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Synaptic Organization of GABAergic Neurons and GABA_A Receptors in the Lateral Geniculate Nucleus and Visual Cortex

Peter Somogyi

*Medical Research Council
Anatomical Neuropharmacology Unit
South Parks Road
Oxford OX1 3QT
United Kingdom*

Gamma-aminobutyric acid (GABA), acting on type-A and type-B receptors, is the main inhibitory transmitter in the mammalian visual system. The organization of circuits, the localization of GABAergic synaptic terminals, and the expression of GABA_A receptors show selective distribution according to neuronal classes and visual channels. For example, it has been shown that in the dorsal lateral geniculate nucleus (dLGN) of the cat, a class of small relay cells, tentatively correlated with the “lagged” X cells, particularly strongly expresses the receptor complex. In the dLGN and in the cortex, the GABA_A receptor complex is present both at synaptic and at nonsynaptic sites in the plasma membrane of neurons. It is suggested that this broad distribution enables the GABAergic system to keep the dynamic range of the postsynaptic cell optimal at widely varying intensities of excitatory drive.

In the visual cortex GABAergic neurons show great diversity in their input and output selectivity and neurochemical characteristics, reflecting the different roles of subsets of cells using the same transmitter. The possible neuronal basis of two receptive-field properties, directional selectivity and end inhibition, is examined in light of new data on connectivity. Data are presented for the hypothesis that the action of different cortical afferents involve subsets of GABAergic neurons selectively regulating the gain of particular inputs on common target cells. The requirements of the particular pathways and the separation of physiologically different streams in the same pathway explain the diversity of GABAergic neuronal circuits.

Introduction

From retina to thalamus and then on to cortex, visual pathways use excitatory, chemically mediated synaptic signals for transmitting information.

From the first synapse established by the photoreceptors in the retina up to the last stage of visual perception, at each excitatory synaptic transfer, local GABA-releasing elements gate and modify the responses evoked in the

postsynaptic cells by the excitatory signal. GABA acts pre- and postsynaptically on at least two different receptors: GABA_B receptors gating Ca⁺⁺ and/or K⁺ channels and GABA_A receptors gating Cl⁻ channels. Whatever the receptor mechanism, the activation of GABA receptors leads to a reduced or substantially modified response in the postsynaptic cell to the excitatory signal.

The structural organization of GABA-containing circuits along the visual pathway has been extensively studied thanks to the availability of antibodies that selectively mark GABAergic neurons. The retina and the LGN are notable successes in the delineation of GABAergic circuits, while in the cortex the task has proved more complex than anticipated. The functional organization of GABA-mediated interactions has recently also been studied with a GABA_B receptor antagonist¹ and much more extensively with selective GABA_A antagonists.² Although in some cases it has been possible to correlate the action of GABA with a particular link in the circuit, in most situations GABA-mediated events cannot be interpreted in terms of the structural framework. Nowhere is this more evident than in the visual cortex.

Most information about the role of GABA along the visual pathway derives from pharmacological manipulation of the GABA_A receptor. This receptor and its endogenous agonist GABA have been implicated in such fundamental phenomena as center-surround inhibition,³ directional,⁴ and ori-

entation⁵ selectivity, and binocular interaction.⁶ While most, if not all, neurons express GABA_A receptors, the cellular and subcellular localization of the receptor is not well known. In an attempt to relate GABA_A receptor-mediated functional phenomena to circuits that can be shown to contain GABA, we have begun the high resolution immunocytochemical localization of the receptor complex in the visual pathway. In this paper, concentrating on the dorsal LGN and the visual cortex, I will summarize our on-going studies on the organization of GABAergic circuits, present preliminary results on the distribution of the GABA_A receptor complex, and discuss emerging principles that may help us to understand GABAergic mechanisms.

Dorsal Lateral Geniculate Nucleus

Origin and Role of GABAergic Input

GABA-mediated inhibition in the LGN of the cat has been shown to play a major role in gating, modifying and preserving visual information from the retina.⁷ Neurons in the LGN receive GABAergic input from axons of the perigeniculate nucleus, from axons of intrinsic neurons (both corresponding to the so called F1 morphological class of terminal, Figure 1) and from presynaptic dendrites (corresponding to F2 class of terminals, Figure 2) of these same intrinsic neurons.⁸ *In vivo* microelectrode studies revealed the existence

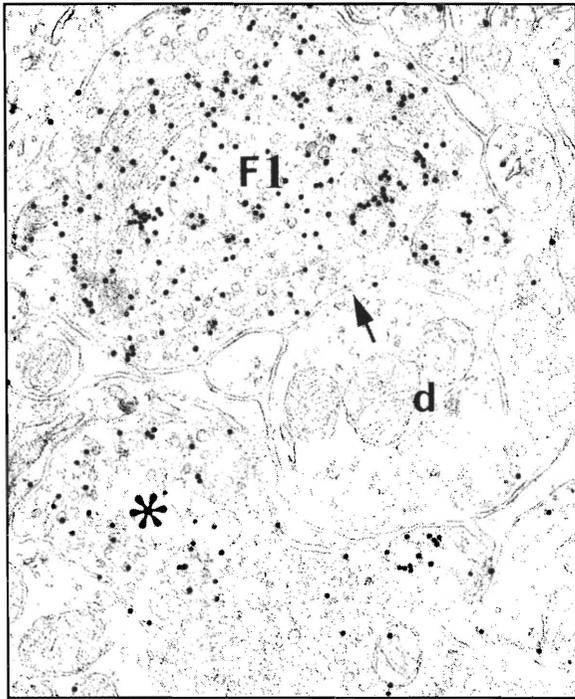


Figure 1. Electron micrograph of a GABA-immunoreacted section from the dLGN of cat. An F1-type terminal making a synaptic contact (arrow) with a relay cell dendrite (d), and another bouton (asterisk) are immunogold labeled, demonstrating the presence of GABA. Most F1 terminals originate from the perigeniculate nucleus and from local axon terminals of GABAergic interneurons. Scale: 0.5 μ m.

of optic-tract-activated inhibition, and combined electrophysiological and pharmacological studies using the GABA_A antagonist bicuculline demonstrated that, for example, binocular inhibition,⁹ inhibition responsible for orientation bias,¹⁰ center-surround inhibition,¹¹ spatial frequency tuning differences of X and Y cells,¹² and global lateral inhibition outside the antagonistic receptive field¹³ all involve GABA_A receptors. It is thus apparent that different types of cells can be recognized on the basis of properties that depend on GABA-mediated inhibitory influences. It is generally assumed that the differences in response properties are brought about by variation in circuitry, but molecular distinction between the cell classes could also be responsible. We therefore studied the cellular and subcellular distribution of one of the main

components of the inhibitory mechanism, the GABA_A/benzodiazepine receptor/chloride channel complex.¹⁴

Cellular and Subcellular Localization of Benzodiazepine/GABA_A Receptor/Cl⁻ Channel Complex

A monoclonal antibody bd-24, specific for an α -subunit of the receptor complex,¹⁵ and light- and electron-microscopic immuno-peroxidase methods¹⁶ were used.

Subcellular localization of the receptor complex. Immunoreactivity was always associated with membranes. Intracellularly the endoplasmic reticulum, the Golgi apparatus and multivesicular bodies showed immunoreactivity representing respectively the biosynthesis, glycosylation and degrada-

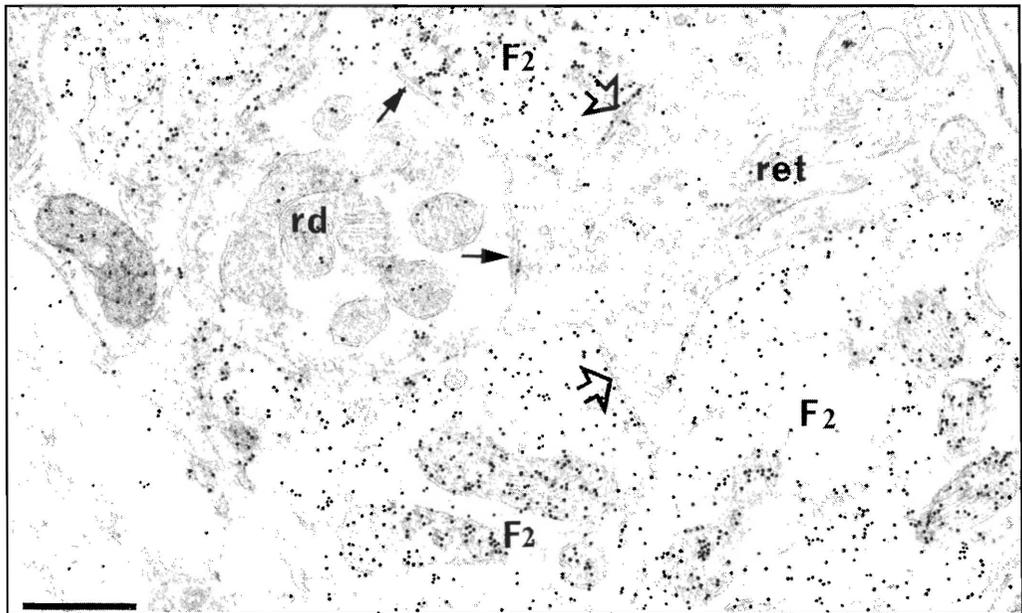


Figure 2. Electron micrograph of a retinal terminal (ret) in the dLGN of cat making synapses with two of three F2 terminals (open arrows) as well as with a relay cell dendrite (rd, lower small arrow). One of the F2 terminals also makes a synapse (upper arrow) with the same relay cell dendrite, closing a triadic arrangement. The F2 terminals originate from the dendrites of local GABAergic cells and are immunopositive for GABA as shown by the heavy immunogold labeling. Scale: 0.5 μ m.

tion of the protein (Figure 3). Presumably, the degree of intracellular receptor immunoreactivity is positively correlated with receptor turnover. Extracellularly, receptor immunoreactivity was present on plasma membranes of dendrites and neuronal somata. We assume that the degree of immunoreactivity correlates positively with receptor density. Interestingly, the level of intracellular and plasma membrane immunoreactivity is not well correlated in the dLGN. Thus some classes of neuron, such as large relay cells in the dLGN, can have strong immunoreactivity on their plasma membrane but negligible immunoreactivity intracellularly.

Differential expression of the receptor complex by different classes of

neuron. In the dLGN of the cat physiologically distinct cell populations can be delineated by size distribution of their somata,¹⁷ and it is known that local circuit neurons contain GABA while relay cells are GABA-negative. When appropriate conditions were used to visualize individual cells, four classes could be recognized as defined by: (1) immunoreactivity for the GABA_A receptor complex, (2) immunoreactivity for GABA, (3) area of somata, and (4) presence or absence of cellular lamellar bodies (CLB).

1. GABA-negative neurons with the smallest soma area showed the strongest immunoreactivity for the receptor complex, mainly in the endoplasmic reticulum and also on the somatic plasma membrane (Figures 3

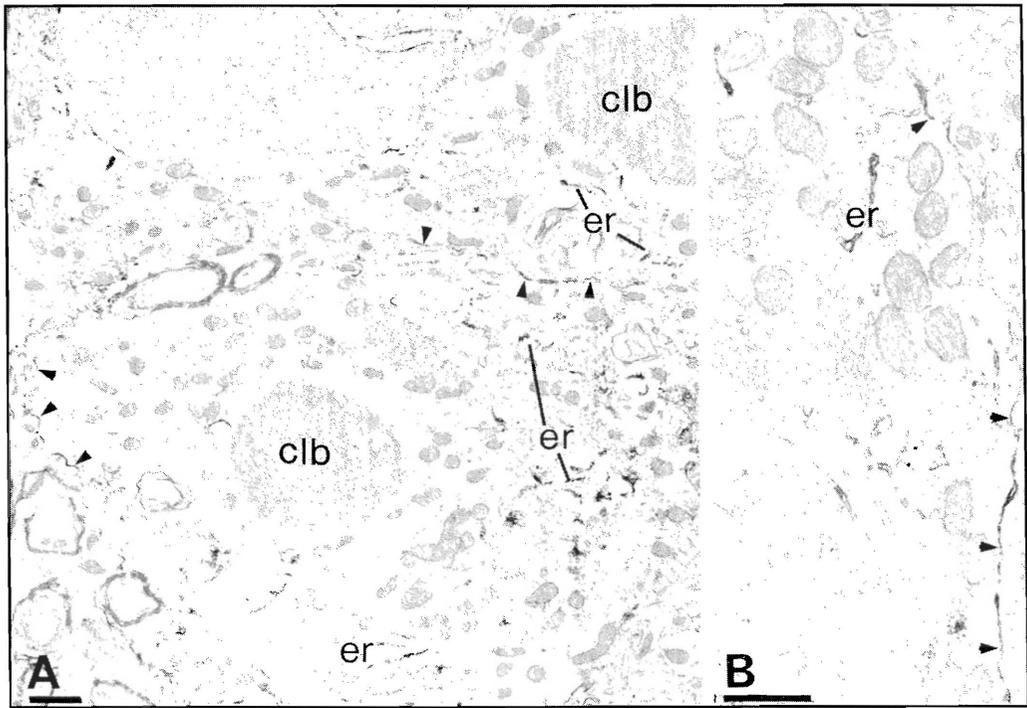


Figure 3. Immunoreactivity for the GABA_A receptor complex in the LGN of cat. Electron micrographs of relay cells, containing cytoplasmic lamellar bodies (clb), and showing strong immunoreactivity in the endoplasmic reticulum (er) and along nonsynaptic plasma membrane (arrow heads) for the α -subunit of the receptor. Scales: 0.5 μ m.

and 4). These cells often seemed to occur in groups of three or four, and they could be observed in all three laminae of the dLGN. The majority of these neurons contained CLBs (Figure 3). The reverse correlation was even stronger. Neurons having CLBs were almost invariably strongly immunoreactive for the α -subunit of the receptor complex. The somatic "area" of strong cells was $201 \pm 56\mu\text{m}^2$ in laminae A-A₁.

This type of cell found by immunostaining may correspond to the so called "lagged" X cells (X_L) studied recently for their physiological response properties.¹⁸ They have a soma area distribution almost identical to that of our strongly receptor immunopositive cells

(Figure 5). We calculated¹⁹ that in the A laminae of the LGN of the cat about 20 percent of neurons may have X_L properties, the same proportion that is thought to contain CLBs,²⁰ a prominent organelle in strongly receptor immunopositive cells. In addition, CLB-containing cells also have some size distribution ($196 \pm 25.1\mu\text{m}^2$) similar²¹ to that of the X_L cells ($236 \pm 66\mu\text{m}^2$)²² and strongly receptor-immunoreactive cells.

The identifying property of X_L neurons is that they show an early, short duration inhibition in their response to visual stimulus, and this is thought to be produced by intrageniculate inhibitory interneurons.¹⁸ The latency and duration of inhibition is very similar to that of the short latency, short duration, Cl-

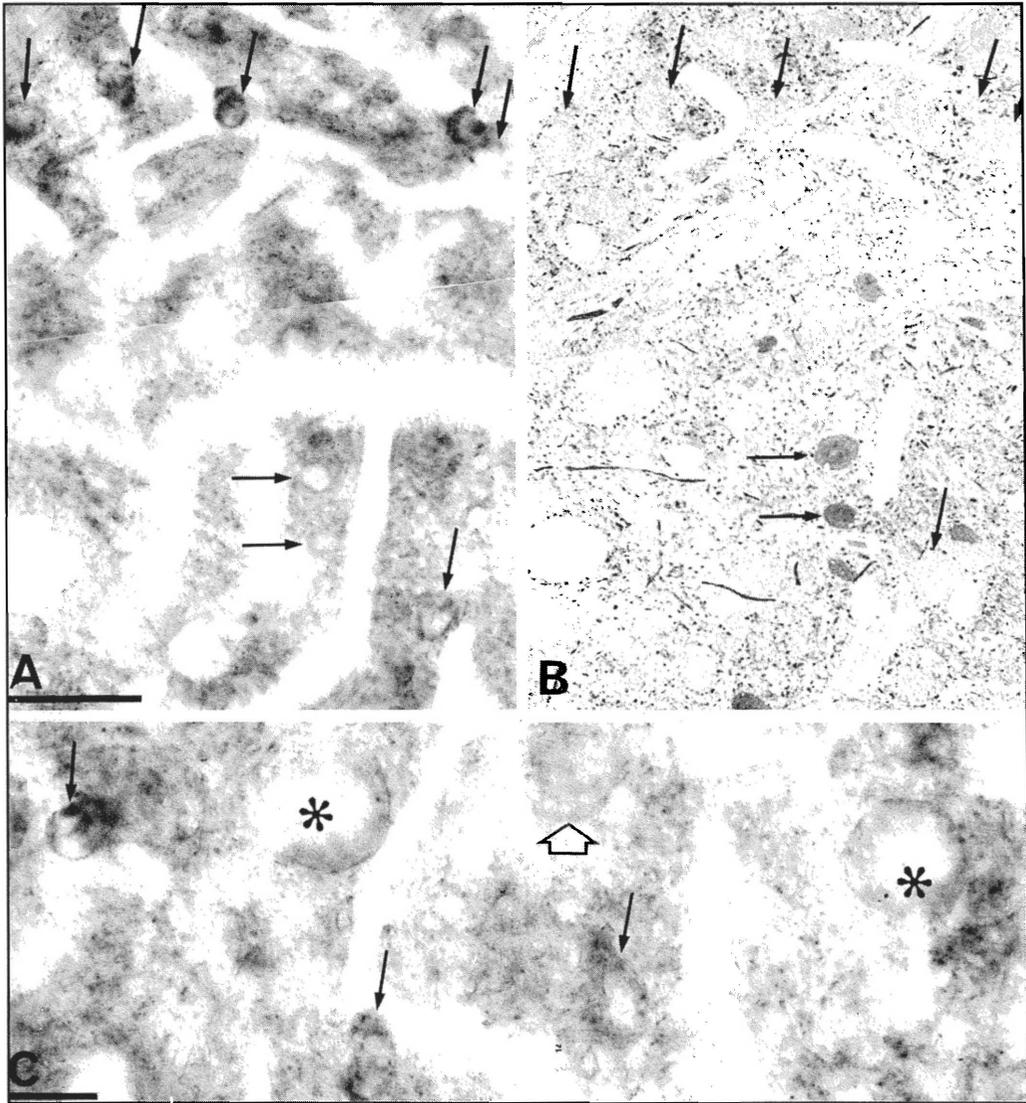


Figure 4. Light micrographs of the A lamina of the dLGN of cat reacted for GABA_A receptor (A and C, α-subunit, 50µm thick osmium-treated sections) and GABA (B, 0.5µm thick sections). A and B show the same area. The strongly receptor-positive cells in A (oblique arrows) are GABA-negative in B. GABA-positive cells (horizontal arrows in B) show little immunoreactivity for the receptor. Some cells (asterisks in C) are outlined by receptor immunoreactivity and these are much larger than neighboring strongly immunoreactive cells (oblique arrows). A medium size cell (open arrow) shows no immunoreactivity. Scales: A and B, 50µm; C, 10µm.

-dependent and bicuculline-sensitive GABA_A-inhibitory postsynaptic potential (IPSP) described in the dLGN both *in vivo*²³ and *in vitro*.²⁴ Therefore, it seems reasonable to suggest that the dip in firing of X_L cells is caused by the activation of GABA_A receptors. The

strong immunoreactivity for the GABA_A receptor of cells which have similar somatic size to X_L cells, may underline the enhanced turnover and expression of the GABA_A receptor as well as the greater sensitivity of these cells to GABAergic inhibition.

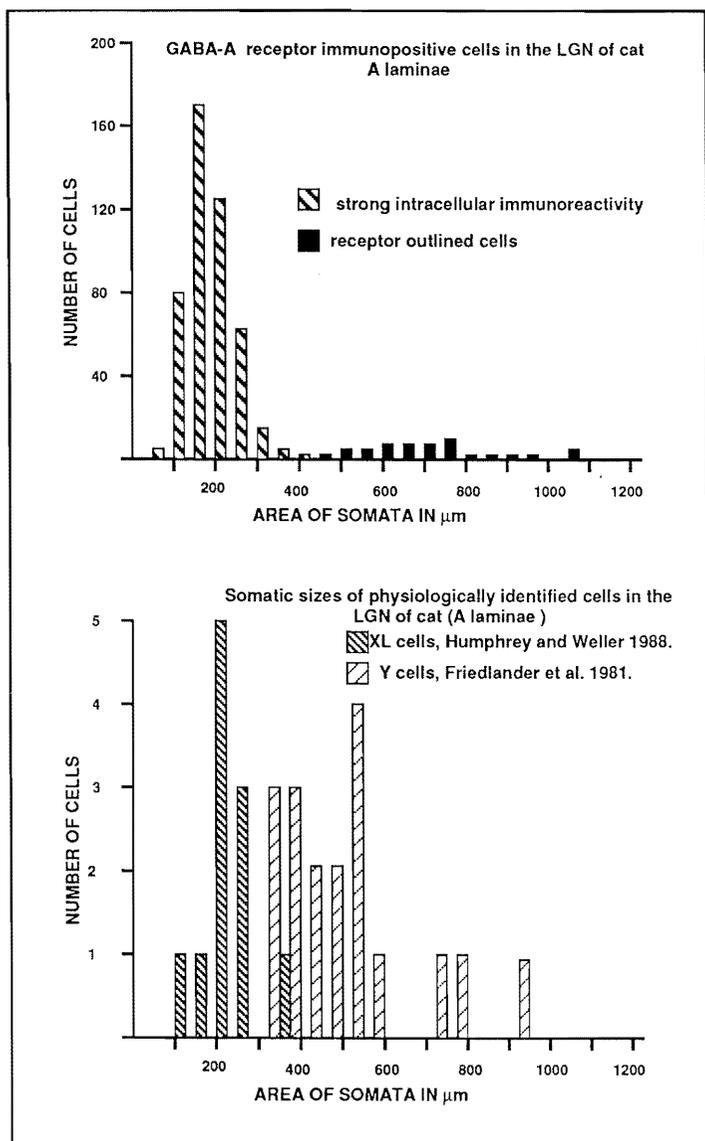


Figure 5. Somatic size distribution of GABA_A receptor-positive neurons in comparison to physiologically identified neurons published previously.^{22,91} Strongly immunopositive cells have similar size to lagged X cells while receptor outlined cells have sizes similar to larger Y cells.

2. Large, GABA-negative cells were strongly immunoreactive on the plasma membrane, their somata and proximal dendrites outlined by receptor immunoreactivity (Figure 4). However they showed scant, if any, intracellular immunoreactivity. Their somata were significantly larger ($691 \pm 149\mu\text{m}^2$ in lam. A-A₁) than those of the previous category of neuron (Figure 5), and CLBs were never observed in these

cells. These cells may correspond to physiologically identified medium and large Y cells. A similar large cell population has been marked by the monoclonal antibody Cat-301.²⁵

3. Most other GABA-negative cells showed no receptor immunoreactivity detectable by light microscopy. Electron microscopy also showed no or very weak immunoreactivity for the α -subunit detected under our conditions.

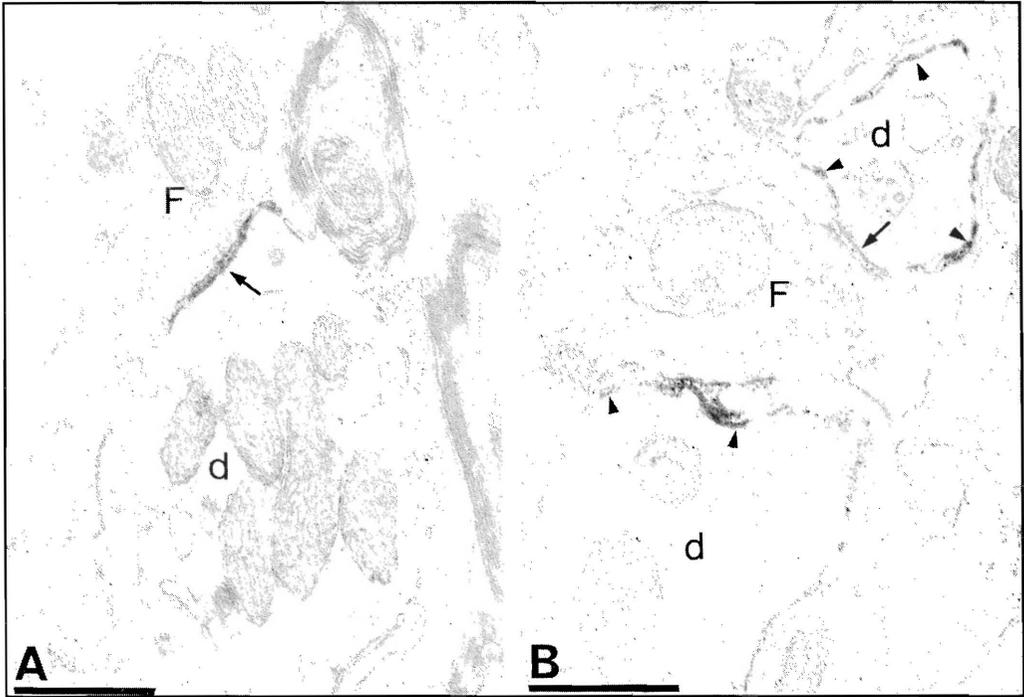


Figure 6. Synaptic and nonsynaptic GABA_A receptor immunoreactivity on dendrites (d). **A.** The synaptic junction (arrow) of an F-type terminal is immunopositive. **B.** The synaptic junction of an F terminal is immunonegative (arrow) but the non-junctional plasma membrane is immunopositive (arrowheads). Scales: 0.5 μ m.

They may correspond to the smaller Y-cell population and to the medium-sized possibly non-lagged X cells.

4. GABA-positive cells showed only weak intracellular immunoreactivity and negligible, if any, immunoreactivity at the somatic and proximal dendritic plasma membrane (Figure 4). We have not found CLBs in GABA-positive cells. The very low levels of extracellular receptor on interneurons in the dLGN are consistent with the absence of short duration IPSPs following optic tract stimulation.²⁶ Although other explanations are possible, this may indicate that neither axonal nor dendro-dendritic interaction between interneurons involve GABA_A receptors.

Synaptic and nonsynaptic recep-

tor immunoreactivity. Surprisingly, the receptor complex is present both at synaptic specializations and at non-junctional sites on the somatic and the dendritic membranes (Figure 6). One advantage of the LGN is that the origin of many pre- and postsynaptic elements can be identified with the use of electron microscopy.²⁷ This provides an opportunity for correlation of receptor distribution at the synaptic junctions with their origin and their chemistry.

In the neuropil the dendrites of projection cells showed immunoreactivity on the plasma membrane both inside and outside the glomeruli. The synaptic junctions formed by many F1 axonal varicosities, most of which have been shown to contain GABA,²⁸ were

immunopositive, as were junctions formed by GABAergic presynaptic dendrites (F2), establishing symmetrical synapses with dendrites of relay cells. Many axo-somatic F1 junctions were also immunoreactive. However, immunoreactivity for the receptor/channel complex was also widely distributed on nonsynaptic plasma membranes of somata and dendrites. The distribution was not uniform even on the same cell, since synaptic junctions of retinal and so-called RSD (round vesicle dark, mostly cortical) boutons, which are not GABAergic, were almost without exception immunonegative under our conditions.

Neurons in the LGN are thought to receive synapses from the mostly GABAergic F terminals on or close to the cell body.²⁹ The inhibitory input to X cells is thought to be predominantly of the F2 (presynaptic dendrite) type, forming triadic synaptic arrangements on dendritic appendages (see Figure 2). In addition some F1 terminal (axonal) input can also be found both on the somata and proximal dendrites.³⁰ Y cells which do not seem to receive F2 input have more F1 terminals on or close to the soma.³¹ Thus, the strong immunoreactivity on the soma and proximal dendritic membrane of the large receptor-outlined cells might reflect this larger somatic GABAergic input of Y cells.

Conclusion. The degree and the mode of expression of the GABA_A-receptor complex is characteristic to neuronal classes of the dLGN. This could provide the basis for differential sensitivity to GABA-mediated inhibition and to GABAergic drugs, adding a

level of molecular specialization to the differences in retinal input and circuits. Together they provide the LGN cells with the machinery to produce the functional differences necessary for transmitting channel specific information. The possible functional significance of the widespread receptor distribution on the plasma membrane will be discussed later.

Visual Cortex

Connections of GABAergic Neurons

Approximately every fifth neuron and 15 percent of synaptic terminals in the visual cortex of cat synthesize GABA.³² Most of the GABAergic synapses are on dendritic shafts (58.1 percent), dendritic spines (26.2 percent), and much less on the somata (13.1 percent) and axon initial segments (2.5 percent) of neurons.³³ The real sophistication of GABAergic circuits however only becomes apparent when one studies the connections of single identified GABAergic cells. Both GABA and its synthetic enzyme glutamate decarboxylase (GAD) can be localized immunocytochemically. However immunocytochemistry alone does not allow the allocation of GABAergic boutons to any particular type of cell. To overcome this limitation we have developed the combination of immunocytochemistry with Golgi impregnation and intracellular iontophoresis of horseradish peroxidase (HRP),³⁴ in order to visualize the dendritic and axonal arborizations of the GABA-containing

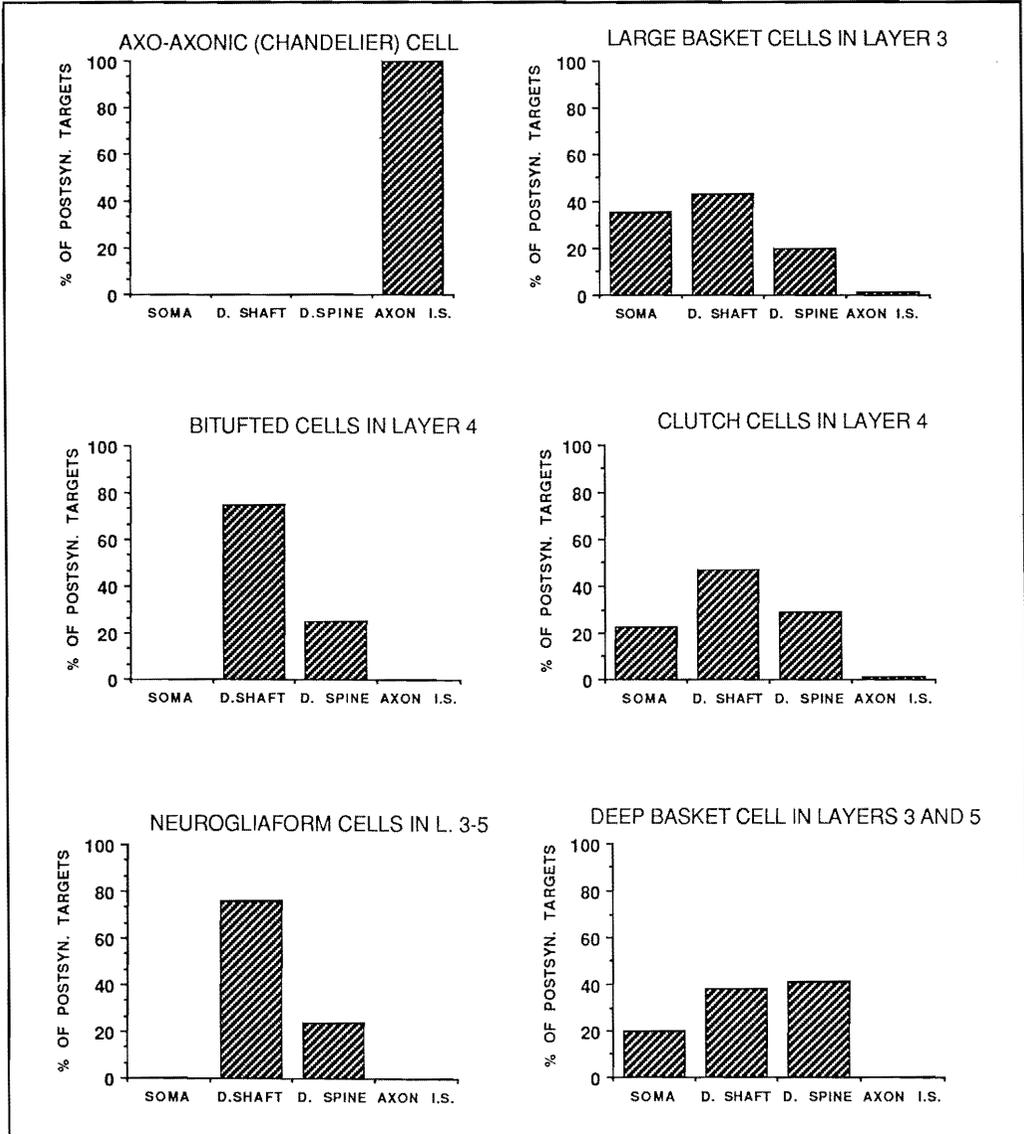


Figure 7. Postsynaptic target selectivity of identified GABAergic neurons in the striate cortex of cat. Note the absolute selectivity of chandelier cells for the axon initial segments and the substantial proportion of dendritic spines contacted by all other types of cell. The numerical density of the different cells is not known, but the majority of all GABAergic synaptic contacts is on dendrites and spines. Data summarized from several studies.⁹²

cells. This approach enabled us to trace the efferent synaptic connections of identified types of GABAergic cortical cells and to evaluate their target selectivity (Figure 7).

Neuronal diversity in cortex, as represented by differences in form, has

been recognized by the great pioneers of cortical microcircuitry Ramon y Cajal, Lorente de No, Szentagothai and others.³⁵ As predicted by them, the combined neurochemical and connectivity characterization outlined above revealed that subsets of neurons synthe-

sizing the same transmitter, namely GABA, have specialized synaptic connections. Only a few types of cell can be briefly mentioned here.

Axo-axonic or chandelier cell. Discovered by Szentagothai,³⁶ these neurons make synapses exclusively with the initial axon segments of pyramidal and some spiny stellate cells.³⁷ Each cell terminates on 200-400 pyramidal cells and each pyramidal cell receives input from 3-5 GABAergic axo-axonic cells.³⁸ This is the only cortical neuron for which reasonably accurate convergence and divergence figures are available. Axo-axonic cells are ubiquitous in all cortical areas including the hippocampus³⁹ and amygdala.⁴⁰ Since they provide input to the initial segment of the axon where the axonally propagated action potentials are generated, they are in a most effective position to control the firing of pyramidal cells. The control of cortical output by a specialized GABAergic neuron acting on the action-potential generating site of pyramidal cells is a unique feature of cortical circuitry. This system could have evolved in response to the other uniquely developed cortical feature: the extensive excitatory interconnections of the excitatory output cells. The chandelier cells, because of the location of their synapses on the axon initial segments, control the final common paths and so their action occurs after all the other inputs have been integrated by the output neurons.

Basket cells. Basket cells have been major candidates as "the" GABAergic inhibitory cells in the cortex, and they were thought to terminate

in pericellular baskets on the somata.⁴¹ Their selectivity has only been studied quantitatively in the visual cortex of cat, using physiologically recorded, intracellularly HRP-injected neurons.⁴² Contrary to previous predictions, only about 20-40 percent of their synapses were on the somata of other neurons, the remainder contacting dendrites. Surprisingly, 20-40 percent of their synapses were on dendritic spines. Confirming strong indirect evidence, GABA has been demonstrated in their synaptic terminals⁴³ (Figure 8). Interestingly, the supra- and infragranular pyramidal cells receive input from separate populations of basket cells, with axons running tangentially up to about one millimeter from the soma, but shorter range varieties also exist in the superficial layers. Layer 4 has a shorter range basket cell, the so-called clutch cell. Thus in the visual cortex of the cat at least a three-tier GABAergic neuronal system exists. In the monkey striate cortex the lamination is probably even more complex; layer-selective basket cells with different lateral territories could be stacked up in the same column.⁴⁴ The laminar segregation strongly implies that the functional properties brought about by basket-cell inhibition have to be produced or maintained at each level of cortical processing.

A noteworthy feature of the basket cells is that in addition to providing a significant input to somata, where their inhibitory effect would be nonselective, many of their synapses on dendritic shafts and spines would probably inhibit strongly those inputs distal

to them.⁴⁵ Most of the contacts on dendritic shafts are close to the soma and thus the influence may be similar to the somatic contacts. The basket-cell synapses on spines are discussed below.

GABA-containing neurons terminating mainly on dendritic shafts and spines. Preliminary examination of the targets of two other types of GABA-containing cells shows that they terminate only on small diameter, presumably distal, dendritic shafts and on dendritic spines.⁴⁶ One of them is the so-called “neurogliaform cell” with a small spherical dendritic field and a dense axonal field arborizing spherically in and around the dendrites in a space around 300 μ m in diameter.⁴⁷ The axonal boutons and synaptic contacts are very small.

The other cell is the so-called “bitufted cell” with sparsely spiny dendrites oriented mainly radially. The axon runs in arcades of collaterals loosely passing through several layers and forms large synaptic terminals. Both cells are numerous in the middle layers but neither their terminals nor their axons seem to respect laminar boundaries and, because their targets cannot be followed back to the parent cells, it is not known what governs the spatial distribution of their processes.

Why make inhibitory contacts on distal dendrites, and in particular on dendritic spines? These contacts may not affect the membrane potential at the level of the somata, but probably inhibit excitatory input arriving at more distal location. In particular the GABAergic synapses located on the necks of spines may only affect the

excitatory input received by the head of the same spine.⁴⁸ The inhibition exerted by these cells would thus be the most selective, because excitatory postsynaptic potential (EPSP) generated in more proximal dendrites or in dendrites not receiving input from the particular GABAergic cell will not be affected.

Accepting this suggestion raises the question of why basket cells invest the same or more synaptic effort into selectively influencing spines as they do into the non-selective inhibition of somata. An attractive explanation for the more peripheral effect of basket cells, as well as for other spine-contacting GABAergic neurons, could be that they do not randomly pick spines, but selectively associate with spines that receive part of a particular excitatory input. This input would then be regulated by the given GABAergic neuron. During ontogenesis the location of inhibitory synapses may be stabilized according to their efficacy in interacting with the particular input. Thus, the effect of a basket-cell terminal, if it was paired with the right excitatory synapse on a spine, could be as effective as on the more proximal dendritic shaft.

Input of GABAergic neurons. In order to understand the role of diversity in the termination of GABAergic neurons we should know under what conditions the particular neurons are activated, that is, what is their input? Unfortunately little information is available on this point. Thalamic afferents have been shown to terminate on GABAergic neurons, which is not unexpected in light of reports that every

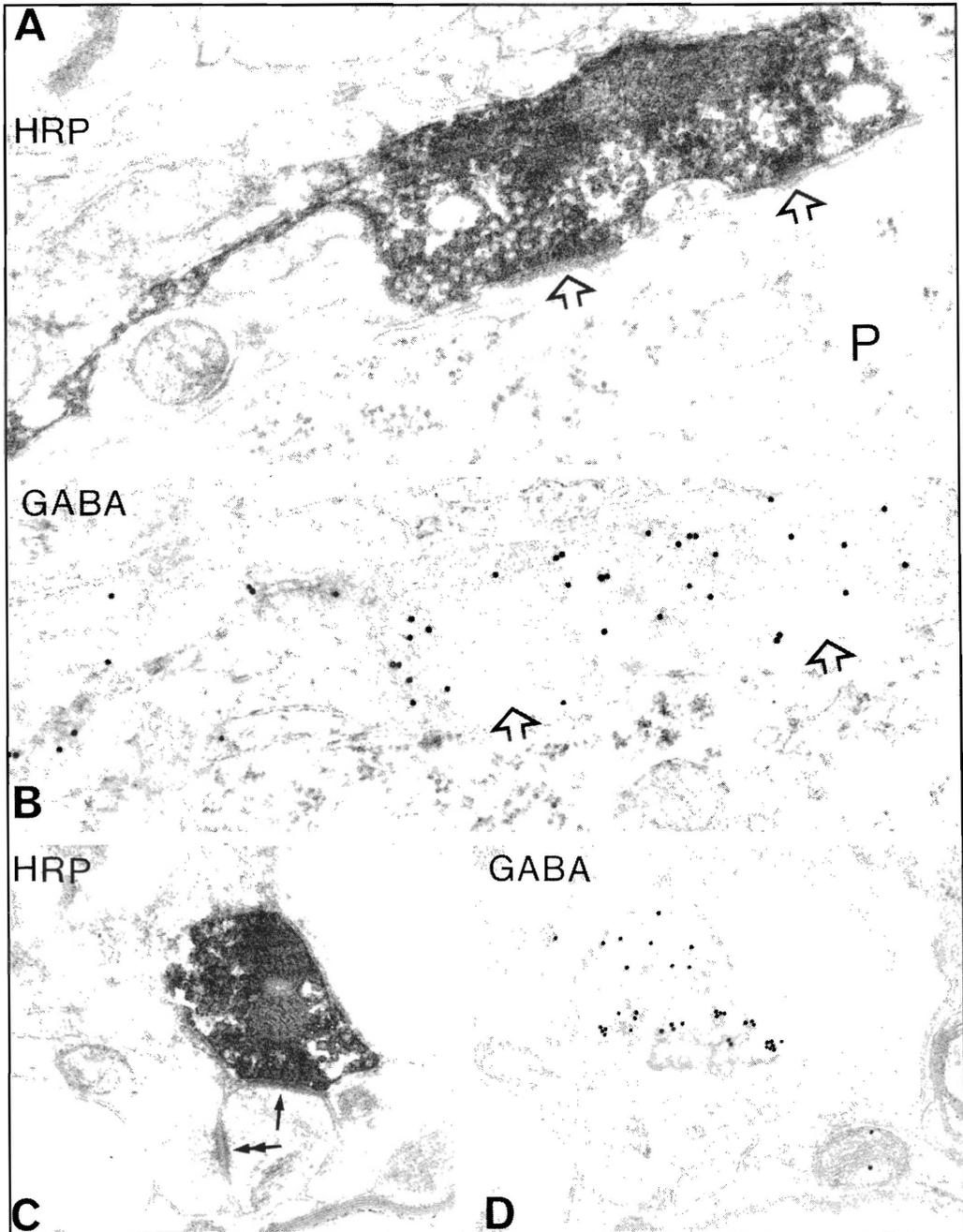


Figure 8. Immunogold demonstration of GABA in synaptic boutons of a large basket cell (A,B serial sections) and a clutch cell (C,D serial sections), both recorded intracellularly and filled with HRP by Kevan Martin and David Whitteridge in the striate cortex of cat. Sections B and D were immunoreacted. The selective distribution of electron-dense gold particles over the boutons demonstrates the presence of GABA in them. The basket terminals make synapses (open arrows) with the soma of a pyramidal cell (P). Only about 13 percent of GABAergic synapses in the cortex are on the somata of neurons. The clutch-cell terminal makes a synapse with a spine (arrow) which also receives a synapse from another bouton (double arrow). About 25 percent of GABAergic synapses are on spines where they can modify the excitatory input coming to the same spine. Data from several studies.^{33,43} Scales: 0.5 μ m.

cell within the termination zone of geniculate axons can be activated monosynaptically.⁴⁹ A more surprising aspect of thalamic termination is that the few synapses that geniculate axons establish with neuronal somata are exclusively on GABAergic cells.⁵⁰ The size and structural features of these neurons were very similar to basket and clutch cells, and it was proposed that their somatic input ensures high reliability of activation. Interestingly, the cells contacted by X-type thalamic afferents in the cat were significantly smaller than cells contacted by Y-type afferents, providing structural evidence that the two streams of visual afferents activate separate sets of inhibitory cells.

There is another example of selective innervation of a subpopulation of GABAergic neurons by subcortical afferents. It has been shown that some serotonin-containing fibers from the brain stem form pericellular nets in the cat cortex, establishing numerous synaptic contacts around some GABAergic neurons while ignoring others nearby.⁵¹ The serotonin-positive fibers also establish contacts with non-GABAergic neurons.

It has been found recently that another subcortical system, afferents from the basal forebrain, some cholinergic,⁵² some GABAergic as shown by Freund and Gulyas,⁵³ also heavily innervate GABAergic neurons. Cholinergic terminals form synapses at least three times⁵⁴ more frequently on GABA-containing cells than the overall population of synapses, although we do not yet know if these cholinceptive cells are a subset of the GABAergic neurons.

Most of the synaptic contacts in cortex are formed by local and interareal axons of spiny, excitatory cells, the pyramidal cells and spiny stellate cells. The question is, to what extent do these cortico-cortical pathways participate in the activation of local, GABAergic neurons? Most of the latter have few dendritic spines or none at all.⁵⁴ Thus, on the one hand, if the main role of the spiny cell axons was the mono- and disynaptic activation of the target areas, then one would expect most of the pyramidal axon boutons to give synapses onto dendritic spines of the recipient spiny, presumably also excitatory, neurons. On the other hand, if the main role was the disynaptic inhibition of the target cell groups, then one would expect the pyramidal cell boutons to terminate mainly on dendritic shafts and somata of GABA-containing inhibitory neurons.

Data about the local targets of HRP-filled pyramidal cells of layer III of cat striate cortex⁵⁵ showed that the overwhelming majority of their terminals, both in layer III and in layer V, in the column of the cell as well as in the distant clumps, made synapses with spines of other pyramidal cells (Figure 9). Of the few dendritic shafts that were contacted, less than half were immunoreactive for GABA and thus overall less than 5 percent of the targets belonged to inhibitory cells (Figure 9). These results, in agreement with other studies,⁵⁶ suggest that the primary role of local as well as interareal axonal systems of spiny neurons is the activation of other excitatory cells. The proportion of inhibitory cells may vary

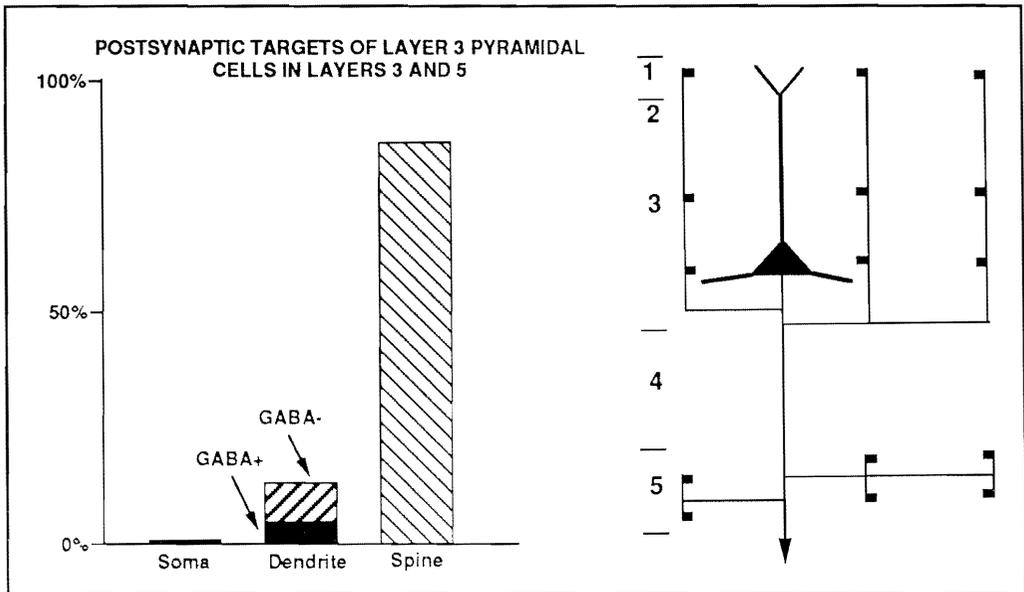


Figure 9. Quantitative distribution of synaptic connections of layer 3 pyramidal cells in the cat's striate cortex. The vast majority of synapses are on dendritic spines of other pyramidal cells. GABA-positive dendrites of inhibitory cells are rarely contacted. Targets were similar in both layers 3 and 5, in the column of the parent cell as well as in distant clumps of terminals. Data based on a study by Kisvarday *et al.*⁵⁵

depending on the role of the particular circuit. Layer 5 pyramidal cells may contact an even smaller proportion,⁵⁷ while some layer 6 pyramidal cells may contact a higher proportion of GABAergic targets⁵⁸ (see also below).

The sparse input from single pyramidal cells to GABA-containing cells suggests that these cells could be activated only through a strong convergence of pyramidal cells. It is possible that this convergence takes place onto a select population of inhibitory neurons, since distributing the few contacts to GABAergic cells randomly would make little impact. Which types of GABAergic cell are involved in the cortico-cortical and local pyramidal connections remains to be established.

We have demonstrated directly the presence of GABA in some of the tar-

gets of pyramidal cell collaterals, thalamic and other subcortical afferents in the visual cortex as discussed above. Indirect evidence indicates that, to different degrees, all other cortical pathways examined so far at the synaptic level may be connected in the same way.⁵⁹ Thus, pathways in the cortex always contact both putative excitatory and inhibitory neurons and the latter can provide feedforward inhibition. This arrangement is highly suited for evolutionary change required for different tasks. By changing the proportion of input to GABAergic neurons and by differential placement of the excitatory and inhibitory synapses, the basic design shows differences between species, areas and layers. The specialization of different channels in afferent pathways, such as the different tha-

lamic streams, or the addition of new cortico-cortical pathways,⁶⁰ resulted in the modification of the basic plan to suit particular needs. Two processes, the separate use of subsets of inhibitory neurons by different afferent systems, and the modifications required by channel specialization molded the structural and functional diversity of GABAergic cells as we see them today (Figure 10).

The Rules of Cortical GABAergic Circuits

The following conclusions may help to guide future work:

1. Most GABAergic-presumed inhibitory synapses in cortex are on dendritic shafts followed by dendritic spines, somata and axon initial segments.³³ This shows that most inhibitory synapses are likely to interact selectively with other inputs converging onto the same dendrites and spines.

2. Different neuronal types, as shown by their form, have different target selectivity. Consequently different parts of cortical neurons receive separate inhibitory inputs from distinct sets of cells.

3. Neurons with similar target selectivity may differentiate into layer-specific subtypes (such as basket cells), each providing input to a separate subset of cells in a column.

4. Different functional streams use different subpopulations of the same cell type (see above the termination of X and Y axons in the cat).

5. There are neurochemical differences between subpopulations of

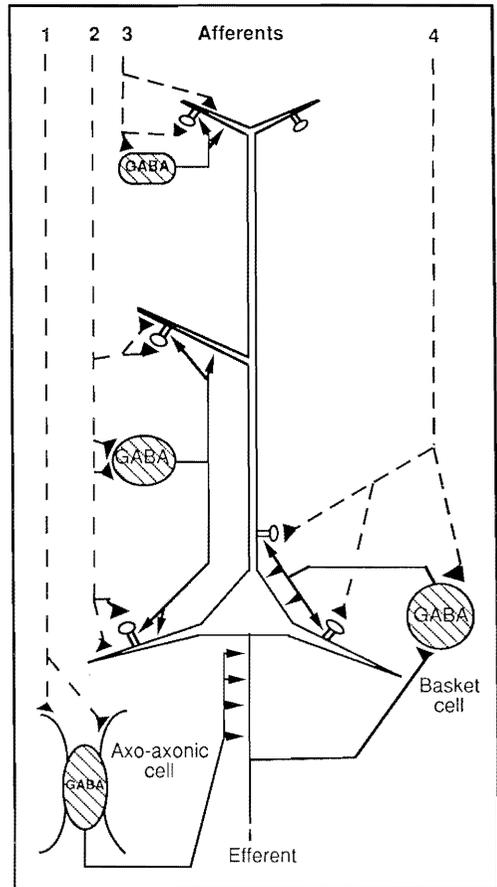


Figure 10. Schematic representation of the innervation of cortical neurons by subcortical and intracortical afferents (one-four). According to the hypothesis different afferents terminate on different parts of the same cell and each afferent system uses subsets of GABAergic neurons, many of which terminate in association with the afferents. This associated termination would make it possible for the GABAergic cells to regulate the gain of inputs selectively. Only few types of inhibitory cell would receive recurrent input from the efferent neurons as shown here for the basket cell. Naturally, the GABAergic neurons receive other input as well, which influences their activity relative to the afferent shown in the diagram. Axo-axonic or chandelier cells control the final output without interacting with other inputs to the pyramidal cells.

GABAergic neurons. The first evidence for this conclusion came from the demonstration of cholecystinin and somatostatin-immunoreactive material in

separate populations of GABA-containing cortical and hippocampal cells.⁶¹ The presence of other neuroactive peptides, the differential expression of cell surface molecules and Ca⁺⁺ binding proteins have been reviewed recently.⁶² We have also found differences in GABA_A receptor expression (see below), and some GABA-containing terminals also contain choline acetyltransferase, the enzyme synthesizing ACh.⁵² These biochemical differences demonstrate the molecular differentiation of GABAergic subsystems.

These conclusions, based on work carried out over the last several years, illustrate the changing concepts of the organization of cortical inhibitory circuits. In place of the general and overall inhibition, thought to be exerted on the soma and proximal dendrites of neurons, a new picture is emerging that shows multiple and selective influences, both on different parts of the same cells and on separate populations of cells, as summarized in Figure 10.

Physiological Correlates of GABAergic Circuits

Since every cortical cell receives GABAergic input, it is not surprising that the response properties of cells change if this input is not operating normally. However, due to the diversity of GABAergic neurons, it is difficult to determine which particular GABAergic circuit is responsible for a property that depends on inhibition. For example, in spite of much attention devoted to the role of inhibition in the maintenance of cortical orientation

selectivity, little progress has been made with its explanation in terms of the known connections.⁶³

Directional selectivity. Basket cells, although forming only a portion of GABAergic synapses, feature prominently in inhibitory schemes because of their relatively long tangential axonal spread, suitable for mediating lateral inhibition. Of all the possible functions of basket cells in the visual cortex, the maintenance of directional selectivity, through a mechanism proposed by Barlow and Lewick⁶⁴ for the rabbit retina and for the cortex,⁶⁵ is compatible with several of their structural features:

1. Basket cells contain GABA and interference with GABAergic neurotransmission abolishes, or reduces, directional selectivity.⁶⁶

2. Directional selectivity is produced by divisive type of inhibition,⁶⁷ and somatic inhibition has been proposed to be divisive.⁶⁸

3. Some basket cells have axons elongated in particular directions and running tangentially for up to a millimeter from the soma. These tangentially oriented inhibitory projections may produce directional selectivity depending on the direction of stimulus sweep relative to the position of the dendritic tree and axonal elongation of the basket cell.

4. Inactivation studies suggest that directional selectivity does not depend on interlaminar connections,⁶⁹ but may depend on horizontal connections.⁷⁰ The three-tier tangential basket-cell system suits such a requirement.

5. Tangential mapping of directional selectivity pattern in the visual cortex revealed isodirectional patches

1-2mm in extent and frequent shifts in preferred direction, with 180° reversals.⁷¹ A system of basket-cell axons can provide this pattern, with reversals coinciding with the somata of basket-cell groups. Clearly it would be important to reconstruct the oriented axonal fields of basket cells in relation to the directional selectivity map in the same piece of cortex. The inhibitory mechanism would probably operate together with horizontal excitatory connections.

End-inhibition. The most explicit hypothesis for the role of a circuit in the elaboration of a receptive-field property that depends on inhibitory interactions has been proposed by Charles Gilbert, Torsten Wiesel and their associates⁷² for the length tuning of some cortical receptive fields. This property is of particular importance in light of our interests in this volume in trying to find the basis of neuronal mechanisms in visual perception. Length tuning or end-stopping has been proposed as the possible basis of curvature detection.⁷³ According to the hypothesis a long inhibitory receptive field is superimposed by GABAergic neurons on the shorter excitatory receptive field of simple cells in layer 4. The inhibitory neurons in layer 4 are activated mainly by pyramidal cells from layer 6, which are known to have long receptive fields. One of the key structural bases of the hypothesis is that some layer 6 pyramidal cells have been shown to project selectively to layer 4 where they terminate mainly on dendritic shafts of presumed inhibitory cells, in contrast to geniculate terminals which mainly terminate on dendritic spines of presumed excitatory cells.⁷⁴

We have tested this structural requirement for the implementation of end-inhibition in the cat's visual cortex by determining the proportion of dendrites containing GABA amongst the targets of a simple cell situated in layer 6.⁷⁵ A post-embedding immunocytochemical procedure was used on electron-microscopic sections of the synaptic contacts of the simple cell recorded intracellularly and marked by HRP (Figure 11). We confirmed the results of McGuire *et al.*⁷⁶ that the majority of targets were dendritic shafts, but only about 14 percent of all postsynaptic targets, all dendritic shafts, contained GABA and therefore belonged to putative inhibitory neurons (Figure 11). The rest of the targets were GABA-negative dendrites (56 percent) and dendritic spines (30 percent). Although our method may somewhat underestimate the proportion of targets belonging to inhibitory neurons,⁷⁷ the results make it unlikely that GABAergic inhibitory neurons would be the major target of the recurrent collateral axon system of pyramidal cells in layer 6. A more likely scenario is that most of the spines and dendritic shafts belong to layer 4 spiny excitatory cells, in line with the predominantly excitatory effect attributed to the projection from layer 6 to layer 4.⁷⁸ Nevertheless the 14 percent of synapses given to GABAergic neurons is the highest found so far for any pathway in cortex (except the basal forebrain projection⁷⁹), and more than three times higher than that found for the axon collaterals of pyramidal cells in layer 3.⁵⁵ Thus, in addition to the monosynaptic excitation, an inhibitory effect can be mediated by this link

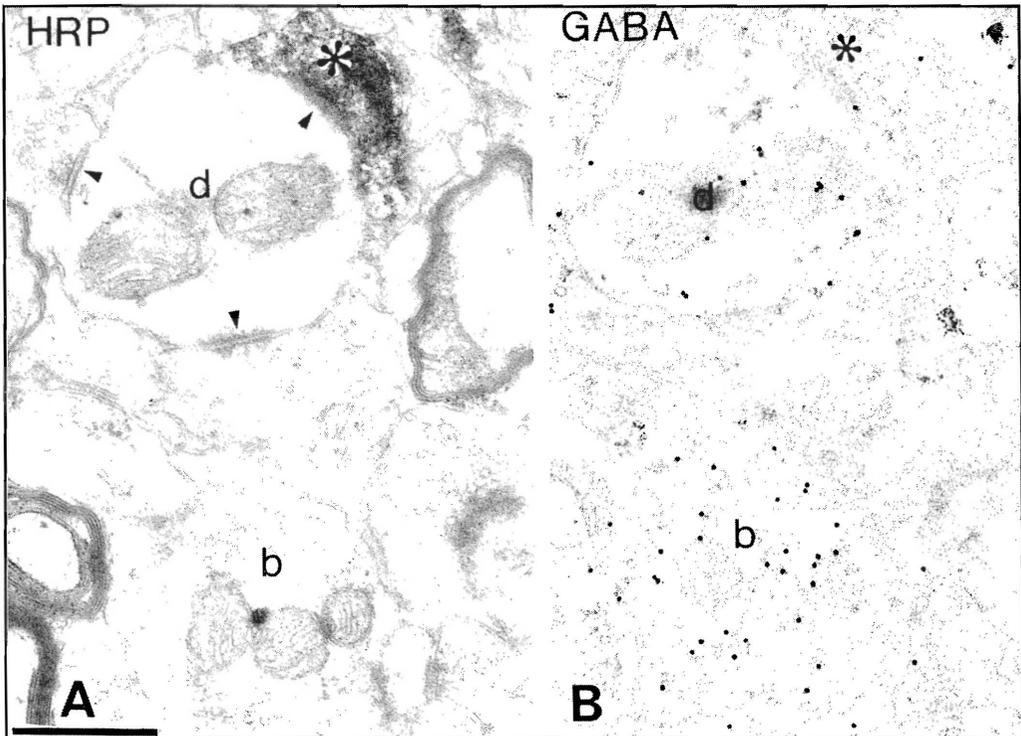


Figure 11. Immunogold demonstration of GABA in a dendrite (d) postsynaptic to the HRP-filled terminal (asterisk) of a layer 6 pyramidal cell. **A** and **B** are serial sections. The dendrite is in layer 4 where most of the intracortical terminals of these layer 6 cells are found. The same dendrite receives other synapses as well (arrow heads). Colloidal gold particles on the dendrite and a bouton (b) in **B** demonstrate their GABA content. The layer 6 pyramidal cell had simple receptive-field properties. Micrographs courtesy of Dr. M. Antal. Scale: 0.5 μ m.

as proposed originally.⁷² However, end-inhibition may not be the only role for this pathway, since the same strong projection also exists from layer 6 to layer 4cB of the monkey striate cortex, where end-inhibition is not a prominent property of the receptive fields.

The LGN has also been proposed as an alternative site for the generation of length tuning,⁸⁰ by an inhibitory mechanism activated by input from cortex. Certainly cortical terminals originating from the same layer 6 cells that project to layer 4 are known to innervate local GABAergic cells in the LGN but, as in the cortex, they also directly innervate the excitatory relay cells.⁵² Thus the cortical and thalamic

ends of the layer 6 cell projection seem to be similarly organized.

These results are not necessarily contradictory. The same mechanisms may synergistically operate in both the LGN and the cortex. One might assume that the inhibitory cells have a higher threshold for activation and require converging input from many layer 6 cells. Using a short bar as stimulus, both the recurrent cortical collaterals in layer 4 and the terminals in the LGN would primarily exert background excitation to the excitatory neurons, providing positive feedback. Lengthening the bar would increasingly activate GABAergic inhibitory cells, either because more layer 6 input would reach them, or

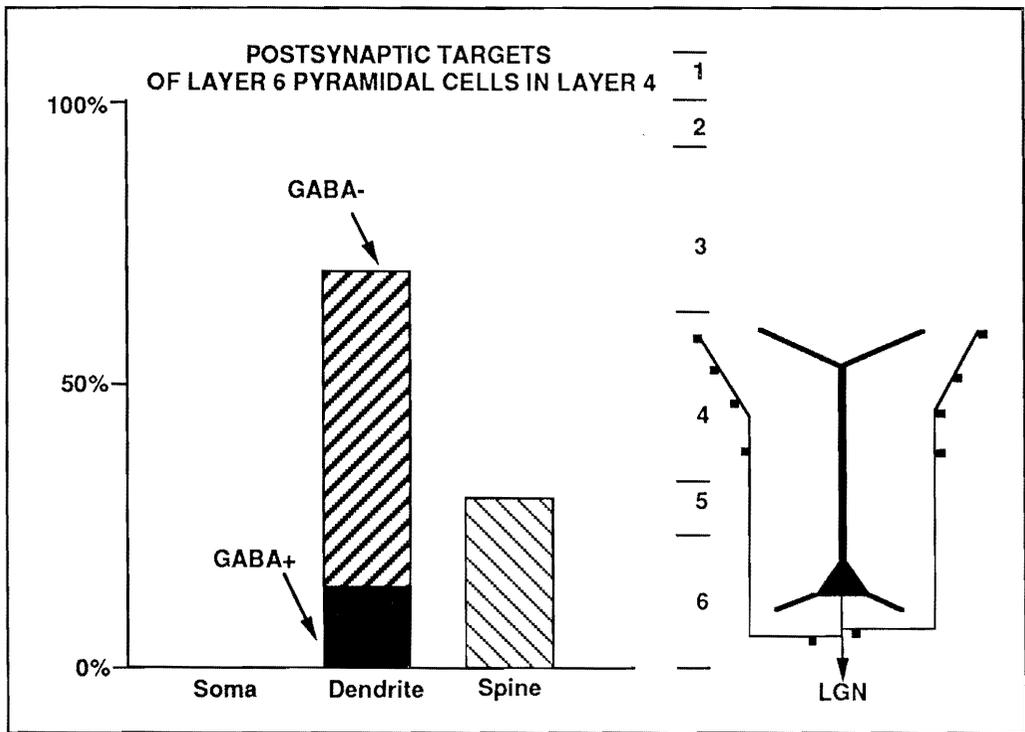


Figure 12. Quantitative distribution of synaptic connections of a layer 6 pyramidal cell with recurrent axons to layer 4 in the cat's striate cortex. The majority of synapses were on dendritic shafts and less on dendritic spines. Only a small proportion of dendrites were GABA-positive as detected by immunocytochemistry. In comparison with layer 3 pyramidal cells the proportion of inhibitory neurons as targets is higher (see Figure 9). Data courtesy of Dr. M. Antal, unpublished results.⁷⁵

because their input/response function rises more steeply than that of the excitatory cells. This hypothesis would basically keep both the cortical and the LGN mechanism proposed for the generation of length tuning and in addition, would also take into account the predominant direct termination of layer 6 cells on putative excitatory neurons, as outlined here (Figure 12).

Localization of GABA_A Receptors

Whatever circuits produce inhibition, some of the effect is mediated by the GABA_A receptors which can now

be localized at the subcellular level. In the striate cortex of the cat, four monoclonal antibodies to either the α - (bd-24, bd-28, 62-3G1) or the β -subunit (bd-17) of the receptor complex were used to reveal intra- and extracellular receptor proteins.⁸² As in the LGN, intracellular immunoreactivity was associated with the endoplasmic reticulum, Golgi apparatus and multivesicular bodies suggesting respectively the synthesis, glycosylation, and degradation of the receptor complex. Extracellular immunoreactivity was associated with the plasma membrane of neuronal somata, dendritic shafts and spines. In the cortex, as elsewhere, the density of

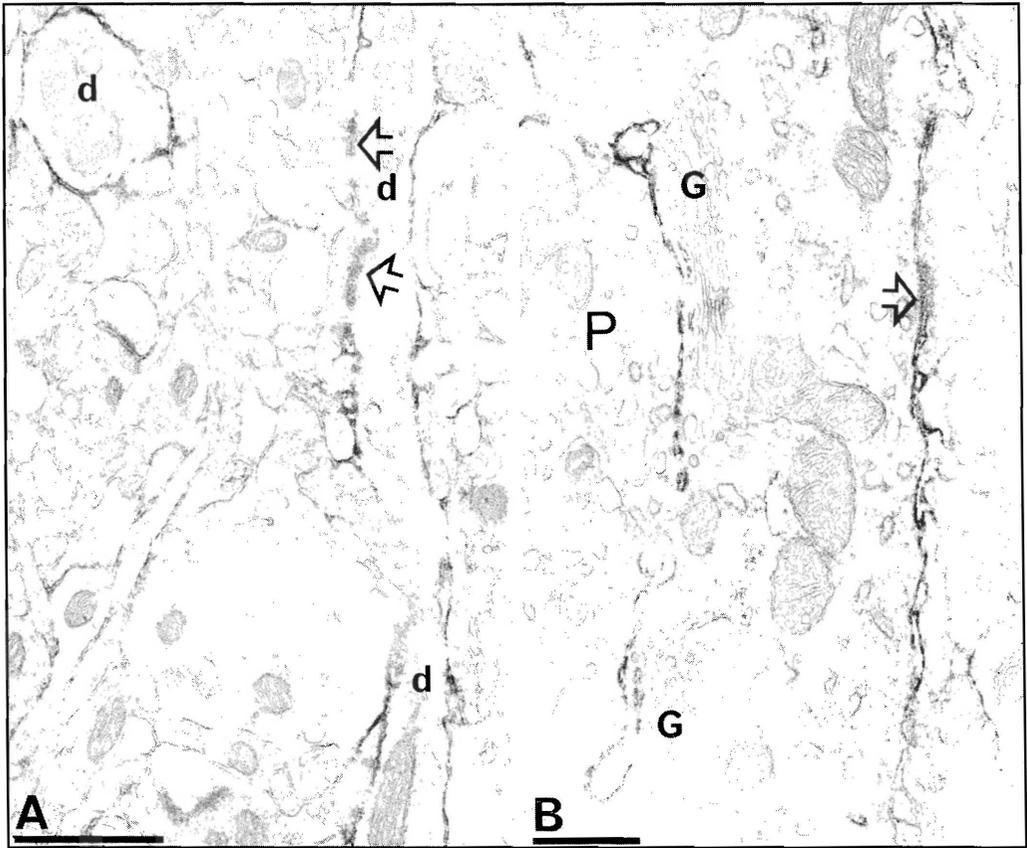


Figure 13. Synaptic and nonsynaptic immunoreactivity for the GABA_A receptor complex in the striate cortex of cat. **A.** Two dendrites (d) are strongly immunopositive along their entire plasma membrane including two synaptic junctions (open arrows). **B.** Immunoreactivity for the receptor complex in the Golgi apparatus (G) of a pyramidal neuron (P) as well as along its plasma membrane both at the synaptic (open arrow) and at the non-junctional plasma membrane. Data.⁸² Scales: **A,** 1 μ m; **B,** 0.5 μ m.

immunoreactivity does not appear to be enhanced at presumed GABAergic synaptic junctions, and it also covers non-junctional plasma membrane (Figure 13).

Immunoreactive receptor density is highest in layer 4. Throughout the cortex different classes of neuron express the receptor complex to different degrees. The highest density of receptor was found on the somatic and dendritic plasma membrane of some GABA-containing cells. Among these cells are the vast majority, if not all, of

the largest GABA-containing neuron. These resemble the large basket cells (see above) in dendritic and somatic features (Figure 14). Some medium and small GABA-positive cells also show very strong receptor immunoreactivity.

Some GABA-negative cells, especially in layer 4, show very strong intracellular reactivity suggesting a high turnover of the protein. Some pyramidal cells in the infragranular layers also show above-average immunoreactivity along the plasma membrane. How-

ever, the majority of pyramidal cells in all layers show less immunoreactivity for the receptor complex than neighboring GABAergic neurons. The membranes of some dendritic spines were also immunoreactive irrespective of whether they received a presumed GABAergic synapse.

The results predict differential sensitivity of different neuronal classes to GABA, which could act at both synaptic and nonsynaptic receptor sites. In particular it should be ascertained whether the increased expression of the GABA_A receptor complex by large basket cells, which feature prominently in schemes of cortical inhibition, is accompanied by an increased sensitivity to GABAergic inhibition. Basket cells do not seem to receive heavier GABA innervation than other types of cell. Thus here again, as in the LGN, receptor density differences rather than wiring may produce different physiological effects. Basket cells are the primary candidates for producing lateral inhibition, and they act on proximal processes of neurons, probably evoking robust effects. A higher threshold of activation, produced by the high density of GABA receptor on their membrane providing tonic inhibition, may represent a mechanism for activation only at high level of cortical activity.

Relationship of GABA-Releasing Terminals and GABA_A Receptors

The most striking feature of the lo-

calization of the GABA_A receptor is that the receptor proteins are present not only at the synaptic junctions but also at non-junctional sites in every area of the brain examined so far.^{16,82} As discussed previously,^{16,83} cross-reactivity of the antibodies with membrane constituents other than the receptor complex is an unlikely explanation for our results. It is also improbable that the distribution results from a diffusion artifact inherent in the immunocytochemical method, because non-junctional plasma membrane is often immunoreactive in the absence of any junctional reaction. The genuine non-junctional localization of the receptor complex is particularly evident on the somata of cerebellar granule cells where they never receive GABAergic synapses.⁸⁴ This is in agreement with the demonstration of functional, somatic GABA-gated Cl⁻ channels demonstrated with the patch-clamp technique (Cull-Candy, personal communication).

The GABA-receptor complex may be unusual amongst neurotransmitter receptors with regard to its localization. Electron microscopic studies of a closely related amino-acid-gated receptor/channel complex, the glycine receptor, show that it is mainly associated with synaptic junctions.⁸⁵

The explanation for the widespread, extrasynaptic distribution of the receptor complex may lie in the general role of the GABA-gated Cl⁻ channel as a regulator of the dynamic range of neurons. The synaptic and nonsynaptic receptors may be used under different levels of neuronal activity, providing a mechanism for adaptation. Cells proba-

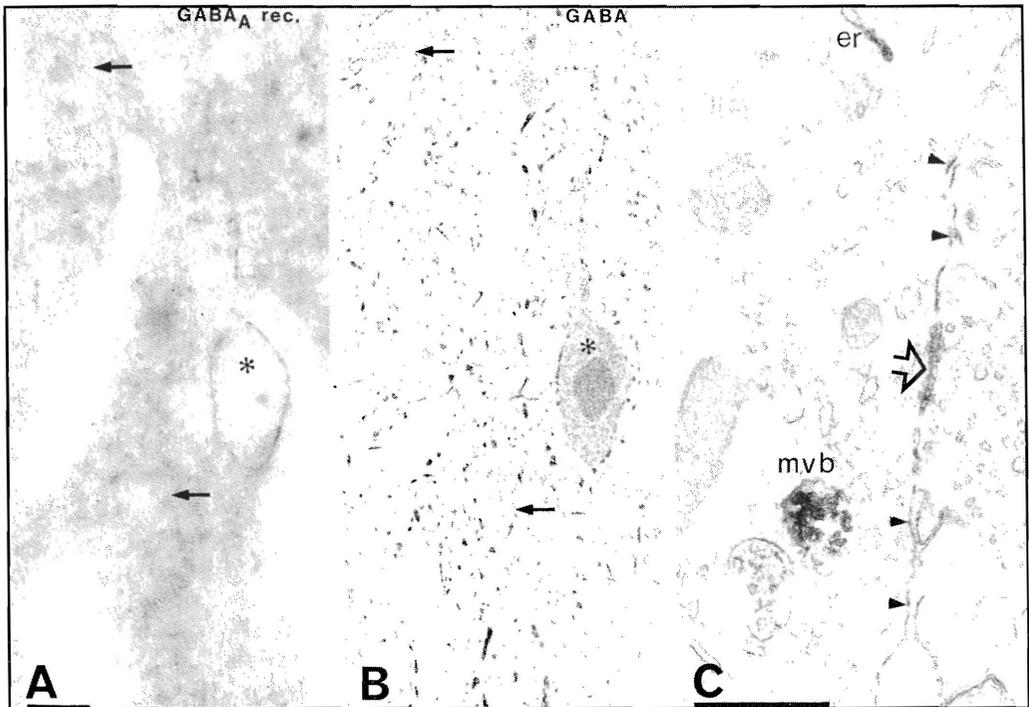


Figure 14. A large layer 3 neuron (asterisk) is strongly immunopositive for the GABA_A receptor complex (A, 50µm thick section), as well as for GABA (B, 0.5µm thick section). The characteristics of this cell show that it is probably a large basket cell. Other cells (horizontal arrows) show no detectable immunoreactivity. The receptor immunoreactivity (arrow heads) was found along most of the cell's plasma membrane, including some synaptic junctions (open arrow) as shown in an electron micrograph in C. Intracellularly receptor immunoreactivity was found in the endoplasmic reticulum (er) and in multivesicular bodies (mvb). Scale: A, B, 10µm; C, 0.5µm.

bly receive some GABA at all times. At low levels of neuronal activity, GABA acting at the synaptic junctions provides adequate inhibitory control of the neurons. The moderate amounts of GABA released can be removed by reuptake or glial uptake⁸⁶ without reaching the extrasynaptic sites and without causing desensitization of the receptors. However, at increased excitatory input, the number of channels operating at the junctions either may not be adequate, or a substantial proportion of receptors may be desensitized⁸⁷ and, as a result, synaptic receptors would not be able to keep the activity of the cell in the range of optimal sensitivity. Under

these conditions channels at extrasynaptic sites would be opened by GABA diffusing from the release sites.⁸⁸ Such lateral diffusion of transmitter to receptors other than the ones facing the releasing terminal has already been proposed as the basis for synergism between simultaneously activated inhibitory synapses using glycine as the transmitter.⁸⁹ Extrasynaptic receptors may also be influenced by GABA released from glial cells under certain conditions.⁹⁰

One consequence of the hypothesis presented above is that the topography of GABAergic effects would primarily be regulated not by the pre-

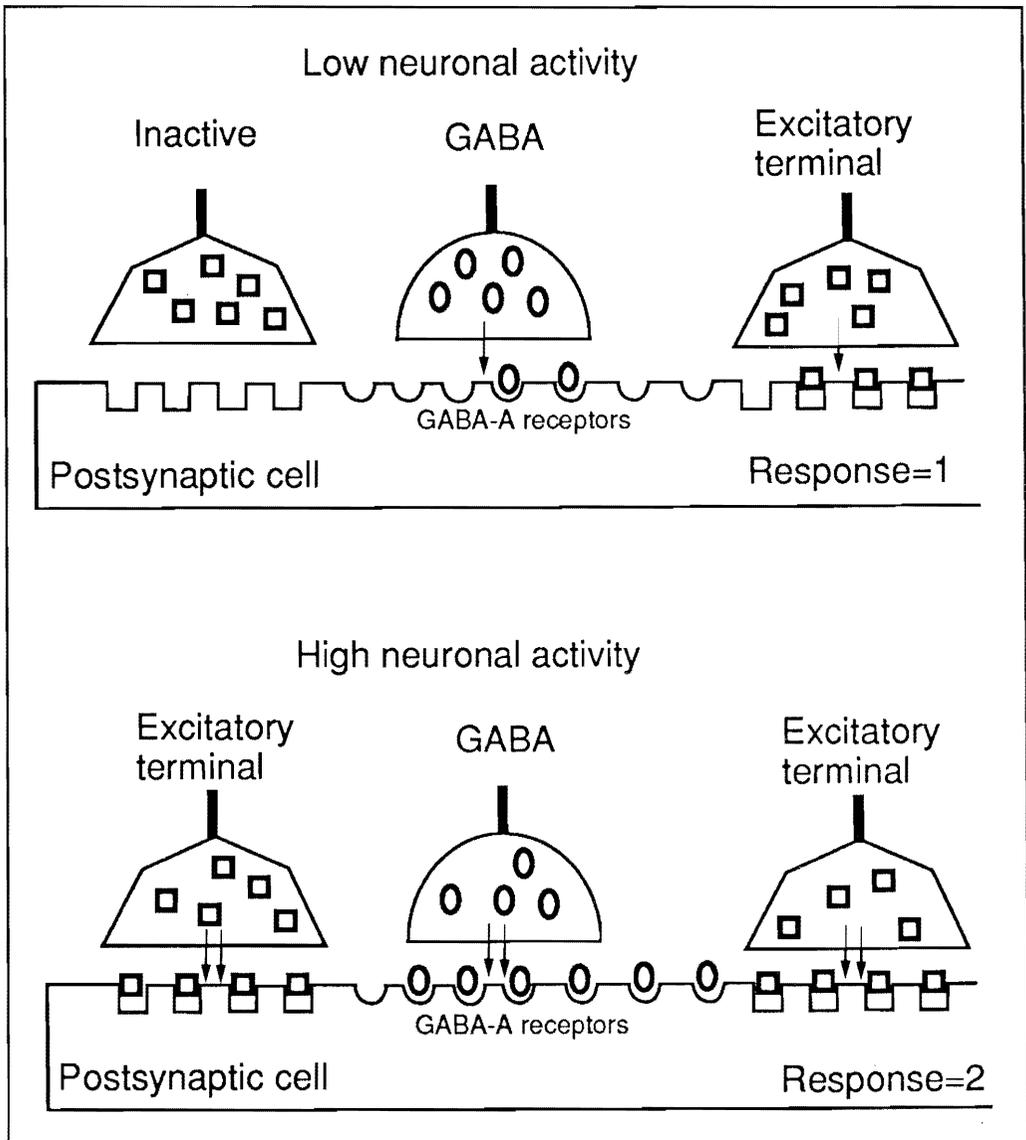


Figure 15. Schematic summary of the possible role of synaptic and extrasynaptic GABA_A receptors as regulators of the dynamic range of the postsynaptic cell's response. The interactions between excitatory and inhibitory input should not be linearly additive as simplified here. See text for detail.

cise placement of receptors but by the precise placement of GABA-releasing synaptic terminals and by the amount of GABA released. This could explain the great degree of spatial differentiation in the location of GABAergic synaptic terminals in stations of the visual pathway and particularly in the cortex. Another consequence of the

hypothesis is that the neurons that provide the GABA-releasing terminals to a certain location on the postsynaptic cell should receive information about both the intensity of the excitatory input to that part of the neuron and about the resulting activity in the postsynaptic cell. Converging feedforward and feedback connections with different gains

could provide the GABAergic cells with the ability to release GABA in proportion to the efficacy of the excitatory drive reaching their target cells (see above and Figure 15).

It is noteworthy that layer 4 of the primary visual cortex, which receives the majority of primary afferent synapses, shows the highest level of GABA_A receptors. Thus the GABAergic system, through the synaptic and, at increased excitatory input, through the non-junctional receptors, could not only set the activity of the individual cells but at the system level could also regulate the activity of the cortical net by regulating cortical input via layer 4.

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