

**Diverse role of neurogliaform cells
in cortical microcircuits**

Ph.D. thesis

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Szeged

2006

Szeged

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1. GENERAL INTRODUCTION

Cortical circuits consist of two major types of neurons: the excitatory pyramidal cells and the inhibitory interneurons. While pyramidal cells mostly do not differ from each other in their morphological and physiological features, interneurons show diversity of great extent. The different interneuron populations can be described with distinct morphology and in accordance with this with distinct firing patterns. These inhibitory cells innervate their postsynaptic targets with GABAergic synapses in contrast with pyramidal cells which operate via glutamatergic synapses.

During my Ph.D. studies I focused on scrutinizing the peculiar role of one of the numerous interneurons, the neurogliaform cells in information processing of neocortical circuits.

Morphological and electrophysiological diversity of neocortical neurons

The principal cells of the neocortex are the pyramidal cells (Zigmond et al., 1999). They are also the numerically dominant cell type of the cortex. In the association cortex pyramidal cells form about 80% of the neurons (Sloper et al., 1979; Winfield et al., 1980). They can be found in all cortical layers except layer I. Their common characteristic features are the spiny dendrites. A typical pyramidal cell has a triangular cell body, a thick, radially oriented apical dendrite, forming a terminal tuft in the most superficial cortical layer and a set of basal dendrites. Despite all of these, the term “pyramidal cells” is referred to a diverse cell group, where these principal cells differ from each other not only by their appearance but by their connectivity as well. Their variability in cell size, dendritic arborization and the presence of their axonal collaterals depends on the laminar localization of the neuron. Using glutamate as neurotransmitter, they are responsible for the majority of excitatory postsynaptic potentials (EPSP) evoked in their postsynaptic cell-pairs. Pyramidal cells mainly innervate dendritic spines of other pyramidal cells and dendritic shafts of aspiny interneurons through asymmetric synapses. No excitatory synapses are made on the somata of pyramidal cells.

GABAergic interneurons constitute only a minor fraction of the total number of neurons in the neocortex (15–25%) (Fairén et al., 1984; Peters and Kara, 1987), but are crucial for normal brain function (Berman et al., 1992; McBain and Fisahn, 2001). These GABAergic cells have influenced both the origin and the backpropagation of the action potentials, and play an important role in the synchronization of the activity of different cell-populations. Despite their small numbers, these interneurons are extremely diverse in their morphological, electrophysiological and molecular properties (Fairén et al., 1984; White et al., 1989; DeFelipe, 1993; Cauli et al., 1997; Kawaguchi and Kubota, 1997; Gupta et al., 2000), therefore it is possible to classify between them on the basis of these characteristics.

Functional characterization is based on action potential firing patterns (Kawaguchi and Kubota, 1996, 1997; Gupta et al., 2000) and the facilitating or depressing nature of postsynaptic potentials evoked by short trains of presynaptic action potentials (Reyes et al., 1998). Physiological classes could be correlated with certain morphological cell types described in the frontal cortex (Kawaguchi, 1993; Kawaguchi and Kubota, 1993, 1996, 1997). Great variety of these cells are also reflected in their complex synaptic relations and their efferent connectivity (Gulyas et al., 1993; Buhl et al., 1994). On the basis of these facts the interneurons could be classified not only by their various cell-type preference (Miles et al., 1996; Cobb et al., 1997), but by their spatially selective innervations of the surface of the postsynaptic cell (Halasy et al., 1996).

The axo-axonic cell exclusively innervates the axon initial segments of the pyramidal cells. The axon has the lowest threshold for action potential generation in neurons; thus, axo-axonic cells are considered to be strategically placed inhibitory neurons controlling neuronal output. 4-8 axo-axonic cells innervate a single pyramidal cell and roughly 300 pyramidal cells are innervated by a single axo-axonic cell (Somogyi, 1977; Somogyi et al., 1982). This special cell type is unique among the GABAergic cells eliciting action potentials on pyramidal cells despite of releasing GABA (Szabadics et al., 2006). Axo-axonic cells can depolarize pyramidal cells and can initiate stereotyped series of synaptic events in cortical networks because of a depolarized reversal potential for axonal relative to perisomatic GABAergic inputs. In addition, there is also emerging recognition of the involvement of this unique cell type in several neurological diseases including epilepsy and schizophrenia (Howard et al., 2005).

Typical members of the GABAergic cells are the basket cells. The name was given by Ramon y Cajal to a distinct type of cerebellar neurons (Ramon y Cajal, 1911). Their

axon terminals surround the somata of the Purkinje cells, creating „baskets” around the cell bodies. Interneurons that form a high fraction of axo-somatic synapses have therefore traditionally been classified as basket cells (Somogyi et al., 1983; Hendry and Jones, 1985). This name in the cerebral cortex denotes a heterogeneous cell population, its members have different firing properties, different axonal arborizations and different neurochemical characteristics (Kawaguchi and Kubota, 1993; Kawaguchi, 1995; Kawaguchi and Kubota, 1997; Kawaguchi and Shindou, 1998). Two unambiguous subclasses of basket cells are the classical large basket cells and small basket cells. In the neocortex, large basket cells are generally large, aspiny multipolar neurons that place ~20–40% of their synapses on target cell somata (Somogyi et al., 1983; Kisvarday et al., 1993). Their axons usually originate from the pial aspect of the soma, and typically ascend to give rise to many long horizontally and vertically projecting axon collaterals that traverse neighboring columns (generic ~300 µm diameter columns are used in this study to refer to the local micro-circuitry) and can extend through all cortical layers and might mediate the GABAergic effects within the columns (Tamas et al., 1997).

There are many types of dendrite-targeting cells which can modulate the diverse plasticity processes and electrogenesis of postsynaptic dendrites of pyramidal cells, such as among others Martinotti cells, bitufted cells, neurogliaform cells.

Sources of slow inhibition

Neurogliaform cells were first described by Ramon Y Cajal at the end of the XIX. Century, in 1899 as *cellule neurogliaforme* (Ramón y Cajal, 1899). He observed small neuroglial cell-like neurons in both the striatum and the II layer of the human cerebral cortex, though they are found in all layers and are especially common in the deeper layers. He also described them in human visual and auditory cortex and in cat auditory cortex as well. According to Ramón y Cajal, the neurogliaform cell is very small and a large number of fine, radiating dendrites that are short, varicose, and rarely branched. The axon is extremely thin and shortly after its origin it breaks up into a very dense, highly ramified arborization composed of delicate branches (Peters, 1984). He called these cells as *spiderweb cells* and *dwarf cells* as synonyms.

Neurogliaform cells were identified from several areas of the nervous system on the base of their morphology: from rat visual cortex (Hedlich and Werner, 1986; Hestrin and Armstrong, 1996), rat frontal cortex (Kawaguchi and Kubota, 1997), medial part of the rat

prefrontal cortex (Gabbott et al., 1997), rat striatum (Sancesario et al., 1998), guinea-pig (*Cavia porcellus*) visual cortex (Hedlich and Werner, 1986), cat visual cortex (Naegele and Katz, 1990), primates prefrontal cortex (Gabbott and Bacon, 1996), primate striatum (Fox and Rafols, 1971), and human neocortex (Marin-Pallida, 1985; Kisvarday et al., 1990; Kalinichenko et al., 2006).

With aim of whole cell patch clamp recordings different types of cells could be separated from each other by the detailed analysis of their membrane- and firing properties (Kawaguchi, 1995). The electrophysiological properties of neurogliaform cells have been investigated profoundly nowadays (Kisvarday et al., 1990; Kawaguchi, 1993, 1995; Hestrin and Armstrong, 1996; Vida et al., 1998; Tamas et al., 2003; Markram et al., 2004; Krimer et al., 2005; Price et al., 2005; Zsiros and Maccaferri, 2005; Ashwell and Phillips, 2006)

Although dual patch clamp recordings revealed several classes of interneurons evoking fast, GABA_A receptor mediated responses in the postsynaptic cells, it was not clear until recently 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, 1998)) and several studies suggested that dendritic inhibition is mediated by GABA_B receptors and possibly by a discrete group of interneurons (Lacaille and Schwartzkroin, 1988; Benardo, 1994) that can modulate dendritic excitability (Larkum et al., 1999; Perez-Garci et al., 2006). Moreover, it was suggested that repetitive firing of interneurons and/or cooperation of several interneurons is necessary for the activation of GABA_B receptors (Mody et al., 1994; Thomson et al., 1996; Kim et al., 1997; Thomson and Destexhe, 1999; Scanziani, 2000) 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; Scanziani, 2000). My colleagues reported that single action potentials evoked in neurogliaform cells are sufficient to elicit combined GABA_A and GABA_B receptor mediated responses (Figure 1.1) and they determined the site of postsynaptic action as dendritic spines and shafts (Tamas et al., 2003). This result detects neurogliaform cells as first and so far the only known identified sources of slow inhibition in neocortical microcircuits. Another significant paper deals with functional role that neurogliaform cells can play in cortical information processing (Szabadics et al., 2007), which shows that these cells can evoke slow GABA_A receptor mediated postsynaptic responses in pyramidal cells

which are intermediate between tonic and phasic inhibition. This kind of inhibition partly requires spillover effects of released GABA.

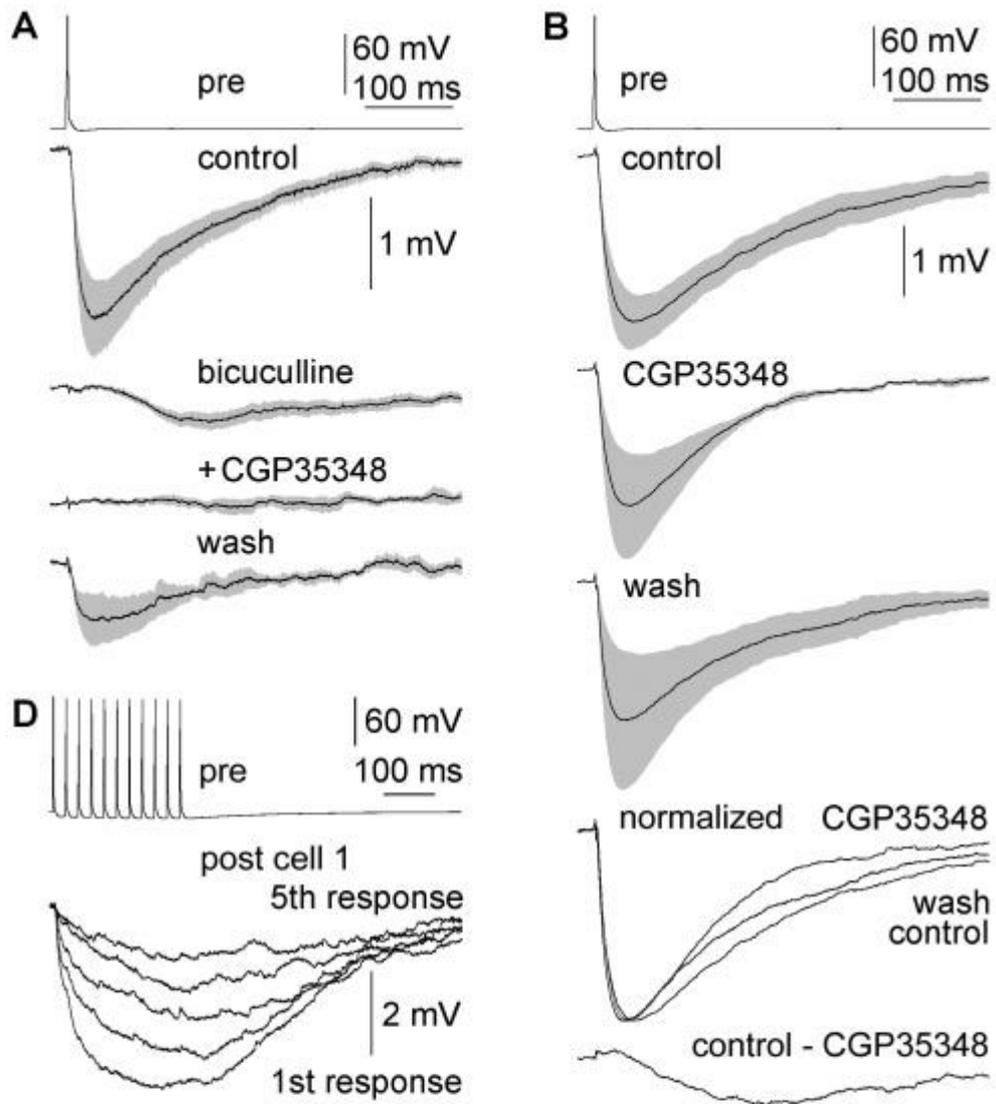


Figure 1.1 Slow inhibition between neurogliaform and pyramidal cells. Traces show averages \pm SEM (gray) of several pairs. **(A)** The initial component of control IPSPs elicited by single presynaptic action potentials (top) was blocked by bicuculline (10 μ M), and the late phase of IPSP was abolished by the subsequent addition of CGP35348 (60 μ M). The IPSPs showed recovery after 30 min of washout. **(B)** The decay of the IPSPs evoked by single spikes in NGFCs (top) could be shortened by application of CGP35348 (60 μ M), and this effect could be partially reversed by returning to the control solution. Superimposed traces are shown normalized to the amplitude of control IPSPs (bottom). **(D)** Rapid use-dependent exhaustion of NGFC-to-pyramid connections demonstrated by a triple recording with a single presynaptic and two postsynaptic cells. The first five consecutive postsynaptic responses (single sweeps) to presynaptic spike trains elicited at 40 Hz once in 4 min.

Nonsynaptic transmission modes in cerebral cortex

At the dawn of neuroscience it was widely believed that the nervous system was a reticulum, or a connected meshwork, rather than a system made up of discrete cells (Kandel et al., 2000). The most prominent advocate of this continuity hypothesis was Camillo Golgi, inventor of well known staining technique. At the end of the 1880's, Ramon y Cajal was using Golgi's microscopic technique, and began to elaborate his neuron doctrine, stating that individual neurons communicate by contact (contiguity theory) (Ramon y Cajal, 1904). The basis of this neuron doctrine is still widely accepted mainly due to discovering chemical synapses between individual neurons by electron microscopic studies in the 1950s. However, results, showing that electrical synapses are more common in the central nervous system than previously thought, give some functionally continuous aspects to neuron doctrine.

In the last 30 years evidence has accumulated that beyond synaptic transmission neurons of the peripheral and central nervous system are capable of broader transmission to various cellular targets through what has come to be known as diffuse or volume transmission. The concept of volume transmission was initially proposed based on electron microscope autoradiographic observations on the serotonin and noradrenaline innervations in adult rat cerebral cortex (Descarries et al., 1975; Descarries et al., 1977; Beaudet and Descarries, 1978; Vizi et al., 1981; Descarries et al., 1982; Descarries and Aznavour, 2003). These nerve terminals (axonal boutons or varicosities) were then shown to often lack the plasma membrane specializations (junctional complexes) that are the hallmark of synapses. It was inferred from this unexpected finding that the transmitter released from such non-junctional (non-synaptic) terminals would diffuse in the extracellular space and reach relatively remote targets to exert a variety of effects (Vizi et al., 1983; Vizi, 2003). A recently published review summarized different possible forms of volume transmission (Agnati et al., 2006). These are the following: synaptic spillover of vesicular release leading to activation of receptors nearby the synapse; extrasynaptic vesicular release without contribution of classical chemical synapse; non-junctional varicosities mainly in case of release of catecholamines; constitutive release of neuropeptides; signaling with gaseous transmitters such as nitric oxide or carbon oxide; reverse functioning of uptake mechanism of main neurotransmitters such as glutamate and GABA (Figure 1.2). Effective volume transmission needs contribution of several important supporting factors (Barbour

and Hausser, 1997): beginning with ultrastructural constraints of it; through presence of extrasynaptic receptors and uptake mechanism with appropriate affinity for released neurotransmitters (Sodickson and Bean, 1996; Vizi, 2000) and at last but not least ambient level of neurotransmitters should be released. Such volume transmission was found in cases of neuromodulators, such as acetylcholine (Umbriaco et al., 1994; Umbriaco et al., 1995; Avendano et al., 1996; Contant et al., 1996; Smiley et al., 1997; Mechawar et al., 2002), serotonin (Umbriaco et al., 1995; Smiley and Goldman-Rakic, 1996), dopamine (Goldman-Rakic et al., 1992; Smiley et al., 1992), noradrenaline (Vizi, 1980, 1990; Umbriaco et al., 1995), NO (Dawson et al., 1994) and classical neurotransmitters like glutamate (Asztely et al., 1997; Kullmann and Asztely, 1998; Kullmann et al., 1999; Rusakov et al., 1999; Kullmann, 2000; Lehre and Rusakov, 2002; Rusakov and Lehre, 2002; Porter and Nieves, 2004; Szapiro and Barbour, 2007) and GABA (Isaacson et al., 1993; Kullmann, 2000; Scanziani, 2000; Ma et al., 2002; Mann-Metzer and Yarom, 2002; Sakaba and Neher, 2003; Lacey et al., 2005; Moldavan et al., 2006; Thompson et al., 2006; Astori and Kohr, 2008) as well. Diffusion of released signaling molecules can have different effects: they can activate extrasynaptic receptors located on somatodendritic domains of innervated cells and on presynaptic terminals as well. Such effects of presynaptic terminals can be homosynaptically when operation of own terminals is modulated leading to alteration of own release machinery, and heterosynaptically when boutons of other cells in vicinity are modulated exerting diffuse, spatially unspecific impacts on nearby neurotransmission (Wu and Saggau, 1997).

Availability of extrasynaptic GABA receptors by volume transmission is well documented. Besides extrasynaptic, δ -subtype containing GABA_A receptors which are responsible for causing tonic inhibition on postsynaptic cells (Mody et al., 1994a; Yeung et al., 2003; Mody and Pearce, 2004; Glykys et al., 2008), presynaptic GABA_B receptors are reported to be activated by spillover effects of neighboring interneurons, but train of single neuron and inhibiting GABA uptake at the same time (Mitchell and Silver, 2000; Astori and Kohr, 2008) or simultaneous firings of several interneurons are required to do this phenomenon (Aroniadou-Anderjaska et al., 2000; Mitchell and Silver, 2000; Giustizieri et al., 2005; Kaneda and Kita, 2005). Some studies suggest expressing of presynaptic GABA_A receptors, as well, but it is not well established yet.

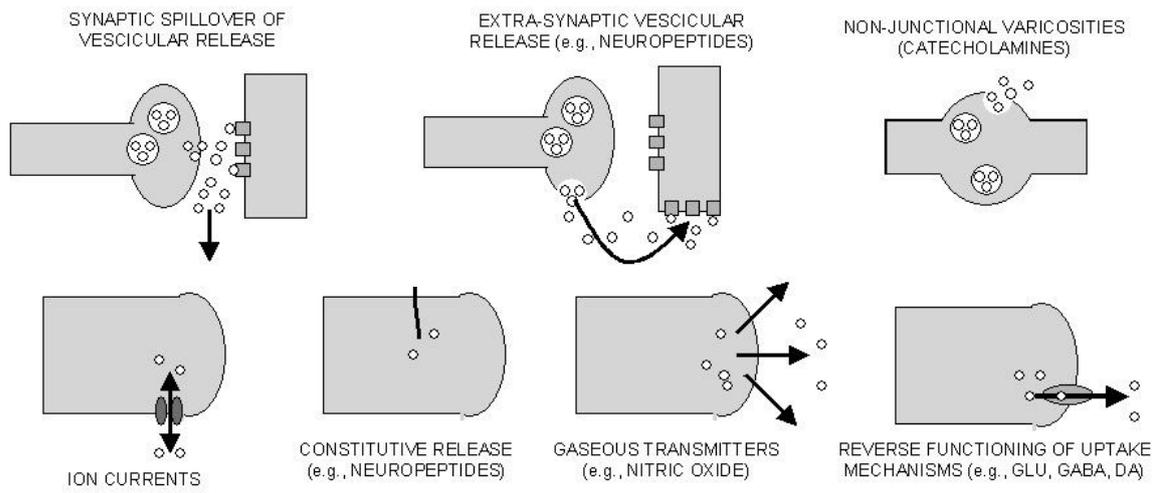


Figure 1.2 Main types of volume transmission in cortical microcircuits (from (Agnati et al., 2006))

2. AIMS

This thesis studies the contribution of special GABAergic interneurons, the neurogliaform cells to information flow in neocortical microcircuits. The main questions of our experiments were the following:

1. What type(s) of GABAergic interneuron do neurogliaform cells establish chemical connections with? What kind of kinetics describe these chemical connections?
2. What types of connections are established in human cortical microcircuits by neurogliaform cells?
3. What is the background mechanism of slow inhibitory synaptic potentials established by neurogliaform cells?
4. Is there a presynaptic effect of neurogliaform cells on nearby connections in the microcircuit?

3. CONTRIBUTIONS

Correlated light- and electron microscopy was performed by Anna Simon and Gábor Tamás. I carried out the majority of electrophysiological recordings and data analysis.

4. MATERIAL AND METHODS

Electrophysiology

All procedures were performed according to the Declaration of Helsinki with the approval of the University of Szeged Ethical Committee and in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals. Young (P16–P35) Wistar rats were anaesthetized by halothane, and following decapitation coronal slices (350 μm) were prepared from their somatosensory cortex. Slices were cut with Leica VT1000S or Microm HM650 vibratome in cold (3–6 $^{\circ}\text{C}$) 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 $^{\circ}\text{C}$, 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 $\text{M}\Omega$) 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). Whole cell patch clamp recordings were obtained at $\sim 32^{\circ}\text{C}$ from concomitantly recorded pairs, triplets or quadruplets of layer II/II putative interneurons and/or pyramidal cells visualized by infrared differential interference contrast (DIC) videomicroscopy (Olympus BX60WI microscope, Hamamatsu CCD camera). Micropipettes (5–7 $\text{M}\Omega$) were filled with 126 mM K-gluconate, 4 mM KCl, 4 mM ATP-Mg, 0.3 mM GTP- Na_2 , 10 mM HEPES, 10 mM creatine phosphate and 8 mM biocytin at pH 7.25 and 300 mOsm. Signals were recorded in fast current clamp or whole cell mode (Luigs & Neumann Infrapatch set-up and two HEKA EPC 10/double patch-clamp amplifiers) and were filtered at 5 kHz, digitised at 10 kHz and analysed with PULSE software (HEKA, Lambrecht/Pfalz, Germany).

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. 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 10-30 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 offsetted 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. SPSS for Windows software package was used for statistical analysis. The significance level for all comparisons was set at $p < 0.05$.

Presynaptic neurogliaform cells were stimulated with brief (2 ms) suprathreshold pulses at > 90 s intervals to avoid exhaustion of transmission, other cell types were stimulated at 0.1 Hz. We applied the same paradigm throughout the study for consistency. Postsynaptic cells were held at -51 ± 4 mV membrane potential. Unless specified, traces shown are averages of 10-50 episodes. The amplitude of postsynaptic events was defined as the difference between the peak amplitude and the baseline value measured prior to the postsynaptic potential onset. Firing-probability plots were constructed from 50–100 consecutive trials as follows: within a given cycle (interval separating two presynaptic action potentials), postsynaptic spike latencies were measured from the peak of the preceding presynaptic action potential. Subsequently, unless otherwise specified, the data were pooled regardless of the position in the presynaptic spike train. Controls were collected before the onset of the presynaptic spike train using an identical cycle length. Data are given as mean \pm s. d. Mann-Whitney U-test, Wilcoxon-test and Friedman-test was used to compare datasets, differences were accepted as significant if $p \leq 0.05$.

Testing of gap junctional coupling of neurogliaform cells

Detecting electrical coupling between recorded neurons was carried out by two main protocols. In subthreshold circumstances hyperpolarizing current pulses (-100 pA, 150 ms) were injected into either of the neurons and the other neurons were monitored whether they

responded with hyperpolarizing potentials and this was done in the opposite direction as well. In suprathreshold conditions single action potentials were injected into either of the neurons and the other were monitored. Signals travel through gap junctions almost without delay making discrimination of gap junctional potentials from synaptic derived postsynaptic potentials easier. Besides, at gap junctions, cells approach within about 3.5 nm of each other (Kandel et al., 2000), a much shorter distance than the 20 to 40 nm distance that separates cells at chemical synapse.

Testing of postsynaptic effects of NGFCs

All procedures on human tissue were performed with the approval of the Ethical Committee of the University of Szeged and in accordance with the Declaration of Helsinki. Human slices were derived from association cortices which had to be removed to gain access for the surgical treatment of deeper brain areas with written informed consent of the patients (aged 18-73 years) prior to surgery. Anaesthesia was induced with intravenous midazolam and fentanyl (0.03 mg/kg, 1-2 Lg/kg respectively). A bolus dose of propofol (1-2 mg/kg) was administered intravenously. To facilitate endotracheal intubation, the patients received 0.5 mg/kg rocuronium. After 120 seconds the trachea was intubated and the patients were ventilated with a mixture of O₂-N₂O at a ratio of 1:2. Anaesthesia was maintained with sevoflurane at MAC volume of 1.2-1.5. Rise times of postsynaptic potentials were measured at 10-90% of amplitudes of IPSPs. The decay of evoked IPSPs could not be fitted with single or double exponential functions, we thus measured the half-width of IPSPs for statistical comparison. The amplitude of postsynaptic events was defined as the difference between the peak amplitude and the baseline value measured before the postsynaptic potential onset. In all experiments the contribution of the different types of GABA receptor were tested by applying 10 µM gabazine, a GABAA receptor antagonist and 10 µM CGP35348, a GABAB receptor antagonist in the recording solution. In all cases standard deviations are indicated at statistical data.

Testing of presynaptic effects of NGFCs

To test the presynaptic effects of neurogliaform cells, I applied a prepulse paradigm in both homosynaptical and heterosynaptical conditions. When homosynaptical presynaptic inhibition was investigated, I elicited an action potential (a prepulse) in a

neurogliaform cell, which is in chemical connection with another cell of any type, and an action potential pair (the test pulse) in the same cell followed the first spike by 100-120 ms to yield the maximum effect of GABA_B inhibition. In these conditions the effect of GABA_B autoceptors located on presynaptic terminals of neurogliaform cells were tested. To gain heterosynaptical presynaptic inhibition, I elicited an action potential (a prepulse) in a neurogliaform cell, which preceded by 100-120 ms the elicited action potential in a presynaptic element of a cell pair with chemical connection between them. In both conditions the presynaptic inhibitory effect of neurogliaform cell was showed in the reduced amplitudes and in the altered paired pulse ratios of the tested postsynaptic responses (Harney and Jones, 2002; Kaneda and Kita, 2005).

The contribution of GABA_B receptors in these modulatory effects of neurogliaform cells was showed by adding GABA_B receptor blocker CGP35348 (40 μM) in recording solution. In a further experiment I tested the presynaptic effects of neurogliaform cell by applying 50 μM *N*-ethylmaleimide (NEM), a sulfhydryl alkylating agent, selectively uncouples pertussis toxin-sensitive G proteins from receptors (Isaacson, 1998). In the presence of NEM GABA released by neurogliaform cells could not exert inhibitory effect by the help of GABA_B receptors. In order to show that diffusion of GABA is required to exert presynaptic effects of neurogliaform cells, I added GABA uptake inhibitor NO711 (40 μM) in recording solution, which can increase the concentration of GABA in extracellular space.

Histology

I have not contributed to the anatomical investigation of recorded cells. Visualization of biocytin was carried out by Éva Tóth, Anna Simon, Gergely Komlósi, light microscopic reconstructions were performed by Gábor Tamás, Éva Tóth, Anna Simon and Gergely Komlósi and the correlated electron microscopy by Gábor Tamás and Anna Simon.

Light microscopic investigations

Histology and anatomical evaluation were performed as described in Tamas et al. 1997. Depolarizing current pulses employed during recording resulted in an adequate filling of neurons by biocytin. Slices were sandwiched between two Millipore filters to

avoid deformations and fixed in 4% paraformaldehyde, 1.25 % glutaraldehyde and 15 % picric acid in 0.1 M phosphate buffer (PB) (pH 7.4) for at least 12 hours. After several washes in 0.1 M PB, slices were cryoprotected in 10%, then 20 % sucrose in 0.1 M PB. Slices were freeze-thawed in liquid nitrogen, then embedded in 10 % gelatine. 300 μ m thick slices embedded in gelatine blocks were resectioned at 60 μ m thicknesses. Sections were incubated in avidin-biotin-horseradish peroxidase (ABC; Vector Labs) complex made in TBS (1:100, pH 7.4) at 4° C overnight. The enzyme reaction was revealed by 3'3'-diaminobenzidine tetrahydrochloride (0.05 %) as chromogen and 0.01% H₂O₂ as oxidant. Sections were postfixated with 1 % OsO₄ in 0.1 M PB. After several washes in distilled water, sections were stained in 1 % uranyl acetate, dehydrated in ascending series of ethanol. Sections were infiltrated with epoxy resin (Durcupan, Fluka) overnight and embedded on glass slides.

Three-dimensional light microscopic reconstructions of recovered cells were carried out using NeuroLucida (MicroBrightfield, Colchester, VT) with 100x objective from the 60 μ m thick serial sections. Dendrogram constructions, synaptic distance measurements were aided by Neuroexplorer (MicroBrightfield) software. Synaptic distances were measured along the dendrites of the postsynaptic cell. The entire somatodendritic surface of recorded cells was tested for close appositions with filled axons or filled dendrites, each of which was traced back to the parent soma. Representative reconstructions were made from the fully recovered neurogliaform cells.

Electron microscopic investigations

Since the resolution of light microscope (200 nm) is not sufficient for the precise identification of synaptic connection, all of the mapped close appositions were tested in the electron microscope. Light micrographs taken from each close apposition in different focal depth were used for the exact identification of these presumed synaptic connections under electron microscope. Blocks containing the cells were cut out from the sections on slides and reembedded. 70 nm serial sections were cut with an ultramicrotome (Leica Ultracut R; Leica Microsystems, Vienna, Austria and RMC MTXL; Boeckeler Instruments, Tucson, Arizona) and mounted on Pyeliform-coated copper grids and stained with lead citrate (EM Stain; Leica Microsystems). Light microscopically detected presumed synapses were checked on the ultrathin sections in electron microscope (TEM, Philips).

We used the following criteria for the identification of chemical synapses: 1, accumulation of synaptic vesicles in the presynaptic terminal; 2, rigid membrane apposition between the pre- and postsynaptic membranes, with the characteristic widening of extracellular space (synaptic cleft). Direct membrane appositions alone didn't predict the presence of synaptic junction.

Gap junctions were identified as follows: membranes of the coupled and stained cells had to be arranged in parallel. The distance between the two electron-lucent stripes was 7-9 nm. 3D electron microscopic reconstructions were made with the help of Reconstruct software which was created with funding, in part, from the National Institutes of Health and the Human Brain Project.

5. RESULTS

5.1 Gap-Junctional Coupling between Neurogliaform Cells and Various Interneuron Types in the Neocortex

Summary

Electrical synapses contribute to the generation of synchronous activity in neuronal networks. Several types of cortical GABAergic neurons acting via postsynaptic GABAA receptors also form electrical synapses with interneurons of the same class, suggesting that synchronization through gap junctions could be limited to homogenous interneuron populations. Neurogliaform cells elicit combined GABAA and GABAB receptor-mediated postsynaptic responses in cortical pyramidal cells, but it is not clear whether neurogliaform cells are involved in networks linked by electrical coupling. We recorded from pairs, triplets, and quadruplets of cortical neurons in layers 2 and 3 of rat somatosensory cortex (postnatal day 20–35). Neurogliaform cells eliciting slow IPSPs on pyramidal cells also triggered divergent electrical coupling potentials on interneurons. Neurogliaform cells were electrically coupled to other neurogliaform cells, basket cells, regular-spiking nonpyramidal cells, to an axoaxonic cell, and to various unclassified interneurons showing diverse firing patterns and morphology. Electrical interactions were mediated by one or two electron microscopically verified gap junctions linking the somatodendritic domain of the coupled cells. Our results suggest that neurogliaform cells have a unique position in the cortical circuit. Apart from eliciting combined GABAA and GABAB receptor-mediated inhibition on pyramidal cells, neurogliaform cells establish electrical synapses and link multiple networks formed by gap junctions restricted to a particular class of interneuron. Widespread electrical connections might enable neurogliaform cells to monitor the activity of different interneurons acting on GABAA receptors at various regions of target cells.

Introduction

Electrical coupling between neocortical interneurons is firmly established by anatomical studies and electrophysiological experiments (Sloper, 1972; Galarreta and

Hestrin, 1999; Bennett and Zukin, 2004; Connors and Long, 2004; Sohl et al., 2005). Dendrodendritic or dendrosomatic gap junctions or gap junction-like structures were shown between morphologically identified interneurons (Sloper, 1972; Tamas et al., 2000; Szabadics et al., 2001; Fukuda and Kosaka, 2003), and actual measurements of electrical coupling were performed between neighboring GABAergic interneurons (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000; Venance et al., 2000; Szabadics et al., 2001; Blatow et al., 2003; Chu et al., 2003). Electrical coupling between neocortical interneurons appears to be highly specific: GABAergic neurons establish electrical coupling almost exclusively with interneurons of the same type forming several distinct networks linking homologous interneurons (Galarreta and Hestrin, 2001; Bennett and Zukin, 2004; Connors and Long, 2004).

The most prominent function demonstrated thus far for electrical coupling is the increased synchrony of firing between the coupled cells (Galarreta and Hestrin, 2001; Bennett and Zukin, 2004; Connors and Long, 2004). Synchronization within networks of particular interneurons is then propagated downstream in cortical microcircuits by the concerted action of GABAergic synapses entraining “postsynaptic” firing (Tamas et al., 2000; Szabadics et al., 2001; Blatow et al., 2003; Buhl et al., 2003). Several GABAergic cell types, homologously interconnected by gap junctions, are also effective in timing postsynaptic action potentials, suggesting that multiple pathways of synchronization operate in parallel within the cortical circuit (Cobb et al., 1995; Tamas et al., 2004). However, distinct cell types in neural circuits are known to establish electrical coupling and could perform special functions. For example, electrical coupling between rods and cones in the retina allows the summation of inputs to different cells and can improve resolution by reducing noise (Guldenagel et al., 2001; Demb and Pugh, 2002; Sohl et al., 2005). Sporadic evidence for heterologous electrical coupling in the neocortex shows coupling of spiny stellate cells and fast-spiking (fs) GABAergic interneurons and occasional coupling between different GABAergic cells (Venance et al., 2000; Chu et al., 2003), but the position of several known cortical cell types is not clear in gap-junctionally linked networks.

Neurogliaform (ngf) interneurons were first described by Ramon y Cajal (1904) as dwarf or spiderweb cells, referring to their compact dendritic tree and dense axonal arborization (Valverde, 1971; Jones, 1975; Kisvarday et al., 1990; Hestrin and Armstrong, 1996; Kawaguchi and Kubota, 1997). Neurogliaform cells have a thus far unique position among cortical interneurons, because they are capable of eliciting slow IPSPs in

postsynaptic pyramidal cells through a combined recruitment of GABAA and GABAB receptors (Tamas et al., 2003). Here, we investigate the electrical synapses of this cell type and identify a multitude of interneuron types gap-junctionally coupled to neurogliaform cells using a combination of simultaneous patch-clamp recordings and correlated light and electron microscopy. Analysis of chemical synapses established by neurogliaform cells on various interneurons types requires a separate study.

Results

Electrical coupling between neurogliaform cells

Neurogliaform cells ($n = 94$) were identified during simultaneous dual, triple, and quadruple recordings of neurons in layer 2/3 of rat somatosensory cortex based on their firing characteristics, followed by light microscopic evaluation of their dendritic and axonal morphology. Similarly to neurogliaform cells identified by previous studies (Hestrin and Armstrong, 1996; Kawaguchi and Kubota, 1997; Tamas et al., 2003), neurogliaform interneurons in our sample responded to long (800 ms), just above threshold current injections with late-spiking firing pattern or with an accelerating train of spikes when activated from resting membrane potential (-64 ± 3 mV). This firing behavior was changed to a decelerating pattern when applying larger current pulses. All neurogliaform cells showed similar axonal features: small boutons frequently occurred along the thin axonal collaterals, forming a dense meshwork within the compact axonal field.

When looking for connections to neurons closely located (<100 μm) to the presynaptic neurogliaform cells, we sampled pyramidal cells as well as several types of interneuron. According to our previous studies showing strong frequency sensitivity of postsynaptic responses elicited by neurogliaform cells, we triggered single action potentials with an interval of 90 s to avoid the loss of responses. Simultaneous triple recordings showed that neurogliaform cells formed divergent connections, which were transmitted by electrical and chemical synapses. Neurogliaform cells eliciting slow, presumably GABAA and GABAB receptor-mediated IPSPs on pyramidal neurons ($n = 7$) also triggered spikelets on other neurogliaform cells ($n = 3$) and other types of interneuron ($n = 4$), which could be followed by long-lasting IPSPs ($n = 2$ on neurogliaform cells and $n = 2$ on other interneurons). Electrical coupling indicated by spikelets in response to presynaptic action potentials was confirmed by the passage of hyperpolarizing signals

between the coupled neurons. These observations encouraged us to search for electrically coupled pairs of neurogliaform cells and interneurons representing various types.

Based on previous experiments showing widespread evidence for electrical coupling between similar GABAergic cells (Galarreta and Hestrin, 2001; Bennett and Zukin, 2004; Connors and Long, 2004), we proceeded by characterizing homologous electrical connections between neurogliaform cells (Figure 5.1.1). Of the 16 pairs of neighboring neurogliaform cells tested for electrical coupling, we confirmed electrical connections between neurogliaform cells in eight cases, indicating a 50% rate for coupling. All electrical connections between ngf cells were reciprocal. Electrical coupling potentials in response to presynaptic spikes showed a relatively wide range in amplitude ($0.09 - 2.08$ mV; 0.62 ± 0.79 mV) at -50 ± 2 mV membrane potential. They followed presynaptic action potentials with a delay of 0.34 ± 0.19 ms, measured as the period spanning the maximal rates of rise of the presynaptic action potential and the electrical coupling potential, respectively. The average amplitude ratio (coupling coefficient) for spikelets and presynaptic potentials was 0.17 ± 0.06 and $1.89 \pm 0.77\%$ when eliciting action potentials and applying long current steps (-200 pA, 200 ms duration) in the first neuron to elicit a response in the second neuron, respectively. Coupling strength was similar in both directions and did not correlate with the distance between the recorded cell bodies (39 ± 13 μ m). From the 16 presynaptic ngf cells, 11 triggered spikelets, which were followed by long-lasting IPSPs; these IPSPs will be characterized in a separate study. Three-dimensional light microscopic reconstruction and mapping of an electrically connected pair of neurogliaform cells indicated one close apposition between a proximal and a distal dendrite (4 and 57 μ m from the somata), suggesting a single place for electrical communication.

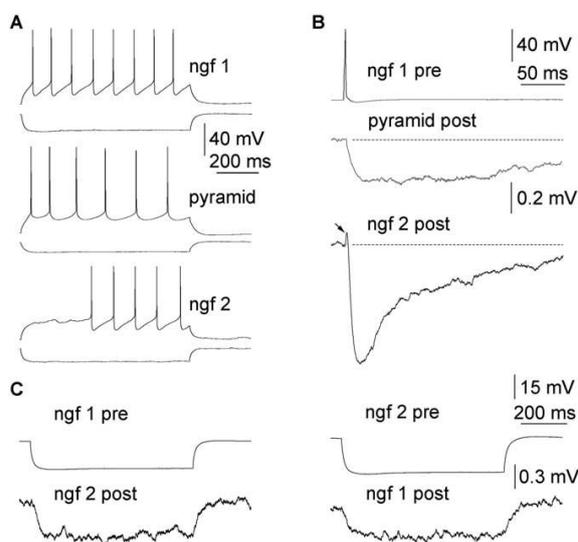


Figure 5.1.1 Neurogliaform cells eliciting slow IPSPs establish homologous electrical synapses. **A**, Firing pattern of two neurogliaform cells (ngf 1 and 2) and a pyramidal cell (pyramid) recorded in the same slice. **B**, Single action potentials in the presynaptic neurogliaform cell (ngf 1) elicited a slow IPSP in the pyramidal cell and spikelet (arrow) followed by a long-lasting IPSP in the other neurogliaform cell. Note that the IPSPs do not return to baseline (dashed line) 250 ms after the presynaptic spike. **C**, Hyperpolarizing current injections into either neurogliaform cells were transmitted to the other cell, confirming electrical coupling. From the 16 ngf-ngf cell connections tested, we confirmed electrical coupling in eight pairs (50%). pre, Presynaptic; post, postsynaptic.

Gap-junctional coupling between neurogliaform and fast-spiking basket cells

Apart from homologous electrical connections between ngf cells, we detected heterologous gap junctions linking ngf cells and fs cells. Fast-spiking basket cells were identified according to their firing characteristics followed by light microscopic evaluation of their dendritic and axonal morphology. Similarly to basket cells identified by previous studies (Tamas et al., 1997; Galarreta and Hestrin, 1999; Gibson et al., 1999; Gupta et al., 2000), fs basket interneurons responded to just above threshold current injections with “stuttering” groups of spikes and with a nonaccommodating train of spikes when applying increased current steps. Membrane time constants of fs cells were also similar to values published previously (Kawaguchi, 1995; Tamas et al., 2003). All fs basket cells showed characteristic axonal branches frequently surrounding somata with large boutons relative to those of ngf cells. Electron microscopic sampling of postsynaptic targets of randomly selected fs cells ($n = 3$) confirmed the identity of these neurons as basket cells showing a target preference toward somata ($28 \pm 5\%$) and dendritic shafts ($68 \pm 7\%$) and occasionally innervating dendritic spines ($3 \pm 3\%$).

From the 31 connections tested between closely spaced ngf and fs cells, we confirmed electrical coupling in six pairs, indicating a 19% rate for interaction. All electrical connections between ngf and fs cells were reciprocal, and, in three pairs, ngf cells elicited IPSPs on postsynaptic fs cells (Figure 5.1.2). Electrical coupling potentials in response to presynaptic spikes showed amplitudes of 0.52 ± 0.23 mV (range, 0.17 – 0.87 mV) at -50 ± 3 mV membrane potential. The duration of electrical coupling potentials measured at half amplitude was longer in fs cells ($n = 3$) than in ngf ($n = 6$) cells (9.11 ± 2.95 vs 3.80 ± 1.23 ms; $p < 0.02$; Mann–Whitney U test), presumably because of the faster afterhyperpolarization in fs cells (1.33 ± 0.13 vs 8.49 ± 0.68 ms, measured from the peak of action potential to the maximum of afterhyperpolarization). Spikelets followed presynaptic action potentials with a latency of 0.28 ± 0.12 ms. Amplitude ratios of presynaptic and postsynaptic potentials did not show direction selectivity. Coupling coefficients for spikelets and presynaptic potentials were 0.39 ± 0.23 and $3.76 \pm 1.42\%$ when eliciting action potentials and applying long current steps in the first neuron to elicit a response in the second neuron, respectively. Thus, electrical coupling appears stronger in our limited sample of ngf–fs than in ngf–ngf connections measured by transferring relatively long-lasting hyperpolarization ($p < 0.002$; Mann–Whitney U test). The strength of coupling did not correlate with the distance measured between the somata of connected

cells ($31 \pm 16 \mu\text{m}$). Three-dimensional light microscopic reconstruction of two electrically connected ngf–fs pairs suggested one close apposition in each connection between the somata of the fs cells and proximal dendrites of the ngf cells (9 and $40.2 \mu\text{m}$ from the somata). Subsequent electron microscopy confirmed a single gap junction as the morphological correlate of electrical communication in both connections.

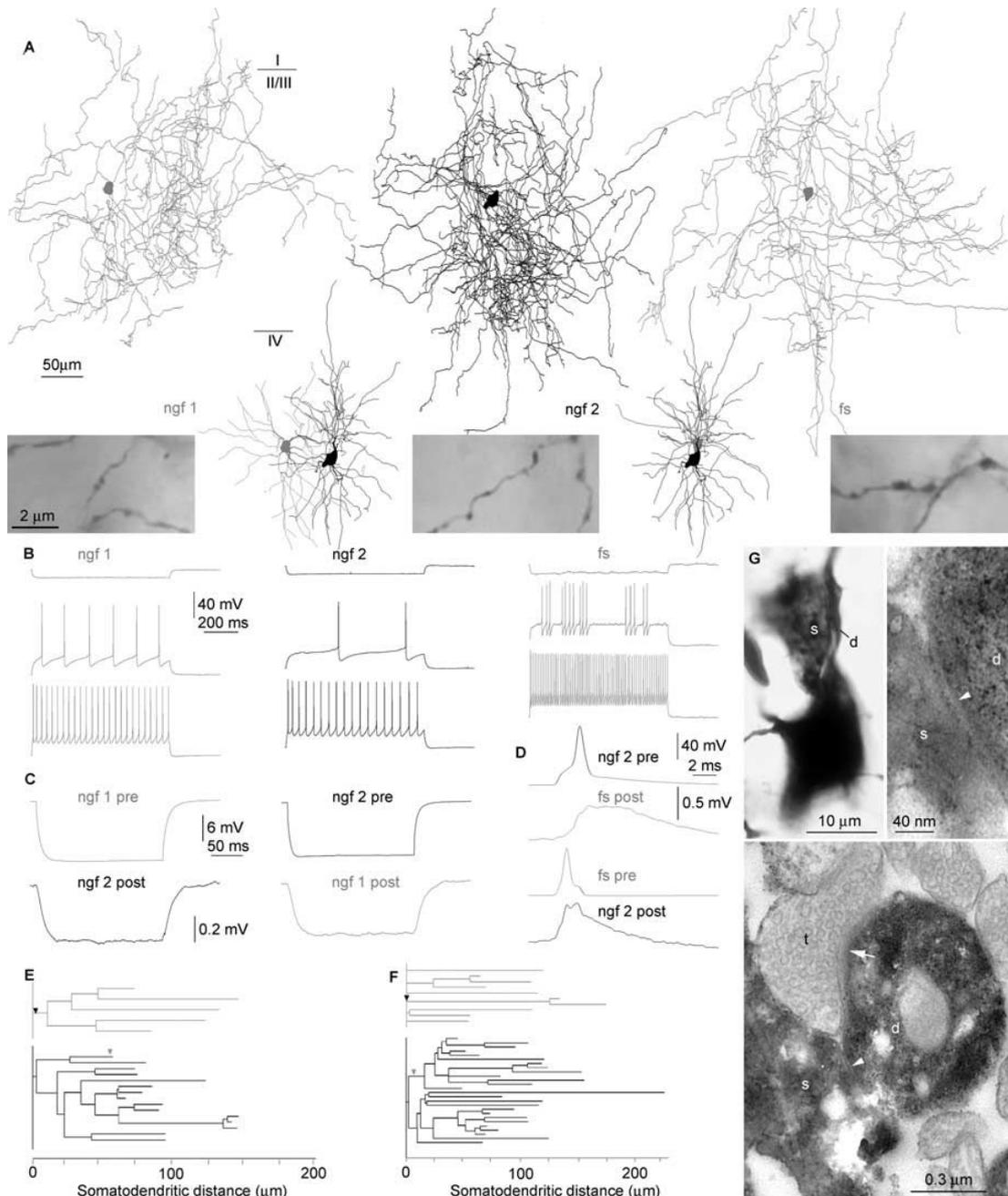


Figure 5.1.2 Heterologous gap junctions link the network of neurogliaform cells and fast-spiking basket cells. **A**, Reconstructions of two neurogliaform cells (ngf 1, gray; ngf 2, black) and a fast-spiking basket cell (gray). Axonal and dendritic arborizations are shown separately for clarity. The position of cells is shown relative to the dendrites of ngf 2, and the laminar position is shown relative to the axons. Light micrographs illustrate morphological differences between axon terminals. **B**, Responses of the three cells to hyperpolarizing (top) and depolarizing current pulses (middle and bottom). **C**, Transmission of

hyperpolarizing current injections into one of the cells confirmed electrical coupling between the neurogliaform cells. **D**, Action potentials elicited in neurogliaform cell 2 triggered spikelets in the fast-spiking cell and vice versa. The rising phase of the spikelets corresponded to the rise of action potentials, indicating electrical coupling. From the 31 ngf–fs cell connections tested, we confirmed electrical coupling in six pairs (19%). **E**, **F**, Dendrograms representing three-dimensional distances measured from the somata to the presumed gap junctions (arrowheads) mediating the interactions between the neurogliaform cells (**E**) and between neurogliaform cell 2 and the fast-spiking cell. **G**, Correlated light and electron microscopy identified a single gap junction as the site of interaction between the soma (s) of the fast-spiking cell and a proximal dendrite (d) of the neurogliaform cell 2. The dendrite of the neurogliaform cell also receives a synaptic junction (arrow) from an unidentified terminal (t). pre, Presynaptic; post, postsynaptic.

Gap-junctional coupling between neurogliaform and regular-spiking cells

Finding heterologous gap junctions between ngf and basket cells propelled us to see whether ngf cells are electrically coupled to a multitude of interneuron types. We detected regular-spiking (rs) nonpyramidal cells as the second type of interneuron forming heterologous gap junctions with ngf cells. Similar to rs cells identified previously (Cauli et al., 1997; Kawaguchi and Kubota, 1997; Cauli et al., 2000; Szabadics et al., 2001; Tamas et al., 2004) they responded to suprathreshold current injections with a regular-spiking firing pattern. The morphology of sparsely spiny dendrites and individual axonal branches of all rs cells appeared similar showing undulating axon collaterals with relatively large, bead-like boutons. The overall axonal arborization of rs cells was restricted to a part of layer 2/3 or in addition to the arborization around the soma, rs cells sent a loose bundle of radially oriented branches toward layer 6. (Szabadics et al., 2001) Confirming previous results (Szabadics et al., 2001), electron microscopic samples of postsynaptic targets of randomly selected rs cells ($n = 3$) showed a target preference toward dendritic shafts ($53 \pm 13\%$) and dendritic spines ($47 \pm 13\%$) and did not target postsynaptic somata. When testing connections between pairs ($n = 30$) of ngf and rs-nonpyramidal cells, we detected heterologous coupling in six cases, indicating a 20% rate for electrical synapses between these cell populations. All electrical connections between ngf and rs cells were mutual and all six ngf cells involved in electrical coupling elicited IPSPs on the postsynaptic rs cells (Figure 5.1.3). Electrical coupling potentials in response to presynaptic spikes showed amplitudes of 0.45 ± 0.38 mV (range, 0.18 – 0.72 mV) at -50 ± 3 mV membrane potential. Spikelets followed presynaptic action potentials with a latency of 0.32 ± 0.12 ms. Electrical coupling strength was symmetrical between ngf and rs cells. Coupling coefficients for spikelets and presynaptic potentials were 1.01 ± 0.60 and $4.59 \pm 3.98\%$ when eliciting spikes and applying hyperpolarizing current steps in the first neuron to elicit

a response in the second neuron, respectively. The strength of coupling was not correlated with the distance measured between the somata of connected interneurons ($28 \pm 19 \mu\text{m}$). Three-dimensional light microscopic mapping and subsequent electron microscopy of an electrically connected ngf–rs pair determined two gap junctions mediating the interaction between proximal dendrites equidistant ($31.025 \pm 3.83 \mu\text{m}$) from the somata.

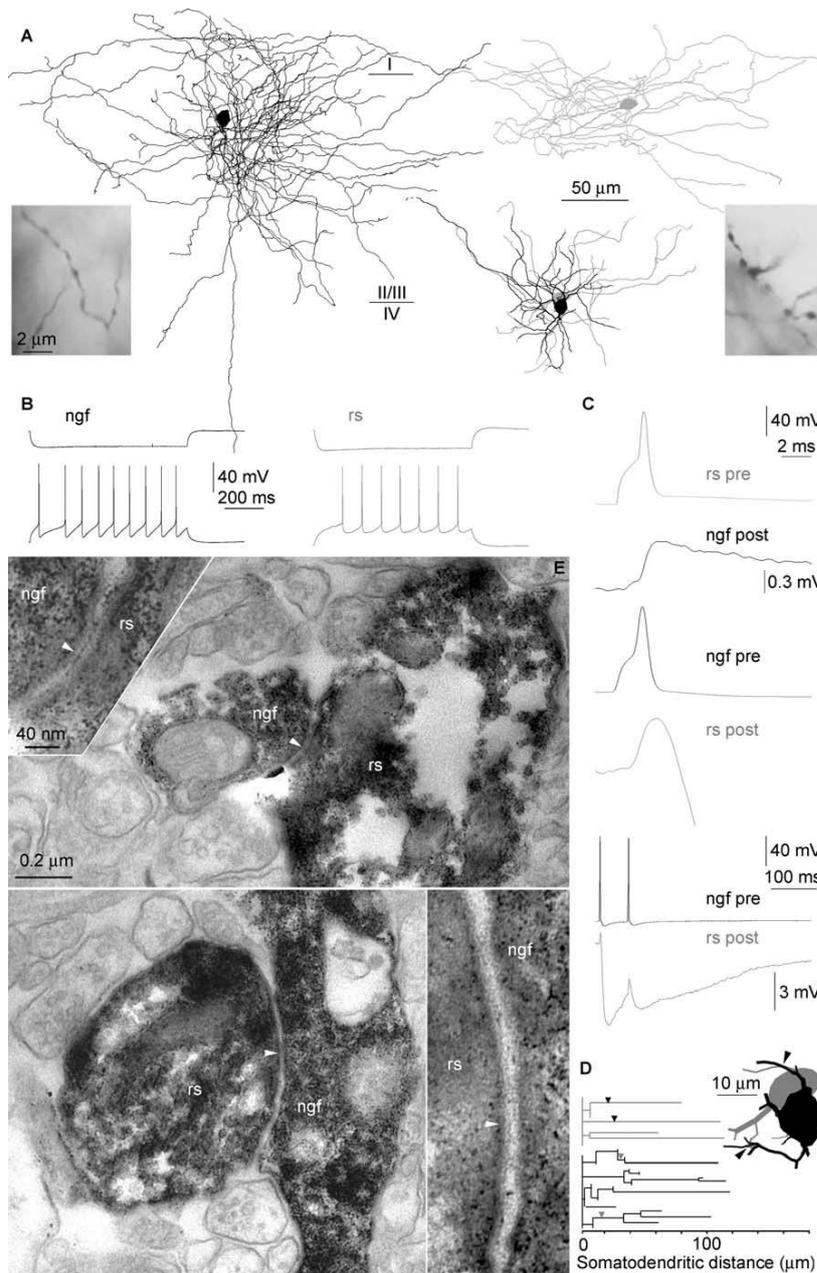


Figure 5.1.3 Heterologous gap junctions connect the network of neurogliaform cells with regular-spiking cells. **A**, Reconstructions of the neurogliaform cell (black) and the regular-spiking cell (gray). Axonal and dendritic arborizations are shown separately for clarity. Dendritic reconstructions show the relative position of the cells. The laminar position is shown relative to the axons. Light micrographs illustrate morphological differences between axon terminals. **B**, Responses of the cells to hyperpolarizing (top) and depolarizing current pulses (bottom). **C**, Action potentials elicited in the regular-spiking cell elicited gap-junctional potentials or spikelets postsynaptically (top). Presynaptic spikes in the neurogliaform cell triggered a spikelet (middle) followed by a longlasting IPSP (bottom). Of the 30 ngf–rs cell connections tested, we confirmed electrical coupling in six pairs (20%). **D**, Dendrograms representing three-dimensional distances measured from the somata to the gap junctions (arrowheads) mediating the interactions between the neurogliaform cell (black) and the regular-spiking cell (gray). Inset, The route of dendrites from the somata to the gap junctions (arrowheads) between proximal dendrites of the ngf and rs cells. **E**, Electron microscopic verification of the two gap junctions (arrowheads) between proximal dendrites of the ngf and rs cells. pre, Presynaptic; post, postsynaptic.

Neurogliaform cell–axoaxonic cell electrical coupling

In search for electrically coupled partners for ngf cells, we recorded a single ngf cell–axoaxonic (aa) cell pair, and this cell pair was electrically coupled (Figure 5.1.4). Although this was a single example, the unique output specificity of aa cells among cortical interneurons and the relatively limited number of aa cell connections studied thus far encouraged us to present the relevant data. Only the somata and axons were recovered from the cell pair, and we determined the identity of the aa cell based on the presence of characteristic cartridges or candles formed by axonal boutons. Moreover, the aa cell innervated a pyramidal cell, which was also recorded in the same slice exclusively on the axon initial segment through five electron microscopically verified synaptic junctions. The aa cell had a firing pattern similar to fs cells. Electrical coupling between the aa and ngf cell was tested with presynaptic action potentials only; postsynaptic spikelets with onsets during the rising phase of presynaptic action potentials, the matching polarity of presynaptic and postsynaptic signals, and the reciprocity of connection ensured electrical coupling. The spikelet triggered by ngf cell was followed by an IPSP, which was not characterized pharmacologically.

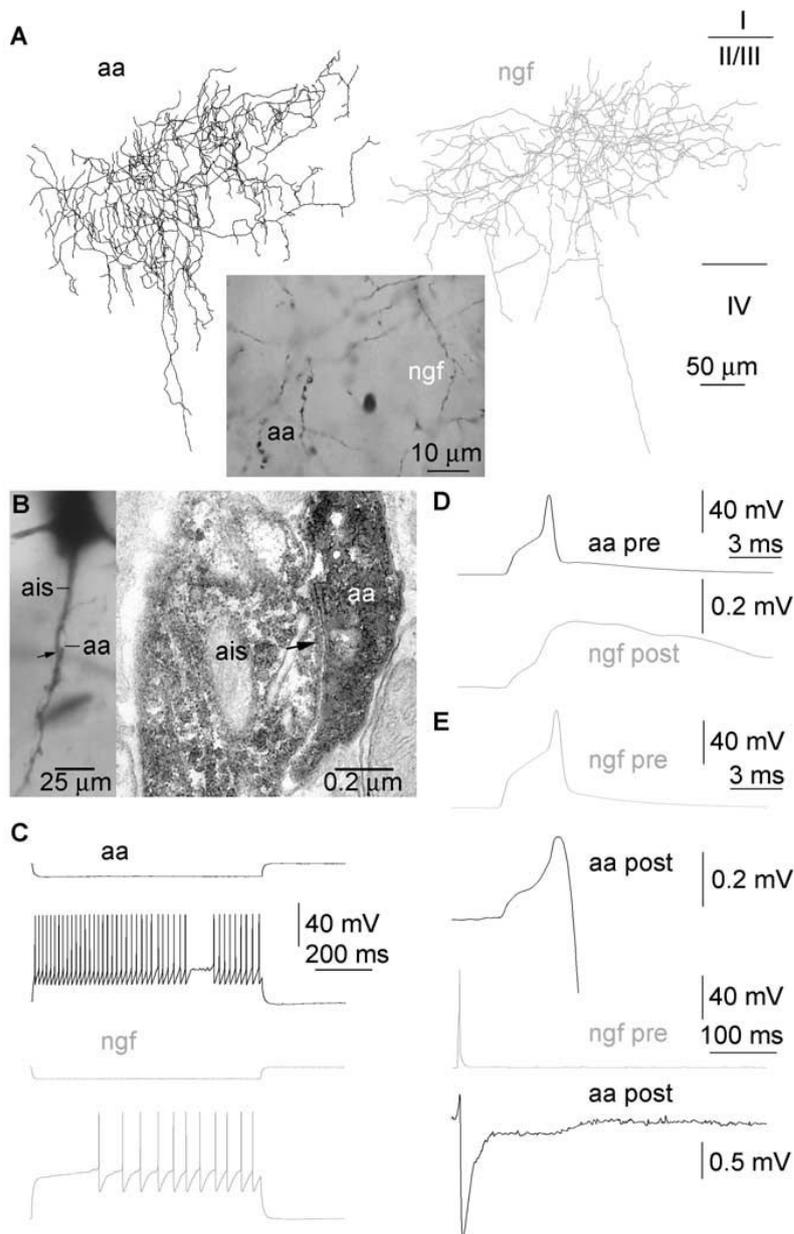


Figure 5.1.4 Heterologous electrical coupling between a neurogliaform cell and an axoaxonic cell. **A**, Reconstructions of the ngf cell (gray) and the aa cell (black). Only axons were recovered from both cells. The light micrographs show morphological features of aa and ngf axon terminals. **B**, Innervation of a pyramidal cell by the axoaxonic cell. Left, The axon of the axoaxonic cell (aa) forms a cartridge around the axon initial segment (ais) of the pyramidal cell. Right, Example of one of the five electron microscopically verified synaptic junctions (arrow) established by the axoaxonic cell on the axon initial segment of the pyramidal cell. **C**, Responses of the cells to hyperpolarizing (top) and depolarizing current pulses (bottom). **D**, Action potentials elicited in the axoaxonic cell elicited gap-junctional potentials or spikelets postsynaptically. **E**, Presynaptic spikes in the neurogliaform cell triggered a spikelet (top, expanded timescale) followed by an IPSP (bottom). pre, Presynaptic; post, postsynaptic.

Electrical synapses between neurogliaform cells and other interneurons

Electrical coupling between ngf and interneurons with firing properties and morphological characteristics distinct from ngf, fs, rs, and aa cells was tested in 26 pairs (data not shown). In 13 pairs, the interneurons recorded simultaneously with ngf cells responded to depolarizing current pulses with a so-called lowthreshold spiking firing pattern and showed dendritic and axonal features of bitufted (bt) cells (Gibson et al., 1999; Tamas et al., 2003; Tamas et al., 2004). We identified two ngf– bt cell pairs and two ngf cell-unidentified interneuron pairs connected by electrical synapses, indicating an average coupling rate of 15%. Apart from the spikelets evoked by the electrically coupled interneurons, an ngf cell elicited IPSPs in a bitufted cell, and an unidentified interneuron evoked an IPSP in an ngf cell.

Discussion

We showed that ngf cells eliciting slow IPSPs on pyramidal cells also triggered divergent electrical coupling potentials on interneurons. In line with previous results showing widespread electrical coupling between interneurons of the same type, ngf cells formed homologous electrical synapses with other neurogliaform cells. The major point of this study is that ngf cells established heterologous electrical coupling with several types of interneuron. We also identified the sites of electrical interaction between different cell types and provided ultrastructural evidence for gap junctions linking somatodendritic sites of the coupled cells.

Previous results of our lab on homologous electrical coupling between interneurons representing the same class identified the site of coupling ultrastructurally as gap junctions between the somata and/or dendrites of electrophysiologically recorded cells (Tamas et al., 2000; Szabadics et al., 2001). The possible axoaxonal electrical coupling between hippocampal pyramidal cells suggests that the formation of gap junctions could follow cell type or connection-specific subcellular patterns (Schmitz et al., 2001; Traub et al., 2003; Hamzei-Sichani et al., 2007). However, heterologous electrical coupling identified here with combined recordings and electron microscopy also operates via somatodendritically placed gap junctions. Thus, it appears to be a generalized feature of cortical circuits that individual cells in electrically coupled networks of cortical interneurons are located within the dendritic arborization of each other and communicate through the dendrites. This could

limit the size of electrically coupled networks, but the relatively uniform and moderate number of gap junctions identified within a single connection thus far allows the formation of widespread electrically interconnected circuits (Tamas et al., 2000; Deans et al., 2001; Szabadics et al., 2001). Ultrastructural identification of gap junctions between coupled cells recorded electrophysiologically also suggests that interneurons of the cerebral cortex are not coupled by cytoplasmic continuity, which can result from artifacts induced by slice cutting (Bennett and Zukin, 2004). This is also supported by previously documented absence of dye coupling between cortical interneurons known to form electrically coupled microcircuits (Galarreta and Hestrin, 2001; Bennett and Zukin, 2004; Connors and Long, 2004). The lack of dye coupling between interneurons of the same type and between ngf cells and other interneurons suggests that permeability properties and possibly the molecular composition of homologous and heterologous gap junctions of interneurons might be similar and involve connexin 36 (Venance et al., 2000). It should be added, however, that ngf cell-like dwarf neurons in the striatum of young rats can be dye coupled with neurons of different classes (Sancesario et al., 1998), but dye coupling may not be a reliable measure of gapjunction coupling among postnatal cortical neurons (Gutnick and Prince, 1981; Knowles et al., 1982; Connors et al., 1984; Roerig and Feller, 2000).

Several types of interneurons eliciting GABAA receptor-mediated postsynaptic responses are known to form electrically coupled networks with interneurons of the same class (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000; Venance et al., 2000; Szabadics et al., 2001; Blatow et al., 2003; Chu et al., 2003; Bennett and Zukin, 2004). Fast IPSPs triggered by members of these networks are elicited on separate subcellular domains. It appears that the somatodendritic domain of postsynaptic cells is targeted by several separate networks of basket or basket-like cells intrinsically linked by gap junctions (Galarreta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000). Similarly, the dendritic region of postsynaptic neurons also receives a multitude of inputs from a number cell populations (rs cells, bitufted or low-threshold spiking cells, and layer 1 interneurons) which were shown to form electrical synapses restricted to the same type of interneuron (Gibson et al., 1999; Venance et al., 2000; Szabadics et al., 2001; Chu et al., 2003). Despite targeting the dendritic domain of postsynaptic neurons and forming electrical synapses within the cell class, ngf cells could have an exceptional position in the cortical architecture. From the multitude of cortical GABAergic interneurons, ngf cells represent the first cell type capable of eliciting slow IPSPs composed of GABAA and GABAB receptor-mediated postsynaptic responses with single presynaptic spikes (Tamas

et al., 2003). Moreover, neurogliaform cells establish electrical synapses and link multiple networks formed by gap junctions restricted to a particular class of interneuron (Figure 5.1.5).

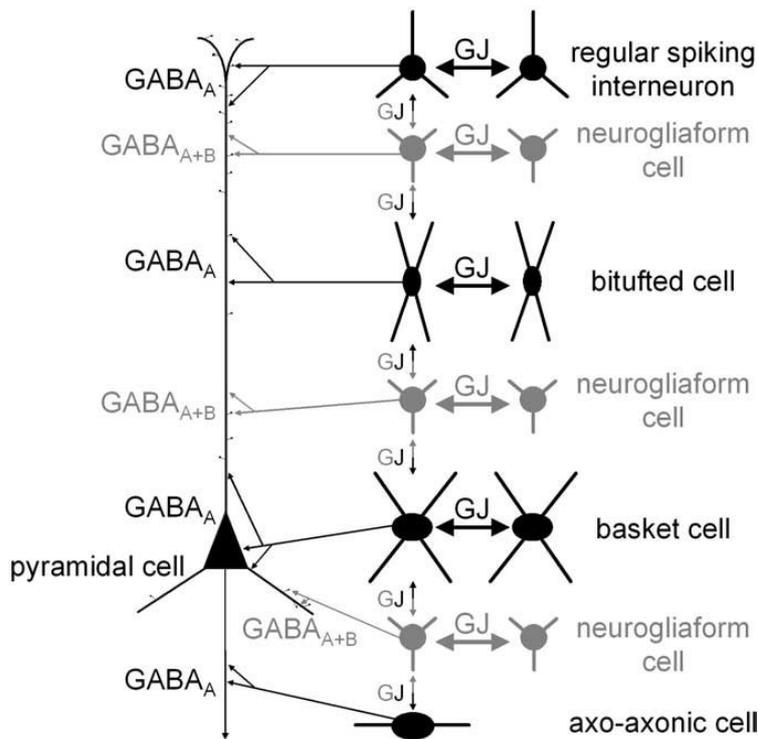


Figure 5.1.5. Neurogliaform cells have a unique position in the cortical microcircuit. To date, neurogliaform cells are the only interneuron type capable of eliciting slow GABAA and GABAB IPSPs on pyramidal cells. Several types of interneurons eliciting GABAA receptor-mediated postsynaptic responses are known to form electrically coupled networks with interneurons of the same class. We found that neurogliaform cells are electrically coupled to other neurogliaform cells (gray), but, unlike other interneurons, neurogliaform cells also establish heterologous gap junctions (at a lower rate of coupling) with regular-spiking nonpyramidal cells, bitufted cells, basket cells, and axoaxonic cells. Thus, heterologous electrical synapses of neurogliaform cells link multiple networks formed by gap junctions restricted to a particular class of interneuron. Widespread electrical connections might enable neurogliaform cells to monitor the activity of different interneurons acting on GABAA receptors at various regions of target cells. GJ, Gap junction.

In-depth analysis of the function of electrical synapses established by ngf cells awaits additional experiments. Homologous electrical synapses within a multitude of cell populations were found to promote synchronization of the coupled cells (Hestrin and Armstrong, 1996; Bennett and Zukin, 2004; Connors and Long, 2004; Sohl et al., 2005). Unlike other known types of interneuron, ngf cells elicit GABAA and GABAB receptor-mediated postsynaptic potentials on pyramidal cells (Tamas et al., 2003) and synchronous release of GABA from several presynaptic interneurons was suggested to be involved in the activation of postsynaptic GABAB receptors (Mody et al., 1994; Kim et al., 1997; Thomson and Destexhe, 1999). Although single spikes in ngf cells are sufficient for eliciting the GABAB receptor-mediated response, we did not detect spike transmission through gap junctions linking ngf cells, in agreement with previous reports showing that

synchronization is promoted by electrical coupling but is usually too weak for spike-to-spike coupling (Tamas et al., 1997; Galarreta and Hestrin, 1999; Gibson et al., 1999; Szabadics et al., 2001; Blatow et al., 2003). However, unlike other known networks of electrically coupled interneurons, ngf cells are embedded into a widespread mesh of electrical synapses linking multiple interneuron classes. Our results suggest that ngf cells form electrical connections with one-half of the neurogliaform cells and with every fifth interneuron representing at least three distinct types within the range of their relatively compact dendritic field. The strength of coupling is similar in homologous and heterologous electrical synapses established by ngf cells and is comparable with values published previously for homologous coupling in networks of other interneurons (Galarreta and Hestrin, 1999; Gibson et al., 1999). Whether heterologous electrical coupling of neurogliaform cells has a specific function similar to retinal gap junctions between dissimilar components of the circuit remains to be seen (Guldenagel et al., 2001; Demb and Pugh, 2002; Lee et al., 2003; Hornstein et al., 2004; Sohl et al., 2005). Theoretical studies suggest that electrical synapses could stabilize synchronization by compensating network heterogeneity (Sherman and Rinzel, 1992; Chow and Kopell, 2000; Kopell and Ermentrout, 2004). Thus, it is conceivable that heterologous coupling between ngf cells and other interneuron types could contribute to operational state-dependent and simultaneous recruitment of ngf cells, which can boost their efficacy in reaching GABAB receptors. In contrast, widespread homologous and heterologous electrical coupling counteracts the spike-triggering effectiveness of excitatory inputs arriving to ngf cells by siphoning current into coupled cells. It was estimated that approximately one-third to one-half of input conductance of neurons involved in electrical coupling occurs via gap junctions to neighboring cells (Amitai et al., 2002; Long et al., 2002). Such postsynaptic conductance load could contribute to the late-spiking firing characteristics of ngf cells and might regulate firing behavior during network operations favoring sporadic and delayed firing of ngf neurons paralleled by the prominent frequency sensitivity of ngf output (Tamas et al., 2003). Thus, neurogliaform cells eliciting slow IPSPs and forming homologous and heterologous gapjunctional connections appear well positioned to monitor the activity of different interneurons acting on GABAA receptors at various regions of target cells.

5.2 Output of neurogliaform cells to various neuron types in the human and rat cerebral cortex

Summary

Neurogliaform cells in the rat elicit combined GABAA and GABAB receptor-mediated postsynaptic responses on cortical pyramidal cells and establish electrical synapses with various interneuron types. However, the involvement of GABAB receptors in postsynaptic effects of neurogliaform cells on other GABAergic interneurons is not clear. We measured the postsynaptic effects of neurogliaform cells *in vitro* applying simultaneous whole-cell recordings in human and rat cortex. Single action potentials of human neurogliaform cells evoked unitary IPSPs composed of GABAA and GABAB receptor-mediated components in various types of interneuron and in pyramidal cells. Slow IPSPs were combined with homologous and heterologous electrical coupling between neurogliaform cells and several human interneuron types. In the rat, single action potentials in neurogliaform cells elicited GABAB receptor-mediated component in responses of neurogliaform, regular spiking, and fast spiking interneurons following the GABAA receptor-mediated component in postsynaptic responses. In conclusion, human and rat neurogliaform cells elicit slow IPSPs and reach GABAA and GABAB receptors on several interneuron types with a connection-specific involvement of GABAB receptors. The electrical synapses recorded between human neurogliaform cells and various interneuron types represent the first electrical synapses recorded in the human cortex.

Introduction

Neurogliaform cells were first described by Ramon y Cajal as spiderweb cells according to their distinctive morphology: multiple dendrites of these cells spread radially from a small, round soma in all directions and their dense axonal plexus composed of very fine branches with small, frequently occurring boutons resembled webs of spiders (Ramon y Cajal, 1904; Kisvarday et al., 1990; Kawaguchi and Kubota, 1997; Krimer et al., 2005; Kawaguchi et al., 2006). The occurrence of neurogliaform cells is reported in all layers of cerebral cortex (Kisvarday et al., 1990; Hestrin and Armstrong, 1996; Kawaguchi and Kubota, 1997; Sancesario et al., 1998; Krimer et al., 2005). Golgi studies confirmed the

presence of neurogliaform cells in samples of human association cortex (Kisvarday et al., 1990). Neurogliaform cells were found in dorsolateral prefrontal cortex of monkeys (Krimmer et al., 2005) and in various cerebral structures of rats including striatum (Sancesario et al., 1998), CA1 area of hippocampus (Price et al., 2005; Zsiros and Maccaferri, 2005; Price et al., 2008) and visual (Hestrin and Armstrong, 1996) and frontal cortex (Kawaguchi and Kubota, 1997).

Neurogliaform cells have a unique position among cortical interneurons because they establish electrical synapses not only with each other but also with other interneuron types in the neocortex and in the CA1 region of the hippocampus (Price et al., 2005; Simon et al., 2005; Zsiros and Maccaferri, 2005; Price et al., 2008). Thus, neurogliaform cells link multiple networks of interneurons and were suggested to play a central role in generating and shaping synchronized activity of neuronal circuits (Simon et al., 2005; Zsiros and Maccaferri, 2005). All but one type of interneuron trigger fast inhibitory postsynaptic potentials (IPSPs) in their postsynaptic target cells mediated by GABAA receptors (Buhl et al., 1994; Gupta et al., 2000; Tamas et al., 2003). By contrast, neurogliaform cells are the only known type of interneuron capable of eliciting slow, long-lasting IPSPs through a combined activation of GABAA and GABAB receptors (Tamas et al., 2003). To date, this effect of neurogliaform cells has been demonstrated only on postsynaptic pyramidal cells (Tamas et al., 2003) and a relatively small contribution of GABAB receptors to neurogliaform cell evoked responses was demonstrated on neurogliaform cells in the CA1 area of the hippocampus (Price et al., 2005; Price et al., 2008). The expression pattern and immunocytochemical localization suggest that GABAB receptors can be found in the neocortex on postsynaptic pyramidal cells and on interneurons (Lopez-Bendito et al., 2002; Lopez-Bendito et al., 2004; Perez-Garci et al., 2006b; Vigot et al., 2006). Electrophysiological experiments also suggest the presence of GABAB receptors on GABAergic neurons (Mott et al., 1999; Martina et al., 2001), but the cellular components of the neocortical microcircuit reaching the GABAB receptors on interneurons are not known. To investigate the role of neurogliaform cells in human cerebral cortex and their postsynaptic effects on interneurons in rat cerebral cortex, we performed simultaneous dual, triple, and quadruple recordings of neurons in layers I–IV of human associational cortices and rat somatosensory cortex *in vitro* with correlated light microscopic analysis.

Results

Similar to our earlier experiments, the postsynaptic IPSPs elicited by neurogliaform cells were highly sensitive to the firing rate of the presynaptic cells (Tamas et al., 2003). 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 at least 1.5 minute. Accordingly, all single action potential-evoked responses were collected at especially low presynaptic firing rates (one spike in 90 second).

Human experiments

We recorded 13 connections in layers 2/3 in slices derived from human association cortices in which the presynaptic neurons were neurogliaform cells. Like the neurogliaform cells identified previously, all neurogliaform cells showed similar anatomical features (Tamas et al., 2003; Simon et al., 2005). Their somata were relatively small and mostly spherical. Multiple dendrites radiated from all parts of the soma and were arranged to a relatively small dendritic field. Small boutons frequently occurred along thin axonal collaterals, forming a dense meshwork within the compact axonal field.

In general, firing parameters of human neurogliaform cells were similar to what was described previously in the rat. Neurogliaform cells in the human sample responded to long (800 ms) suprathreshold current injections with late spiking firing pattern or with an accelerating train of spikes when activated from resting membrane potential (-62.4 ± 4.7 mV). This firing behavior was changed to a decelerating pattern when applying larger current pulses. The time constant and the input resistances of human neurogliaform cells were 8.7 ± 1.5 ms and $219.1 \pm 55.1 M_{\Omega}$, respectively. Human neurogliaform cells showed a characteristic sag in response to hyperpolarizing current injections, which was not typical of neurogliaform cells in rat cerebral cortex. Postsynaptic cells were pyramidal cells ($n = 4$) and various interneurons ($n = 7$) in the human sample. We identified these cells based on firing characteristics and dendritic and axonal morphology. All four postsynaptic pyramidal cells showed regular spiking firing pattern, their input resistances and membrane time constants were $150.5 \pm 42.1 M_{\Omega}$ and 11.9 ± 1.5 ms, respectively. Electrophysiological properties of postsynaptic interneurons were variable; input resistances and time constants were $255.3 \pm 88.2 M_{\Omega}$ and 13.3 ± 6.9 ms, respectively. Five out of seven postsynaptic human

interneurons responded with a sag to hyperpolarizing current injections and, in two cells showed rebound spikes following the hyperpolarization. The sag and rebound spiking was apparent in postsynaptic human interneurons exhibiting various firing behavior (i.e., fast spiking and accommodating) in response to depolarizing current injections.

Human neurogliaform cells evoked long-lasting inhibition in postsynaptic cells (Figure 5.2.1). The rise times and half-width of IPSPs in these connections were 16.5 ± 8.5 and 184.9 ± 96.5 ms, respectively. When comparing IPSPs arriving to postsynaptic pyramidal cells (not shown) and interneurons in the human sample, we could not detect significant differences in 10–90% rise times and half-widths (20.1 ± 9.8 ms and 12.7 ± 6.8 ; 230.4 ± 64.6 ms and 148.6 ± 108.4 , respectively). For comparison, 10–90% rise times and half-width of IPSPs evoked by neurogliaform cells in rat pyramidal cells were 23.4 ± 9.8 ms and 183.9 ± 82.5 . The maximal amplitudes of IPSPs evoked by human neurogliaform cells were 0.94 ± 0.88 mV.

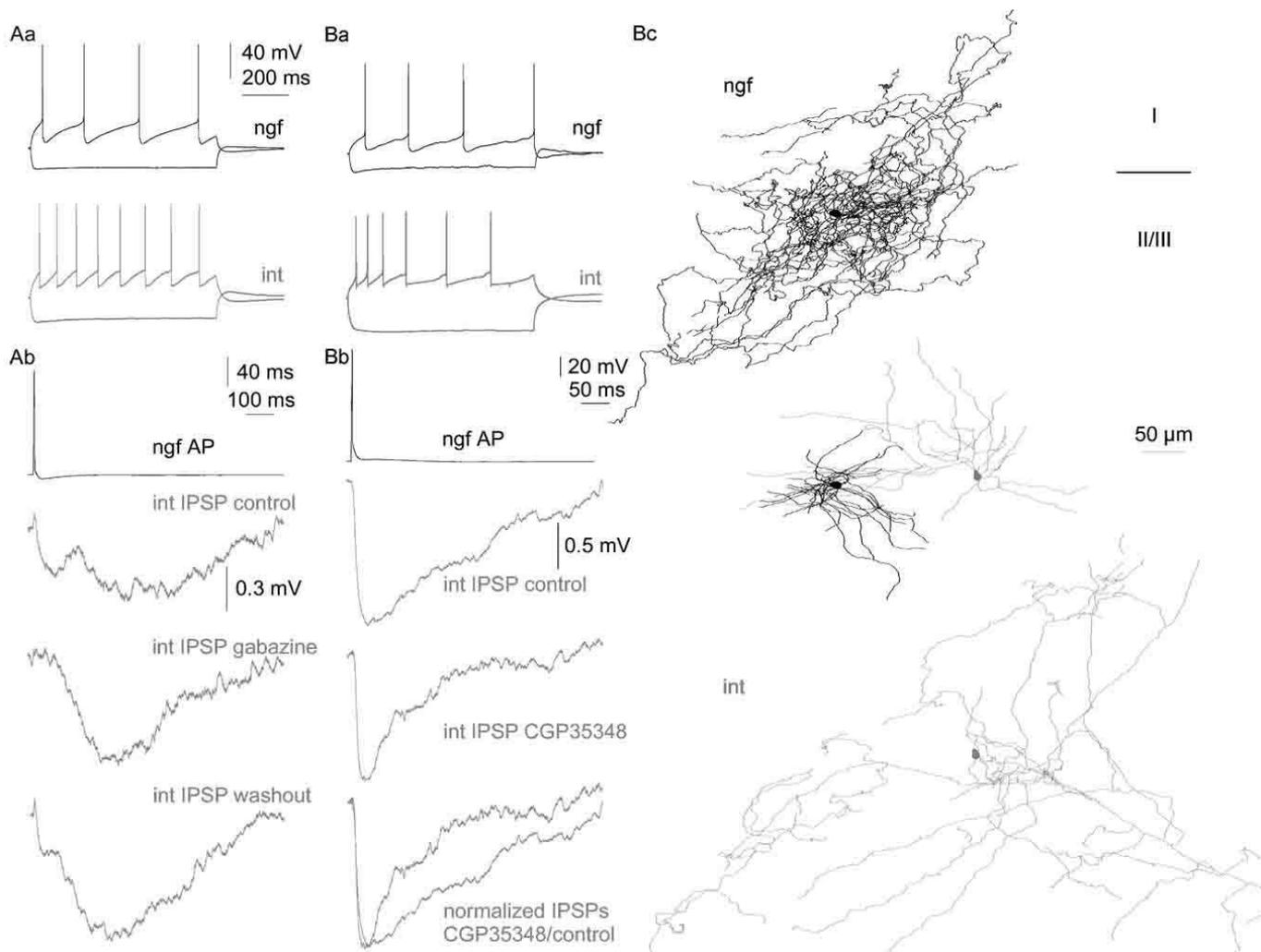


Figure 5.2.1 The effect of human neurogliaform cells is mediated by GABAA and GABAB receptors on postsynaptic interneurons. (Aa) Firing pattern of a human neurogliaform cell (ngf) and a postsynaptic

interneuron (int). (Ab) Single action potentials of the neurogliaform cell elicited biphasic IPSPs in the interneuron in control conditions (top). The initial component of the IPSP was reversibly blocked by the GABAA receptor antagonist gabazine (10 μ M, middle and bottom). (Ba) Firing properties of a presynaptic neurogliaform cell (ngf) and postsynaptic interneuron (int) recorded in human association cortex. (Bb) The postsynaptic cell responded with a long lasting IPSP to single action potentials evoked in the neurogliaform cell (top). The decay of IPSP could be shortened by the GABAB receptor blocker CGP35348 (60 μ M, middle and bottom). (Bc) Three dimensional light microscopic reconstructions of the presynaptic neurogliaform cell (black) and the postsynaptic interneuron (gray). Axonal (top and bottom) and dendritic (middle) reconstructions are separated for clarity, the relative position of the two cells is shown by the dendritic arborizations.

We applied pharmacological blockers to dissect the contribution of the two GABA receptor subtypes to the inhibition evoked by human neurogliaform cells. Addition of the GABAB receptor blocker CGP35348 (60 μ M) into the bath solution could shorten the duration of slow IPSPs evoked by neurogliaform cells ($n = 2$; postsynaptic cells were interneurons). When testing the connections by applying the GABAA receptor blocker gabazine (10 μ M), we could isolate the slow component of the IPSPs ($n = 2$; postsynaptic cells were interneurons). In addition to chemical neurotransmission elicited by human neurogliaform cells, we detected homologous electrical connections from neurogliaform cells to other neurogliaform cells ($n = 2$, not shown) and a heterologous electrical connection between a neurogliaform cell and a different type of interneuron which was combined with a slow IPSP triggered by the neurogliaform cell (Figure 5.2.2). Electrical synapses were detected as spikelets in the postsynaptic cells in response to presynaptic action potentials. When injecting hyperpolarizing current pulses into either of the connected cells, the other cell responded with an electrical coupling potential of similar polarity confirming electrical interaction. The strength of electrical coupling was symmetrical in the homologous and heterologous electrical synapses showing subthreshold coupling coefficients of 0.036 ± 0.004 .

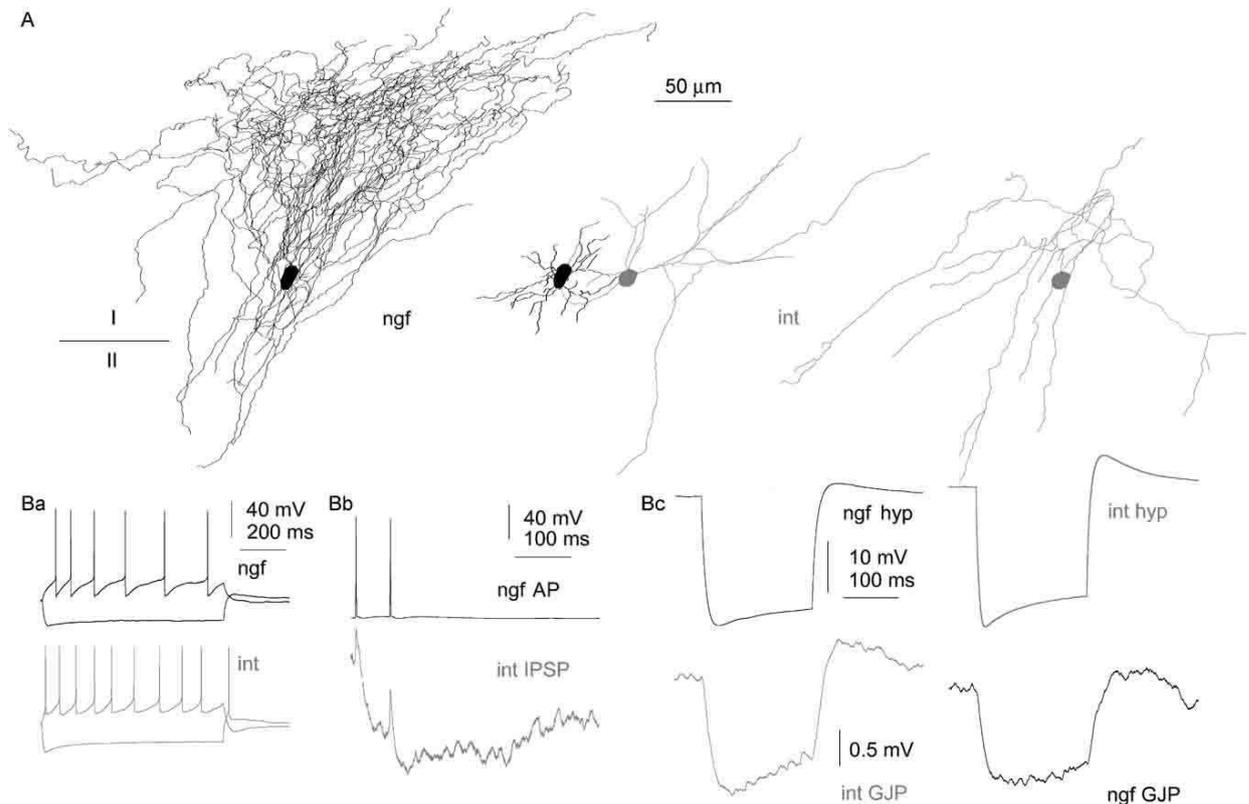


Figure 5.2.2 Human neurogliaform cells are involved in electrical coupling. (A) Three dimensional light microscopic reconstructions of an electrically coupled neurogliaform cell (ngf, black) and a different type of interneuron (int, gray) in layer I of the human cerebral cortex. Axonal (left and right) and dendritic (middle) reconstructions are separated for clarity, the dendritic arborizations show the original position of the interneurons. (Ba) Firing patterns of the neurogliaform cell and the interneuron. (Bb) Action potentials in the neurogliaform cell triggered postsynaptic spikelets in the interneuron followed by long lasting IPSPs. (Bc) Reciprocal transmission of hyperpolarizing current pulses injected into the neurogliaform cell (left) or the interneuron (right) confirmed electrical coupling between the two cells.

Rat experiments

Following our findings in the human cortex based on a relatively limited sample, we performed supporting experiments in slices taken from the rat cerebral cortex. We recorded 48 pairs of neurons in which neurogliaform cells established chemical connections with various types of interneuron (neurogliaform cells, regular spiking cells, fast spiking basket cells, fast spiking axo-axonic cells, and unclassified interneurons). We identified these cells based on their firing characteristics and by light microscopic evaluation of dendritic and axonal morphology.

The input resistances of postsynaptic neurogliaform cells ($n = 12$) were $182.7 \pm 55.2 \text{ M}\Omega$. The time constants of neurogliaform cells in our sample were $9.9 \pm 1.1 \text{ ms}$. There was no significant difference between time constants of human and rat neurogliaform cells ($p = 0.792$). Input resistance in rat neurogliaform cells was

significantly lower than that of in the human ($p < 0.01$). Neurogliaform cells responded with longlasting IPSPs to single action potentials of presynaptic neurogliaform cells Figure 5.2.3). The rise times and half-width of IPSPs of neurogliaform cells were 6.5 ± 1.5 and 44.4 ± 10.6 ms. To determine the extent of GABAB receptor contribution to IPSPs elicited by neurogliaform cells, we blocked GABAB receptor-mediated component with CGP35348 in three neurogliaform cell to neurogliaform cell connections. The decay phase of neurogliaform cell to neurogliaform cell IPSPs were shortened by CGP35348 resulting in a $16 \pm 7.9\%$ decrease in the half-width of IPSPs showing the involvement of GABAB receptors in these connections.

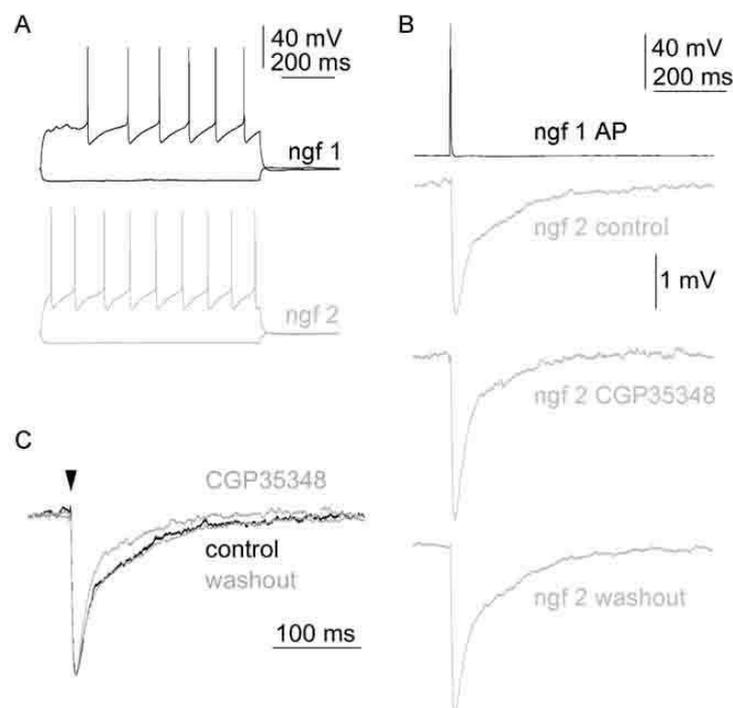


Figure 5.2.3 GABAB receptors contribute to IPSPs between neurogliaform cells in the rat cortex. (A) Firing patterns of two neurogliaform cells (ngf 1 and ngf 2). (B) Single action potentials in the presynaptic neurogliaform cell elicited slow IPSPs in the postsynaptic neurogliaform cell. The decay of the IPSP could be shortened by the application of the GABAB receptor blocker CGP35348 (60 μ M). The late component of IPSP showed recovery after 30 min of washout. (C) Superimposed traces are shown normalized to the control IPSP.

Neurogliaform cell innervated postsynaptic interneurons ($n = 12$) that responded to suprathreshold current injections with a regular-spiking firing pattern with accommodating train of spikes (Figure 5.2.4). The input resistance and membrane time constant of regular spiking nonpyramidal cells was $249.1 \pm 31.2 \text{ M}\Omega$ and 13.7 ± 10.4 ms, respectively. Their anatomical features were similar to regular spiking nonpyramidal cells identified by previous studies (Kawaguchi and Kubota, 1997; Szabadics et al., 2001; Krimer et al., 2005). The morphology of sparsely spiny dendrites and individual axonal branches of these cells with relatively large boutons were in line with their physiological similarity. The IPSPs from neurogliaform cells to regular spiking interneurons were the longest among

neurogliaform to interneuron connections ($p < 0.002$ for neurogliaform to neurogliaform, $p < 0.001$ for neurogliaform to fast spiking cell connections, see below). The differences detected between the duration of IPSPs triggered by neurogliaform cells on regular spiking, neurogliaform and fast spiking cells could not be accounted for differences in postsynaptic membrane time constants. The rise time and half-width of IPSPs in neurogliaform to regular spiking nonpyramidal cell connections were 16.6 ± 10.8 and 135.7 ± 51.1 ms. Application of the GABAA receptor blocker gabazine ($n = 4$) and the GABAB receptor blocker CGP35348 ($n = 2$) showed that neurogliaform to regular spiking nonpyramidal cell connections were composed of GABAA and GABAB receptor-mediated components. When GABAA receptors were blocked, we could detect residual hyperpolarizing responses with a late onset (41.8 ± 2 ms) and slow decay times. Blockade of GABAB receptors could shorten the duration of IPSPs by $42.9 \pm 21.1\%$.

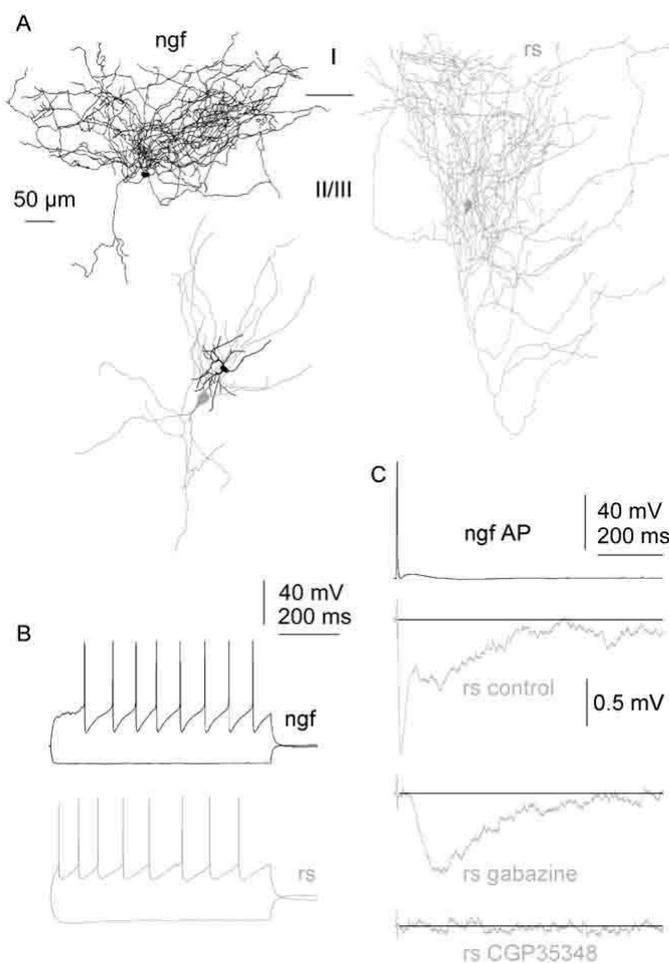


Figure 5.2.4 GABAA and GABAB receptors mediate neurogliaform cells (ngfc) to regular spiking interneuron (rs) connections. (A) Three dimensional light microscopic reconstructions of the presynaptic neurogliaform cell (black) and the postsynaptic regular spiking interneuron (gray). Axonal (left and right) and dendritic (middle) reconstructions are separated for clarity, the relative position of the two cells is shown by the dendritic arborizations. (B) Firing patterns of the two cells. (C) Single action potentials in the neurogliaform cell elicited slow IPSPs with two components in the regular spiking interneuron. The early component could be blocked by the GABAA receptor blocker gabazine ($10 \mu\text{M}$) and the late phase of IPSP was abolished by the subsequent addition of CGP35348 ($60 \mu\text{M}$).

Postsynaptic fast spiking cells responded with high frequency, nonaccommodating trains of spikes to depolarizing current pulses. Axonal terminals of eight postsynaptic fast spiking cells showed close appositions on unlabeled somata, thus we classified these

neurons as basket cells (Karube et al., 2004). In the remaining two cases, axon terminals of the postsynaptic cells were arranged into cartridges or candles characteristic to chandelier or axo-axonic cells. Input resistance and membrane time constants of basket and axo-axonic fast spiking interneurons were similar, thus we pooled the data (110.8 ± 25.2 and 7.1 ± 1.2 ms, respectively). The kinetics of neurogliaform to basket and neurogliaform to axo-axonic cell connections were also similar and showed relatively small slow component (Figure 5.2.5). The rise times and half-width of IPSPs elicited by neurogliaform cells in fast spiking cells were 3.5 ± 0.9 and 25.8 ± 14.6 ms. The IPSPs from neurogliaform cells to fast spiking interneurons were the shortest among neurogliaform to interneuron connections ($p < 0.03$ for neurogliaform to neurogliaform, $p < 0.001$ for neurogliaform to regular spiking cell connections). Pharmacological dissection could be performed in three neurogliaform to basket cell connections. A small residual IPSP component was detected during GABAA receptor blockade with gabazine ($n=2$, $10 \mu\text{M}$) and, when blocking GABAB receptor activation with CGP35348 ($n=1$, $60 \mu\text{M}$), the half-width of response changed only slightly (from 72 to 62.3 ms).

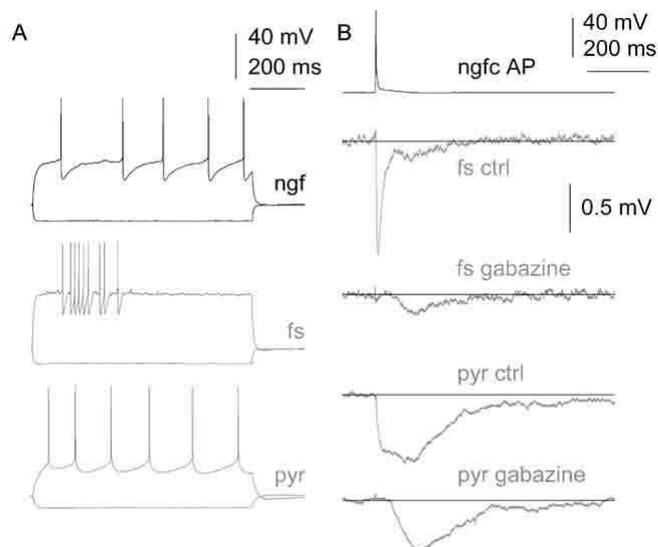


Figure 5.2.5 Divergent connections of a neurogliaform cell (ngfc) to postsynaptic fast spiking (fs) and pyramidal (pyr) cells. (A), Firing properties of the recorded cells. (B), Single action potentials in the neurogliaform cell elicited IPSPs with complex kinetics in the fast spiking as well as in the pyramidal cell. A residual slow component was detectable in both postsynaptic neurons during application of the GABAA receptor blocker gabazine ($10 \mu\text{M}$).

Discussion

The first simultaneous multiple recordings of human neurogliaform cells and their postsynaptic targets revealed that single spikes in neurogliaform cells elicit long-lasting unitary IPSPs composed of GABAA and GABAB receptor-mediated components in

various types of interneuron. We confirmed these results in the rat cortex and, moreover, our experiments showed that human neurogliaform cells, similar to those tested in the rat (Tamas et al., 2003), evoke long-lasting IPSPs in pyramidal cells. These results suggest that neurogliaform cells represent a widespread source for slow IPSPs targeting multiple cell types in microcircuits of the human and rat neocortex and are in line with earlier experiments showing a relatively small GABAB receptor component in unitary connections between neurogliaform cells of the hippocampus (Price et al., 2005; Price et al., 2008).

The effect of neurogliaform cells in the rat (Vida et al., 1998; Tamas et al., 2003) and human (Kisvarday et al., 1990) cerebral cortex is targeted towards the dendritic compartment of the postsynaptic cells arriving to dendritic spines, spine necks, and dendritic shafts but some evidence suggests that postsynaptic somata might also receive synapses from neurogliaform cells (Kawaguchi and Kubota, 1997). Dendritic spines receive the majority of glutamatergic inputs arriving to cortical pyramidal cells and simulations suggested that inhibition arriving to spines is more effective through GABAB than GABAA receptors in reducing EPSPs through powerful a hyperpolarizing effect (Qian and Sejnowski, 1990; Calver et al., 2002; Tamas et al., 2003). Metabotropic GABAB receptors are coupled to G-protein-coupled inwardly rectifying K⁺ channels (Kir3 channels) (Calver et al., 2002). Quantitative analysis of immunogold particles labeling Kir3.2 revealed an enrichment of the protein around putative glutamatergic synapses on dendritic spines, similar to that of GABAB receptors and a high degree of co-clustering of Kir3.2 and GABA(B1) was revealed around excitatory synapses by freeze–fracture replica immunolabeling (Kulik et al., 2006). Interestingly, GABAB receptors and Kir3.2 channels were found to be mainly segregated on dendritic shafts (Kulik et al., 2006) and thus the postsynaptic machinery of neurogliaform input on dendritic shafts of pyramidal cells and on smooth dendrites of GABAergic interneurons known to express GABAB receptors (Lopez-Bendito et al., 2002; Kulik et al., 2003) is not clear. However, more recent experiments provide evidence for a differential molecular composition of presynaptic and postsynaptic GABAB receptors, but dendritic receptors were found to contain the GABAB1b isoform in the hippocampus and in the neocortex (Perez-Garci et al., 2006b; Vigot et al., 2006) suggesting a similar mechanism of action on dendritic shafts and spines for neurogliaform cells. Postsynaptic GABAB receptor-mediated inhibition can powerfully depress Ca²⁺-spikes in apical dendrites of neocortical pyramidal cells (Perez-Garci et al., 2006). Back-propagating action potentials modulate Ca²⁺-signaling in hippocampal and

neocortical interneurons (Wang and Kelly, 2001; Goldberg et al., 2003c; Goldberg et al., 2003a; Goldberg et al., 2003b; Saraga et al., 2003) and thus GABAB receptor-mediated inhibition of neocortical interneurons by neurogliaform cells can interact with dendritic Ca²⁺-dynamics in GABAergic cells.

The cooperation between GABAB receptors and dendritic excitability might be influenced by the differential availability of GABAB receptors on postsynaptic GABAergic dendrites. The differences detected between the duration of IPSPs triggered by neurogliaform cells on regular spiking, neurogliaform, and fast spiking cells suggest a minor relative contribution of GABAB receptors on fast spiking basket and axo-axonic cells compared to postsynaptic regular spiking and neurogliaform cells. Heterogeneous GABAB receptor involvement in IPSCs from various types of GABAergic cells were recorded in response to a molecular layer stimulus in hippocampal dentate-hilus border interneurons (Mott et al., 1999). In the neocortex, synaptically released and exogenous GABA evoked IPSPs were recorded without a detectable GABAB component in fast spiking cells whereas compound GABAA and GABAB receptor-mediated responses were found in regular spiking cells (Martina et al., 2001). Although our method of recording unitary IPSPs elicited by neurogliaform cells allowed the identification of GABAB receptors expressed by fast spiking basket cells, it appears that the actual ratio of GABAA and GABAB receptor involvement in the output of neurogliaform cells could be connection specific and primarily determined by the postsynaptic cell type.

Our experiments represent the first electrical synapses recorded electrophysiologically in the human cerebral cortex. Although electrical coupling is firmly established between cortical interneurons in juvenile animals (Galarreta and Hestrin, 2001; Bennett and Zukin, 2004; Connors and Long, 2004; Sohl et al., 2005), electrophysiological evidence for electrical synapses from adult cerebral cortex is very limited (Galarreta et al., 2004) due to technical difficulties, i.e., the quality of images using infrared videomicroscopy is influenced by axonal myelination resulting in a decreased transparency of slices. Nonpyramidal cells form gap junctions in the adult hippocampus (Kosaka and Hama, 1985) and neocortex (Sloper, 1972; Peters, 1980; Connors et al., 1983; Peinado et al., 1993; Tamas et al., 2000), but the probability of dye coupling and gap junctions is reportedly affected by development. Our recordings demonstrate that simultaneous quadruple recordings from visually identified neurons can be performed in the adult human cortex and that electrical coupling is present between mature human interneurons. The human recordings are in line with data obtained in the rat showing that human

neurogliaform cells form homologous electrical synapses with other neurogliaform cells (Chu et al., 2003; Price et al., 2005; Simon et al., 2005; Price et al., 2008) and, moreover, human neurogliaform cells can establish heterologous electrical coupling with other types of interneuron (Simon et al., 2005; Zsiros and Maccaferri, 2005). We provide evidence that long-lasting IPSPs are combined with homologous and heterologous electrical coupling between neurogliaform cells and several human and rat interneuron types. Synaptic potentials propagate through electrically coupled networks of interneurons (Zsiros et al., 2007), and the slow kinetics of neurogliaform cell triggered IPSPs are particularly well suited for effectively spreading in the network through electrical synapses which act as low pass filters. Neurogliaform cells represent a unique element in human and rat microcircuits of the cerebral cortex. Embedded into an extensive network of homologous and heterologous electrical synapses linking several interneuron classes, neurogliaform cells are able to monitor the sub- and suprathreshold activity of coupled neurons and can transform this activity to long-lasting chemical signaling through metabotropic GABA_B receptors on multiple neuron populations.

5.3 Single interneuron driven GABAergic volume transmission in the cerebral cortex

Summary

The predominant inhibitory neurotransmitter GABA acts in or around specifically placed synaptic junctions between cortical neurons. However, ultrastructural analysis of functional GABAergic neurogliaform cell to interneuron connections could not detect synapses linking the cells. There are no synapses on axon terminals in the cortex, yet GABA, released from individual neurogliaform cells by single spikes, could reach presynaptic GABAB receptors on synapses linking other neurons suppressing excitatory and inhibitory communication. This modulatory effect was enhanced by GABA transporter blockade, it was ineffective beyond the axonal cloud of individual neurogliaform cells and could not be reproduced by basket cells. Thus, neurogliaform interneurons use single cell driven volume transmission and release GABA for paracrine, nonsynaptic and spatially unspecific effects providing widespread suppression of communication in the local circuit.

Introduction

Gamma-aminobutyric acid (GABA) is predominantly released by local interneurons in the cerebral cortex. A fundamental aspect of cortical GABAergic circuits is the presynaptic cell type-specific targeting of synapses: interneurons form connections restricted to particular subcellular domains of their target cells (Freund and Buzsaki, 1996; Klausberger and Somogyi, 2008). This suggests that compartmentalized, synaptic site specific action of GABA is required in cortical networks for phasic inhibition (Farrant and Nusser, 2005; Glykys and Mody, 2007; Klausberger and Somogyi, 2008). However, GABA released at the synaptic cleft diffuses to receptors outside the postsynaptic density and thus tonically activates extrasynaptic GABAA and reaches GABAB receptors, which include subtypes of both receptor families especially sensitive to low concentrations of GABA (Nusser et al., 1998; Moss and Smart, 2001; Fritschy and Brunig, 2003; Farrant and Nusser, 2005; Glykys and Mody, 2007). The synaptic and extrasynaptic action of GABA is in line with idea that neurons of the brain use synaptic (or wiring) transmission and nonsynaptic (or volume) transmission for communication (Barbour and Hausser, 1997; Vizi, 2000). However, reuptake mechanisms restrict the spatial extent of extrasynaptic GABAergic effects

(Guastella et al., 1990; Overstreet and Westbrook, 2003) and it was proposed that concerted action of several presynaptic interneurons, sustained firing of individual cells or increased release site density is required to reach ambient GABA levels sufficient to activate extrasynaptic receptors (Barbour and Hausser, 1997; Mitchell and Silver, 2000; Scanziani, 2000; Overstreet and Westbrook, 2003; Glykys and Mody, 2007). We show that individual interneurons of a cell class specialize in using GABA in a nonsynaptic, paracrine way resulting in modulation of target elements not receiving synapses in the surrounding microcircuit.

Uniquely among neocortical interneurons, neurogliaform cells evoke long-lasting inhibition composed of an unusually slow GABA_A receptor-mediated component and GABA_B receptor mediated responses in their target neurons (Tamas et al., 2003; Szabadics et al., 2007; Price et al., 2008). A distinctive feature of neurogliaform cells among cortical interneurons is the very dense axonal arborization in which presynaptic boutons on the same or neighboring collaterals can be found a couple micrometers from one another (Fig. 1) (Tamas et al., 2003; Karube et al., 2004). GABA can activate receptors located up to several micrometers from the release site (Overstreet and Westbrook, 2003). We hypothesized that the high density of neurogliaform axons could help in counteracting transmitter reuptake mechanisms and release of the transmitter from neurogliaform cells might reach the majority of GABA receptors expressed in the tissue intermingled by the axonal cloud.

Results

We simultaneously recorded from functionally coupled pairs of neurons in which a target neuron responded to action potentials triggered in a neurogliaform cell and searched for the morphological correlates of the connection. Correlated light and electron microscopic analysis of combined GABAergic and electrical neurogliaform cell to interneuron connections confirmed the presence of gap junctions in all tested connections (n=3 presented in Simon et al., 2005), but further assessment of these specimens could not detect chemical synaptic junctions between the coupled cells even though ultrastructural identification of gap junctions between labeled neurons is more difficult than that of synapses (Fig. 5.3.1). When tracing neurogliaform axons on serial ultrathin sections along their approach to functionally coupled neurons, the most closely placed synapses established by the boutons of the neurogliaform cells were $2.7 \pm 1.6 \mu\text{m}$ (range, 1.1-5.3 μm)

from the target dendrites. This suggests that neurogliaform axons do not require a synaptic contact for eliciting inhibitory postsynaptic potentials (Figure 5.3.1). Similarly, we could identify synapses between neurogliaform cells and interneurons linked by IPSPs only neither in the rat (n=5) cortex nor in humans (n=3). Supporting these and earlier results (Tamas et al., 2003), ultrastructural analysis of GABAergic neurogliaform cell to pyramidal cell interactions in the rat cortex showed synaptic junctions in two pairs, but identified no synapses in three other pairs tested. Potentially nonsynaptic communication suggests a very high rate of functional coupling between neurogliaform cells and neighboring neurons. Indeed, when searching our database containing 183 simultaneously recorded pairs of neurogliaform cells and other neurons with somata located <100 μm apart, we detected hyperpolarizing effects of neurogliaform cells in 157 (86 %) of tested cells, a ratio unprecedented in paired recordings of cortical neurons (Markram et al., 2004).

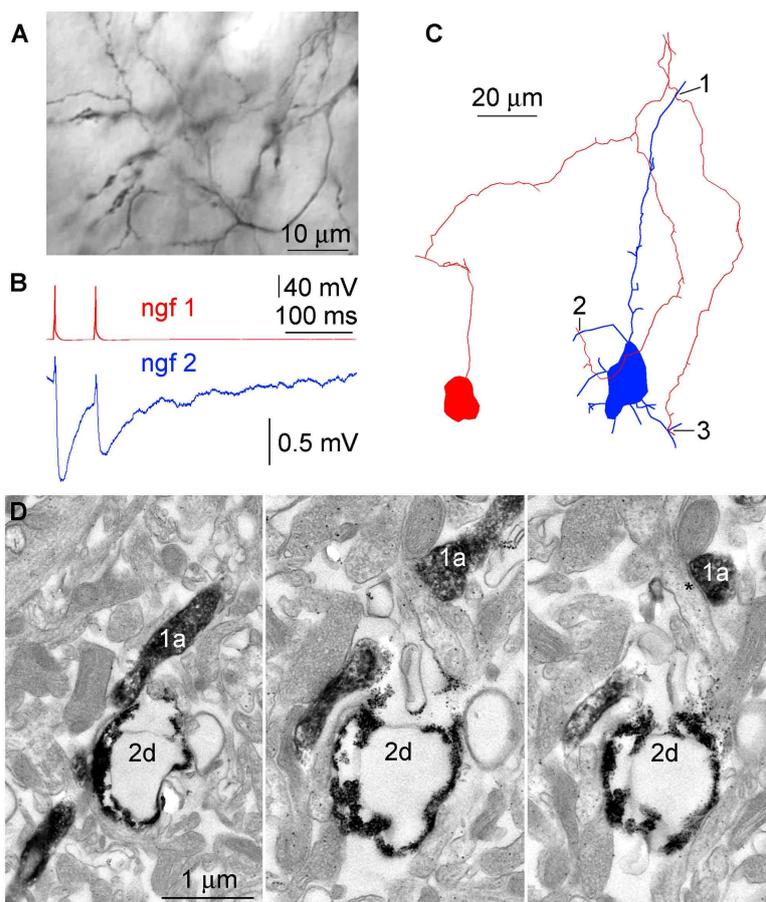


Figure 5.3.1 Neurogliaform cells do not require direct synaptic junctions to elicit IPSPs on target cells. **(A)** Light micrograph of the dense axonal cloud formed by a single biocytin labeled neurogliaform cell. **(B)** Presynaptic action potentials in neurogliaform cell 1 (ngf 1, red) elicited electrical coupling potentials (spikelets) combined with IPSPs in neurogliaform cell 2 (ngf 2, blue). **(C)** Route of the axon of neurogliaform cell 1 (red) cell to light microscopically close appositions (1-3) with the dendrites of the neurogliaform cell 2 (blue). None of these appositions could be verified as synaptic junctions. **(D)** One of the nonsynaptic close appositions (1 on panel B) in three consecutive serial electron microscopic sections (1a, axon of neurogliaform cell 1, 2d dendrite of the neurogliaform cell 2). The axon of neurogliaform cell 1 forms a synaptic junction (asterisk) on an unlabeled dendritic shaft approximately 1.5 μm from the dendrite of neurogliaform cell 2.

Functional testing of single cell initiated nonsynaptic signaling is best on potential targets not contacted by synapses at all. Thus, we asked whether GABA, released from

neurogliaform cells, modulates axon terminals which do not receive synaptic junctions in the cerebral cortex (Peters, 1991) but frequently express GABAB receptors (Isaacson et al., 1993; Mitchell and Silver, 2000; Sakaba and Neher, 2003; Price et al., 2008; Guetg et al., 2009). First, we tested if neocortical neurogliaform cells could modulate their own axon terminals via GABAB receptors similar to hippocampal neurogliaform cells (Price et al., 2008) (Figure 5.3.2). The paired pulse ratio of slow IPSPs elicited by neurogliaform cells in their target cells could be reversibly altered with the GABAB receptor blocker CGP35348 (40 μ M; n=8; 0.17 ± 0.06 , 0.45 ± 0.17 and 0.16 ± 0.10 in control, CGP35348 and wash, respectively, $p<0.001$). Thus, GABA has homosynaptic or autocrine action on neurogliaform axon terminals involving presynaptic GABAB receptors which contribute to the massive downregulation of neurogliaform output lasting for more than a minute (Tamas et al., 2003; Szabadics et al., 2007). In contrast, the paired pulse ratio in output connections of fast spiking basket cells was not changed in CGP35348 indicating that locally released GABA could not find GABAB receptors on presynaptic terminals of these cells (n=6; 0.69 ± 0.04 , 0.66 ± 0.06 and 0.66 ± 0.09 in control, CGP35348 and wash, respectively; Figure 5.3.2).

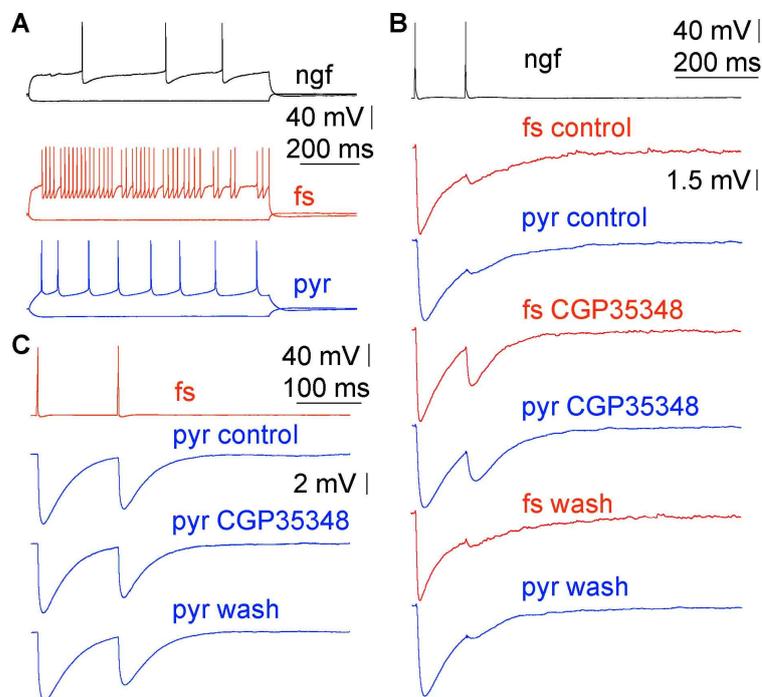


Figure 5.3.2 GABAB receptor mediated homosynaptic output suppression is present on neurogliaform axons but absent on terminals of basket cells. (A-C) Triple recording of a neurogliaform cell (ngf), a pyramidal cell (pyr) and a fast spiking basket cell (fs). (A) Firing properties of the three neurons. (B) Powerful suppression of the second postsynaptic responses detected in control conditions is reversibly lifted during the application of the GABAB receptor antagonist CGP35348 suggesting homosynaptic inhibitory modulation of neurogliaform axon terminals through presynaptic GABAB receptors. (C) In contrast, the output of basket cell terminals targeting the pyramidal cell is not modulated by GABAB receptor blockade.

Modulatory action of neurogliaform cells was not limited to homosynaptic downregulation of axon terminals. Heterosynaptic or paracrine effects of neurogliaform

cells on axons were also suggested by experiments in which we simultaneously recorded from three neurons consisting of a pyramidal cell to an interneuron connection (test EPSPs) and a neighboring neurogliaform cell activated 60 and 120 ms before the first and second test EPSP, respectively (Figure 5.3.3). Alternating trials with and without the activation of neurogliaform cells were recorded at a very low frequency (once in every two minutes) to avoid activity dependent loss of neurogliaform cell output (Tamas et al., 2003; Szabadics et al., 2007; Price et al., 2008). As expected from the high rate of coupling from neurogliaform cells to other neurons, the decay phase of the hyperpolarizing effect of the neurogliaform cells overlapped with the test EPSPs and corresponding input resistance changes of the neurons postsynaptic to the EPSPs were 10 ± 5 % and 4 ± 5 % measured at 60 and 120 ms after the spike in the neurogliaform cells, respectively. Accordingly, amplitudes of EPSPs and IPSPs reported below were corrected with the corresponding changes in input resistance. Switching on the action potential in the neurogliaform cells 60 ms before the spike in the pyramidal cell could not change the amplitude of the first test EPSPs ($n=5$; 98 ± 4 %) relative to control, i.e. when the spike in the neurogliaform cell was not elicited (Figure 5.3.3). This indicates that tonic inhibition through GABAA receptors potentially activated by neurogliaform cells (Pearce, 1993; Szabadics et al., 2007) did not interfere significantly with test EPSPs apart from contributing to input resistance changes. However, the neurogliaform cells were effective in decreasing the amplitude of the second test EPSPs timed 120 ms after the spike in the neurogliaform cells to 77 ± 5 % ($p < 0.03$) of control (Figure 5.3.3). The differential action of neurogliaform cell output on the first and the second test EPSPs induced changes in their paired pulse ratio (from 85 ± 20 to 69 ± 22 %, $p < 0.01$). Moreover, neurogliaform cells, activated 120 ms prior to test IPSPs triggered by other neurogliaform cells, successfully suppressed the amplitude of test IPSPs to 74 ± 4 % ($n=10$; $p < 0.02$) of control (Figure 5.3.3). This resulted in a peculiar activation sequence dependent cross modulation of IPSP amplitudes between closely located neurogliaform cells. Thus, the experiments on test EPSPs and IPSPs showed that individual action potentials in neurogliaform cells could suppress appropriately delayed responses elicited by other neurons.

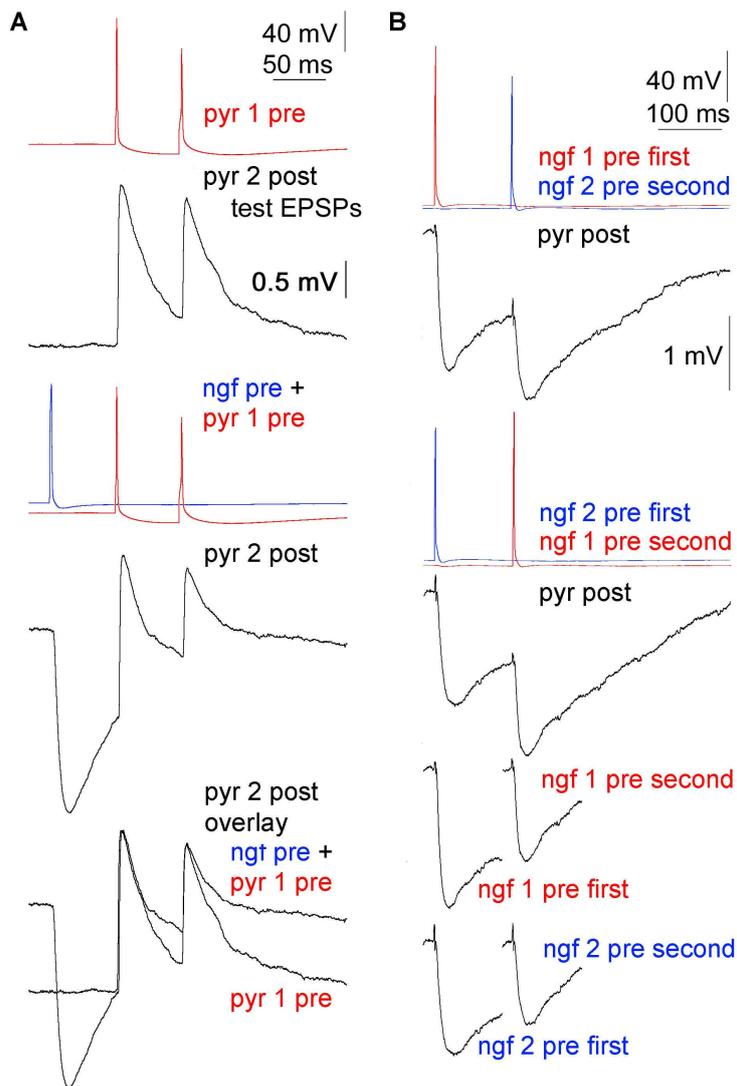


Figure 5.3.3 (A) Single neurogliaform cells heterosynaptically modulate unitary glutamatergic connections between linking other neurons. Simultaneous triple recording showing a pyramidal cell 1 (pyr 1) to pyramidal cell 1 (pyr 2) connection (test EPSPs) while switching the output of a neurogliaform cell on and off 60 ms before the first pyramidal spike. Preceding action potentials of the neurogliaform cell suppressed the amplitude of the second, but not the first EPSPs evoked by pyramidal cell 1. (B) Activation sequence dependent cross modulation of unitary IPSP amplitudes between closely located neurogliaform cells. Top, Activation of neurogliaform cell 1 or 2 (ngf 1 or ngf 2) followed by a spike in neurogliaform cell 2 or 1 resulted in sequential IPSPs in the postsynaptic pyramidal cell (pyr). Bottom, Comparison of the amplitude of preceding and following IPSPs elicited by neurogliaform cell 1 or 2 indicates effective suppression of follower IPSPs.

The metabotropic GABAB receptor mediated part of responses triggered by neurogliaform cells begins and peaks approximately 70 ms and 120 ms after the presynaptic action potential, respectively (Tamas et al., 2003; Price et al., 2008) suggesting that the differential regulation of test responses timed at various delays following action potentials in neurogliaform cells could depend on GABAB receptors. Accordingly, we investigated whether preceding activation of neurogliaform cells could modulate the test connections in the presence of the GABAB receptor blocker CGP35348 (40 μ M). The amplitudes of test EPSPs (n=3) or test IPSPs (n=10) under these conditions remained unchanged (101 \pm 4% and 98 \pm 8%, respectively) by switching on and off the spike in the neurogliaform cells showing that GABAB receptors were required for the heterosynaptic modulatory action of neurogliaform cells (Figure 5.3.4). These experiments also provide

further evidence that GABAA receptors, which remained functional in these experiments, are not sufficient for the suppression of test connections.

GABAB receptors are located on axon terminals as well as dendritic compartments of cortical neurons (Kulik et al., 2003), thus we investigated whether GABA released by neurogliaform cells acts on GABAB receptors located pre- or postsynaptically. We introduced the G-protein uncoupler N-ethyl-maleimide (100 μ M) (Shapiro et al., 1994) into the intracellular solution used for postsynaptic cells of test IPSPs in order to block the effect of GABAB receptors postsynaptically but not presynaptically. In spite of the broad spectrum blockade of G-protein coupled receptors in the postsynaptic cells, neurogliaform cells remained effective in suppressing the amplitude of test IPSPs to 77 ± 6 % of control ($n=6$; $p<0.04$). Furthermore, when examining possible modulatory effects of neurogliaform cells on presynaptic terminals of fast spiking basket cells without GABAB receptors, test IPSPs were not suppressed ($n=6$; 99 ± 4 % of control) showing that presynaptic GABAB receptors are sufficient to produce the modulatory effect of neurogliaform cells in the local microcircuit. All experiments presented above involved triple recordings of closely spaced (<100 μ m) neurons. Anatomical assessment of additional test connections with somata located outside the axonal cloud of the simultaneously recorded neurogliaform cell ($n=12$) provided further support in favor of presynaptic modulation (Figure 5.3.4). The area of overlap between the dendritic field of postsynaptic cells in test connections and the territory of neurogliaform axons did not correlate with the level of suppression in test responses ($R=-0.27$, $p<0.38$). However, only those test connections were suppressed ($n=8$ out of 12, 82 ± 9 %, $p<0.03$) in which potential synaptic sites mediating test responses were within the axonal cloud of neurogliaform cells ($n=8$) and the rest of test connections were not modulated ($n=4$, 102 ± 3 %). Thus, the modulatory effect of GABA released from neurogliaform cells appears confined to the axonal cloud and requires the presynaptic element of suppressed connections.

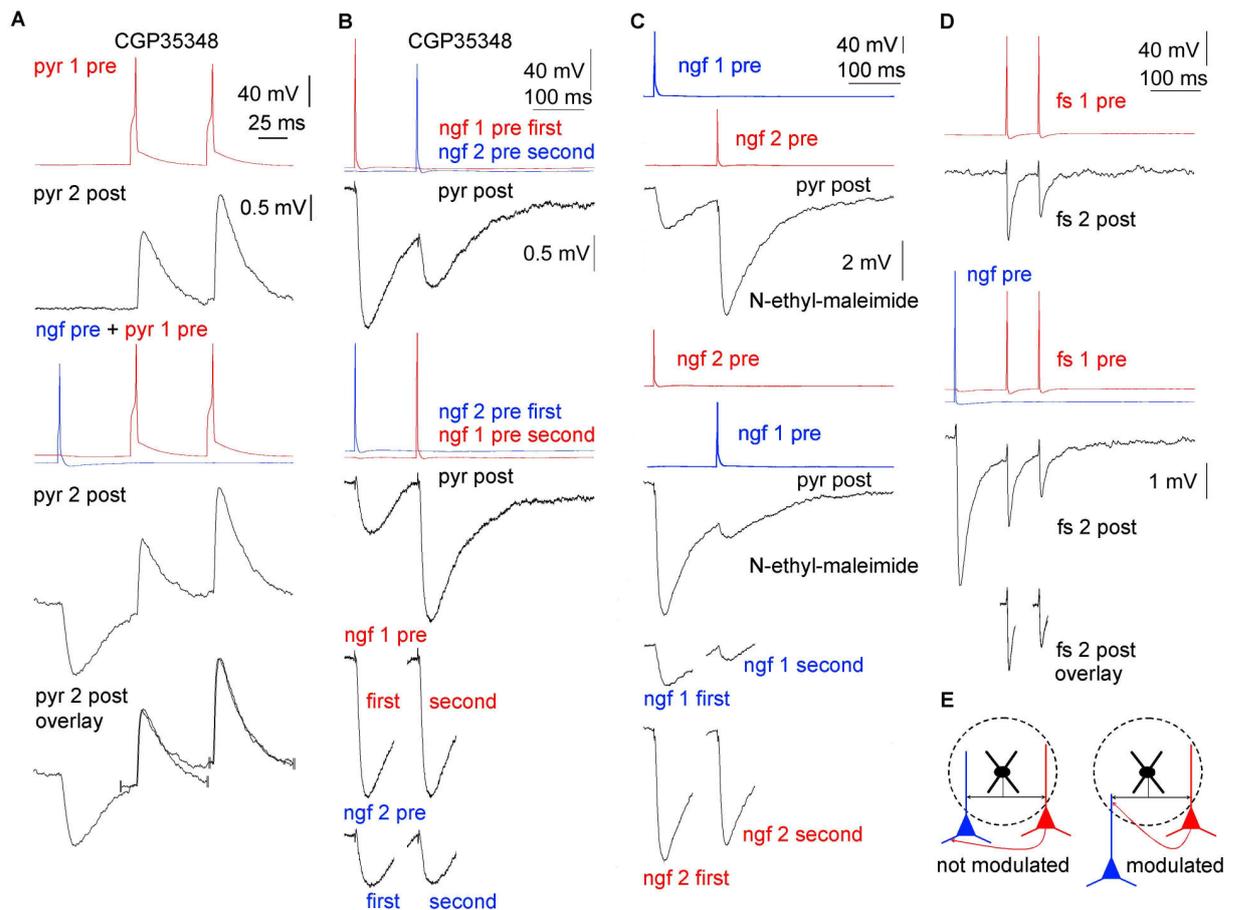


Figure 5.3.4 (A-B) The modulatory effect of neurogliaform cells requires GABAB receptor activation. **(A)** Testing the modulatory effect of a neurogliaform output on a pyramidal cell (pyr 1) to pyramidal cell (pyr 2) connection by switching the neurogliaform cell (ngf) on and off 60 ms before the first pyramidal spike. Preceding action potentials of the neurogliaform cell could not suppress EPSPs in the presence of the GABAB receptor antagonist CGP35348. **(B)** Activation of neurogliaform cell 1 or 2 (ngf 1 or ngf 2) followed by a spike in neurogliaform cell 2 or 1 resulted in sequential IPSPs in the postsynaptic pyramidal cell (pyr). Comparison of the amplitude of preceding and following IPSPs elicited by neurogliaform cell 1 or 2 indicates that the GABAB receptor antagonist CGP35348 prevents suppression of follower IPSPs. **(C-E)** Heterosynaptic suppression of synaptic response amplitude by neurogliaform output is targeted to presynaptic sites. **(C)** Responses of a postsynaptic pyramidal cell (pyr) filled with the G-protein uncoupler N-ethyl-maleimide to sequential activation of two neurogliaform cells (ngf 1 and ngf 2). Postsynaptic blockade of G-protein coupled GABAB receptors did not eliminate powerful suppression of follower IPSPs as shown by the comparison of the amplitude of preceding and following IPSPs elicited by ngf cell 1 or 2. **(D)** Presynaptic axon terminals of fast spiking basket cells are not modulated by GABAB receptors and, accordingly, preceding activation of a neurogliaform cell (ngf) fails to modulate the amplitude of IPSPs in a test connection linking a presynaptic and a postsynaptic fast spiking basket cells (fs 1 and fs 2, respectively). The three cells were also linked by electrical synapses as shown by spikelets before IPSPs in postsynaptic responses. **(E)** Neurogliaform cells (black) modulate synaptic responses only if the terminals (arrows) of presynaptic cells (red) of test connections reside within their axonal territory.

The experiments presented above suggest that single neurogliaform cells release GABA in ways which overcome the capacity of GABA reuptake mechanisms available. For further testing of this idea, we blocked the major neocortical plasma membrane GABA transporter (GAT-1) (Guastella et al., 1990) known to regulate the spillover of synaptically released GABA (Scanziani, 2000; Overstreet and Westbrook, 2003) to see if the

modulatory effect of neurogliaform cells could be enhanced. Adding the GAT-1 blocker NO711 (40 μ M) into the extracellular solution produced a profound modulatory effect of neurogliaform cells on test IPSPs (n=8; 59 ± 19 % of control, Figure 5.3.5). The heterosynaptic suppression of test connections during GABA reuptake blockade was significantly ($p<0.025$) enhanced compared to recordings without NO711 confirming our hypothesis that the amount of extrasynaptically available GABA released from neurogliaform cells contributes to the modulatory effect.

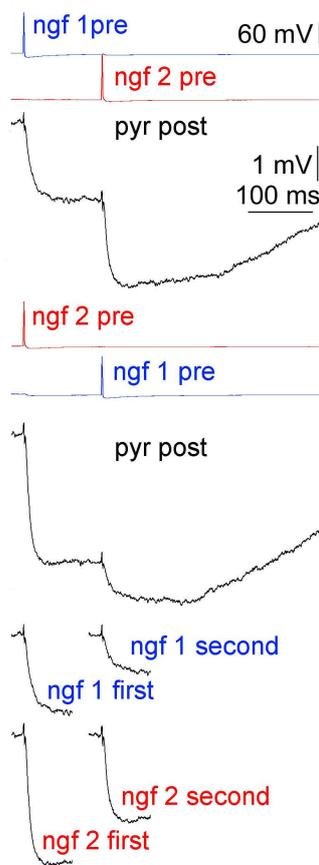


Figure 5.3.5 Blockade of GABA reuptake enhances the modulatory effect of neurogliaform cells. (A) Top and middle, Activation of neurogliaform cell 1 or 2 (ngf 1 or ngf 2) followed by a spike in neurogliaform cell 2 or 1 resulted in sequential IPSPs in the postsynaptic pyramidal cell (pyr). Bottom, Comparison of the amplitude of preceding and following IPSPs elicited by neurogliaform cell 1 or 2 indicates powerful suppression of follower IPSPs in the presence of the GABA transporter GAT-1 blocker NO711. NO711 significantly enhanced the suppression of postsynaptic potentials by neurogliaform cells.

Finally we asked if a different type of GABAergic interneuron could modulate the surrounding microcircuit similar to neurogliaform cells. We applied the same experimental regime probing the potential modulatory effect of fast spiking basket cells, but single spikes in these interneurons failed to modulate the amplitude of test EPSPs elicited by pyramidal cells in the axonal field of fast spiking basket cells (n=6, 97 ± 6 % relative to control, $p>0.34$; Figure 5.3.6). It was suggested that interneurons can reach extrasynaptic receptors when firing repeatedly (Isaacson et al., 1993; Destexhe and Sejnowski, 1995; Kim et al., 1997; Glykys and Mody, 2007), thus we tested if trains of action potentials in

fast spiking basket cells would suffice in modulating the test connections. Again, fast spiking basket cells firing several action potentials could not alter the amplitude of test EPSPs ($n=6$, $99\pm 8\%$ relative to control, $p>0.71$), not even if trains of spikes of fast spiking basket cells were combined with the application of the GABA reuptake blocker NO711 ($40\ \mu\text{M}$) on test EPSPs ($n=4$, $97\pm 9\%$ relative to control, $p>0.63$; Figure 5.3.6).

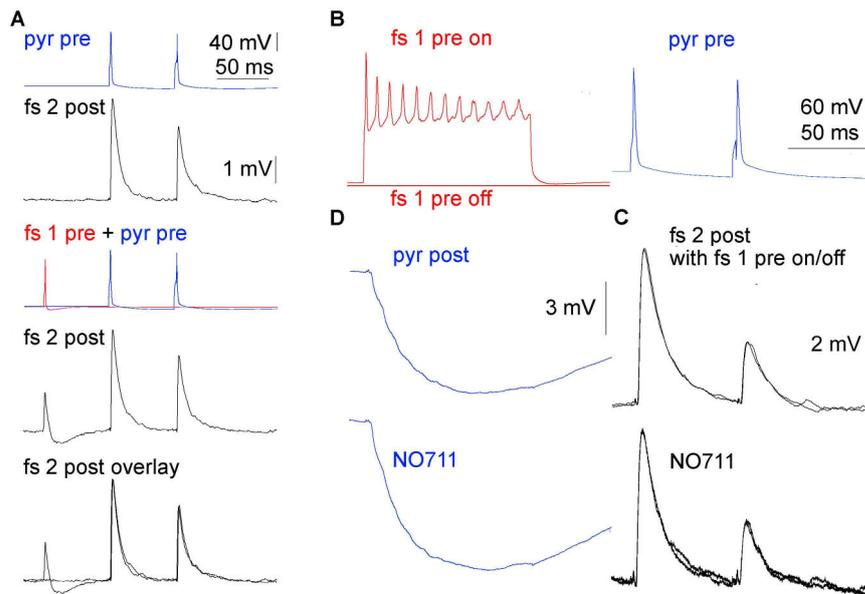


Fig. 5.3.6 Individual fast spiking basket cells are not capable of heterosynaptic modulation. **(A)** Simultaneous triple recording showing a pyramidal cell 1 (pyr 1) to fast spiking basket cell (fs 2) connection while switching the output of a presynaptic fast spiking basket cell (fs 1) on and off 60 ms before the first pyramidal spike. Preceding action potentials of the presynaptic fast spiking cell could not modulate

the amplitude of EPSPs evoked by pyramidal cell 1. **(B-D)** Trains of action potentials in single fast spiking basket cells are not sufficient for heterosynaptic modulation. **(B)** A train of action potentials in a fast spiking cell (fs 1) is followed by two presynaptic spikes in a pyramidal cell (pyr). **(C)** Top, EPSPs in a postsynaptic fast spiking cell (fs 2) triggered by the pyramidal cell show no amplitude change while switching on and off the train of spikes in the presynaptic fast spiking cell. Bottom, EPSP amplitudes remain unmodulated during blockade of GABA reuptake with NO711. **(D)** Postsynaptic IPSPs recorded in the pyramidal cell (pyr) elicited by the spike train in the presynaptic fast spiking cell (fs 1, as shown in panel B) ensure that the output of the presynaptic fast spiking cell was functional in normal extracellular solution (top) and during addition of NO711 (bottom).

Discussion

Unlike the rest of interneuron types characterized to date which form highly specific circuits in placing their synapses on somatodendritic compartments and the axon initial segment of postsynaptic cells (Freund and Buzsaki, 1996; Klausberger and Somogyi, 2008), neurogliaform cells follow a different strategy of spatial unspecificity and provide nonsynaptic input to the entire surface of target cells in addition to conventional synaptic junctions (Tamas et al., 2003). Neurogliaform cells are capable of flooding the volume of their axonal field with GABA in effective concentrations and target the overwhelming

majority of nearby neurons which, in turn, selectively express receptors sensitive to low concentrations of the neurotransmitter on their various compartments (Kulik et al., 2003; Kullmann et al., 2005; Guetg et al., 2009). Although presynaptic mechanisms producing the GABA cloud in neurogliaform axonal arborizations are not understood, they might involve a unique release machinery with an unconventional calcium dependence (Sakaba and Neher, 2003; Price et al., 2008). Solitary spikes in a single neurogliaform cell might replace the concerted action potentials of interneuron populations in modulating presynaptic terminals and postsynaptic domains expressing GABA receptors at certain operational states of the microcircuit (Barbour and Hausser, 1997; Mitchell and Silver, 2000; Scanziani, 2000; Overstreet and Westbrook, 2003; Glykys and Mody, 2007; Klausberger and Somogyi, 2008). Such modulation involves pyramidal and some GABAergic terminals targeting neurogliaform cells leading to retrograde self-regulation of afferents specific to this cell type. The spatial extent of neurogliaform axons (Tamas et al., 2003; Karube et al., 2004; Simon et al., 2005) suggest that solitary spikes in individual neurogliaform cells provide means for synchronized changes in the efficacy of synaptic connections in conjunction with regulating dendritic excitability (Perez-Garci et al., 2006) across a couple of hundred micrometers in the microcircuit. Further studies are required to determine the detailed temporal dynamics of neurogliaform cell activity induced changes in synaptic strength at the network level, but the long lasting suppressed or silent periods of neurogliaform terminals (Tamas et al., 2003; Price et al., 2008) are complementary to the slow activation and inactivation of GABAB receptors targeted pre- and postsynaptically. Although GABAA receptors are not sufficient in producing the heterosynaptic modulation of responses without the contribution of presynaptic GABAB receptors, neurogliaform cells are likely to be involved in supplying extracellular GABA levels required for GABAA receptor mediated tonic inhibition in the microcircuit (Farrant and Nusser, 2005; Kullmann et al., 2005; Glykys and Mody, 2007) also suggested by the combined phasic and tonic activation of GABAA receptors on postsynaptic dendrites in response to input from neurogliaform cells (Pearce, 1993; Szabadics et al., 2007). In conclusion, neurogliaform cells do not require synapses to produce long lasting and synchronized inhibitory responses in neurons located in the space intermingled by their axons. In addition to network hyperpolarization, single neurogliaform cells simultaneously provide widespread suppression of communication in the local circuit acting on axons formed by resident neurons or modulating terminals of long range projections at their arrival.

6. GENERAL DISCUSSION AND OUTLOOK

This thesis is focused on specific role that neurogliaform cells play in neocortical microcircuits. The background of my work was a recently published paper of our lab in which my colleagues found that single action potential of neurogliaform cells can evoke compound GABA_A and GABA_B receptor mediated slow inhibition in postsynaptic pyramidal cells. These cells are the only identified sources of this kind of inhibition so far. Thus, it is important to know whether interneurons are innervated by means of either electrical or chemical synapses by neurogliaform cells as well; what kind of mechanism underlies the capability of these cells to reach postsynaptic GABA_B receptor; whether presynaptic GABA_B receptors can also be activated by GABA released by these cells.

First, we have shown that neurogliaform cells establish homologous gap junctions with each other, which is common among interneurons (Galarreta and Hestrin, 1999; Gibson et al., 1999; Beierlein et al., 2000; Galarreta and Hestrin, 2001; Szabadics et al., 2001), because several other types of GABAergic cells are known to do this, including fast spiking basket cells (Galarreta and Hestrin, 1999, 2001), cannabinoid-1 receptor expressing irregular spiking interneurons (Galarreta et al., 2004), regular spiking nonpyramidal cells (Szabadics et al., 2001) and multipolar bursting cells (Blatow et al., 2003) as well. Besides, we shown that neurogliaform cells are capable of establishing heterologous gap junctions as well, that is electrical synapses with interneurons from different classes, which is very unique in neocortical networks. Neurogliaform cells establish homologous electrical connections with 50% rate of coupling and heterologous electrical connections with basket cells and regular spiking cells with 19-20% rate for interactions. Other studies provided proofs that neurogliaform cells are the most abundant cell type participating in heterologous gap junctional couplings (Zsiros and Maccaferri, 2005; Zsiros et al., 2007), besides - like in neocortex - they can also form homologous electrical connections in hippocampus (Price et al., 2005).

Some theoretical studies suggest that electrical couplings between different types of interneurons can lead to antiphase or asynchronous firing (Sherman and Rinzel, 1992; Chow and Kopell, 2000; Pfeuty et al., 2003, 2005; Pfeuty et al., 2007). Specifically, the ability of electrical coupling to synchronize the rhythm of spiking neurons depends on coupling strength of coupling and on frequency of firing rates. At low coupling strengths

and high firing rates the synchronized state is likely to be unstable, whereas at lower firing frequencies the synchronized and antisynchronized states are bistable. Regarding possible low firing rates of neurogliaform cells (Zhu et al., 2004), which is supported by our results on pronounced depression of their outputs, intensive gap junctional couplings of neurogliaform cells alone (especially if they are mainly dendritic) make the interconnected network susceptible to operate in asynchronous state (Sherman and Rinzel, 1992; Chow and Kopell, 2000). However, in majority of our recordings neurogliaform cells evoked IPSPs in cells which were innervated by electrical couplings by them. Modeling studies tell us that electrical couplings are prone to induce synchrony to greater extent when they are paired with strong inhibition, even if they hinder it in absence of inhibition (Chow and Kopell, 2000; Pfeuty et al., 2003, 2005; Pfeuty et al., 2007). This may be the way neurogliaform cells use to promote synchrony in coupled networks of interneurons. However, it is not known yet that what are the functional consequences (regarding synchrony) of pairing gap junctional potentials with slow and not fast inhibition. Besides, gap junctional couplings give possibility for participating cells to harmonize their metabolic state due to leakage of various factors through electrical synapses.

The second point of my results is in line with interpretation of previous paragraph. We found that similarly to pyramidal cells, interneurons responded with compound GABA_A and GABA_B receptor mediated slow inhibition to single action potential of neurogliaform cells. This finding is in correlation with results of other labs, because it is reported that these types of cells can establish slow inhibitory network with each other in CA1 hippocampal area (Price et al., 2005). We showed that other interneuron subtypes are also innervated by neurogliaform cells including regular spiking nonpyramidal cells, fast spiking basket and axoaxonic cells beyond other neurogliaform cells. The contribution of activation of GABA_B receptors in responses these cells were connection specific possible due to different availability of GABA_B receptors on various postsynaptic cells which can be underlied by different expression pattern (Martina et al., 2001) or various postsynaptic dependent spatial arrangements of synapses. It is well known that GABA_B receptors are expressed mainly extrasynaptically (Kulik et al., 2003; Kulik et al., 2006), activation of them therefore calls for leakage of GABA from synaptic cleft or other spillover effects. This spillover effects can be strongly modulated by spatial parameters of synapses. On the other hand, we could not find any classical synaptic structure between connected pairs of recorded neurogliaform cells and interneurons, however, electrical synapses are harder to find than chemical ones, nonetheless we could detect them between these pairs in several

cases. This also suggests existence of spillover transmission between neurogliaform cells and interneurons. There are some functional consequences of such a widespread slow inhibition. First, it can lower overall excitability and specifically dendritic excitability modulating powerfully electrogenesis in dendrites (Perez-Garci et al., 2006). Second, it can give way to various G protein related signaling mechanism having impact on dendritic operation.

Besides, these results provide the first simultaneous recordings from pairs and triplets of human neocortical neurons, and at the same time the first recordings from human neurogliaform cells and their innervated target neurons. These experiments revealed that functional role human neurogliaform cells can play in neocortical microcircuits is very similar to role that neurogliaform cells play in rodent cortical microcircuits. They are capable of eliciting GABA_A and GABA_B receptor mediated slow inhibition with single action potential of them in GABAergic interneurons and pyramidal cells as well. Moreover, we could detect human neurogliaform cells participating in heterologous gap junctional couplings revealing first signs of possible functional role of electrical synapses in human cortical operations.

The main finding of this dissertation is that GABA released by single action potential of neurogliaform cells can reach not only classical postsynaptic receptors but presynaptic GABA_B receptors as well. It is known that GABA_B receptors are expressed on presynaptic terminals (Charara et al., 2000; Lopez-Bendito et al., 2002; Ma et al., 2002; Kulik et al., 2003; Charara et al., 2004; Galvan et al., 2004; Lopez-Bendito et al., 2004; Porter and Nieves, 2004; Lacey et al., 2005; Perez-Garci et al., 2006; Price et al., 2008) and they can be activated by ambient level of GABA in extrasynaptic space (Isaacson, 1998; Aroniadou-Anderjaska et al., 2000; Shen and Johnson, 2001; Molyneaux and Hasselmo, 2002; Sakaba and Neher, 2003; Porter and Nieves, 2004; Kaneda and Kita, 2005; Moldavan et al., 2006; Thompson et al., 2006; Vigot et al., 2006; Alle and Geiger, 2007; Astori and Kohr, 2008; Price et al., 2008). Most of these works left unanswered the question that what the source of ambient GABA is (Isaacson, 1998; Shen and Johnson, 2001; Molyneaux and Hasselmo, 2002; Porter and Nieves, 2004; Moldavan et al., 2006) or presynaptic inhibitory mechanism in some of them requires sustained stimulation of cells which are sources of GABA (Kaneda and Kita, 2005; Alle and Geiger, 2007; Astori and Kohr, 2008).

Hippocampal neurogliaform cells are reported to innervated each other by compound GABA_A and GABA_B receptor mediated slow inhibition (Price et al., 2005; Price et al.,

2008), which transmission is downregulated by presynaptic GABA_B autoreceptor, but mechanism of this homosynaptic modulation remained unclear, because the existence of it was proved by bath application of GABA_B receptor antagonist. We showed that neocortical neurogliaform cells can modulate presynaptic terminals via GABA_B receptors both homosynaptically and heterosynaptically and heterosynaptic modulation takes place on both GABAergic and glutamatergic terminals. The revealed mechanism of this impact is based on volume transmission of GABA which is supported by the facts that blocking GABA transporters, which raises concentration of extrasynaptic GABA, enhances this effect and there are much more ectopic release sites on neurogliaform axons than release sites paired with classic postsynaptic structures. Such volume transmission plays significant but not main role in ability of neurogliaform cells to evoke slow GABA_A receptor mediated transients in postsynaptic pyramidal cells (Szabadics et al., 2007). Significant results of paper by Marco Capogna's lab regarded processes in presynaptic terminals mediated by presynaptic GABA_B receptors (Price et al., 2008). They found that activation of presynaptic GABA_B receptors by baclofen could modulate unitary IPSCs but did not alter Ca²⁺-signals measured by two-photon Ca²⁺-imaging in axons of recorded presynaptic neurogliaform cells in contrast with other interneurons. One possible mechanism is proposed in case of glutamatergic terminals, that is G protein activated cAMP dependent hindering of recruitment of vesicles in presynaptic terminals which did not permit the increase of Ca²⁺-concentration to stimulate vesicle priming (Sakaba and Neher, 2003). It is remained to be investigated whether this mechanism takes place in our case, as well.

It would be interesting to find out that what the key features of neurogliaform cells make them suitable for sources of such ambient level of GABA that can activate extrasynaptic receptors in surroundings of terminals. Possible underlying mechanism can be 1. peculiar arrangements of synapses of neurogliaform cells that favors escaping GABA from synaptic cleft; 2. greater amount of GABA released from terminals of neurogliaform cells; 3. verified numerous ectopic release sites of these cells; 4. denser axonal arborisation of these cells than in case of other interneurons.

It is also remained to be unrevealed whether human neurogliaform cells are capable of modulating presynaptically robust propagating of activation in human neocortical microcircuits detected recently (Molnar et al., 2008).

These effects of neurogliaform cells can also be mediated by neuropeptide Y (NPY), which is known to be expressed by these cells from previous studies (Kawaguchi and

Kubota, 1997; Price et al., 2005) and from our preliminary immunocytochemical experiments which detected NPY in neurogliaform cells eliciting slow IPSPs in pyramidal neurons. NPY was shown to modulate excitatory and inhibitory transmission in the cerebral cortex presumably at presynaptic sites (Bacci et al., 2002) therefore the action of NPY possibly released by neurogliaform cells could contribute to the modulatory role of this cell type in local networks.

7. ÖSSZEFOGLALÁS

Az agykérgünket felépítő több tíz milliárdnyi neuron igen erőteljes morfológiai és funkcióbeli változatosságot mutat. Főbb sejttípusai a glutamátot felszabadító, serkentő hatású, a kéreg fő kimenetét adó piramissejtek és a GABA-t felszabadító, gátló hatású, a piramissejtek működését befolyásoló interneuronok. Bár a piramissejtek is nagyfokú változatosságot mutatnak, ennél jóval nagyobb morfológiai és funkcióbeli heterogenitás jellemzi az interneuronok populációját. Talán a legfőbb különbség az egyes interneuron-típusok között abban rejlik, hogy posztszinaptikus célsejtjeik melyik doménjét idegzik be. Ennek megfelelően beszélhetünk dendritcélzó, szomatikus gátlást közvetítő és közvetlenül a piramissejtek axon iniciális szegmentumát beidegző interneuronokról.

Jelen disszertáció egy dendritcélzó interneurontípus, a neurogliaform sejt azon - sok tekintetben különleges - szerepéről szól, amit az alapvető agykérgi hálózatbeli működésekben betölt. A következőkben részletezett eredmények kiindulási alapját laborunk egy korábbi munkája képezi, melyben kollégáim azt mutatták ki, hogy a neurogliaform sejtek egyetlen akciós potenciálja GABA_A és GABA_B receptor mediált lassú gátlást váltott ki posztszinaptikus piramissejteken. Ezek a sejtek az eddig ismert egyetlen forrásai a kérgi lassú gátlásnak. Ezek alapján adódott a kérdés, hogy vajon piramissejteken kívül posztszinaptikus interneuronokat is beidegeznek-e (akár elektromos szinapszisokkal is) neurogliaform sejtek; emellett fontos tudnunk, hogy milyen mechanizmus teszi képessé ezeket a sejteket arra, hogy az általuk felszabadított GABA posztszinaptikus GABA_B receptorokat aktiváljon; végül pedig felmerül az a kérdés is, hogy vajon preszinaptikus GABA_B receptorok is aktiválódhatnak-e neurogliaform sejtek által felszabadított GABA hatására.

Először is kimutattuk, hogy neurogliaform sejtek homológ rés kapcsolatokat létesítenek egymás között, ez más interneuronokra is jellemző (Galarreta and Hestrin, 1999; Gibson et al., 1999; Beierlein et al., 2000; Galarreta and Hestrin, 2001; Szabadics et al., 2001), úgy is mint gyorsan tüzelő kosársejtekre, cannabinoid-1 receptort expresszáló szabálytalanul tüzelő sejtekre, szabályosan tüzelő interneuronokra és multipoláris bürsztölő sejtekre. Emellett vizsgálataink szerint a neurogliaform sejtek képesek heterológ elektromos szinapszisokat is létesíteni más típusú interneuronokkal is, ez viszont már egyedülálló tulajdonsága ezen sejteknek. Összes egymás közti kapcsolataik 50%-ában

képeztek homológ elektromos szinapszisokat, és összes kosársejtekkel és szabályosan tüzelő interneuronokkal létesített kapcsolataik 18-20%-a bizonyult heterológ elektromos szinapszissal. Más tanulmányok megerősítették, hogy a neurogliaform sejtek a leggyakoribb sejtek, melyek heterológ elektromos szinapszisokban vesznek részt, emellett a neokortexen kívül hippocampus-ban is jelentettek homológ réskapcsolatokat neurogliaform sejtek között.

Elméleti tanulmányok azt sugallják, hogy különböző interneuronok között létesített elektromos kapcsolatok antifázikus vagy aszinkron tüzeléshez vezethetnek (Sherman and Rinzel, 1992; Chow and Kopell, 2000; Pfeuty et al., 2003, 2005; Pfeuty et al., 2007). Pontosabban, az elektromos kapcsolatok azon képessége, hogy a résztvevő neuronok tüzelését szinkronizálja a kapcsolat erősségétől és a tüzelési frekvenciától együttesen függ. Alacsony kapcsoltsági együttható és magas tüzelési frekvencia esetén a szinkronizált állapot kevésbé stabil, míg alacsony tüzelési frekvenciákon a szinkronizált és antiszinkronizált állapotok lehetnek stabilak. Tekintve a neurogliaform sejtek valószínű alacsony tüzelési frekvenciáját (Zhu et al., 2004), amit a neurogliaform sejtek kimenetének erőteljes depresszióját mutató eredményeink is megerősítenek, önmagában ezen sejtek intenzív heterológ elektromos kapcsolatai (különösen azok döntően dendritikus volta) a kapcsolt hálózatot aszinkron állapotú működésmódra hajlamosíthatják. Ugyanakkor az esetek többségében a neurogliaform sejtek gátló posztzinaptikus potenciálok is keltnek azokban a sejtekben, amelyekkel elektromos kapcsolatokat is létesítenek. Modellezési vizsgálatok azt mutatják, hogy ilyen esetekben ezek a réskapcsolatok nagyobbfokú szinkronitást képesek előidézni (Sherman and Rinzel, 1992; Chow and Kopell, 2000). Emellett az elektromos szinapszisok azt is lehetővé teszik a résztvevő sejtek számára, hogy metabolikus állapotukat is valamelyest szinkronizálják azáltal, hogy számos metabolit számára átjárhatóak.

Egy másik fontos következtetése a disszertációnak az, hogy a piramissejtekhez hasonlóan a posztzinaptikus interneuronok is $GABA_A$ és $GABA_B$ receptor mediált lassú gátlással válaszolnak közeli neurogliaform sejtek egyetlen akciós potenciáljának hatására. Más laborok eredményei alátámasztják ezt, mert korábban kimutatták ezekről a sejtekről, hogy egymást lassú gátlással idegzik be a hippocampus CA1 régiójában (Price et al., 2005). A mi eredményeink annyiban újak ehhez képest, hogy neurogliaform sejteken kívül még más interneuronok is: szabályosan tüzelő nempiramissejtek, Martinotti sejtek és részben gyorsan tüzelő kosár- és axoaxonikus sejtek is lassú gátlást kapnak preszinaptikus neurogliaform sejtek felől. A $GABA_B$ receptor mediált komponens amplitúdója

kapcsolatspecifikusnak bizonyult feltehetően a különböző posztsinaptikus GABA_B receptor expresszióinak vagy az egyes szinapszisok eltérő téri elrendeződésének köszönhetően. Az ismert, hogy a GABA_B receptorok jobbra extraszinaptikus kifejeződésűek (Kulik et al., 2003; Kulik et al., 2006), emiatt aktivációjukhoz szükséges a felszabadított GABA kiszivárgása a szinaptikus résből vagy más diffúzió alapú hatások (spillover). Ezeket jelentősen befolyásolják a szinapszis térbeli jellemzői. Másrészt nem találtunk egyetlen egy esetben sem klasszikus kémiai szinapszist neurogliaform sejtek és interneuronok között, míg a nehezebben fellelhető elektromos szinapszisokat elektronmikroszkópos vizsgálatok során sikerült kollégáimnak megtalálni. Ez szintén spillover hatások jelenlétére utal neurogliaform és interneuronok kapcsolataiban. Ha az ilyen széleskörű lassú gátlás funkcionális következményeit tekintjük, először is a serkentetőség általános csökkenését okozhatja, másrészt ezen belül a dendritik serkentetőségét, amivel erőteljes modulációját okozza a dendritikus elektrogenézisnek (Perez-Garci et al., 2006). Emellett számos eddig feltáratlan G fehérjékkel kapcsolatos szignalizációs mechanizmust képes elindítani.

Végül de nem utolsósorban ezen eredmények szolgáltak példaként az első szimultán elvezetésekre humán kérgi sejtek párjaiból és tripleteiből, és ezen belül neurogliaform sejtekből és posztsinaptikus célsejtjeikből. Ezek a kísérletek arra szolgáltattak bizonyítékot, hogy a neurogliaform sejtek által neokortikális hálózatokban betöltött funkcionális szerep nagyon hasonló ahhoz, amit rágcésélők kérgi neurogliaform sejtjei betöltenek: GABA_A és GABA_B receptor mediált lassú gátlást keltenek egyetlen akciós potenciállal posztsinaptikus piramissejteken és interneuronokon. Emellett kimutattuk, hogy humán neurogliaform sejtek heterológ elektromos szinapszisokban is részt vesznek patkánybeli társaikhoz hasonlóan.

Ezen disszertáció legfőbb eredménye az, hogy kimutattuk, hogy a neurogliaform sejtek egyetlen akciós potenciálja által felszabadított GABA neurotranszmitter molekulák képesek nemcsak a klasszikus posztsinaptikus receptoraikat elérni, hanem ezen túlmenően a preszinaptikus GABA_B receptorokat is. Az ismert, hogy ezen receptorokat a preszinaptikus terminálisok is kifejezik (Charara et al., 2000; Lopez-Bendito et al., 2002; Ma et al., 2002; Kulik et al., 2003; Charara et al., 2004; Galvan et al., 2004; Lopez-Bendito et al., 2004; Porter and Nieves, 2004; Lacey et al., 2005; Perez-Garci et al., 2006; Price et al., 2008), és hogy aktivációjukhoz az extraszinaptikus tér megfelelő nagyságú GABA koncentrációja szükséges (Isaacson, 1998; Aroniadou-Anderjaska et al., 2000; Shen and Johnson, 2001; Molyneaux and Hasselmo, 2002; Sakaba and Neher, 2003; Porter and

Nieves, 2004; Kaneda and Kita, 2005; Moldavan et al., 2006; Thompson et al., 2006; Vigot et al., 2006; Alle and Geiger, 2007; Astori and Kohr, 2008; Price et al., 2008). Ugyanakkor korábbi tanulmányok azt a kérdést megválaszolatlanul hagyták, hogy mi a forrása ezen extraszinaptikus GABA-nak. Más tanulmányok pedig azzal érveltek (Isaacson, 1998; Shen and Johnson, 2001; Molyneaux and Hasselmo, 2002; Porter and Nieves, 2004; Moldavan et al., 2006), hogy a preszinaptikus gátlás létrejöttéhez szükséges GABA koncentráció GABAerg interneuronok nagy frekvenciájú hosszan tartó tüzelésével érhető el (Kaneda and Kita, 2005; Alle and Geiger, 2007; Astori and Kohr, 2008).

Hippocampális neurogliaform sejtekről leírták, hogy egymást GABA_A és GABA_B receptor mediált lassú gátlással idegzik be, mely kapcsolatok modulálhatóak GABA_B autoreceptorokkal (Price et al., 2005; Price et al., 2008), de a mechanizmus – a GABA forrása – itt is tisztázatlan maradt, mert ebben az esetben is GABA_B receptor antagonistá használata szolgált bizonyítékkal. Ezen túlmenően a mi kísérleteink azt is mutatják, hogy neokortikális neurogliaform sejtek képesek gátolni a környező preszinaptikus terminálisokat preszinaptikus GABA_B receptorok segítségével mind homoszinaptikusan mind heteroszínaptikusan, azaz saját terminálisokat és más sejtek terminálisait, köztük GABAerg illetve GABAerg terminálisokat egyaránt. A leírt mechanizmus a GABA diffúzió alapú térfogati transzmissziója, melyet azok a tények is alátámasztanak, amely szerint a GABA transzporter molekulák gátlása, amely növeli az extraszinaptikus GABA koncentrációt, fokozza ezt a hatást, és amely szerint a neurogliaform sejtek axonjai jóval több ektópikus transzmitter-felszabadulási helyet tartalmaznak mint olyan felszabadulási helyet, amelyet klasszikus poszt-szinaptikus struktúra kísér. Az ilyen térfogati transzmisszió részt vesz, bár nem kizárólagos szerepet játszik egy korábban publikált jelenségben, amely szerint a neurogliaform sejtek képesek lassú GABA_A-áramokat kelteni poszt-szinaptikus piramis-sejteken (Szabadics et al., 2007). Egy másik fontos eredmény szerint a preszinaptikus GABA_B receptorok általi moduláció neurogliaform sejtek terminálisán szamben más terminálisokkal nem változtatja meg preszinaptikus Ca²⁺-jeleket (Price et al., 2008). Egy lehetséges magyarázó mechanizmus serkentő terminálisok esetén merült fel, mely szerint a terminálisban lévő vezikulák toborzásának G fehérje aktivált cAMP-függő gátlása tehető felelőssé az észlelt hatásért (Sakaba and Neher, 2003). További kutatások kellenek ahhoz, hogy meggyőződjünk arról, hogy neurogliaform sejtek terminálisán is ez a helyzet.

Érdekes lenne megvizsgálni azt is, hogy milyen specifikus jellemzők teszik lehetővé a neurogliaform sejtek számára egy olyan hatásos extraszinaptikus GABA koncentráció

létrehozását, amely mindenféle extraszinaptikus (dendritikus és axonális egyaránt) GABA_B receptort aktiválni tud. Lehetséges mechanizmusok lehetnek: 1. neurogliaform sejtek szinapszisainak különleges térbeli elrendeződése, mely kedvez a GABA kidiffundálásának a szinaptikus résből; 2. nagyobb a neurogliaform sejtek általa felszabadított GABA mennyisége; 3. igazolt számos ektópikus felszabadulási hely neurogliaform sejtek axonján; 4. sűrűbb axonális felhő igazolt megléte.

Emellett az is tisztázásra vár, hogy vajon a humán neurogliaform sejtek képesek-e hasonló térfogati transzmisszióval preszinaptikusan gátolni a humán kérgi hálózatokra jellemző erőteljes aktivációs hullámok terjedését (Molnar et al., 2008).

A neurogliaform sejtek ilyen hatásai ugyanakkor felvetik azt a lehetőséget is, hogy esetleg ezen sejtek képesek a GABA-val együtt neuropeptid-Y-t (NPY) is felszabadítani (Kawaguchi and Kubota, 1997; Price et al., 2005), melyeket előzetes immunhisztokémiai vizsgálataink szerint expresszálnak. Az NPY-ról ismert, hogy képes az agykéregben a serkentő és gátló neurotranszmissziót gátolni feltételezhetően preszinaptikus terminálisokon hatva (Bacci et al., 2002), ezért a neurogliaform sejtek által feltételezhetően felszabadított NPY-molekulák hozzájárulnak ezen sejtek preszinaptikus moduláló hatásához.

8. ACKNOWLEDGEMENT

First of all I would like to thank my supervisor Dr. Gábor Tamás for giving me the possibility to join his research group and work with him. During the last years, he has always taught me the best techniques and his stimulating suggestions helped me to find the strict scientific way of thinking. Many thanks to the members of Gábor's laboratory. Andrea Lőrincz, Anna Simon, Éva Tóth, Rita Báldi, Eszter Boldog, Judith Baka, Anikó Tóth, János Szabadics, Gábor Molnár, Miklós Füle, Gergely Komlósi, Csaba Varga NF, Sándor Lovas, Tamás Cseppentő, Márton Rózsa for the friendly and inspiring environment. Anna, Éva and Gergő performed the anatomical experiments presented in this dissertation.

I'm always proud to be the scholar of Boehringer Ingelheim Foundation during my Ph.D. period. This scholarship, besides the enormous financial support, gave me more motivation to be in accordance with their confidence and expectations. I wish to thank the late Hermann Fröhlich, the former director of the BIF, Monika Beutelspacher, the secretary of the BIF and Claudia Walters, present director of the BIF for their help and kindness.

I would like to thank all the members of Department of Physiology, Anatomy and Neuroscience in University of Szeged.

I feel deep gratitude for my parents and for my sister, Zsanett. The special spirit of my family still provides a persistent inspiration for my life and work.

Especially, I would like to give my special thanks to my girlfriend, Andi whose love enabled me to complete this work.

9. REFERENCES

- Agnati LF, Leo G, Zanardi A, Genedani S, Rivera A, Fuxe K, Guidolin D (2006) Volume transmission and wiring transmission from cellular to molecular networks: history and perspectives. *Acta Physiol (Oxf)* 187:329-344.
- Alle H, Geiger JR (2007) GABAergic spill-over transmission onto hippocampal mossy fiber boutons. *J Neurosci* 27:942-950.
- Amitai Y, Gibson JR, Beierlein M, Patrick SL, Ho AM, Connors BW, Golomb D (2002) The spatial dimensions of electrically coupled networks of interneurons in the neocortex. *J Neurosci* 22:4142-4152.
- Aroniadou-Anderjaska V, Zhou FM, Priest CA, Ennis M, Shipley MT (2000) Tonic and synaptically evoked presynaptic inhibition of sensory input to the rat olfactory bulb via GABA(B) heteroreceptors. *J Neurophysiol* 84:1194-1203.
- Ashwell KW, Phillips JM (2006) The Anterior Olfactory Nucleus and Piriform Cortex of the Echidna and Platypus. *Brain Behav Evol* 67:203-227.
- Astori S, Kohr G (2008) Sustained granule cell activity disinhibits juvenile mouse cerebellar stellate cells through presynaptic mechanisms. *J Physiol* 586:575-592.
- Asztely F, Erdemli G, Kullmann DM (1997) Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. *Neuron* 18:281-293.
- Avendano C, Umbriaco D, Dykes RW, Descarries L (1996) Acetylcholine innervation of sensory and motor neocortical areas in adult cat: a choline acetyltransferase immunohistochemical study. *J Chem Neuroanat* 11:113-130.
- Bacci A, Huguenard JR, Prince DA (2002) Differential modulation of synaptic transmission by neuropeptide Y in rat neocortical neurons. *Proc Natl Acad Sci U S A* 99:17125-17130.
- Barbour B, Hausser M (1997) Intersynaptic diffusion of neurotransmitter. *Trends Neurosci* 20:377-384.
- Beaudet A, Descarries L (1978) The monoamine innervation of rat cerebral cortex: synaptic and nonsynaptic axon terminals. *Neuroscience* 3:851-860.
- Beierlein M, Gibson JR, Connors BW (2000) A network of electrically coupled interneurons drives synchronized inhibition in neocortex. *Nat Neurosci* 3:904-910.
- Benardo LS (1994) Separate activation of fast and slow inhibitory postsynaptic potentials in rat neocortex in vitro. *J Physiol* 476:203-215.
- Bennett MV, Zukin RS (2004) Electrical coupling and neuronal synchronization in the Mammalian brain. *Neuron* 41:495-511.
- Berman NJ, Douglas RJ, Martin KA (1992) GABA-mediated inhibition in the neural networks of visual cortex. *Prog Brain Res* 90:443-476.
- Blatow M, Rozov A, Katona I, Hormuzdi SG, Meyer AH, Whittington MA, Caputi A, Monyer H (2003) A novel network of multipolar bursting interneurons generates theta frequency oscillations in neocortex. *Neuron* 38:805-817.
- Buhl DL, Harris KD, Hormuzdi SG, Monyer H, Buzsaki G (2003) Selective impairment of hippocampal gamma oscillations in connexin-36 knock-out mouse in vivo. *J Neurosci* 23:1013-1018.
- Buhl EH, Halasy K, Somogyi P (1994) Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature* 368:823-828.

- Calver AR, Davies CH, Pangalos M (2002) GABA(B) receptors: from monogamy to promiscuity. *Neurosignals* 11:299-314.
- Cauli B, Porter JT, Tsuzuki K, Lambolez B, Rossier J, Quenet B, Audinat E (2000) Classification of fusiform neocortical interneurons based on unsupervised clustering. *Proc Natl Acad Sci U S A* 97:6144-6149.
- Cauli B, Audinat E, Lambolez B, Angulo MC, Ropert N, Tsuzuki K, Hestrin S, Rossier J (1997) Molecular and physiological diversity of cortical nonpyramidal cells. *J Neurosci* 17:3894-3906.
- Charara A, Heilman TC, Levey AI, Smith Y (2000) Pre- and postsynaptic localization of GABA(B) receptors in the basal ganglia in monkeys. *Neuroscience* 95:127-140.
- Charara A, Galvan A, Kuwajima M, Hall RA, Smith Y (2004) An electron microscope immunocytochemical study of GABA(B) R2 receptors in the monkey basal ganglia: a comparative analysis with GABA(B) R1 receptor distribution. *J Comp Neurol* 476:65-79.
- Chow CC, Kopell N (2000) Dynamics of spiking neurons with electrical coupling. *Neural Comput* 12:1643-1678.
- Chu Z, Galarreta M, Hestrin S (2003) Synaptic interactions of late-spiking neocortical neurons in layer 1. *J Neurosci* 23:96-102.
- Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378:75-78.
- Cobb SR, Halasy K, Vida I, Nyiri G, Tamas G, Buhl EH, Somogyi P (1997) Synaptic effects of identified interneurons innervating both interneurons and pyramidal cells in the rat hippocampus. *Neuroscience* 79:629-648.
- Connors BW, Long MA (2004) Electrical synapses in the mammalian brain. *Annu Rev Neurosci* 27:393-418.
- Connors BW, Benardo LS, Prince DA (1983) Coupling between neurons of the developing rat neocortex. *J Neurosci* 3:773-782.
- Connors BW, Benardo LS, Prince DA (1984) Carbon dioxide sensitivity of dye coupling among glia and neurons of the neocortex. *J Neurosci* 4:1324-1330.
- Contant C, Umbriaco D, Garcia S, Watkins KC, Descarries L (1996) Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. *Neuroscience* 71:937-947.
- Dawson TM, Dawson VL, Snyder SH (1994) Molecular mechanisms of nitric oxide actions in the brain. *Ann N Y Acad Sci* 738:76-85.
- Deans MR, Gibson JR, Sellitto C, Connors BW, Paul DL (2001) Synchronous activity of inhibitory networks in neocortex requires electrical synapses containing connexin36. *Neuron* 31:477-485.
- DeFelipe J (1993) Neocortical neuronal diversity: chemical heterogeneity revealed by colocalization studies of classic neurotransmitters, neuropeptides, calcium-binding proteins, and cell surface molecules. *Cereb Cortex* 3:273-289.
- DeFelipe J, Fairen A (1982) A type of basket cell in superficial layers of the cat visual cortex. A Golgi-electron microscope study. *Brain Res* 244:9-16.
- Demb JB, Pugh EN (2002) Connexin36 forms synapses essential for night vision. *Neuron* 36:551-553.
- Descarries L, Aznavour N, eds (2003) Diffuse (volume) transmission. Amsterdam: Elsevier Science.
- Descarries L, Beaudet A, Watkins KC (1975) Serotonin nerve terminals in adult rat neocortex. *Brain Res* 100:563-588.
- Descarries L, Watkins KC, Lapiere Y (1977) Noradrenergic axon terminals in the cerebral cortex of rat. III. Topometric ultrastructural analysis. *Brain Res* 133:197-222.

- Descarries L, Watkins KC, Garcia S, Beaudet A (1982) The serotonin neurons in nucleus raphe dorsalis of adult rat: a light and electron microscope radioautographic study. *J Comp Neurol* 207:239-254.
- Destexhe A, Sejnowski TJ (1995) G protein activation kinetics and spillover of gamma-aminobutyric acid may account for differences between inhibitory responses in the hippocampus and thalamus. *Proc Natl Acad Sci U S A* 92:9515-9519.
- Dutar P, Nicoll RA (1988) A physiological role for GABAB receptors in the central nervous system. *Nature* 332:156-158.
- Fairen A, DeFelipe J, Regidor J (1984) Nonpyramidal neurons: general account. New York: Plenum Press.
- Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci* 6:215-229.
- Feldman ML, Peters A (1978) The forms of non-pyramidal neurons in the visual cortex of the rat. *J Comp Neurol* 179:761-793.
- Fox CA, Rafols JA (1971) Observations on the oligodendroglia in the primate striatum. Are they Ramon y Cajal's "dwarf" or "neurogliaform" neurons? *J Hirnforsch* 13:331-340.
- Freund TF, Buzsaki G (1996) Interneurons of the hippocampus. *Hippocampus* 6:347-470.
- Fritschy JM, Brunig I (2003) Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacol Ther* 98:299-323.
- Fukuda T, Kosaka T (2003) Ultrastructural study of gap junctions between dendrites of parvalbumin-containing GABAergic neurons in various neocortical areas of the adult rat. *Neuroscience* 120:5-20.
- Gabbott PL, Bacon SJ (1996) Local circuit neurons in the medial prefrontal cortex (areas 24a,b,c, 25 and 32) in the monkey: I. Cell morphology and morphometrics. *J Comp Neurol* 364:567-608.
- Gabbott PL, Dickie BG, Vaid RR, Headlam AJ, Bacon SJ (1997) Local-circuit neurones in the medial prefrontal cortex (areas 25, 32 and 24b) in the rat: morphology and quantitative distribution. *J Comp Neurol* 377:465-499.
- Galarreta M, Hestrin S (1999) A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature* 402:72-75.
- Galarreta M, Hestrin S (2001) Spike transmission and synchrony detection in networks of GABAergic interneurons. *Science* 292:2295-2299.
- Galarreta M, Erdelyi F, Szabo G, Hestrin S (2004) Electrical coupling among irregular-spiking GABAergic interneurons expressing cannabinoid receptors. *J Neurosci* 24:9770-9778.
- Galvan A, Charara A, Pare JF, Levey AI, Smith Y (2004) Differential subcellular and subsynaptic distribution of GABA(A) and GABA(B) receptors in the monkey subthalamic nucleus. *Neuroscience* 127:709-721.
- Gibson JF, Beierlein M, Connors BW (1999) Two networks of electrically coupled inhibitory neurons in neocortex. *Nature* 402:75-79.
- Gilbert CD, Wiesel TN (1979) Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. *Nature* 280:120-125.
- Giustizieri M, Bernardi G, Mercuri NB, Berretta N (2005) Distinct mechanisms of presynaptic inhibition at GABAergic synapses of the rat substantia nigra pars compacta. *J Neurophysiol* 94:1992-2003.
- Glykys J, Mody I (2007) Activation of GABAA receptors: views from outside the synaptic cleft. *Neuron* 56:763-770.

- Glykys J, Mann EO, Mody I (2008) Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? *J Neurosci* 28:1421-1426.
- Goldberg JH, Tamas G, Yuste R (2003a) Ca²⁺ imaging of mouse neocortical interneurone dendrites: Ia-type K⁺ channels control action potential backpropagation. *J Physiol* 551:49-65.
- Goldberg JH, Yuste R, Tamas G (2003b) Ca²⁺ imaging of mouse neocortical interneurone dendrites: contribution of Ca²⁺-permeable AMPA and NMDA receptors to subthreshold Ca²⁺dynamics. *J Physiol* 551:67-78.
- Goldberg JH, Tamas G, Aronov D, Yuste R (2003c) Calcium microdomains in aspiny dendrites. *Neuron* 40:807-821.
- Goldman-Rakic PS, Lidow MS, Smiley JF, Williams MS (1992) The anatomy of dopamine in monkey and human prefrontal cortex. *J Neural Transm Suppl* 36:163-177.
- Guastella J, Nelson N, Nelson H, Czyzyk L, Keynan S, Miedel MC, Davidson N, Lester HA, Kanner BI (1990) Cloning and expression of a rat brain GABA transporter. *Science* 249:1303-1306.
- Guetg N, Seddik R, Vigot R, Turecek R, Gassmann M, Vogt KE, Brauner-Osborne H, Shigemoto R, Frotscher M, Kulik A, Bettler B (2009) The GABAB1a Isoform Mediates Heterosynaptic Depression at Hippocampal Mossy Fiber Synapses. *J Neurosci* 29:1414-1423.
- Guldenagel M, Ammermuller J, Feigenspan A, Teubner B, Degen J, Sohl G, Willecke K, Weiler R (2001) Visual transmission deficits in mice with targeted disruption of the gap junction gene connexin36. *J Neurosci* 21:6036-6044.
- Gulyas AI, Miles R, Hajos N, Freund TF (1993) Precision and variability in postsynaptic target selection of inhibitory cells in the hippocampal CA3 region. *Eur J Neurosci* 5:1729-1751.
- Gupta A, Wang Y, Markram H (2000) Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. *Science* 287:273-278.
- Gutnick MJ, Prince DA (1981) Dye coupling and possible electrotonic coupling in the guinea pig neocortical slice. *Science* 211:67-70.
- Halasy K, Buhl EH, Lorinczi Z, Tamas G, Somogyi P (1996) Synaptic target selectivity and input of GABAergic basket and bistratified interneurons in the CA1 area of the rat hippocampus. *Hippocampus* 6:306-329.
- Hamzei-Sichani F, Kamasawa N, Janssen WG, Yasumura T, Davidson KG, Hof PR, Wearne SL, Stewart MG, Young SR, Whittington MA, Rash JE, Traub RD (2007) Gap junctions on hippocampal mossy fiber axons demonstrated by thin-section electron microscopy and freeze fracture replica immunogold labeling. *Proc Natl Acad Sci U S A* 104:12548-12553.
- Harney SC, Jones MV (2002) Pre- and postsynaptic properties of somatic and dendritic inhibition in dentate gyrus. *Neuropharmacology* 43:584-594.
- Hedlich A, Werner L (1986) Classification of neurons of the visual cortex of the guinea pig (*Cavia porcellus*). A Golgi study. *J Hirnforsch* 27:651-677.
- Hendry SH, Jones EG (1985) Morphology of synapses formed by cholecystokinin-immunoreactive axon terminals in regio superior of rat hippocampus. *Neuroscience* 16:57-68.
- Hestrin S, Armstrong WE (1996) Morphology and physiology of cortical neurons in layer I. *J Neurosci* 16:5290-5300.
- Hornstein EP, Verweij J, Schnapf JL (2004) Electrical coupling between red and green cones in primate retina. *Nat Neurosci* 7:745-750.

- Howard A, Tamas G, Soltesz I (2005) Lighting the chandelier: new vistas for axo-axonic cells. *Trends Neurosci* 28:310-316.
- Isaacson JS (1998) GABAB receptor-mediated modulation of presynaptic currents and excitatory transmission at a fast central synapse. *J Neurophysiol* 80:1571-1576.
- Isaacson JS, Solis JM, Nicoll RA (1993) Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron* 10:165-175.
- Jones EG (1975) Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. *J Comp Neurol* 160:205-268.
- Jones EG (1984) Laminar distribution of cortical efferents, Peters, A.//Jones, E.G. Edition. New York: Plenum Press.
- Kalinichenko SG, Pushchin, II, Dyuzhen IV (2006) Neurochemical diversity of neurogliaform cells in the human primary motor cortex. *J Chem Neuroanat*.
- Kandel E, Schwartz J, Jessell T (2000) Principles of neural science: McGraw-Hill Professional.
- Kaneda K, Kita H (2005) Synaptically released GABA activates both pre- and postsynaptic GABA(B) receptors in the rat globus pallidus. *J Neurophysiol* 94:1104-1114.
- Karube F, Kubota Y, Kawaguchi Y (2004) Axon branching and synaptic bouton phenotypes in GABAergic nonpyramidal cell subtypes. *J Neurosci* 24:2853-2865.
- Kawaguchi Y (1993) Groupings of nonpyramidal and pyramidal cells with specific physiological and morphological characteristics in rat frontal cortex. *Journal of Neurophysiology* 69:416-431.
- Kawaguchi Y (1995) Physiological subgroups of nonpyramidal cells with specific morphological characteristics in layer II/III of rat frontal cortex. *J Neurosci* 15:2638-2655.
- Kawaguchi Y, Kubota Y (1993) Correlation of physiological subgroupings of nonpyramidal cells with parvalbumin- and calbindinD28k-Immunoreactive neurons in layer V of rat frontal cortex. *J Neurophysiol* 70:387-396.
- Kawaguchi Y, Kubota Y (1996) Physiological and morphological identification of somatostatin- or vasoactive intestinal polypeptide-containing cells among GABAergic cell subtypes in rat frontal cortex. *J Neurosci* 16:2701-2715.
- Kawaguchi Y, Kubota Y (1997) GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cereb Cortex* 7:476-486.
- Kawaguchi Y, Shindou T (1998) Noradrenergic excitation and inhibition of GABAergic cell types in rat frontal cortex. *J Neurosci* 18:6963-6976.
- Kawaguchi Y, Karube F, Kubota Y (2006) Dendritic branch typing and spine expression patterns in cortical nonpyramidal cells. *Cereb Cortex* 16:696-711.
- Kim U, Sanchez-Vives MV, McCormick DA (1997) Functional dynamics of GABAergic inhibition in the thalamus. *Science* 278:130-134.
- Kisvarday ZF, Beaulieu C, Eysel UT (1993) Network of GABAergic large basket cells in cat visual cortex (area 18): implication for lateral disinhibition. *J Comp Neurol* 327:398-415.
- Kisvarday ZF, Martin KA, Whitteridge D, Somogyi P (1985) Synaptic connections of intracellularly filled clutch cells: a type of small basket cell in the visual cortex of the cat. *J Comp Neurol* 241:111-137.
- Kisvarday ZF, Gulyas A, Beroukas D, North JB, Chubb IW, Somogyi P (1990) Synapses, axonal and dendritic patterns of GABA-immunoreactive neurons in human cerebral cortex. *Brain* 113:793-812.
- Klausberger T, Somogyi P (2008) Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321:53-57.

- Knowles WD, Funch PG, Schwartzkroin PA (1982) Electrotonic and dye coupling in hippocampal CA1 pyramidal cells in vitro. *Neuroscience* 7:1713-1722.
- Kopell N, Ermentrout B (2004) Chemical and electrical synapses perform complementary roles in the synchronization of interneuronal networks. *Proc Natl Acad Sci U S A* 101:15482-15487.
- Kosaka T, Hama K (1985) Gap junctions between non-pyramidal cell dendrites in the rat hippocampus (CA1 and CA3 regions): a combined Golgi-electron microscopy study. *J Comp Neurol* 231:150-161.
- Krimer LS, Zaitsev AV, Czanner G, Kroner S, Gonzalez-Burgos G, Povysheva NV, Iyengar S, Barrionuevo G, Lewis DA (2005) Cluster analysis-based physiological classification and morphological properties of inhibitory neurons in layers 2-3 of monkey dorsolateral prefrontal cortex. *J Neurophysiol* 94:3009-3022.
- Kulik A, Vida I, Lujan R, Haas CA, Lopez-Bendito G, Shigemoto R, Frotscher M (2003) Subcellular localization of metabotropic GABA(B) receptor subunits GABA(B1a/b) and GABA(B2) in the rat hippocampus. *J Neurosci* 23:11026-11035.
- Kulik A, Vida I, Fukazawa Y, Guetg N, Kasugai Y, Marker CL, Rigato F, Bettler B, Wickman K, Frotscher M, Shigemoto R (2006) Compartment-dependent colocalization of Kir3.2-containing K⁺ channels and GABAB receptors in hippocampal pyramidal cells. *J Neurosci* 26:4289-4297.
- Kullmann DM (2000) Spillover and synaptic cross talk mediated by glutamate and GABA in the mammalian brain. *Prog Brain Res* 125:339-351.
- Kullmann DM, Asztely F (1998) Extrasynaptic glutamate spillover in the hippocampus: evidence and implications. *Trends Neurosci* 21:8-14.
- Kullmann DM, Min MY, Asztely F, Rusakov DA (1999) Extracellular glutamate diffusion determines the occupancy of glutamate receptors at CA1 synapses in the hippocampus. *Philos Trans R Soc Lond B Biol Sci* 354:395-402.
- Kullmann DM, Ruiz A, Rusakov DM, Scott R, Semyanov A, Walker MC (2005) Presynaptic, extrasynaptic and axonal GABAA receptors in the CNS: where and why? *Prog Biophys Mol Biol* 87:33-46.
- Lacaille JC, Schwartzkroin PA (1988) Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. I. Intracellular response characteristics, synaptic responses, and morphology. *J Neurosci* 8:1400-1410.
- Lacey CJ, Boyes J, Gerlach O, Chen L, Magill PJ, Bolam JP (2005) GABA(B) receptors at glutamatergic synapses in the rat striatum. *Neuroscience* 136:1083-1095.
- Larkum ME, Zhu JJ, Sakmann B (1999) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* 398:338-341.
- Lee EJ, Han JW, Kim HJ, Kim IB, Lee MY, Oh SJ, Chung JW, Chun MH (2003) The immunocytochemical localization of connexin 36 at rod and cone gap junctions in the guinea pig retina. *Eur J Neurosci* 18:2925-2934.
- Lehre KP, Rusakov DA (2002) Asymmetry of glia near central synapses favors presynaptically directed glutamate escape. *Biophys J* 83:125-134.
- Long MA, Deans MR, Paul DL, Connors BW (2002) Rhythmicity without synchrony in the electrically uncoupled inferior olive. *J Neurosci* 22:10898-10905.
- Lopez-Bendito G, Shigemoto R, Kulik A, Paulsen O, Fairen A, Lujan R (2002) Expression and distribution of metabotropic GABA receptor subtypes GABABR1 and GABABR2 during rat neocortical development. *Eur J Neurosci* 15:1766-1778.
- Lopez-Bendito G, Shigemoto R, Kulik A, Vida I, Fairen A, Lujan R (2004) Distribution of metabotropic GABA receptor subunits GABAB1a/b and GABAB2 in the rat

- hippocampus during prenatal and postnatal development. *Hippocampus* 14:836-848.
- Lund JS, Lewis DA (1993) Local circuit neurons of developing and mature macaque prefrontal cortex: Golgi and immunocytochemical characteristics. *J Comp Neurol* 328:282-312.
- Ma CL, Kelly JB, Wu SH (2002) Presynaptic modulation of GABAergic inhibition by GABA(B) receptors in the rat's inferior colliculus. *Neuroscience* 114:207-215.
- Mann-Metzer P, Yarom Y (2002) Pre- and postsynaptic inhibition mediated by GABA(B) receptors in cerebellar inhibitory interneurons. *J Neurophysiol* 87:183-190.
- Marin-Pallida M (1985) Neurogenesis of the climbing fibers in the human cerebellum: a Golgi study. *J Comp Neurol* 235:82-96.
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci* 5:793-807.
- Martina M, Royer S, Pare D (2001) Cell-type-specific GABA responses and chloride homeostasis in the cortex and amygdala. *J Neurophysiol* 86:2887-2895.
- McBain CJ, Fisahn A (2001) Interneurons unbound. *Nat Rev Neurosci* 2:11-23.
- Mechawar N, Watkins KC, Descarries L (2002) Ultrastructural features of the acetylcholine innervation in the developing parietal cortex of rat. *J Comp Neurol* 443:250-258.
- Miles R, Toth K, Gulyas AI, Hajos N, Freund TF (1996) Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* 16:816-823.
- Mitchell SJ, Silver RA (2000) GABA spillover from single inhibitory axons suppresses low-frequency excitatory transmission at the cerebellar glomerulus. *J Neurosci* 20:8651-8658.
- Mody I, Pearce RA (2004) Diversity of inhibitory neurotransmission through GABA(A) receptors. *Trends Neurosci* 27:569-575.
- Mody I, De Koninck Y, Otis TS, Soltesz I (1994) Bridging the cleft at GABA synapses in the brain. *Trends Neurosci* 17:517-525.
- Moldavan MG, Irwin RP, Allen CN (2006) Presynaptic GABA(B) receptors regulate retinohypothalamic tract synaptic transmission by inhibiting voltage-gated Ca²⁺ channels. *J Neurophysiol* 95:3727-3741.
- Molnar G, Olah S, Komlosi G, Fule M, Szabadics J, Varga C, Barzo P, Tamas G (2008) Complex Events Initiated by Individual Spikes in the Human Cerebral Cortex. *PLoS Biol* 6:e222.
- Molyneaux BJ, Hasselmo ME (2002) GABA(B) presynaptic inhibition has an in vivo time constant sufficiently rapid to allow modulation at theta frequency. *J Neurophysiol* 87:1196-1205.
- Moss SJ, Smart TG (2001) Constructing inhibitory synapses. *Nat Rev Neurosci* 2:240-250.
- Mott DD, Li Q, Okazaki MM, Turner DA, Lewis DV (1999) GABA(B)-Receptor-mediated currents in interneurons of the dentate-hilus border. *J Neurophysiol* 82:1438-1450.
- Naegele JR, Katz LC (1990) Cell surface molecules containing N-acetylgalactosamine are associated with basket cells and neurogliaform cells in cat visual cortex. *J Neurosci* 10:540-557.
- Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different GABA(A) receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci* 18:1693-1703.
- Overstreet LS, Westbrook GL (2003) Synapse density regulates independence at unitary inhibitory synapses. *J Neurosci* 23:2618-2626.
- Pearce RA (1993) Physiological evidence for two distinct GABA(A) responses in rat hippocampus. *Neuron* 10:189-200.

- Peinado A, Yuste R, Katz LC (1993) Gap junctional communication and the development of local circuits in neocortex. *Cereb Cortex* 3:488-498.
- Perez-Garci E, Gassmann M, Bettler B, Larkum ME (2006) The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca²⁺ spikes in layer 5 somatosensory pyramidal neurons. *Neuron* 50:603-616.
- Peters A (1980) Morphological correlates of epilepsy: cells in the cerebral cortex. *Adv Neurol* 27:21-48.
- Peters A, Kara DA (1987) The neuronal composition of area 17 of rat visual cortex. IV. The organization of pyramidal cells. *J Comp Neurol* 260:573-590.
- Peters AaJ, E. G., ed (1984) *Cerebral Cortex. Cellular components of the cerebral cortex.* New York: Plenum Press.
- Peters AP, S. L./Webster, H. (1991) *The Fine Structure of the Nervous System, Third Edition* Edition: Oxford University Press, New York.
- Pfeuty B, Mato G, Golomb D, Hansel D (2003) Electrical synapses and synchrony: the role of intrinsic currents. *J Neurosci* 23:6280-6294.
- Pfeuty B, Mato G, Golomb D, Hansel D (2005) The combined effects of inhibitory and electrical synapses in synchrony. *Neural Comput* 17:633-670.
- Pfeuty B, Golomb D, Mato G, Hansel D (2007) Inhibition potentiates the synchronizing action of electrical synapses. *Front Comput Neurosci* 1:8.
- Porter JT, Nieves D (2004) Presynaptic GABAB receptors modulate thalamic excitation of inhibitory and excitatory neurons in the mouse barrel cortex. *J Neurophysiol* 92:2762-2770.
- Price CJ, Scott R, Rusakov DA, Capogna M (2008) GABA(B) receptor modulation of feedforward inhibition through hippocampal neurogliaform cells. *J Neurosci* 28:6974-6982.
- Price CJ, Cauli B, Kovacs ER, Kulik A, Lambolez B, Shigemoto R, Capogna M (2005) Neurogliaform neurons form a novel inhibitory network in the hippocampal CA1 area. *J Neurosci* 25:6775-6786.
- Qian N, Sejnowski TJ (1990) When is an inhibitory synapse effective? *Proc Natl Acad Sci USA* 87:8145-8149.
- Ramon y Cajal S (1904) *Textura del Systema Nervioso del Hombre y los Vertebrados.* In. Madrid, Spain: Moya.
- Ramon y Cajal S (1911) *Histologie du systeme nerveux de l'homme et des vertebres.* Paris: Maloine.
- Ramón y Cajal S (1899) Estudios sobre la corteza cerebral humana. *Corteza visual Rev Trim Microgr* 4:1-63.
- Reyes A, Lujan R, Rozov A, Burnashev N, Somogyi P, Sakmann B (1998) Target-cell-specific facilitation and depression in neocortical circuits. *Nat Neurosci* 1:279-285.
- Roerig B, Feller MB (2000) Neurotransmitters and gap junctions in developing neural circuits. *Brain Res Brain Res Rev* 32:86-114.
- Rusakov DA, Lehre KP (2002) Perisynaptic asymmetry of glia: new insights into glutamate signalling. *Trends Neurosci* 25:492-494.
- Rusakov DA, Kullmann DM, Stewart MG (1999) Hippocampal synapses: do they talk to their neighbours? *Trends Neurosci* 22:382-388.
- Sakaba T, Neher E (2003) Direct modulation of synaptic vesicle priming by GABA(B) receptor activation at a glutamatergic synapse. *Nature* 424:775-778.
- Sancesario G, Pisani A, D'Angelo V, Calabresi P, Bernardi G (1998) Morphological and functional study of dwarf neurons in the rat striatum. *Eur J Neurosci* 10:3575-3583.

- Saraga F, Wu CP, Zhang L, Skinner FK (2003) Active dendrites and spike propagation in multi-compartment models of oriens-lacunosum/moleculare hippocampal interneurons. *J Physiol* 552:673-689.
- Scanziani M (2000) GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. *Neuron* 25:673-681.
- Schmitz D, Schuchmann S, Fisahn A, Draguhn A, Buhl EH, Petrasch-Parwez E, Dermietzel R, Heinemann U, Traub RD (2001) Axo-axonal coupling. a novel mechanism for ultrafast neuronal communication. *Neuron* 31:831-840.
- Shapiro MS, Wollmuth LP, Hille B (1994) Modulation of Ca²⁺ channels by PTX-sensitive G-proteins is blocked by N-ethylmaleimide in rat sympathetic neurons. *J Neurosci* 14:7109-7116.
- Shen KZ, Johnson SW (2001) Presynaptic GABA(B) receptors inhibit synaptic inputs to rat subthalamic neurons. *Neuroscience* 108:431-436.
- Sherman A, Rinzel J (1992) Rhythmogenic effects of weak electrotonic coupling in neuronal models. *Proc Natl Acad Sci U S A* 89:2471-2474.
- Simon A, Olah S, Molnar G, Szabadics J, Tamas G (2005) Gap-junctional coupling between neurogliaform cells and various interneuron types in the neocortex. *J Neurosci* 25:6278-6285.
- Sloper JJ (1972) Gap junctions between dendrites in the primate neocortex. *Brain Res* 44:641-646.
- Sloper JJ, Hiorns RW, Powell TP (1979) A qualitative and quantitative electron microscopic study of the neurons in the primate motor and somatic sensory cortices. *Philos Trans R Soc Lond B Biol Sci* 285:141-171.
- Smiley JF, Goldman-Rakic PS (1996) Serotonergic axons in monkey prefrontal cerebral cortex synapse predominantly on interneurons as demonstrated by serial section electron microscopy. *J Comp Neurol* 367:431-443.
- Smiley JF, Morrell F, Mesulam MM (1997) Cholinergic synapses in human cerebral cortex: an ultrastructural study in serial sections. *Exp Neurol* 144:361-368.
- Smiley JF, Williams SM, Szigeti K, Goldman-Rakic PS (1992) Light and electron microscopic characterization of dopamine-immunoreactive axons in human cerebral cortex. *J Comp Neurol* 321:325-335.
- Sodickson DL, Bean BP (1996) GABAB receptor-activated inwardly rectifying potassium current in dissociated hippocampal CA3 neurons. *J Neurosci* 16:6374-6385.
- Sohl G, Maxeiner S, Willecke K (2005) Expression and functions of neuronal gap junctions. *Nat Rev Neurosci* 6:191-200.
- Somogyi P (1977) A specific 'axo-axonal' interneuron in the visual cortex of the rat. *Brain Res* 136:345-350.
- Somogyi P, Freund TF, Cowey A (1982) The axo-axonic interneuron in the cerebral cortex of the rat, cat and monkey. *Neuroscience* 7:2577-2607.
- Somogyi P, Kisvarday ZF, Martin KA, Whitteridge D (1983) Synaptic connections of morphologically identified and physiologically characterized large basket cells in the striate cortex of cat. *Neuroscience* 10:261-294.
- Somogyi P, Tamas G, Lujan R, Buhl EH (1998) Salient features of synaptic organisation in the cerebral cortex. *Brain Res Rev* 26:113-135.
- Szabadics J, Lorincz A, Tamas G (2001) β and γ frequency synchronization by dendritic GABAergic synapses and gap junctions in a network of cortical interneurons. *J Neurosci* 21:5824-5831.
- Szabadics J, Tamas G, Soltesz I (2007) Different transmitter transients underlie presynaptic cell type specificity of GABA_A,slow and GABA_A,fast. *Proc Natl Acad Sci U S A* 104:14831-14836.

- Szabadics J, Varga C, Molnar G, Olah S, Barzo P, Tamas G (2006) Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science* 311:233-235.
- Szapiro G, Barbour B (2007) Multiple climbing fibers signal to molecular layer interneurons exclusively via glutamate spillover. *Nat Neurosci* 10:735-742.
- Tamas G, Buhl EH, Somogyi P (1997) Fast IPSPs elicited via multiple synaptic release sites by distinct types of GABAergic neuron in the cat visual cortex. *J Physiol (Lond)* 500:715-738.
- Tamas G, Buhl EH, Lorincz A, Somogyi P (2000) Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. *Nat Neurosci* 3:366-371.
- Tamas G, Lorincz A, Simon A, Szabadics J (2003) Identified sources and targets of slow inhibition in the neocortex. *Science* 299:1902-1905.
- Tamas G, Szabadics J, Lorincz A, Somogyi P (2004) Input and frequency-specific entrainment of postsynaptic firing by IPSPs of perisomatic or dendritic origin. *Eur J Neurosci* 20:2681-2690.
- Tarczy-Hornoch K, Martin KA, Stratford KJ, Jack JJ (1999) Intracortical excitation of spiny neurons in layer 4 of cat striate cortex in vitro. *Cereb Cortex* 9:833-843.
- Thompson SE, Ayman G, Woodhall GL, Jones RS (2006) Depression of glutamate and GABA release by presynaptic GABAB receptors in the entorhinal cortex in normal and chronically epileptic rats. *Neurosignals* 15:202-215.
- Thomson AM, Destexhe A (1999) Dual intracellular recordings and computational models of slow inhibitory postsynaptic potentials in rat neocortical and hippocampal slices. *Neuroscience* 92:1193-1215.
- Thomson AM, West DC, Hahn J, Deuchars J (1996) Single axon IPSPs elicited in pyramidal cells by three classes of interneurons in slices of rat neocortex. *J Physiol* 496 (Pt 1):81-102.
- Traub RD, Pais I, Bibbig A, LeBeau FE, Buhl EH, Hormuzdi SG, Monyer H, Whittington MA (2003) Contrasting roles of axonal (pyramidal cell) and dendritic (interneuron) electrical coupling in the generation of neuronal network oscillations. *Proc Natl Acad Sci U S A* 100:1370-1374.
- Umbriaco D, Garcia S, Beaulieu C, Descarries L (1995) Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum radiatum of adult rat hippocampus (CA1). *Hippocampus* 5:605-620.
- Umbriaco D, Watkins KC, Descarries L, Cozzari C, Hartman BK (1994) Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. *J Comp Neurol* 348:351-373.
- Valverde F (1971) Short axon neuronal subsystems in the visual cortex of the monkey. *Int J Neurosci* 1:181-197.
- Venance L, Rozov A, Blatow M, Burnashev N, Feldmeyer D, Monyer H (2000) Connexin expression in electrically coupled postnatal rat brain neurons. *Proc Natl Acad Sci U S A* 97:10260-10265.
- Vida I, Halasy K, Szinyei C, Somogyi P, Buhl EH (1998) Unitary IPSPs evoked by interneurons at the stratum radiatum-stratum lacunosum-moleculare border in the CA1 area of the rat hippocampus in vitro. *J Physiol* 506 (Pt 3):755-773.
- Vigot R, Barbieri S, Brauner-Osborne H, Turecek R, Shigemoto R, Zhang YP, Lujan R, Jacobson LH, Biermann B, Fritschy JM, Vacher CM, Muller M, Sansig G, Guetg N, Cryan JF, Kaupmann K, Gassmann M, Oertner TG, Bettler B (2006) Differential Compartmentalization and Distinct Functions of GABA(B) Receptor Variants. *Neuron* 50:589-601.

- Vizi ES (1980) Modulation of cortical release of acetylcholine by noradrenaline released from nerves arising from the rat locus coeruleus. *Neuroscience* 5:2139-2144.
- Vizi ES (1990) Synaptic and nonsynaptic cross talk between neurons. Role of presynaptic alpha 2-receptors in mental disorders. *Ann N Y Acad Sci* 604:344-352.
- Vizi ES (2000) Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the central nervous system. *Pharmacol Rev* 52:63-89.
- Vizi ES (2003) Non-synaptic interaction between neurons in the brain, an analog system: far from Cajal-Sherringtons's galaxy. *Bull Mem Acad R Med Belg* 158:373-379; discussion 379-380.
- Vizi ES, Harsing LG, Jr., Zsilla G (1981) Evidence of the modulatory role of serotonin in acetylcholine release from striatal interneurons. *Brain Res* 212:89-99.
- Vizi ES, Gyires K, Somogyi GT, Ungvary G (1983) Evidence that transmitter can be released from regions of the nerve cell other than presynaptic axon terminal: axonal release of acetylcholine without modulation. *Neuroscience* 10:967-972.
- Wang JH, Kelly P (2001) Calcium-calmodulin signalling pathway up-regulates glutamatergic synaptic function in non-pyramidal, fast spiking rat hippocampal CA1 neurons. *J Physiol* 533:407-422.
- White WF, Brown KL, Frank DM (1989) Glycine binding to rat cortex and spinal cord: binding characteristics and pharmacology reveal distinct populations of sites. *J Neurochem* 53:503-512.
- Winfield DA, Gatter KC, Powell TP (1980) An electron microscopic study of the types and proportions of neurons in the cortex of the motor and visual areas of the cat and rat. *Brain* 103:245-258.
- Wu LG, Saggau P (1997) Presynaptic inhibition of elicited neurotransmitter release. *Trends Neurosci* 20:204-212.
- Yeung JY, Canning KJ, Zhu G, Pennefather P, MacDonald JF, Orser BA (2003) Tonicly activated GABAA receptors in hippocampal neurons are high-affinity, low-conductance sensors for extracellular GABA. *Mol Pharmacol* 63:2-8.
- Zhu Y, Stornetta RL, Zhu JJ (2004) Chandelier cells control excessive cortical excitation: characteristics of whisker-evoked synaptic responses of layer 2/3 nonpyramidal and pyramidal neurons. *J Neurosci* 24:5101-5108.
- Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR (1999) *Fundamental Neuroscience*. San Diego: Academic Press.
- Zsiros V, Maccaferri G (2005) Electrical coupling between interneurons with different excitable properties in the stratum lacunosum-moleculare of the juvenile CA1 rat hippocampus. *J Neurosci* 25:8686-8695.
- Zsiros V, Aradi I, Maccaferri G (2007) Propagation of postsynaptic currents and potentials via gap junctions in GABAergic networks of the rat hippocampus. *J Physiol* 578:527-544.