptically coupled pairs of three types of presynaptic interneuron and postsynaptic pyramidal cells combined with correlated light and electron microscopy were performed (Tamas et al., 2000). $GABA_B$ receptor localization studies indicated a gradient-like immunoreactivity for $GABA_B$ receptors with stronger labelling in the upper layers (Lopez-Bendito et al., 2002). We thus tried to identify the sources of slow inhibition in layers 2-3 of rat somatosensory cortex. Neurogliaform cells (NGFCs, n =

78) were identified based on late spiking firing pattern and their axonal and dendritic morphology (Cajal, 1904; Valverde, 1971; Jones, 1975; Kisvarday et al., 1990; Hestrin and Armstrong, 1996; Kawaguchi and Kubota, 1997) (Fig. 3.1A and D). Basket cells (n = 19) showed fast spiking firing pattern, received depressing unitary EPSPs arriving from pyramidal cells (n = 5), immunoreactivity for parvalbumin (n = 4 out of 4 tested) and they preferentially innervated postsynaptic somata (31 %), dendritic shafts (66 %) and occasionally spines (3 %). Bitufted cells (n = 15) responded to depolarizing current pulses with a so-called low-threshold spiking firing pattern (Kawaguchi and Kubota, 1997; Reyes et al., 1998), received facilitating EPSPs from neighboring pyramidal cells (n = 3), placed their synapses onto dendritic shafts and spines (74 %; 26 %; n = 45), and contained somatostatin (n = 4 out of 4 tested). Postsynaptic potentials in pyramidal neurons elicited by NGFCs 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 (Fig. 3.1B). The decay of NGFC 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 NGFC 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 NGFC, basket and bitufted to pyramid connections, respectively). Voltage clamp experiments confirmed the conclusions of these recordings (Fig. 3.1C).

Random electron microscopic sampling of postsynaptic targets showed that NGFCs predominantly innervated dendritic spine necks (30 %), spine heads (41 %) and dendritic shafts (29 %, n = 65 target profiles; Fig. 3.1D) (Kisvarday et al., 1990; Kawaguchi and Kubota, 1997). Three-dimensional light microscopic mapping of NGFC to pyramid connections (n = 8) confirmed these results and predicted 10 ± 6 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 μm ± 27 μm (range, 25 - 92 μm) from the soma (Fig. 3.1E-F).

NGFC to pyramid IPSPs were composed of two components (n = 21, Fig. 3.2A-B). The early component could be blocked by bicuculline (10 μM, n = 10) or gabazine (20 μM, n =3) indicating the involvement of $GABA_A$ receptors (Fig. 3.2A). Bicuculline or gabazine blockade alone never abolished the response completely and revealed a residual slow component of neurogliaform IPSPs with onset latencies of 60.6 ± 17.3 ms. This late component contribued to the integral of the control IPSPs by 32.1 ± 19.8 % and could be

blocked by further addition of the $GABA_B$ receptor antagonist CGP35348 (60 μM). The presence of a postsynaptic $GABA_B$ receptor mediated slow component was confirmed by experiments in which the decay of NGFC to pyramid IPSPs was reversibly shortened by CGP35348 (n = 8, Fig. 3.2B). Although CGP35348 decreased the amplitude of the early component in 3 out of 8 connections, the difference was not significant for the whole dataset. The early component was absent at -72 ± 1 mV (n = 8), the expected reversal potential for mixed chloride and hydrocarbonate conductance (Fig. 3.2C), therefore anion passage through $GABA_A$ receptors was responsible for the early phase in agreement with the bicuculline blockade. Hyperpolarization of the postsynaptic cells near to the equilibrium potential for potassium ions (–87 ± 2 mV) largely eliminated the late component consistent with $GABA_B$ receptor involvement.

The compound IPSPs were highly sensitive to the firing rate of the presynaptic neurons. This could explain why the sources for slow inhibition have remained obscure up to date. We activated the presynaptic neurogliaform cells with single action potentials delivered

at various intervals and stable amplitude of postsynaptic responses could only be achieved if the interval between presynaptic spikes was more than 1.5 minutes. Accordingly, all single action potential evoked responses for kinetics, pharmacology and reversal potentials detailed above were collected at especially low presynaptic firing rates (one spike in 100-120 s). When activating the presynaptic NGFCs with trains of action potentials at 40 Hz, the amplitude of postsynaptic responses decreased rapidly (Fig. 3.2D). Even at a train interval of 4 minutes (n = 7), postsynaptic responses showed rapid decrease in amplitude resulting in complete loss of response after five to eight presynaptic spike trains. After total exhaustion, recovery of IPSP amplitude was tested with a single presynaptic spike in every 15 minutes and showed recovery in all cases. The recovery was initially detectable after 15-45 minutes and reached 31-79 % of control amplitude as measured 90 minutes after exhaustion indicating that the synapses remained functional. Application of high frequency stimulation or presynaptic interspike intervals above 1.5 minutes did not have an effect on the kinetics of single spike evoked events in the same pair.

Discussion

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. Synapses of neurogliaform cells appear specialized for sparse temporal operation tuned for long-lasting metabotropic effects. Although it has been suggested that in some interneuron to pyramidal cell connections, repeated presynaptic activation might be necessary to recruit slow inhibition (Thomson et al., 1996; Thomson and Destexhe, 1999), single action potentials at very low firing rates are sufficient to elicit the metabotropic $GABA_B$ component. We cannot rule out, however, that other type(s) of GABAergic cells might also activate postsynaptic GABAB receptors. GABA uptake mechanisms powerfully remove the transmitter from the extracellular space within a distance restricted to about a micrometer from the release sites (Overstreet et al., 2000). Our results thus suggest that postsynaptic $GABA_B$ receptors could be spatially associated with the synapses formed by NGFCs. Electron microscopic studies revealed extrasynaptically placed $GABA_B$ receptors on dendritic spines and shafts (Fritschy et al., 1999; Li et al., 2001; Kulik et al., 2002; Lopez-Bendito et al., 2002). A possible synaptic enrichment of these receptors remains to be determined. We show that action of NGFCs is predominantly targeted to dendritic spines. The slow rise times of NGFC to pyramidal cell IPSPs and IPSCs might support spines as targets reflecting the filtering effect of the spine

necks. Alternatively, $GABA_A$ receptor subunit composition might influence activation kinetics (Pearce, 1993; Banks et al., 1998). Although we cannot rule out that neurogliaform synapses on dendritic shafts and spines act through different receptors, data from the cerebellum suggest that $GABA_B$ receptors are placed on spines (Fritschy et al., 1999; Lopez-Bendito et al., 2002). Spines receive the majority of excitatory input and simulations showed that if inhibitory synapses found on cortical spines are effective, then they should be mediated through $GABA_B$ receptors providing powerful hyperpolarizing inhibition reducing the excitatory synaptic potentials on the same spine (Qian and Sejnowski, 1990). In addition to hyperpolarizing inhibitory effects, the diffusion barrier provided by the targeted postsynaptic spines can locally enhance metabotropic changes following $GABA_B$ receptor activation. Therefore even sparse temporal operation of NGFCs could result in sustained modulation of excitability.

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

Summary

Theoretical studies predict that the modes of integration of coincident inputs depend on their location and timing. To test these models experimentally, we simultaneously recorded from three neocortical neurons *in vitro* and investigated the effect of the subcellular position of two convergent inputs on the response summation in the common postsynaptic cell. 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 and on the activation state of I_h. The results suggest that, when few inputs are active, the majority of afferent permutations undergo linear integration, 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.

Introduction

The rules of synaptic summation are thought to depend on the dendritic geometry of the postsynaptic cell (Zador et al., 1995; Mainen et al., 1996), on a variety of synaptic and voltage dependent conductances distributed heterogeneously over the dendritic tree (Johnston et al., 1996; Hausser et al., 2000) and on the relative position and timing of inputs (Jack et al., 1975; Shepherd and Brayton, 1987; Rall et al., 1992; Segev et al., 1995; Hausser et al., 2000). Theoretical analysis of dendritic integration began by assuming that passive cables serve as reasonable models of dendrites (Jack et al., 1975; Segev et al., 1995). According to cable theory, electrically isolated inputs sum linearly, whereas closely located inputs produce an attenuated response as a consequence of reduction in the ionic driving force or a decrease in dendritic input resistance leading to shunting of synaptic currents (Jack et al., 1975; Segev et al., 1995). However, dendritic membranes are not passive, as they contain voltage dependent conductances, which could selectively amplify distal inputs or subserve local nonlinear operations (Koch et al., 1983; Mel, 1993). Direct experimental determination of the influence of the location of synaptic inputs on dendritic integration has been relatively sparse.

Electrophysiological analysis *in vivo* showed sublinear summation in motoneurons (Kuno and Miyahara, 1969) and both linear and non-linear modes of integration of responses in the visual system (Douglas et al., 1988; Jagadeesh et al., 1993; Jagadeesh et al., 1997; Borg-Graham et al., 1998; Hirsch et al., 1998; Kogo and Ariel, 1999; Anderson et al., 2000). Experiments in brain slices indicated linear input summation in motoneurons (Skydsgaard and Hounsgaard, 1994) and hippocampal pyramidal cells (Langmoen and Andersen, 1983). Physiological tests without the additional information on the subcellular position of inputs leave dendritic integration rules open to several interpretations (Major et al., 1994; Zador et al., 1995; Mainen et al., 1996). Using local glutamate microiontophoresis and extracellular stimulation onto visualized dendrites, Cash and Yuste (Cash and Yuste, 1999) reported linear and position independent summation of excitatory postsynaptic potentials (EPSPs). Summation of postsynaptic potentials also depends on the ongoing firing rate of neurons because the action potential acts as variable reset of integration (Hausser et al., 2001). Most experiments so far focused on the summation of excitatory inputs; to our knowledge integration properties of convergent GABAergic synapses have not been reported. In order 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 neurons simultaneously recorded in the neocortex. The integration of two glutamatergic or GABAergic inputs was tested as a function of the relative location of synapses, the relative timing of inputs and the activation of the voltage gated conductance, Ih. The results reveal that they all have an influence on the mode of integration of inputs, and that different combinations of inputs, and postsynaptic cells might express distinct integration kinetics.

Results

Out of several hundred multiple recordings med, the physiological analysis of 28 triple cell recordings involving convergent inputs onto the same postsynaptic cell in layers 2/3 of somatosensory cortex was carried out in detail. Of these, satisfactory intracellular labeling of all three cells permitted the unequivocal tracing of afferents back to the parent somata in 12 cases. Pairs of presynaptic pyramidal or FS cells were activated using a cyclic stimulation paradigm (Fig. 4.1B, 4.4B) which evoked simultaneous presynaptic action potentials with a relative peak to peak difference of 0.14 ± 0.12 ms. Postsynaptic cells receiving two convergent inputs represented pyramidal neurons, FS and Bt cells (McCormick et al., 1985; Reyes et al., 1998) (Fig. 4.1A). In agreement with earlier studies (McCormick et al., 1985; Reyes et al., 1998), input resistance and membrane time constant of the postsynaptic cells

were, on average, 207 ± 32 MΩ and 23 ± 5 ms in pyramidal cells (n=15), 127 ± 49 MΩ and 10 ± 3 ms in FS (n=9) cells and 405 ± 137 MΩ and 25 ± 6 ms in Bt (n=4) neurons.

Summation of convergent EPSPs and EPSCs

Integration of convergent unitary EPSPs evoked by local layer 2/3 pyramidal neurons was tested in 9 FS cells, 4 Bt neurons, and 2 pyramidal cells (Fig. 4.1-3). Based on the linearity of summation between synchronous inputs, pyramid to FS connections could be clearly divided into two groups. Unitary EPSPs summed linearly in 5 FS cells, but moderately sublinear summation was detected in 4 postsynaptic FS neurons. In the linearly summating group of cells, amplitudes of the converging smaller and bigger unitary EPSPs were 0.99 ± 0.77 mV and 2.11 ± 1.84 mV measured at -51 ± 2 mV membrane potential (Fig. 4.1A-G). Linear summation (100.9 ± 0.4 %) was apparent when comparing the peak amplitude of experimentally recorded compound responses (3.12 ± 2.31 mV) with the calculated sums of individual inputs (3.10 ± 2.29 mV). In the group showing sublinear summation characteristics, amplitudes of unitary smaller and bigger EPSPs (1.21 ± 1.06 and 2.31 ± 1.82 mV) were similar to those measured in the linear pyramid to FS connections (Fig. 4.2). Experimentally recorded maximal amplitudes of compound EPSPs (3.29 ± 2.71 mV) were consistently smaller than those of corresponding algebraic sums of individual EPSPs (3.54 ± 2.85 mV; $p < 0.05$, Wilcoxon-test), indicating moderately sublinear summation (90.8 ± 5.6 %; $p < 0.04$). The time course of the degree of linearity was distinct from the kinetics of EPSPs or EPSCs since decay time constants of unitary EPSPs (10.7 ± 3.0 ms) and EPSCs (2.6 ± 0.7 ms) were respectively longer and shorter than that of the degree of linearity (6.7 ± 1.9 ms; $p < 0.05$, Mann-Witney U-test; Fig. 4.2A, 4.5C).

In two FS cells, similar experimental paradigms were applied holding the postsynaptic cells in voltage clamp mode (Fig. 4.1C, 4.2B). Although measurements in current clamp mode indicated linear (Fig. 4.1B) or sublinear (Fig. 4.2\) integration, summation of postsynaptic currents was close to linear in both cases, as the amplitudes of recorded composite EPSCs were 99.6 and 97.5 % of the algebraic sums of individually evoked EPSCs (Fig. 4.1C, 4.2B).

To investigate the effect of the relative timing of two EPSPs on their summation characteristics in FS neurons, we tested how asynchronous EPSPs interact by eliciting the smaller unitary responses (0.66 ± 0.38 mV) 5 ms after the bigger EPSPs (1.46 ± 0.52 mV) in 2 triplets showing linear and in 2 triplets showing sublinear summation of synchronous inputs.

Asynchronous activation produced linear summation (100.7 ± 0.9 %) in all four cases, as recorded compound events and calculated sums of unitary psps were similar in amplitude (1.41 ± 0.38 and 1.40 ± 0.39 mV) measured at the peak of the single smaller EPSP (Fig. 4.2c).

Summation characteristics might depend on the amplitude of inputs. Therefore, we examined the degree of linearity as a function of EPSP amplitude measured as the maximal amplitude of the algebraic sums of individual EPSPs and found no correlation in pyramid to FS cell triplets ($n = 9$; Spearman-correlation, $R = 0.13$, $p > 0.5$). Moreover, we tested the summation of EPSPs on Bt cells (n=4), because pyramidal inputs to these cells show relatively strong paired pulse facilitation (Reyes et al., 1998) (Fig. 4.3A, B). Regardless of the amplitude increase from the first to second EPSPs (314 ± 79 %), all four triplets showed linear summation for both the first and second events; therefore, we pooled the data. Amplitudes of the smaller and bigger unitary EPSPs were 0.32 ± 0.29 mV and 0.78 ± 0.79 mV on average (n=8), as measured at -50 ± 3 mV membrane potential. Simultaneous activation of presynaptic cells produced compound EPSPs with amplitudes of 1.10 ± 1.03 mV, similar to that of algebraic sums of individual EPSPs (1.08 ± 1.07 mV). Linear (101.1 ± 2.1 %) summation of EPSPs was apparent when comparing the amplitude of compound recordings with the appropriate calculated sums of individual inputs at the peak of the recorded EPSPs (Fig. 4.3B). Kinetics of the measured and the corresponding calculated compound events were also similar. The two triplets consisting of convergent pyramidal EPSPs to pyramidal cells also showed linear summation characteristics (not illustrated).

Structural analysis of the basis of convergent EPSPs showing *linear* integration properties was carried out in three triplets with two FS cells and two BT cells receiving the pyramidal inputs. On average, unitary innervation was mediated by 2.0 ± 0.8 and 2.8 ± 1.7 predicted contact sites by the axons evoking the smaller and bigger unitary EPSPs, respectively. Measured from the soma, postsynaptic cells were innervated at distances of 79 ± 35 and 76 ± 23 μm by the weaker and stronger inputs, respectively. The two sets of afferents contacted different dendritic segments in all cases, and the contact sites made by the two presynaptic pyramids were relatively distant from each other on the postsynaptic cell (146 ± 42 μm; Fig 4.1E,F). Electron microscopic testing of all suspected contact sites confirmed light microscopic predictions as being synaptic junctions in the triplet presented in Fig. 4.1A-G. Anatomical analysis of pyramid to FS triplets, which showed *sublinear* summation, could be performed in two cases. In contrast to distant input sites in triplets producing linear EPSP summation, boutons evoking EPSPs summating sublinearly were relatively close to one

another. Light microscopic evaluation of the first triplet indicated 5 and 2 contact sites for the two inputs with an average distance of 55 ± 21 µm (n = 10), as measured along the dendrites between all individual predicted synapses of distinct origin. Both presynaptic cells made two contacts on dendritic branches originating from the same stem, and the nearest two synapses made by the two presynaptic axons were 7 µm apart. Analysis of the second triplet revealed that the two inputs targeted the same dendritic segment of the postsynaptic FS cell (Fig. 4.2D). The average distance of the two inputs was 14 ± 3 µm (n = 2), as proved by electron microscopy.

Summation of convergent IPSPs and IPSCs

The data derived from EPSP-EPSP interactions indicated that neighboring input positions might result in sublinear summation. Extensive testing of this hypothesis requires input combinations reliably targeting the same postsynaptic domain. Cortical basket cells show FS firing pattern, they selectively target the perisomatic region of postsynaptic cells and frequently innervate pyramidal cells in their vicinity (Somogyi et al., 1998). Therefore, we focused our efforts on recording convergent FS cell inputs onto pyramidal cells (n = 13). The majority of convergent unitary IPSPs synchronously elicited by FS cells in layer 3 pyramidal neurons produced sublinear summation (n=8), but linear interactions (n=5) were also apparent (Fig. 4.4). In the sublinearly summating group of cells, smaller unitary IPSPs were 1.49 ± 0.86 mV and greater IPSPs were 2.63 ± 1.42 mV in amplitude, measured at –50 ± 1 mV membrane potential (Fig. 4.4B, 4.5A). In these triplets, the degree of linearity was correlated with the maximal amplitude of the algebraic sums of convergent IPSPs (n = 8; R = 0.95; p > 0.001). Simultaneously evoked unitary responses produced compound IPSPs with recorded amplitudes of 3.71 ± 1.61 mV, being consistently smaller (91.8 ± 4.0 %) than that of corresponding algebraic sums of individual IPSPs (4.11 ± 1.94 mV; p < 0.01, Wilcoxon-test). Normalization of composite recorded IPSPs to the amplitude of the calculated IPSPs showed no apparent difference in kinetics. When comparing the kinetics of the degree of linearity with that of unitary IPSPs and IPSCs, the 10 to 90 % rise time (9.8 ± 1.9 ms) was significantly longer than the rise times of IPSPs or IPSCs (5.6 ± 1.2 ms and 0.92 ± 0.03 ms; p < 0.03, Mann-Whitney U-test). Decay time constants of the degree of linearity (55.8 ± 5.7 ms) differed from that of corresponding IPSCs (7.7 ± 0.8 ms; p < 0.01), but were similar to that of IPSPs (60.1 ± 8.4 ms Fig. 4.4B-C, 4.6C). In the FS cell to pyramid triplets (n=5) showing linear summation (Fig. 4.4I), smaller and bigger unitary IPSPs were 0.48 ± 0.14 mV and 2.77

± 2.39 mV in amplitude recorded at –50 ± 2 mV membrane potential. These triplets did not show significant correlation between the degree of linearity and the maximal amplitude of the algebraic sums of convergent IPSPs (n = 5; R = 0.60; p > 0.2). Synchronously activated unitary responses produced compound IPSPs with recorded amplitudes of 3.23 ± 2.35 mV, a value similar (99.6 ± 0.4 %) to that of the amplitude of calculated composite IPSPs (3.25 ± 2.37 mV).

We tested how asynchronous IPSPs interact by eliciting the smaller unitary responses (0.82 ± 0.48 mV) 5 ms after the bigger IPSPs (2.05 ± 1.64 mV) in 2 triplets showing linear and in 3 triplets showing sublinear summation of synchronous inputs (Fig. 4.5). Asynchronous activation did not change the properties of summation as compared to synchronous timing of inputs; the difference in the degree of linearity of the peak response between synchronous and corresponding asynchronous input combinations was 0.6 ± 4.2 % (Fig. 4.5A-B). We also tested synchronous input summation in five pyramidal cells holding the postsynaptic cells in voltage clamp mode (Fig. 4.4D). Although, for these connections, the measurements in current clamp mode indicated either linear (n=2) or sublinear (n=3; Fig. 4.4B) integration, summation of postsynaptic currents was close to linear in all five cases, as the amplitudes of recorded composite IPSCs were 97.0 ± 2.0 % of the algebraic sums of individually evoked IPSCs (Fig. 4.4D).

Summation properties are likely to depend on voltage gated conductances and I_h is thought to be the most prominent current activated by IPSPs in pyramidal cells. Therefore, after completing some of the protocols detailed above in normal extracellular solution, we continued some experiments (n=6) in the presence of the channel blocker ZD7288 (40 μM), (Fig. 4.5). The application of ZD7288 increased the input resistance of pyramidal cells by 38 ± 19 % and the amplitude and decay time constant of unitary IPSPs to 133 ± 25 % and 137 ± 24 % of the control, respectively (all three, $p < 0.05$, Wilcoxon-test). When synchronously activating the smaller and bigger IPSPs (1.02 ± 0.84 mV and 3.19 ± 2.65 mV) in the presence of ZD7288, all six triplets showed sublinear summation, although control measurements indicated 3 linearly and 3 sublinearly summating input combinations (99.7 ± 0.5 % and 93.6 ± 2.1 %, respectively). Overall, ZD7288 significantly increased sublinearity from 96.7 ± 3.5 to 90.4 ± 3.9 % (n = 6, $p < 0.03$, Wilcoxon-test). The time course of the degree of linearity and IPSPs were different (Fig. 4.5C), as shown by the difference in the decay time constants (IPSP, 87.9 ± 9.3 ms vs., linearity 53.5 ± 12.3 ms; $p < 0.05$, Wilcoxon-test). Asynchronous activation of the convergent inputs (n=6) in the presence of ZD7288 changed the degree of

linearity of the peak response from 90.4 ± 3.9 % to 95.9 ± 6.2 % ($p < 0.04$; Wilcoxon-test; Fig. 4.5D) resulting in linearization of 3 input combinations.

Anatomical analysis of the connections providing convergent IPSPs with *sublinear* integration properties could be carried out in four triplets (Fig. 4.4E-H). All eight presynaptic FS cells innervated pyramidal cells on or relatively close to the soma. Predicted and/or electron microscopically verified synapses were 20 ± 21 μm from the postsynaptic somata. The average distance between the two sets of contact sites was 41 ± 13 μm, and the closest synapses/contacts originating from two converging presynaptic cells were 12 ± 14 μm apart measured along the postsynaptic dendrites or estimated on the soma. In five measurements of

basket cell input to pyramidal cells, light microscopic estimates of somatic synapses cannot account for those that are obscured by the soma, therefore, these estimates are less accurate. Electron microscopic serial section analysis of all suspected contact sites in one triplet showed co-alignment of inputs on the soma and proximal dendrites of the postsynaptic cell (Fig. 4.4E-H). One of the presynaptic FS cells in the somatic co-termination case established synapses (n=6) exclusively on the cell body, whereas the other presynaptic FS cell targeted the cell body by four, and the proximal apical dendrite by 3 synapses. The distance between the synapses formed by the two presynaptic cells was, on average, 23 ± 22 µm. From the linearly summating triplets, two triplets could be analyzed anatomically. Light microscopic estimates indicated that the postsynaptic pyramidal cells were innervated by 2 and 2 contact sites by the cells evoking the smaller IPSPs and 14 and 5 presumed synapses by the bigger unitary IPSPs, respectively. The four presynaptic cells innervated the postsynaptic cells at an average distance of 32 ± 14 µm from the soma. The mean dendritic distance of the convergent sets of afferents was 59 ± 16 and 73 ± 24 µm from each other, and the nearest neighbors of distinct sources were 42 and 39 µm distant.

Synapse location dependence of the degree of linearity in input summation

Overall, when comparing the time course of the degree of linearity in converging EPSPs on FS cells and converging perisomatic IPSPs on pyramidal cells showing sublinear summation, both the 10 to 90 % rise time and decay time constant are faster in FS cells than in pyramidal neurons ($p < 0.01$, Mann-Whitney U-test). This indicates that the temporal dynamics of the degree of linearity are connection and/or cell type specific. However, the combined analysis also revealed that EPSP-EPSP and IPSP-IPSP interactions summate similarly, depending on the spatial arrangement of inputs on the postsynaptic neuron (Fig. 4.6D-E). The average somato-dendritic distance of the two sets of afferents and the distance between nearest neighboring synapses made by the two different converging cells correlated with the linearity of input summation ($R = 0.81$ and $R = 0.85$; $p < 0.001$; Spearman-correlation).

Discussion

Our results provide direct experimental evidence that the summation of two convergent unitary inputs follows linear or close to linear summation in cortical neurons *in vitro*. This suggests that, when a small number of afferents are simultaneously active, linear or

moderately sublinear summation dominates the integration of inputs as detected at the soma. Considering total dendritic lengths of 2500-12000 μm (Gulyas et al., 1999) per neuron, a 50 μm relative upper distance limit for significant nonlinear interactions and a uniform input density, input through a particular synaptic junction would sum linearly with 96-99 % of synapses in cortical neurons during low level of activity. Similar estimates for interactions between unitary inputs providing several synaptic junctions cannot be made, because the number and spatial dispersion of unitary synapses could influence the outcome, and these have been experimentally addressed only for a fraction of cortical connections. Linear operations maintain the impact of individual afferents influencing output at a given time, and enable integration of multiple sources of information by an additive interaction of inputs.

Electron microscopic determination of the sites of synaptic junctions provided by the functionally tested converging afferents revealed that the distance between distinct simultaneously active inputs influences the degree of linearity of summation. Supporting predictions made by cable theory (Jack et al., 1975; Rall et al., 1992; Segev et al., 1995), nonlinear interactions were recorded between inputs targeting closely located postsynaptic sites. Compartment specific interactions between afferents targeting closely situated membrane domains could be detected already between two inputs, as suggested earlier (Koch et al., 1983; Shepherd and Brayton, 1987; Bush and Sejnowski, 1994), although sublinearity was moderate. A simultaneous activation of many co-aligned inputs might lead to more significant nonlinear interactions. The co-alignment of inputs of common origin on neurons is prominent in the hippocampus and neocortex, where some glutamatergic inputs terminate on distinct dendritic regions and GABAergic interneurons subdivide the surface of postsynaptic cells leading to a pairing of excitatory and inhibitory inputs on particular subcellular regions (Somogyi et al., 1998).

Nonlinear interactions may increase the computational power of neurons (Koch, 1997; Segev and London, 2000). The degree of linearity of input summation depends on the type of connection, the relative timing of inputs and on the activation state of at least one voltage gated conductance, I_h. Cellular mechanisms underlying the properties of summation remain to be explored in detail. Part of the sublinearity of interactions between closely located sites might be caused by a larger increase in membrane conductance caused by the opening of synaptic receptor channels when both synapses are active, as compared to the conductance increase caused by a single active synapse (Kogo and Ariel, 1999). The observation that asynchronous activation of pyramid to FS cell inputs, characterized by rapid synaptic conductances, resulted in the linearization of summation supports that increase in membrane

conductance could be a factor in producing sublinearity. On the other hand, the time course of sublinearity is more prolonged than that of the EPSC, particularly given that the synaptic current is somewhat filtered, indicating that other factors may contribute to sublinearity. In addition, the strong correlation between the amplitude of sublinearly summating IPSPs and the degree of linearity suggests that the local change in the membrane potential and the resulting drop in the relatively small driving force could contribute to the observed sublinearity. The contribution of local changes in driving force to sublinearity might be less in EPSP-EPSP interactions, in which no correlation was found between EPSP amplitudes and linearity and the EPSPs were of smaller amplitude relative to the driving force than the IPSPs. The uniformly linear summation of currents recorded in voltage clamp mode suggests the involvement of driving force changes in sublinearity detected in postsynaptic potential recordings; the membrane potential is clamped at the holding potential and cannot approach the reversal potential. Sublinear current summation could also result from a lack of diffusible ions on either side of the postsynaptic membrane, but our measurements suggest that changes in driving force are likely responsible for nonlinear summation. Active dendritic conductances are involved in sublinear summation (Hoffman et al., 1997; Cash and Yuste, 1999; Kogo and Ariel, 1999) as well as in supralinear boosting (Gillessen and Alzheimer, 1997; Hoffman et al., 1997) of inputs in pyramidal neurons, and were proposed to make summation paradoxically linear (Cash and Yuste, 1999). It is likely that the difference in the time course of linearity we have found between the summation of EPSPs and IPSPs is to some extent due to the distinct passive and/or active membrane properties of the receiving FS and pyramidal cells. In particular, FS neurons were suggested to act as coincidence detectors (Geiger et al., 1997), and in these cells nonlinear integration of EPSPs appear to be limited to a narrow time window, thus influencing mainly precisely synchronous EPSPs. In contrast, the degree of linearity of IPSP summation was not influenced by a 5 ms difference between the two inputs. The time course of the degree of linearity for EPSPs and IPSPs during I_h blockade was shorter than the postsynaptic voltage response suggesting the involvement of active dendritic properties (Kogo and Ariel, 1999) in unitary input summation in FS as well as in pyramidal cells.

In pyramidal neurons, I_h is likely to be one of the conductances involved in shaping integration properties. Moderate activation of I_h by preceding unitary IPSPs might explain that asynchronous perisomatic IPSPs also showed sublinear summation characteristics. The degree of linearity was correlated with the amplitude of convergent IPSPs, therefore the increased sublinearity during I_h blockade could result from the greater drop in driving force produced by

the bigger IPSPs. However, furher experiments are needed to explain the observation that synchronous and asynchromous IPSPs summed similarly in control conditions but desynchronization reduced sublinearity during ZD7288 application. Our results also indicate that I_h tends to linearize the summation of IPSPs arriving proximally. The scenario for dendritic IPSPs might be different, since I_h and other conductances, which might shape integration properties, are preferentially expressed on distal dendrites (Hoffman et al., 1997; Magee, 1998; Williams and Stuart, 2000; Berger et al., 2001). In addition to I_h examined here, tetrodotoxin sensitive voltage gated sodium conductances might also influence the properties of IPSP summation through IPSP amplification at more depolarized membrane potentials (Stuart, 1999). Moreover, voltage dependent conductances may change integration properties considerably when neurons fire action potentials

The relative weight of linear and nonlinear interactions of inputs in single neurons could be influenced also by the timing of convergent inputs. Although we have not been able to test the wide range of spatiotemporal parameters of previous simulations examining the time course of nonlinear interactions (Koch et al., 1983), the method applied here was sensitive enough to detect differences between synchronous and asynchronous input summation. Our results suggest that the dependence of summation on the relative activation time of inputs is connection type specific and is influenced by differential activation of I_h. Randomly timed and spatially scattered inputs would favor linear integration, but synchronous activation of co-aligned afferents could shift the balance of processing towards nonlinear integration. Orchestrated EPSPs and IPSPs arrive at postsynaptic neurons in experimentally evoked cortical oscillations (Jefferys et al., 1996; Fisahn et al., 1998), suggesting rhythmic alternations in the mode of summation. Postsynaptic potentials arrive at much higher rates in vivo than in the slices studied here, therefore the ratio of nonlinear interactions might be underrepresented in our study compared to more physiological conditions. The degree of linearity in processing might depend on behavioral states, which are accompanied by a variable synchrony in the firing of GABAergic neurons targeting specific cellular compartments (Csicsvari et al., 1998). At high level of activity, time and domain specific nonlinearity of summation in cortical networks might selectively reduce the impact of individual inputs arriving in a synchronous barrage (Destexhe and Pare, 1999). In contrast, at low level of activity, the information content of inputs summating linearly would be maintained.

4.5 Summation of unitary IPSPs elicited by identified axo-axonic interneurons

Summary

We provided recent experimental evidence that coincident unitary events sum slightly sublinearly when targeting closely located postsynaptic sites. Simultaneous activation of many co-aligned inputs might lead to more significant nonlinear interactions especially in compartments of relatively small diameter. The axon initial segment of pyramidal cells has a limited volume and it receives inputs only from a moderate number of axo-axonic interneurons. We recorded the interaction of unitary axo-axonic inputs targeting a layer 4 pyramidal cell and determined the exact number and position of synapses mediating the effects. Both axo-axonic cells established three synaptic release sites on the axon initial segment of the postsynaptic cell which received a total of 19 synapses. The summation of identified IPSPs was slightly sublinear (9.4 %) and the time course of sublinearity was slower than that of the IPSPs. Repeating the experiment while holding the postsynaptic cell in voltage clamp mode showed linear summation of IPSCs, suggesting that a local decrease in driving force could contribute to the sublinear summation measured in voltage recordings. The results indicate that moderate sublinearity during the interaction of neighboring inputs might be preserved in cellular compartments of relatively small volume even if a considerable portion of all afferents converging to the same domain is simultaneously active.

Introduction

Electron microscopic determination of the sites of synaptic junctions provided by the functionally tested converging afferents revealed that the distance between distinct simultaneously active inputs influences the degree of linearity of summation (Tamas et al., 2002). Supporting predictions made by cable theory (Jack et al., 1975; Segev et al., 1995; Koch, 1999), nonlinear interactions were recorded between inputs targeting closely located postsynaptic sites. Compartment specific interactions between afferents targeting closely situated membrane domains have already been detected between two inputs, although sublinearity was moderate. Simultaneous activation of many co-aligned inputs might lead to more significant nonlinear interactions especially in compartments of relatively small diameter, but simultaneous recording of a significant fraction of all inputs targeting the same domain is difficult. The axon initial segment (AIS) of pyramidal cells appears to be the postsynaptic compartment of choice for these experiments since it receives inputs exclusively

from axo-axonic interneurons and it was estimated that only three to six presynaptic axo-axonic cells converge onto the same pyramidal cell (Somogyi, 1989).

Axo-axonic cells (AACs) are local circuit neurons, which are unique elements of the cortical microcircuit (Somogyi et al., 1998). Their widespread occurrence in a variety of cortical regions suggest that AACs contribute to fundamental operations in cortical processing. Several electrophysiological and anatomical characteristics of AACs are similar to other GABAergic interneurons (Soriano and Frotscher, 1989; Gulyas et al., 1993; Buhl et al., 1994b; Kawaguchi, 1995; Pawelzik et al., 1999; Maccaferri et al., 2000; Krimer and Goldman-Rakic, 2001), but AACs are distinct providing uniform innervation of principal neurons exclusively targeting their axon initial segments (Somogyi, 1977). Axo-axonic interneurons elicit fast IPSPs showing paired pulse depression in their target cells (Buhl *et al.* 1994b; Pawelzik *et al.* 1999; Maccaferri *et al.* 2000), but how axo-axonic inputs interact is not known. Here we present a case study of two axo-axonic inputs converging onto a pyramidal cell; this unique example resulted from more than 1700 triple and/or quadruple recordings with 188 convergent inputs targeting the same postsynaptic cell.

Results

We recorded the interaction of unitary axo-axonic inputs targeting a layer 4 pyramidal cell and determined the exact number and position of synapses mediating the effects. The two presynaptic AACs had a fast spiking firing pattern and the postsynaptic pyramidal cell showed a regular spiking behavior (Fig. 5.1A-C). When activating either AACs separately, the postsynaptic pyramidal cell responded with short-latency IPSCs or IPSPs; both presynaptic cells evoked current and voltage responses of similar amplitude (50.1 pA, 58.8 pA and 0.85 mV, 0.90 mV, respectively, Fig. 5.1D-F). Application of a paired pulse protocol (interval, 60 ms) produced depression of the second responses (paired pulse ratios, 79 % and 65 %). Simultaneous activation of the two AACs produced a linear summation of IPSCs (101.4 %) when comparing the peak amplitude of experimentally recorded compound responses (110.5 pA) with the calculated sum of individual inputs (109.0 pA) and the linearity of IPSC summation was maintained during the entire time course of responses as evidenced also by the almost perfectly overlapping recorded and calculated traces (Fig. 5.1D-E). Repeating the experiment in current clamp mode, however, indicated moderately sublinear summation (9.4 %) as the experimentally recorded maximal amplitude of compound IPSP (1.58 mV) was smaller than those of corresponding algebraic sums of individual IPSPs (1.75 mV; Fig. 5.1F-G). The time course of the degree of linearity was distinct from the kinetics of

IPSPs. Both individual and compound IPSPs showed exponential decays (τ = 19.2 ± 2.1 ms), but the kinetics of linearity could not be adequately fitted with conventional functions as the degree of linearity platoed around 92 % for ~ 30 ms before decaying back to baseline (half-width, 62.3 ms).

The somata of all three cells were located in layer 4 of the cortex (Fig. 5.2A-B). The dendrites of pyramidal cell and AAC1 were fully recovered, the distal dendrites of AAC2 could not be traced further than ~100 µm from the soma. Most dendrites of the AACs originated from the upper or lower pole of the cell body and formed radially elongated fields. The axons of each cell were recovered and could be reconstructed in detail. Similar to previously established characteristics (Somogyi, 1977), both AACs gave rise to clusters of radially oriented small axonal branches. Electron microscopic sampling of postsynaptic targets taken from nonoverlapping parts of the axonal arborizations identified both presynaptic cells as AACs since both cells established synaptic junctions exclusively on AISs (n = 13 and 14 for AAC1 and AAC2, respectively). High order branches of both presynaptic axons fromed close appositions with the AIS of the postsynaptic pyramidal cell and both AAC1 and AAC2 established three synaptic junctions in neighboring clusters at distances of 19 ± 3 and 40 ± 13 µm from the soma (Fig. 5.2C-G). Synapses formed by the two presynaptic cells were, on average, 20 ± 12 µm from each other and the distance between the nearest synapses of distinct sources was 6 µm. We determined the total number synaptic junctions (n = 19) on the axon initial segment of the postsynaptic cell between the axon hillock and the first branch point in serial electron microscopic sections. The complete three dimensional reconstruction of the postsynaptic AIS and all presynaptic terminals showed that 11 unidentified presynaptic boutons formed 13 synapses in addition to the five identified boutons and six identified synaptic junctions.

Discussion

Our results provide a direct experimental example that the summation of inputs targeting the AIS follows slightly sublinear summation in cortical neurons *in vitro*. The results indicate that the degree of sublinearity during the interaction of neighboring inputs might be similar in cellular compartments of different volume. In the postsynaptic pyramidal cell, the volume of the AIS measured from the hillock to the origin of the first axonal collateral was approximately 47 μm^3, which corresponded to ~ 1.3 % of the somatic volume (~ 3500 μm^3). It should be noted, however, that the soma may provided a sink for chloride ions entering the AIS during the experiments maintaining the gradient and therefore promoting linearity of

summation. This could be of significant importance since we have showed that the degree of sublinearity between IPSPs targeting the soma and/or proximal dendrites was highly correlated with the amplitude of inputs suggesting the contribution of a drop in local driving force to sublinearity (Tamas et al., 2002). Ultimately, experiments addressing the summation of convergent inputs targeting the same dendritic spine could test interactions minimizing source/sink factors influencing summation properties (Shepherd and Brayton, 1987; Qian and Sejnowski, 1990; Koch, 1999). Furthermore, given the synapses arriving from the two presynaptic cells formed separate proximal and distal clusters on the AIS, our recordings suggest that on-the-path shunting mechanisms (Koch, 1999) were suppressed in the case studied here. This could be due to the relatively high density of voltage gated ion channels on the AIS decreasing the significance of the synaptic conductance changes. The involvement of voltage activated currents, such as de-inactivation of sodium conductances by hyperpolarization might be responsible for the difference in kinetics between the time course of linearity and the postsynaptic potentials.

One of the key factors shaping summation properties of any neuron is the background activity of synaptic conductances. The number of simultaneously active synapses on a particular cellular compartment might change dramatically depending on the behavioral state of the animal. However, the AIS receives inputs solely from AACs and the six synapses identified in this study represent 32 % of all synapses which targeted the recorded pyramidal cell and approximately 20-40 % of all synaptic junctions targeting this compartment on average (DeFelipe et al., 1985; Somogyi et al., 1985; Somogyi, 1989; Farinas and DeFelipe, 1991). This suggests that, even if a significant portion of all afferents converging to the same domain is simultaneously active, linear or moderately sublinear summation might be conserved in the integration of inputs as detected at the soma.

4.6 Contribution of Ih to the summation of dendritic IPSPs

Introduction

Our previous results demonstrated that unitary inputs sum location dependently. This is particularly interesting in case of inhibitory inputs because distinct sources of inhibition subdivide the somato-dendritic surface of postsynaptic pyramidal cells. We showed that perisomatic inputs sum slightly sublinearly, even in small axon initial segments. However, how these summation rules can be applied for dendrites of pyramidal cells, which receive most of the inhibitory synapses, is not known. Dendrites of neurons contain voltage-gated channels which could influence the integration of unitary inputs (Lipowsky et al., 1996; Fricker and Miles, 2000). It was shown that activation of sodium channels by simultaneously arriving EPSPs could underlie supralinear summation. We demonstrated previously that hyperpolarization activated cation channels (Ih) contribute to the sublinear summation of two perisomatic IPSPs. We have found that blockade of this channel by ZD7288 increased the sublinearity of summation by increasing the global inward resistance of postsynaptic membrane and, therefore, amplitude of unitary IPSPs. However, increasing concentration of Ih channels was found from the soma to apical dendrites of cortical pyramidal cells (Lorincz et al., 2002; Strauss et al., 2004). This subcellular distribution suggests input location dependent contribution of this ion channel to the integration of inhibitory inputs originated from distinct GABAergic cell types. To test this hypothesis we compared the effects of Ih channels on the summation of perisomatic and dendritic IPSPs.

Results

Currents through Ih channels show slow activation kinetics; therefore they could influence the decaying phase of PSP more prominently than peak amplitudes (Magee, 1999). Thus, we investigated the changes not only on peak amplitude but also in the inhibition carried in control conditions and in the presence of ZD7288 in narrow time window. We integrated the difference between the measured and expected compound responses in the first 100 ms in control conditions and in the presence of ZD7288 (Fig 6.1C). We grouped the experiments according to the predicted innervated domain of the postsynaptic cell to find correlation between the location of the synapses and the drug effect. We predicted the input localizations relative to soma based on the well-established firing pattern of layer 2/3 GABAergic neurons (see chapter 4.1). Therefore, we formed three groups: combination of two perisomatic (n=21), two dendritic (n=20) or one dendritic and one perisomatic (n=20) inhibitory inputs. In

randomly chosen cases we investigated the localization of inputs on postsynaptic pyramidal cells by light microscopy (n=13). These results confirm that synapses originated from fast-spiking cells are located on the perisomatic region, while bitufted and regular-spiking non-pyramidal cells innervate the dendritic region of postsynaptic pyramidal cells. However, this sample was not enough to determine the correlation of the relative position of individual inputs and pharmacological sensitivity for ZD7288 of IPSP summation. In control conditions we have found similar IPSP summation in all of the three groups: two perisomatic IPSPs 94.8 ± 4.8 %, perisomatic and dendritic IPSPs 93.5 ± 5.0 %, two dendritic IPSPs 94.9 ± 6.4. Blockade of Ih channels by ZD7288 had different effects from experiments to experiments (n=25, Fig 6.1A, C). In 9 cases the area difference of measured and calculated compound IPSP became more prominent indicating more sublinear summation in presence of ZD7288 (-0.503 ± 0.288 ms x mV). Almost all tested perisomatic-perisomatic IPSP-pairs were in this group (n=5 of 6) as well as some dendritic-dendritic (n=2 out of 10) or dendritic-perisomatic IPSP-pairs (n=2 out of 9). However, in other experiments (n=11) the ZD7288 shifted the measured area to the opposite direction, toward linear summation (0.956 ± 0.59 ms x mV). In this group we could not detect any perisomatic-perisomatic IPSP-pairs but dendritic-dendritic (n=6 out of 10) and dendritic-perisomatic (n=5 out of 9). The area difference between the measured and calculated sum did not change significantly (0.02 ± 0.111 ms x mV) in the remaining 5 experiments. The changes of sublinearity at the peaks of IPSPs showed similar results: -5.7 ± 3.4 %, 6.65 ± 8.5 % and –1.6 ± 5.9 %, respectively. In summary, linearization effects of ZD7288 were found only when at least one of the inhibitory inputs was located on the dendrites of the postsynaptic cells. This indicates the location dependent role of the Ih activation in sublinear summation of IPSPs in control conditions.

To clarify the underlying mechanisms of location dependent contribution of Ih in IPSP summation, we measured the effects of blockade of AMPA and NMDA glutamate receptors on the summation of differentially localized IPSPs. Channel noise, background synaptic activity, spillover of glutamate from synaptic cleft or leakage from synaptic terminals opens a small fraction of glutamate receptors persistently causing a constant conductance through these channels. Presumably, blockade of these conductances changes the membrane resistance of the postsynaptic cell and does not affect the active integration of IPSPs. This effect could be more prominent in distal dendrites than closer to the soma because of the higher concentration of glutamate receptors in the distal region (Andrasfalvy and Magee, 2001) and the relatively higher membrane surface of thin dendritic branches. Summation of perisomatic and dendritic IPSP pairs are differentially influenced by glutamate receptors. We did not find

significant area difference deflections in control and drug circumstances at any input combinations. When a perisomatic IPSP was recorded with other perisomatic (n=4) or dendritic (n=9) IPSP, we did not detect differences in the linearity at the peak of IPSPs. In case of two dendritic IPSPs, blockade of glutamate receptors slightly shifts the summation towards sublinearity (-4.4 ± 2.1 %, n=3). These exclusive effect on two dendritic IPSPs indicate more pronounced effects of NBQX and AP-5 on the membrane resistance of dendrites than in the perisomatic region.

Discussion

Interpretation of the results with ZD 7288 on perisomatic IPSPs appears straightforward: blockade of Ih changes the global membrane resistance of the postsynaptic cells, therefore the inputs get virtually closer to each other. In case of dendritic IPSP summation, the opposing ZD 7288 effects can be explained in various ways. First, the higher Ih activity on the dendritic region can cause a constant depolarization relative to the soma. Thus, the amplitudes of the IPSPs decrease when this ion channel is blocked because the membrane potential moves closer to the reversal of IPSPs predominantly mediated by chloride ions and we have shown that summation is amplitude dependent (see chapter 4.4). The second explanation for different ZD 7288 effects on dendritic IPSP summation is that simultaneous activation of two dendritic IPSPs recruit more Ih channels than separate activation of the same inputs. Such excessive Ih channel activation causes depolarization, suppressing the compound amplitude relative to what is expected from single IPSPs. It is likely that NBQX and AP-5 affect the dendritic membrane properties more effectively than in the perisomatic region. In this case, the decrease of dendritic membrane potential and increase of membrane resistance should be similar to the effects of ZD7288. However, we have not found similar position dependent modulation of IPSP summation by glutamate receptors and by Ih channels. Therefore, these results suggest that location dependent modulation of IPSP summation requires active involvement of Ih channels.

4.7 Readout of spatial and temporal input summation by single neocortical interneurons

Summary

Neurons receive thousands of convergent synaptic inputs and generate a characteristic output pattern of action potentials, but how single neuronal output reflects temporally organized input activity is not understood. We compared the interaction of identified, unitary excitatory inputs at sub- and suprathreshold postsynaptic membrane potentials in single, neocortical fast-spiking cells. Differentially timed excitatory inputs showed close to linear summation at subthreshold conditions and the degree of these slight non-linearity was depended on postsynaptic membrane potential and the timing of EPSPs. Suprathreshold interaction of the same inputs, however, was highly nonlinear and depended on presynaptic timing. 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. 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 cells. Transformation of linear subthreshold summation rules to nonlinear and dynamic spike transmission reflects the sequence and synchrony of inputs in single postsynaptic cells and could enhance temporal precision of information flow in cortical networks.

Introduction

The relationship between input and output events of single neurons appears crucial in cortical operation: important findings show that firing rates in cortical neurons reflect particular input patterns (Shadlen and Movshon, 1999; Salinas and Sejnowski, 2001) and that correlated neural activity is a potential neural code underlying sensory processing in the brain (Singer and Gray, 1995; Softky, 1995; Salinas and Sejnowski, 2001). How cortical neurons integrate inputs and how neuronal output reflects input activity are critical for the explanation of cortical function. The rules of subthreshold input summation are crucial in determining postsynaptic output properties: nonlinear interactions may increase the computational power of neurons (Koch et al., 1983; Segev et al., 1995; Koch, 1997; Poirazi and Mel, 2001) and linear operations would maintain the impact of individual afferents influencing output at a given time (Cash and Yuste, 1999; Tamas et al., 2002). Active dendritic conductances are involved in sublinear summation (Hoffman et al., 1997; Cash and Yuste, 1999; Kogo and Ariel, 1999; Tamas et al., 2002), they can detect coincident excitatory inputs (Softky, 1994;

Agmon-Snir et al., 1998), boost input efficacy supralinearly in pyramidal neurons (Gillessen and Alzheimer, 1997; Hoffman et al., 1997; Nettleton and Spain, 2000), and are proposed to make summation paradoxically linear (Cash and Yuste, 1999). The degree of linearity of input summation depends on the type of connection, the relative timing of inputs (Margulis and Tang, 1998; Angulo et al., 1999; Tamas et al., 2002) and could be regulated by preceding action potentials in the postsynaptic cell (Hausser et al., 2001). Action potential triggering properties of EPSP is determined by the active conductances of the postsynaptic cells and by the kinetics of EPSPs (Fricker and Miles, 2000). Cortical neurons are sensitive for the timing of incoming synaptic afferents (Konig et al., 1996) and this can be dynamically adapted to preceding membrane potential changes and the activation state of sodium channels (Stuart and Sakmann, 1995; Margulis and Tang, 1998; Angulo et al., 1999; Azouz and Gray, 2000; Nettleton and Spain, 2000; Azouz and Gray, 2003). Fast spiking interneurons receive extremely fast excitatory inputs (Thomson et al., 1995; Buhl et al., 1997) which can trigger action potentials reliably and with short time lag (Fricker and Miles, 2000; Galarreta and Hestrin, 2001). Single GABAergic neurons can effectively entrain the temporal pattern of postsynaptic action potentials in networks of various brain regions (Cobb et al., 1995; Hausser and Clark, 1997; Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000; Tamas et al., 2004) and therefore contribute in feed-forward cortical circuits (Gibson et al., 1999; Pouille and Scanziani, 2001, 2004). Experiments on dual unitary connections via gap junctions combined with GABAergic synapses within separate populations of cortical interneurons showed that precisely timed and placed convergent inputs might be critical in synchronizing neuronal activity (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000; McBain and Fisahn, 2001). Yet, the functions between subthreshold input interactions and postsynaptic spike generation and between the temporal distribution of postsynaptic action potentials and the pattern of convergent excitatory inputs remain to be elucidated. Therefore, we measured the effects of voltage-gated channels activated by two converging unitary EPSPs and their involvement in suprathreshold integration of same input pairs to determine how temporal sequences of presynaptic network activity can be detected by single FS cell firing.

Results

Voltage-dependent subthreshold EPSP summation

To test the relationship between input integration and output generation, we have simultaneously recorded from triplets (n=30) of neurons consisting of two presynaptic

pyramidal cells converging onto a postsynaptic fast spiking cell in layer 2/3 of rat somatosensory cortex. Recorded cells were identified according to established firing patterns (McCormick et al., 1985; Kawaguchi and Kubota, 1997; Galarreta and Hestrin, 1999; Gibson et al., 1999; Gupta et al., 2000): high frequency, non-accommodating firing pattern with narrow spikes (0.54 ± 0.03 ms half width) and fast, big amplitude AHP. The classification was confirmed by light-microscopic examination (Tamas et al., 2000). The postsynaptic cells formed axonal boutons, which were frequently opposed to somata suggesting that these cells were basket cells. Electron microscopic analysis of synaptic targets confirmed these results and showed that postsynaptic cells (n=4) innervated somata (28 ± 4 %), dendritic shafts (64 ± 5 %) and dendritic spines (8 ± 3 %). We tested the summation of subthreshold EPSPs at two different membrane potentials (-65.2 ± 1.2 mV and -35.1 ± 2.0 mV; n=12; Fig. 7.1A). Similar to our previous experiments (Tamas et al., 2002), the summation of unitary EPSPs was linear or slightly sublinear (96.2 ± 2.7 %, measured at the peak of compound EPSP) in FS cells at relatively hyperpolarized membrane potentials. Depolarization of the postsynaptic cells shifted the linearity of the same interactions to supralinear values (107.7 ± 2.2 %, $p < 0.01$; Fig. 7.1A,C). Asynchronization (5 ms) of the two inputs changed summation towards linearity with 8.4 ± 3.5 % at the depolarized membrane potentials ($p<0.05$, n=6, Fig 7.1B-C).

Transformation of summation rules at suprathreshold level

Spike timing efficacy of single and simultaneous inputs was tested in 23 triple recordings (Fig. 7.2). Postsynaptic FS cells were depolarized to respond with action potentials in the majority (85 ± 3 %) of trials activating both presynaptic cells (Fig. 7.2A, 7.3A). When applying these conditions, FS cells also fired spontaneously at 2.3 ± 0.5 Hz measured in a period of 140 ms prior to the activation of presynaptic cells. When depolarized, FS cells showed spontaneous membrane potential fluctuations in the beta and gamma frequency ranges (Pike et al., 2000; Fellous et al., 2001), but the timing of presynaptic action potentials and postsynaptic spikes was unrelated to phases of ongoing subthreshold activity. Single EPSPs arriving to fast-spiking postsynaptic cells triggered action potentials in 44.2 ± 4.5 % (range, 0-97 %) of trials in a time window of 10 ms following presynaptic activation. The peak of distributions of EPSP-triggered postsynaptic action potentials followed presynaptic spikes by 3.74 ± 0.19 ms (n=41; Fig 7.2A,B). The latency of postsynaptic spikes was not correlated with the amplitude of EPSPs (see also ref. (Galarreta and Hestrin, 2001) but showed a linear correlation with the 10-90 % rise times of EPSPs ($p<0.001$, $R=0.65$) in line with earlier results recorded in pyramidal cells (Fricker and Miles, 2000). Simultaneous

activation of two EPSPs elicited action potentials with a shorter latency by shifting the peaks of postsynaptic spike distributions towards presynaptic spikes by 0.29 ± 0.09 ms ($p<0.01$, Fig. 7.2B). In addition to the faster spike triggering effect, synchronous presynaptic activation improved the precision of spike transmission by narrowing the half width of postsynaptic spike distributions by 19.4 ± 5.9 % relative to the distribution triggered by the more precise individual input ($p<0.01$). This acceleration and decreased temporal jitter of spike transmission by synchronous inputs did not depend on the spike-triggering efficacy of individual inputs (Fig. 7.2A, 7.3A).

The time courses of linearity of subthreshold responses or spike distributions were calculated as the rate of responses to together activation and calculated sum of individual responses, referenced to the maximum of latter. The linearity of subthreshold voltage responses at either postsynaptic membrane potentials was consistently different from spike transmission linearity curves (Fig. 7.2C). The time course of linearity for spike transmission showed a highly supralinear peak 2.95 ms after presynaptic action potentials followed by a drop to sublinearity, which were shaped by shorter latency and improved precision of postsynaptic action potential timing in response to dual activation. The relative numbers of postsynaptic action potentials in response to combined inputs varied when compared to the sum of spikes triggered by individual inputs (111 ± 7 %, range: 53 - 214%) depending on state of postsynaptic cell and amplitude of EPSPs. When single input activation reaches reliably the postsynaptic threshold, together activation could not increase the number of evoked spikes. Otherwise, when peaks of unitary EPSPs remain just below threshold, the number of spikes evoked by simultaneous activation would be much more than expected. Our samples are distributed between these endpoints. When dividing the dataset into three groups based on the ratio between the measured and calculated spike-triggering efficacy of dual presynaptic activation, supralinearity showed a similarly timed peak in all three groups. When more spikes (127.6 ± 3.8 %, n=8) were evoked than expected the peak of supralinearity was at 3.15 ms; in case of similar number (104.4 ± 2.4 %, n=10) of postsynaptic action potential it was at 2.99 ms and when less spikes (75.3 ± 5.7 %, n=6) were elicited by simultaneous activation it was at 2.82 ms. These indicate that reduced temporal jitter and lag are not sensitive for relative amplitude of inputs and can be observed in wide range postsynaptic membrane potential state when EPSPs arriving synchronously.

Differential readout of EPSPs according to preceding activity

We then tested the impact of input asynchronization on spike transmission by activating convergent presynaptic cells in succession with 2, 5 and 10 ms intervals (Fig. 7.3). When preceding inputs evoked a postsynaptic spike, follower inputs could not trigger postsynaptic action potentials at the tested intervals. Therefore EPSPs with lesser spike triggering efficacy (17.7 ± 3.2 %) were the first to be activated and analyzed further (Fig. 7.3A). However, those traces where first EPSP elicited spikes in the postsynaptic cells were not excluded from the analysis allowing us to compare the distribution of action potential in single and together activation. Postsynaptic spike timing was referenced to the action potential of the second presynaptic cell. The distributions of postsynaptic spikes evoked by

asynchronous activation were significantly different from the calculated sums of differentially timed single EPSP triggered spike distributions at all tested intervals (Fig 7.3B, $p<0.01$, Kolmogorov-Smirnov-test). The latency of postsynaptic action potentials was dependent on the interval between presynaptic spikes. When the less reliable input preceded the follower EPSP by 2 ms, the peak of postsynaptic spike distributions were shifted towards the presynaptic spike with 0.4 ± 0.24 ms ($p<0.05$, n=10) relative to single EPSP-evoked spike distributions (Fig. 7.3, black and blue trace). We could not detect similar differences when the interval between presynaptic activation was 5 ms (n=22). 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 ($p<0.05$, n=10) 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 (0.7 ± 9.3, -6.7 ± 11.4, -1.1 ± 13.3 % for 2, 5 and 10 ms respectively, measured as the half-width of distributions, Fig. 7.3A-B black vs. blue). Changes in latency but not in temporal jitter influenced the time course of linearity during asynchronous presynaptic activation characteristically (Fig. 7.3C). The highly supralinear peak observed in response to synchronous activations drops when stimulating slightly asynchronously and reverses to sublinearity when applying 10 ms presynaptic interval. Interestingly, these changes appeared to be limited to relatively narrow time window around two to four ms after the second presynaptic spike. Overall, dual activation of presynaptic cells at 2 or 5 ms interval did not change the number of spikes evoked in a period of 10 ms after the second presynaptic spike relative to the sum of postsynaptic spike distributions in response to separate presynaptic activation (Fig. 7.3B, black vs. green). At 10 ms interval, however, the same comparison indicated a significant drop in the number of postsynaptic spikes elicited during dual presynaptic firing (from 68.3 ± 8.1 % to 50.2 ± 8.3 %, $p<0.01$).

Contribution of sodium channels to time dependent EPSP interaction

Spike threshold is sensitive for preceding depolarization (Fricker and Miles, 2000; Azouz and Gray, 2003). Therefore, in search for mechanisms underlying the temporal profile of spike transmission, we measured the thresholds of action potentials evoked by single, synchronous and asynchronous follower EPSPs. We compared spike thresholds measured in asynchronous paradigms to that of simultaneous activation within the same presynaptic stimulation cycles (Fig. 7.4A). When two EPSPs arrived synchronously to postsynaptic FS cells, the firing threshold was -18.7 ± 0.23 mV. The threshold for action potentials was 0.28 mV more

depolarized when responding to single EPSPs ($p<0.05$, Student's t-test, n=165 spikes). Checking the same EPSPs during dual presynaptic activation as followers, spike thresholds moved to even more depolarized levels (with 0.48, 0.86 and 1.25 mV for 2, 5 and 10 ms intervals, respectively, $p<0.001$, n=207, 170 and 136 spikes, Student's t-test, Fig 7.4B). Threshold potentials for 5 and 10 ms presynaptic intervals were also significantly different from single activation ($p<0.001$).

EPSP-like waveforms were injected in voltage-clamp circumstances into FS cells to study the availability of voltage-gated channels for excitatory inputs during different parts of EPSPs (Fig. 7.4C). The amplitude (3 mV) of these waveforms were in the range of unitary EPSP amplitudes (2.42 ± 0.28 mV, n=42) had activated an inward current (n=9) at depolarized membrane potential (4-6 mV from threshold), which could be blocked by intracellular application of 5 mM sodium channel blocker, QX314 (n=3). The evoked currents reached the peak during the onset of the decaying phase of EPSP. Injection of kinetically similar EPSPs with higher amplitude (>10 mV) activated not only inward but outward currents (data not shown) similarly as described previously in different neuron types (Fricker and Miles, 2000; Axmacher and Miles, 2004). Small amplitude, square pulses (6 mV, approaching 1-2 mV the threshold, 3 ms) activated a fast inward current (Fig. 7.4D) in FS cells (amplitude: -67.3 ± 7.8 pA, integral area: -114 ± 15 pA x ms). When these square pulses were incorporated into different parts of previous EPSP-like voltage commands reaching the same command potential (6 mV), they activated a decreased amount of channel during the EPSP ($p<0.05$, n=8) when compared to solitary square pulses. When square pulse started 2 ms after the onset of EPSPs, the current amplitude was -53.3 ± 7.7 pA, with integral area -102 ± 16 pA x ms during the square pulse part (3ms). The smallest current was measured when pulses were delivered 5 ms after the EPSP onset (amplitude: -38.3 ± 5 pA, integral area: -72 ± 10 pA x ms) with a recovery starting 10 ms after the start of EPSP (amplitude: -43.7 ± 6.5 pA, integral area: -77 ± 12 pA x ms). Currents available to square pulses were similar before and after the EPSP (amplitude: -62.6 ± 7.3 pA, integral area: -107 ± 13 pA x ms, 20 ms after the onset of EPSP). No differences could be detected in the rise of normalized inward currents during the square pulses indicating that activation properties of sodium channels were not affected by EPSPs. No similar currents could be detected when basket cells were recorded in presence of intracellular QX314 (n=3). Differential sodium current availability for EPSPs altered spike generation. The same protocols were applied to FS cells at more depolarized holding potentials reaching the threshold at the square pulse part of the command potential. Currents preceding action potentials were aligned according to the maximal derivative of the

rise of action potentials. The fastest exponential current rise prior to action potential was measured during simple square pulses (time constant: -0.22 ± 0.032 ms). The current rise during EPSPs was significantly slower: -0.334 ± -0.057, -0.457 ± 0.071, -0.362 ± 0.06; 2, 5 and 10 ms after EPSP onset, respectively, $p<0.05$, $n=5$. When square pulses were inserted 20 ms after EPSP onset, the current rise before action potentials recovered: -0.26 ± 0.046 ms.

Discussion

Non-linear properties of EPSP integration

Our results provide evidence that the rules of summation can be switched by the operational state of postsynaptic neurons. These results support earlier experiments showing that subthreshold activation of voltage-gated sodium channels causes suprathreshold EPSP summation in cortical neurons (Margulis and Tang, 1998; Angulo et al., 1999; Nettleton and Spain, 2000). We show that unitary EPSPs are able to activate voltage gated channels that modulate the input summation. Remaining below the threshold, the level of supralinearity at depolarized membrane potentials were independent from the level of sublinearity at rest indicating that contribution of sodium channel activation could mask input-site independently the passive, spatial EPSP summation rules in FS cells (Tamas et al., 2002). Close to linear subthreshold input summation rules can turn to highly nonlinear interactions in spike transmission during a limited time window. Our finding that the probability of action potentials generated by two input individually and together sum non-linearly is predictable, because of non-linear features of action potential threshold. However, here we determined the temporal characterics and event sequence dependence of subthreshold to suprathreshold signalization. Larger input cross action potential threshold more quickly and are enhanced by supralinear EPSP summation together with dynamic spike threshold, therefore favoring non-linear postsynaptic action potential generation. By investigating asynchronously arriving EPSPs we found that temporal distribution of firing of FS cells indicate the relative temporal position of subthreshold EPSPs preceding postsynaptic spikes, and therefore, a single action potential evoked by EPSP carries information about the relative timing of preceding inputs. Differential availability of sodium channels during subthreshold 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. The partial sodium channel inactivation also contribute to decreased supralinearity of summation of asynchronous, subthreshold EPSPs.

Precise readout of EPSP series

The temporal distribution of postsynaptic firing could precisely reflect the results of functions of temporal and spatial inputs summation and their excitability, which is highly determined by preceding input timing. Each input can contribute to the generation of outputs: synchronous inputs enhance the precision of suprathreshold signal propagation, while preceding EPSP decreases the spike transmission efficacy of follower EPSPs. Therefore, output-generating strategies of this cell type is pivoted between coincidence detection and rate coding. Detection of many synchronous inputs is facilitated by the delayed activation of potassium currents by higher amplitude compound EPSP (Fricker and Miles, 2000; Axmacher and Miles, 2004), and by effective propagation in electrically coupled network of FS cells (Galarreta and Hestrin, 2001). Firing followed by the strong after-hyperpolarization in fast spiking cells can reset sodium channel dynamics (Erisir et al., 1999) which seems to be the main postsynaptic factor in the input timing dependent output generation.

From the view of synaptic information efficacy (London et al., 2002), differentially timed preceding EPSPs correspond to the simplest background activity. These input timing dependent non-linear processes could enhance temporal precision of network processes and alter spike-triggering effectiveness of individual inputs. The distribution of postsynaptic action potentials is indicative of presynaptic synchronization at a temporal resolution in the millisecond range and reflects the sequence of asynchronous inputs targeting the same postsynaptic neuron. By this temporal cross talk each input carries some information: preceding input influence the information efficacy of the follower and the follower is influenced by previous inputs. FS cells can reliably read input sequences taking each input in context of others. FS cells *per se* can slightly bias the dinamics of these processing due to the reliably properties of their excitatory inputs (Thomson et al., 1995; Buhl et al., 1997), they can just read it. Forasmuch, the results of these reading is strictly determined by the preceding input activity.

Transmitting the readout

Networks of FS cells coupled by electrical and GABAergic synapses can establish and maintain correlated firing (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000) and the output of FS cells effectively entrains firing of pyramidal neurons (Cobb et al., 1995; Miles et al., 1996; Tamas et al., 2004). It appears that FS cells are prominent in intracortical (Thomson et al., 1995; Buhl et al., 1997; Gupta et al., 2000; Galarreta and Hestrin, 2001; Pouille and Scanziani, 2001, 2004) and ascending feed-forward inhibitory

circuits (Beierlein et al., 2003; Hirsch et al., 2003; Swadlow, 2003) and fire only at well defined temporal segments during rhythmic processes (Klausberger et al., 2003). The characteristic temporal profile of nonlinearity in action potential propagation minimizes the temporal jitter for propagating excitation in cortical feed-forward networks. Consequently, input sequence and synchrony could be dynamically represented by firing distribution of single postsynaptic cells, therefore facilitating the propagation and identification of repeated motifs in cortical information processing (Ikegaya et al., 2004).

5. GENERAL DISCUSSION AND OUTLOOK

This thesis is focused on specific aspects of sequential activity in the divergent and convergent cortical networks. First, we have shown that repeated activity of separately placed inhibitory inputs can differentially entrain the firing of various postsynaptic targets cells allowing parallel information processing. This parallelism is further emphasized by the differential excitation basket and bitufted cells by local pyramidal cells (Reyes et al., 1998; Pouille and Scanziani, 2004). Information transfer from basket and bitufted cells is frequency and connection specific. Postsynaptic regular spiking non-pyramidal cells respond similarly to different presynaptic stimulation frequencies due to the different kinetics of differentially placed GABAergic inputs. Pyramidal cells, however, show a theta phase related nesting oscillations in response to inputs arriving from several interneuron classes. Action potentials of fast-spiking cells are phased in the gamma range irrespective of presynaptic firing frequency and inputs location. Moreover, we showed that dendritic interaction of GABAergic and gap junctional connections could effectively transmit and maintain coherent activity in the network of regular spiking non-pyramidal cells. Our experiments on the effect of bitufted and regular spiking cells provided evidence that dendritic IPSPs can effectively phase the firing of postsynaptic cells. This suggests a novel role for dendritic inhibitory networks in addition to the known local control of excitability and Ca^{2+} electrogenesis (Miles et al., 1996).

We found that single action potentials of neurogliaform cells, in contrast to other GABAergic cells, elicit combined $GABA_A$ and $GABA_B$ receptor mediated responses in postsynaptic pyramidal cells. The slow $GABA_A$ component is always followed by a $GABA_B$ component and that the ratio of the two components is relatively stable from in different neurogliaform to pyramid connections. Our experimental method only allowed the measurement of electrical activity of neurons but it has to emphasized that $GABA_B$ receptor activation triggers a variety of molecular pathways (Bowery et al., 2002) also recruited in the postsynaptic neurons by neurogliaform cells. Since dendritically placed inhibitory inputs are effective in regulating the generation of output spikes, it is conceivable that the dendritic slow inhibition elicited by neurogliaform cells could influence the firing of postsynaptic pyramidal cells for long periods of time, although we focused only on the subthreshold effects of neurogliaform cells.

The interpretation of our results could be complex following reports that dendritic $GABA_A$ responses are excitatory under certain circumstances in mature cortical pyramidal cells (Staley et al., 1995; Rivera et al., 1999; Martina et al., 2001; Gulledge and Stuart, 2003).

In our experiments, postsynaptic cells were depolarized with current injections to a relatively depolarized level to mimick up states occurring spontaneously in vivo (Lampl et al., 1999). In up states, the membrane potential of neurons is above the reversal potential measured for dendritic $GABA_A$ responses, therefore, dendritic GABAergic inputs elicit hyperpolarization. Furthermore, part of the effect of neurogliaform cells is certainly hyperpolarizing and inhibitory regardless of chloride/hydrocarbonate concentrations due to the potassium conductance through $GABA_B$ receptors.

A different factor which should be considered regarding our experiments is the relatively high input frequency, low input resistance and short time constant measured in vivo relative to the in vitro conditions applied here (Steriade et al., 1993b; Borg-Graham et al., 1998; Kamondi et al., 1998; Lampl et al., 1999). The spike timing efficacy of IPSPs is highly dependent on decay kinetics. We used lower beta (19 Hz vs. 15-25 Hz) and gamma (37 Hz vs. 40-80 Hz) frequency ranges in presynaptic stimulations, therefore the kinetics of IPSPs could be speeded up during trains of stimuli resembling in vivo conditions due to preceding unitary IPSPs and convergent polysynaptic inputs likely to be triggered by the applied stimulus. Global membrane resistance changes affect dendritic inputs more prominently than proximal afferents because of the relatively increased filtering effects on signals traveling to the action potential initial zone. Thus, the amplitude of unitary dendritic IPSPs could be paradoxically overestimated when measuring them from the soma in our experiments due to the higher number of silenced dendritic versus somatic inputs in vitro relative to in vivo. The hypothetical decrease in the amplitude of unitary dendritic IPSPs in vivo would increase the significance of synchronization of dendrite targeting inhibitory cells.

The discrepancy between in vivo and in vitro neuronal properties has a potentially greater impact our experiments on input summation. We provided the first direct experimental evidence that two coincident unitary events sum linearly or slightly sublinearly depending on the position of inputs. A number of permanently active leak conductances and persistently active voltage gated channels produce a high background conductance in vitro in addition to synaptic receptors opened by spontaneous synaptic activity and spillover of transmitters to extrasynaptic membrane areas. A single unitary input causes only a relatively small change in the membrane conductance leading to almost linear input summation. Under physiological conditions, persistent synaptic activation further increase the background conductance suggesting that the majority of subthreshold input permutations undergo linear summation maintaining the importance of individual inputs.

Activation of voltage-gated channels influence summation rules in a subcellular input location and timing dependent manner. We showed that the relatively high number of dendritic inhibitory inputs relative to perisomatic inputs could be compensated by the preferential dendritic distribution of Ih channels. Furthermore, activation of voltage-gated channels by convergent inputs can convert linear subthreshold input summation rules to highly nonlinear suprathreshold interactions. Nonlinearity, however, is limited to a relatively narrow time window enhancing temporal precision of network processes and altering spike-triggering effectiveness of individual inputs. Fast-spiking cells can read the sequence of their excitatory inputs as a result of input timing dependent interaction between the temporal summation and voltage-gated channel activation or rather inactivation. Therefore, each input contributes to the output generation of postsynaptic neurons. This result reconciles some opposing views of experimenters and theoreticians on the linearity of input summation and information flow in cortical networks.

Finally, we presented examples that sequential activation of inhibitory inputs lead to location dependent segregation of information processing. Stereotyped sequences of two different responses can be elicited by single sources. Individual inputs maintain their impact on the behavior of postsynaptic cells, however their effects can be rhythmically changed over time. Stereotyped temporal sequences are present in the neocortex and neocortical neurons show a high sensitivity for successive inputs. Sequentiality might govern pathway and subcellular location specific information processing in the neocortical networks.

6. ÖSSZEFOGLALÁS

A legbonyolultabb élő struktúra az emberi agykéreg, amely a magas szintű idegi funkciókért felelős. Ennek a struktúrának az alapvető építőelemei az idegsejtek. Két fő idegsejt típust ismerünk: piramissejtek és helyi interneuronok. A piramissejtek axonális kimenete elhagyja az adott kérgi területet, azonban oldalágakkal a saját kérgi területüket is beidegzik. A piramissejtek glutamátot szabadítanak fel, amely serkenti a posztszinaptikus sejtjeiket. Az interneuronok csoportjába tartozik körülbelül az idegsejtek 20 %-a, melyek GABA-t felszabadítva helyi gátló funkciót töltenek be az agykérgi hálozatokban. Az interneuronok, a piramissejtekkel ellentétben, változatos morfológiai és fiziológiai tulajdonságokkal rendelkeznek. Az agykérgi idegsejtek szinaptikus kapcsolatban állnak egymással és kéreg alatti sejtekkel. Ezek a kémiai kapcsolatok alakítják át a preszinaptikus axon akciós potenciálját posztszinaptikus válasszá. Ezeket a bejövő szinaptikus jeleket, specifikus aktív és passzív tulajdonságokkal kölcsönhatva, a posztszinaptikus sejtek dendritjei dolgozzák fel és küldik tovább az akciós potenciál keletkezésének a helyéhez, az axonhoz. Amikor az egyes idegsejtekben akciós potenciál keletkezik egyidejű szinaptikus bemenetet adnak a posztszinaptikus sejtjeiknek. Ezen kapcsolatok tulajdonságait a pre- és posztszinaptikus sejt típusa határozza meg. Minden egyes agykérgi idegsejt több ezer szinaptikus bemenetet fogad és dolgoz fel, amelynek az eredménye alakítja ki a sejt akciós potenciál kimenetét. Tehát, az idegsejtek egymáshoz való viszonyát a térben szervezett divergens és konvergens kapcsolataik határozzák meg. Azonban, az idegi aktivitás nemcsak térben, de időben is szervezett, mivel az elektroenkefalográfiával megismert ritmusos agyi tevékenység az idegsejtek egyidejű működésének az eredménye. A különböző agyi ritmusok különféle viselkedési mintázatokhoz köthetők. Az egyes idegsejtek követik az adott agyterület ritmusát, azonban általában alacsonyabb frekvenciával váltanak ki akciós potenciáltokat. A különböző típusú idegsejtek tüzelése a ritmus más-más fázisához kötődik. Azonban ma még kevéssé ismert, hogy egy idegsejt hogyan vesz részt ennek a térben és időben rendezett működésnek a kialakításában. Illetve, az is további vizsgálatokat igényel, hogy egy szinaptikus bemenetnek mekkora hatása van egy idegsejt működésére.

Az elvezetett gyorsan tüzelő (FS) sejtek a posztszinaptikus sejttesttel és a közeli dendritágakkal szinaptizáltak (20 ± 16 μm a sejttesttől), ezért ezeket a sejteket kosársejtként azonosítottuk. Ezzel ellentétben a bitufted (Bt) sejtek a távolabbi dendritekre küldték szinapszisaikat, átlagosan 65 ± 25 μm-re a sejttesttől. Ha ezeket a sejttestközeli, illetve dendritikus gátló bemenetek β (15-25 Hz) és γ (40-80 Hz) frekvencia tartományban aktiváltuk

eltérően befolyásolták a különféle posztszinaptikus sejtek tüzelését. Ez a bemenetek elhelyezkedésétől és a preszinaptikus frekvenciától függően határozta meg a piramis- és RSNP sejtek akciós potenciáljainak keletkezését és azok idejét. A különféle preszinaptikus interneuronok γ frekvenciájú stimulációja θ frekvenciájú oszcillációt indukál a piramissejtekben. Azonban azt találtuk, hogy az FS sejtek ezen θ ritmus pozitív, míg a Bt sejtek annak negatív fázisában képesek a posztszinaptikus akciós potenciálokat γ fázisban hatékonyabban időzíteni. Az RSNPC sejtek tüzelését a Bt sejtek képesek γ frekvencián szinkron időzíteni, míg a FS sejtek nem. A posztszinaptikus FS sejtekben γ fázisban csoportosulnak az akciós potenciálok, függetlenül a preszinaptikus stimuláció frekvenciájától és a bemenetek elhelyezkedésétől.

Eredményeink rámutatnak arra, hogy mind a sejttest közeli, mind a dendritikus gátló bemenetek hatékonyan képesek befolyásolni a posztszinaptikus akciós potenciálok időzítését, viselkedési szempontból fontos frekvencia tartományokban. Ezen kapcsolatok konvergenciája és divergenciája növeli az agykérgi hálozatok szerteágazóságát és összetettségét, ezáltal információ feldolgozó képességét. A sejttípus specifikus tulajdonságok kölcsönhatása különféle elhelyezkedésű GABAerg bemenetekkel térben és időben meghatározott idegi működést eredményez és irányíthatja az aktivitás terjedését az agykérgi hálozatokban.

A nem elvezetett posztszinaptikus célelemek vizsgálata azt mutatta, hogy a RSNP sejtek főként dendrit tüskékkel (53 ± 12 %) vagy dendrit törzsekkel (45 ± 10 %) és csak ritkán sejttestekkel (2 ± 4 %) szinaptizálnak. Dendritikus GABAerg kapcsolatok (75 ± 18 μm-re a sejttesttől) RSNP sejtek között képesek β ferkvencián időzíteni a posztszinaptikus sejtek tüzelését, γ frekvenciájú preszinaptikus aktiválás esetén azonban nem. Az RSNP sejtek egymás közötti elektromos kapcsolatát 2-8 réskapcsolat közvetítette a dendrit törzs és/vagy tüskék között (59 ± 21 μm-re a sejttesttől). A β és γ frekvencián kiváltott réskapcsolat potenciálok fáziskéséssel időzítették a posztszinaptikus akciós potenciálokat, habár az erős elektromos kapcsolatok (8 réskapcsolat) szinkronizálták a pre- és posztszinaptikus sejtek működését. Azonban, GABAerg szinapszisokkal és elektromosan is kapcsolt sejtek működése még gyengébb kapcsolatok esetén is szinkronizálódott β és γ frekvencián is.

Tehát elmondhatjuk, hogy az elektromosan és szinaptikusan kapcsolt RSNP sejtek hálózata, a korábban leírt FS és Bt sejtek hasonló hálózatától függetlenül irányíthatja és dolgozhatja fel a ritmikus idegi aktivitást.

A GABAerg neurogliaform sejteket (NGFC) késleltett tüzelési mintázatuk, sűrű axonfelhőjük és rövid, alig elágazó dendritfájuk alapján azonosítottuk. A neurogliaform sejtek elsősorban dendrittüskéket (71%), illetve dendrittörzseket (29%) innerváltak. A

neurogliaform sejtek által, piramissejteken kiváltott posztszinaptikus potenciálok felfutási ideje lassabb ($p < 0.001$, 23.4 ± 9.8 ms, $n = 54$), mint a kosár- (5.8 ± 2.0 ms, $n = 19$) és a bitufted sejtek (6.5 ± 1.7 ms, $n = 15$) által kiváltottak. Az elöbbi IPSP lecsengése nem illeszthető exponenciális függvénnyel. Ezért, a válaszok félszélességét hasonlítottuk össze. A neurogliaform sejtek által kiváltott IPSP-k sokkal hosszabb ideig (183.9 ± 82.5 ms, $p < 0.001$) tartanak, mint más interneuronok által aktiváltak (61.3 ± 16.3 ms, illetve 58.9 ± 17.9 ms). A neurogliaform sejtekről piramissejtekre érkező IPSP-nek két összetevője van. A kezdeti komponens klorid-permeábilis, bicuculline és gabazine érzékeny $GABA_A$ receptor aktiváció eredménye. A késleltetve aktiválódó rész pedig $GABA_B$ receptor működéshez kötött kálium konduktancia következménye.
Eredményeink azt bizonyítják, hogy a neurogliaform sejtek egy akciós potenciálja, ellentétben más agykérgi interneuronokkal, összetett, lassú $GABA_A$ és $GABA_B$ válaszokat vált ki a posztszinaptikus piramissejtekben.

Egyidejüleg vezettünk el három agykérgi idegsejtből, hogy megvizsgáljuk az egy sejtre érkező két szinaptikus bemenet elhelyezkedésének hatását azok összeadódására. A bemenetek forrását és posztszinaptikus elhelyezkedését fény- és elektron-mikroszkópikus vizsgálatokkal határoztuk meg. Két serkentő vagy két gátló bemenet lineárisan adódott össze, ha azok egymástól távol helyezkedtek el a posztszinaptikus sejt felszinén. Az egymáshoz közeli bemenetek összeadódása enyhe szublinearitást mutatott. A linearitás mértéke függött a vizsgált kapcsolatok típusától és egymáshoz viszonyított időzítésétől is. A piramissejt axon iniciális szakaszára érkező gátló bemenetek összeadódása szintén csak enyhe szublinearitást mutatott. Pedig az erre a viszonylag kis térfogatú sejtalkotóra érkező bemenetek jelentős hányadát aktiváltuk.
Eredményeink alapján, amikor csak kevés szinapszis működik egyidejűleg, lineáris vagy attól csak kissé eltérő bemenet összeadódás a jellemző az agykérgi sejteken. Így az egyes bemenetek fenntartják a jelentőségüket. Azonban a közeli szinapszisok közötti, lineáristól eltérő bemenet kölcsönhatás sejttípus-függően növelheti az egyes sejtek információ feldolgozó képességét.

Az Ih csatornák gátlása szublineáris irányba tolja el a sejttest közeli IPSP-k összeadódását a posztszinaptikus piramissejteken. Két dendritikus IPSP összeadódása gyakran szublineáris, pedig a ezek a bemenetek egymástól távol helyezkedtek el. Az Ih blokkoló, ZD 7288-nek, többféle hatását találtunk a gátló bemenetek összeadódására, abban az esetben ha legalább az egyik dendritikus elhelyezkedésű. Néhány kísérletben a ZD 7288 növelte a szublinearitást, hasonlóképpen, mint két sejttest közeli IPSP esetén. Azonban a legtöbb

esetben a ZD 7288 lineáris irányba tolta el az összeadódást, különösen akkor amikor mindkét gátló bemenet a posztszinaptikus sejt dendritjein helyezkedett el. Tehát, az Ih csatornák által okozott szublinearitást csak akkor láttunk amikor a bemenetek közül legalább az egyik a dendriten volt.

Eredményeink rámutatnak arra, hogy a Ih csatornák aktívan résztvesznek a IPSP összeadódásának elhelyezkedés-függő szabályozásában. Ezáltal szabályozhatják a dendritikus és sejttest közelébe érkező gátlás hatékonyságát.

Az FS sejtekre érkező serkentő bemeneteken vizsgáltuk, hogy a küszöb alatti bemenet összeadódási szabályok hogyan érvényesülnek a posztszinaptikus sejtek tüzelésében. Először megmértük a küszöb alatti összeadódás posztszinaptikus membránpotenciál függését. Azt találtuk, hogy az enyhe non-lineáritás mértéke függ egyrészt, a membránpotenciáltól, másrészt a bemenetek időzítésétől. Azonban ugyanezen bemenetek küszöb feletti kölcsönhatásának az eredménye jelentős non-linearitást mutat. Két EPSP egyidejű aktiválása a posztszinaptikus sejtben úgy vált ki akciós potenciálokat, hogy azok hamarabb és rövidebb időablakban fordulnak elő, mint azt várnánk a külön-külön EPSP aktivációk hatásából. Továbbá a posztszinaptikus akciós potenciálok eloszlását pontosan meghatározza, hogy az akciós potenciált kiváltó EPSP elött mennyi idővel volt másik, küszöb alatti EPSP. Más szóval, a preszinaptikus akciós potenciálok relatív időzítésétől függ a kiváltott posztszinaptikus akciós potenciálok latenciája és pontossága. Amikor a két EPSP között 2 ms idő volt a posztszinaptikus akciós potenciálok eloszlásának a csúcsa 0.4 ± 0.24 ms-mal közelebb került a második preszinaptikus akciós potenciálhoz, ahhoz képest amikor az egyedül érkezett. Nem találtunk hasonló eltérést amikor a különbség 5 ms volt. Azonban, ha a két EPSP között 10 ms volt, a posztszinaptikus akciós potenciálok eloszlása 0.75 ± 0.28 ms-mal későbbre tolódott, az egyidejű aktivációjukhoz képest. Az egyidejű aktivációval ellentétben, egyik eltolt idejű, kettős EPSP aktivációnál sem tapasztaltuk, hogy a posztszinaptikus akciós potenciálok eloszlása rövidebb időablakra korlátozódna, az EPSP-k külön aktivációjához képest. Azt találtuk, hogy az EPSP-k alatt a nátrium csatornák elérhetőségének változásai képesek ellensúlyozni a temporális szummáció hatásait és csökkenteni a követő EPSP hatékonyságát a megelőző EPSP időzítése szerint.

Eredményeink arra szolgáltatnak bizonyítékokat, hogy a térbeli és időbeli bemenet összeadódási szabályok és a feszültség-függő ion csatornák működésének kölcsönhatása okozza az bemenetek időzítés függő feldolgozását az agykérgi FS sejtekben. Ezáltal minden egyes bemenet részt vesz a posztszinaptikus sejt kimenetének a kialakításába. A küszöb alatt közel lineárisan összeadódó bemenetek küszöb feletti hatása nagymértékben non-lineáris a

posztszinaptikus akciós potenciál időzitésén, amely tükrözi a bemenetek sorrendjét és egyidejűségét, ezáltal elősegíti az agykérgi információ áramlás pontosságát.

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