# MOLECULAR NEUROANATOMY OF SYNAPSES, CELLS AND SYSTEMS IN THE BRAIN

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Molecular neuroanatomy involves the identification of the molecular machinery of neurons and glia within the framework of connections and physiological properties of cells. It provides information on the physical constraints imposed by the brain on neuronal circuits in meeting their functional demands. Thanks to tremendous technical advances, molecular neuroanatomy often paves the way for functional questions tackled by other disciplines, and it also helps to integrate information from many disciplines, leading to the explanation of behaviour in terms of specific neuronal networks.

Neural networks are thought to be composed of repeating modules, which can be defined as the basic circuit consisting of several types of neurone and associated glial cells. The operational role of each neurone in a class is stereotyped, and is determined by the temporal and spatial distribution of its input and output as well as by the molecular events underlying the transformation of signals. The regular architecture of some areas of the brain allows the use of simple methods for the prediction of the connections and the chemistry of neurons. However, in other areas such as the neocortex, the identification of neuronal signatures can only be based on synaptic level examination of the identified neurons and their molecular composition. Of particular interest are neurotransmitters, second messengers, receptors and ion channels, i.e. the molecular machinery concerned with neural communication.

More often than not, the structural, chemical and dynamic properties of cells are examined separately for technical reasons. In exceptional cases it is possible to relate all these properties to single identifiable neurons. These cells can be recognised on the basis of structural, dynamic or chemical characteristics from specimen to specimen. In most cases however, both in vertebrates and in invertebrates, a role in the network is carried out by a probabilistically equivalent set of cells, members of which we would like to reexamine from experiment to

experiment. Therefore catalogues of properties are used to identify individual members of the set of neurons.

In the following brief survey, some aspects of the organization of cortical, hippocampal and cerebellar circuits will be examined, demonstrating how the localization of molecules and the spatial relationships of synaptic connections can be used to identify equivalent sets of neurons and to predict the mode of cellular interactions. I will concentrate on examples which show how different techniques have helped us to answer questions related to the function of the systems that we have studied; I will not attempt to review the literature in the areas discussed below. Technical details of the procedures can be found in recent reviews (Freund and Somogyi, 1989; Somogyi and Freund, 1989; Somogyi 1990).

When trying to explain the operation of a complex neural network on a realistic basis, the first task is to define the basic circuit that carries out the transformation between input and output. With my colleagues I have been studying the basic circuit of the cerebral cortex, establishing its properties with a rigour allowed by current technology.

## Chemical neuroanatomy of the basic cortical circuit

The cortical circuit is an integral part of all sensory-motor transformation. Sensory information arrives at the cortex via the thalamus, and information about different aspects of the outside world are carried in parallel channels in the same modality. For example in the visual system of the cat at least three physiologically distinct channels, the X, Y and W pathways, have been described from retina to cortex (Sherman, 1985). The thalamic axons involved in these pathways terminate in the middle, small cell populated layers of cortex and the monosynaptic target cells are activated by one of the pathways. Cortical cells are largely silent, in spite of considerable spontaneous activity in the thalamus of both anaesthetized and alert animals. When cortical cells respond they are particular for the parameters of the stimulus; e.g. in the visual domain for the orientation and length of stimulus, direction of movement etc. This specificity in the response is generated to a great extent in cortex, already in the middle layers where thalamic axons terminate. Rich intracortical connections, mediated by both intrinsic neurons and by the local collaterals of output cells, distribute the thalamic input to supra- and infragranular layers in a lamina specific and horizontally patchy manner.

## Excitatory amino acids in cortical cells

The output leaves the cortex via the axons of pyramidal cells, which use excitatory amino acid transmitters and produce monosynaptic activation of their targets, as shown by physiological and pharmacological studies. Anatomical evidence for the excitatory amino acid nature of pyramidal cell transmitter(s) comes from the selective retrograde labelling of pyramidal cells by $^{3}$H-D-aspartate injected into their termination areas (for review see Streit 1991, this volume). Since all pyramidal cells also possess axon collaterals and make synapses within the area where their cell body is located, small intracortical injections of the radiolabelled false transmitter can be used to study their intra- and interlaminar local connections (Kisvarday et al., 1989). This strategy was also used to study the chemical nature of another large population of cortical cells, the spiny stellate cells. Most spiny stellate cells have axons intrinsic to the cortical area where their cell body is located. Physiological and pharmacological methods have not elucidated their chemical nature because it was not possible to activate them selectively without also activating pyramidal cells. The retrograde labelling by $^{3}$H-D-aspartate, injected into the termination of their axons, showed that spiny stellate cells have the molecular machinery for the selective uptake of acidic amino acids, and this indicates that they use such an amino acid as transmitter (Kisvarday et al., 1989).

The endogenous compound that mediates excitatory amino acid interactions is thought to be glutamate, but it is not at all clear whether glutamate can account for all of the interactions, and other compounds have also been suggested (e.g. Do et al., 1986). The development of antibodies to glutamate and aspartate (Storm-Mathisen and Ottersen, 1986) led to numerous studies showing some degree of immunoreactivity in the somata of cortical cells including pyramidal cells. However, somatic immunoreactivity for amino acids, which are also involved in various metabolic pathways and protein synthesis, has little relevance to transmitter function. Indeed it has been demonstrated that the somata of cholinergic motoneurons is strongly immunopositive for glutamate (Ottersen and Storm-Mathisen, 1984), and the highest aspartate immunoreactivity was localized in the somata of GABA-positive, inhibitory cells in the hippocampus and cerebellum (Ottersen and Storm-Mathisen, 1985, Madl et al., 1987).

The presence of glutamate or other excitatory amino acids in the nerve terminals should have more relevance to a transmitter role, if it can be shown that the identified population of terminals contains distinctly higher levels of immunoreactivity than other neurochemically different populations of terminals. On a regional level, sensitive immunoenzyme methods can provide a first step towards

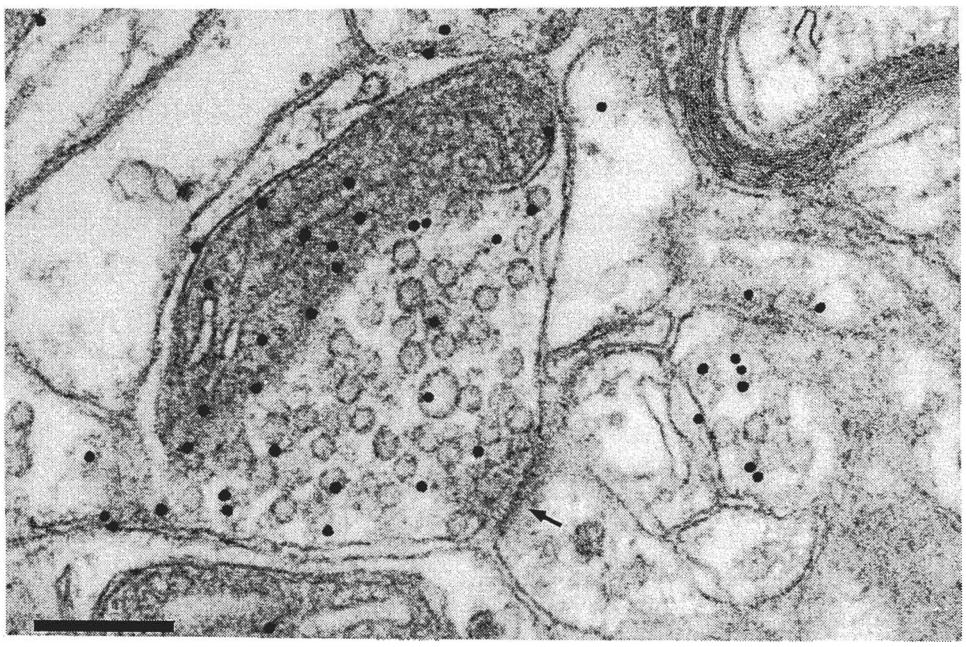


Fig. 1. Glutamate immunopositive bouton establishing a synapse (arrow) with a spine in the striate cortex of cat. Postembedding immunogold method using a rabbit antiserum to glutamate conjugated to bovine serum albumin by glutaraldehyde, and goat anti-rabbit IgG conjugated to colloidal gold. In spite of the impressive accumulation of gold particles over the bouton, suggestions based on such pictures, for the transmitter of terminals remain anecdotal without quantitative comparison of immunogold density over bouton populations, identified by their common origin and/or by their common biochemical character. Scale: 0.2 μm

such a goal (e.g. Liu et al., 1989). However, quantitative comparisons can only be obtained using particulate markers. This was first achieved in the cerebellum using quantitative immunogold localization of glutamate in parallel and mossy fibre terminals, which were shown to contain higher level of immunoreactivity than glial cell processes or GABA-positive terminals (Somogyi et al., 1986). Although individual terminals can be shown to contain immunoreactivity for glutamate in cortex as well (Fig. 1), without quantitative measurement of the immunoreactivity of a population identified with respect to their common origin, such data remain anecdotal. The quantitative postembedding immunogold method, combined with relative measurement of glutamate levels within distinct populations of terminals in the same sections, remains the only valid way to study selective distribution of amino acids which participate in important metabolic pathways as well as in neurotransmission (Ottersen, 1989).

The role of pyramidal cell axon collaterals. In addition to their distant targets pyramidal cells establish hundreds of synapses locally. Depending on the monosynaptic targets of the axons, these collaterals can either activate other excitatory neurons or, by activating local inhibitory cells, can exert disynaptic

inhibition. Quantitative studies on the targets of local terminals of pyramidal cells (Kisvarday et al., 1986) showed that most of the postsynaptic elements were the spines of other pyramidal cells. The origin of the pyramidal cell terminals was identified using intracellular HRP injection of physiologically characterised cells. Subsequent electron microscopic (EM) examination of the synapses, and post-embedding immunogold reaction of the postsynaptic elements for GABA, revealed that less than 5% of the targets originated form GABAergic inhibitory neurons. A similar technique was used for the estimation of GABAergic targets in long cortico-cortical pathways (Lowenstein and Somogyi, 1991). However, instead of intracellular HRP, which does not travel well over long distances within the short survival time of a physiological experiment, *Phaseolus vulgaris* leucoagglutinin (PHA-L) was used to label cortico-cortical terminals. Most of the targets were found to be spines of pyramidal cells and only 17% were dendritic shafts, of which about 70% were GABA-positive (Lowenstein and Somogyi, 1991). These quantitative studies combining physiological recording, intracellular marking and anterograde labelling methods with the immunocytochemical characterization of postsynaptic elements, reveal the cortex as a profusely interconnected network of spiny neurons using excitatory amino acid transmitters. The break for this network is provided by local inhibitory cells which must receive highly convergent input from sets of excitatory cells making up the same pathway. The identification of some of these inhibitory cells has been achieved using a battery of combined techniques.

## Identification of cortical GABAergic neurons

Physiological and pharmacological studies demonstrated that GABA is the major inhibitory neurotransmitter in cortex. Immunolocalization of glutamate decarboxylase (GAD), the main synthetic enzyme for GABA, showed that it was present in non-pyramidal cells (Ribak, 1978), many of which, unlike pyramidal and spiny stellate cells, have smooth or sparsely spiny dendrites. The enzyme was also localized in synaptic boutons establishing type 2 (symmetrical) synaptic contacts (Ribak, 1978). However, immunocytochemistry on its own proved insufficient to identify the origin of any particular terminal because, in the extensive immunoreactivity of the neuropil, the terminal axon branches could not be followed back to the somata of cells. Furthermore the dendritic immunoreactivity was insufficient to relate the GAD-positive cells to well known neuronal classes described by Golgi methods or by intracellular labelling.

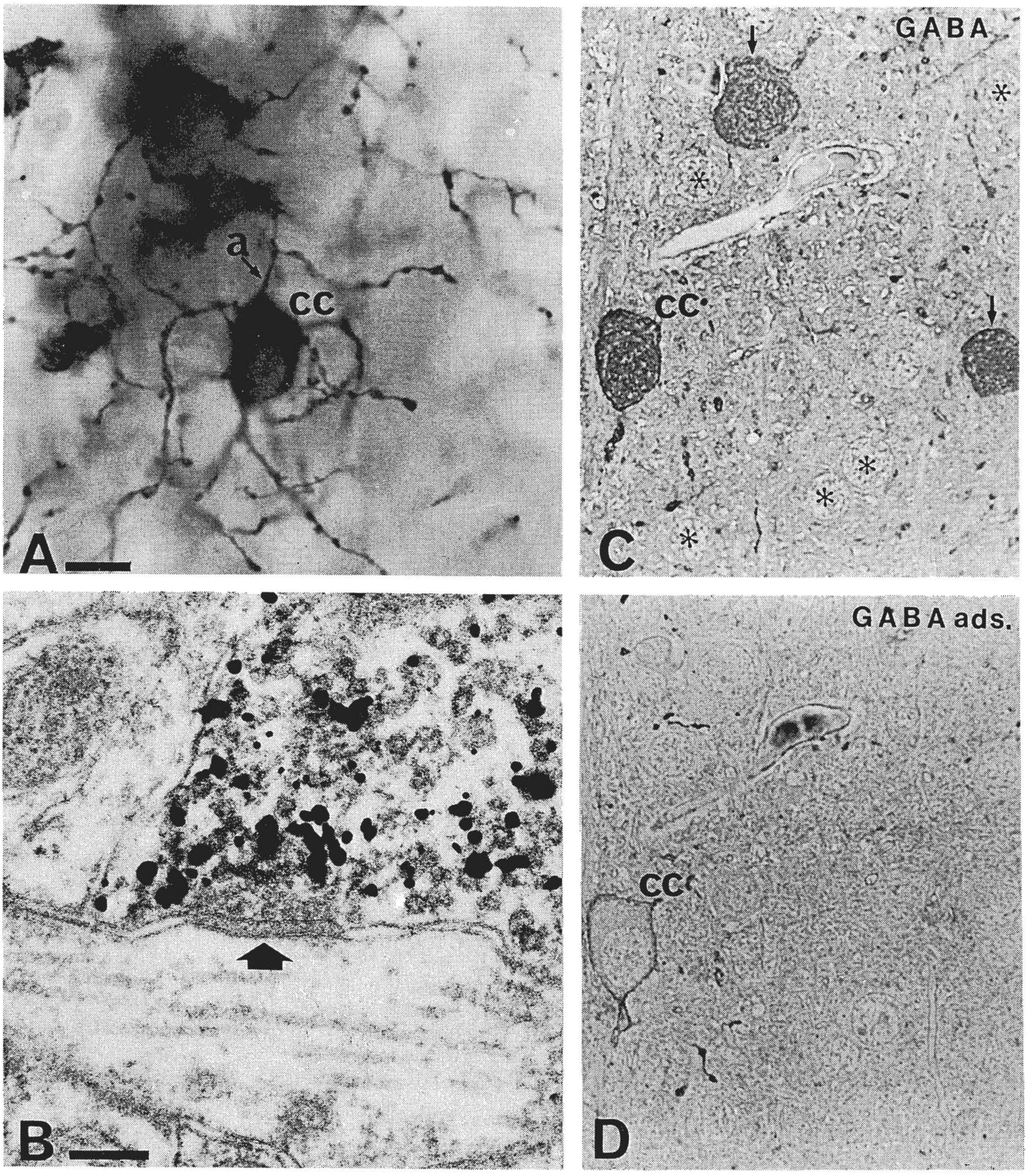



Fig. 2. Immunocytochemistry of a neurone identified as a **clutch cell** (cc) by Golgi impregnation in the striate cortex of monkey. **A.** Resin embedded section (90 µm thick) showing the processes of the cell (axon, a). **B.** One bouton, identified by the gold/silver deposit as originating from this cell, makes a type 2 synapse (arrow). **C.** A 0.5 µm thick section of the somata of the same cell after immunoperoxidase reaction for GABA. Note the peroxidase deposit in the clutch cell and in two more neurons (arrows), but not in others (asterisks). **D.** A consecutive section was reacted in the same way as shown in C, but the anti-GABA serum was preadsorbed with GABA conjugated to polyacrylamide beads. Note that only the thin rim of gold/silver deposit is visible, but the interior of the cell is free of deposit. Data from the work of Kisvarday et al., (1986). Scales: A, C and D, 10 µm ; B, 0.2 µm.

Three combined techniques were developed to identify particular classes of GABAergic cells (for review and detailed methods see Somogyi, 1988; Somogyi and Freund, 1989; Freund and Somogyi, 1989).

1. Pre-embedding immunoenzyme reaction for GAD, followed by Golgi impregnation and gold toning of cells.

2. Golgi impregnation and gold toning of cells, followed by postembedding immunoenzyme demonstration of GABA in semithin sections of their somata (Fig. 2).

3. Intracellular injection of HRP, followed by EM immunogold demonstration of GABA in the terminals of the identified neurons (Fig. 3.).

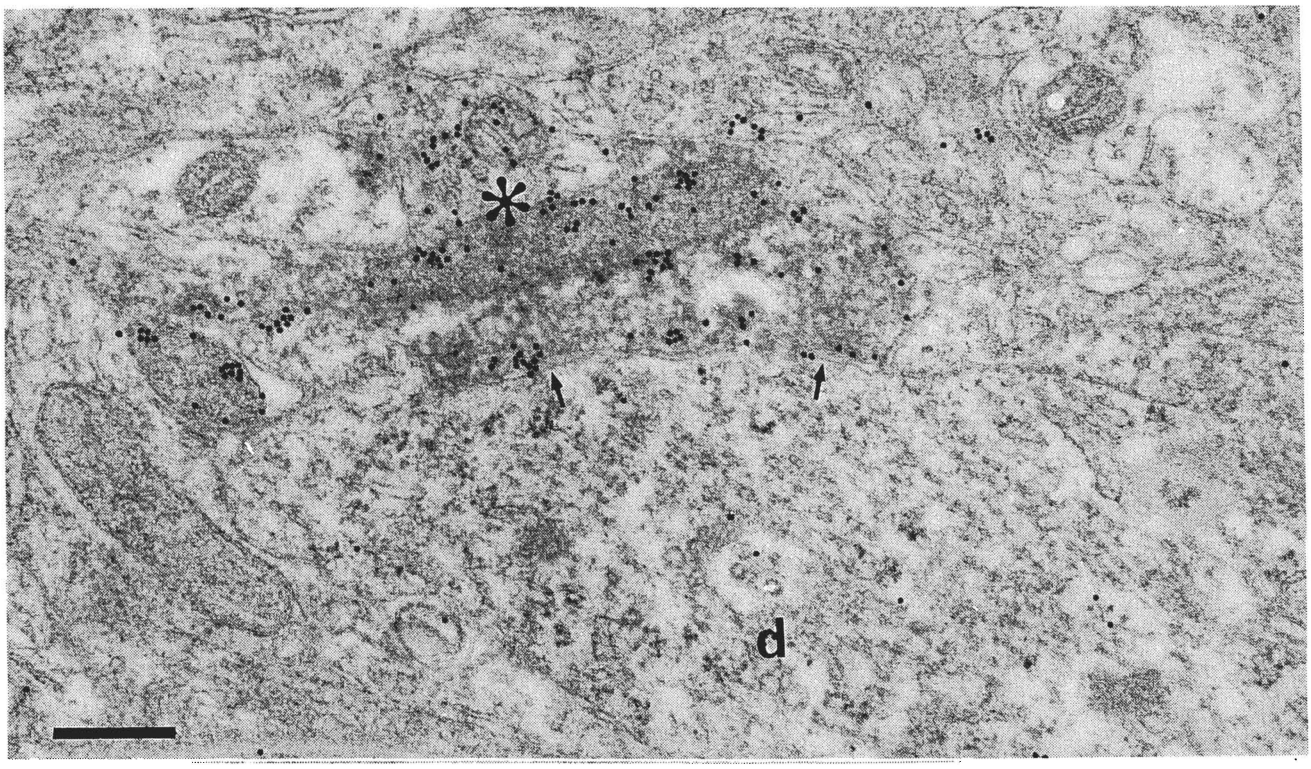



Fig. 3. Postembedding immunogold demonstration of GABA in a bouton (asterisk) of a large basket cell identified by intracellular recording and filling with HRP in the striate cortex of cat (see cell 3 in Somogyi et al., 1983). Note the weak electron density of the peroxidase reaction-product following sodium periodate treatment for the removal of osmium. The bouton makes two synaptic junctions (arrows) with a dendrite (d). For technical details see chapter by Somogyi (1988). Scale: 0.2µm

All three methods result in the localization of a transmitter marker and at the same time provide the opportunity to follow terminals of the axon of the identified cell and sample its postsynaptic targets. The quantitative distribution of postsynaptic targets together with the laminar distribution of the terminals was used as a signature for the delineation of GABAergic neuronal classes (for review see Somogyi, 1989). It was shown that chandelier cells terminate exclusively on the axon initial segment of pyramidal cells. Since the axon initial segment is thought to

generate the propagated action potential, it have been suggested that the strategic location of chandelier cell synapses could provide a mechanism for setting the threshold for firing and/or synchronizing populations of pyramidal cells. Further studies revealed at least three classes of basket cells with distinct laminar patterns of their axons: large basket cells in layers 2-3, clutch cells in layer 4, and deep basket cells in layers 5-6. They all terminate on the somata (20-40%), dendritic spines (20-40%) and dendritic shafts of neurons. Further classes, such as neurogliaform and bitufted cells, terminate exclusively on spines and dendritic shafts of neurons. The overall distribution of postsynaptic elements to all GABAergic boutons was established using post-embedding EM immunogold reaction for GABA (Beaulieu and Somogyi, 1990). The average distribution is different from the distribution profile of postsynaptic targets of identified neuronal classes mentioned above, therefore it is clear that none of the classes of cells examined so far picks its synaptic targets randomly.

From these quantitative studies a new picture of inhibitory influences emerges. Different parts of the same postsynaptic cell receive GABAergic input from distinct populations of local circuit neurons. This is a fundamental feature of the basic cortical circuit as shown by the presence of the same organisation in other cortical areas such as the hippocampus. Chandelier cells containing GABA have been discovered in the hippocampus (Somogyi et al., 1985), and the presence of basket cells and local circuit neurons of the stratum radiatum and moleculare of the hippocampus and the dentate gyrus have been demonstrated by classical Golgi studies. The quantitative determination of the distribution of the axon terminals and the neuronal elements postsynaptic to these GABAergic cells awaits further work.

## Selective inputs to cortical GABAergic neurons

The multiplicity of GABAergic inputs converging onto the same postsynaptic cell was suggested to provide a structural basis for different subcortical and cortical pathways to govern the gain of their own effect through the activation of separate sets of GABAergic cells (Somogyi 1989). Evidence is accumulating that in the cortex as well as in the hippocampus different populations of GABAergic cells receive distinct subcortical input.

**Thalamic afferents.** The first evidence was provided by the measurement of perikaryon sizes of neurons which received monosynaptic somatic input from physiologically identified X and Y-type visual thalamic axons in the striate cortex of cat. Freund et al. (1985) found that all such cells were GABA-positive, and the

somata innervated by Y-type axons were significantly larger than somata innervated by X-type axons. Therefore the two physiologically distinct streams of afferents innervate different populations of cells.

**Serotonergic axons** from the brain stem have been shown to provide dense innervation of the soma and proximal dendrites of a subpopulation of non-pyramidal cells (Mulligan and Tork, 1988). Both GAD and GABA have been demonstrated in some of the densely innervated cells using dual immunocytochemical methods (Tork et al., 1988). In any area only a small proportion of GABAergic cells were densely innervated indicating that they play a special role in mediating serotonergic effects on cortical neurons which themselves received sparse and diffuse input from other or the same afferents.

**Septo-hippocampal pathway.** A GABAergic pathway from the medial septum has been shown to innervate selectively subpopulations of GABAergic cells in the hippocampus (Freund and Antal, 1988). In this elegant study the septo-hippocampal terminals were identified by the anterograde transport of PHA-L; the targets of the boutons (black in nickel intensified DAB reaction) were revealed first by light microscopic pre-embedding immunohistochemistry for calcium binding proteins (brown after DAB reaction), which were present in high concentration in subsets of GABAergic cells. This two colour localization of presynaptic afferents and postsynaptic cells made it possible to demonstrate the selectivity of innervation. Subsequent electron microscopic demonstration of synaptic contacts and an EM immunogold reaction showed the presence of GABA in both the presynaptic terminals and in the postsynaptic somata and dendrites. Interestingly not all GABAergic cells received such input.

**Cholinergic basal forebrain pathway.** Further evidence for the selective innervation of subpopulations of GABAergic cells by subcortical afferents comes from our recent work on the termination of cholinergic synapses in the cortex of cat (Beaulieu and Somogyi, 1991). The cholinergic innervation of the cortical mantle by the basal forebrain plays an important role in the activation of cortical systems (Buzsaki et al., 1988; Vanderwolf, 1988). Receptors for acetylcholine (ACh) modulate the way in which cortical neurons respond to other inputs, and thus have an influence on all cortical activity from the responses to sensory thalamic input to higher integrative functions such as learning and memory.

In the visual cortex of cat the application of ACh to cortical cells enhances the visually evoked responses in about 60% of cells, but indirect GABA-mediated inhibitory influences have also been reported (Muller and Singer, 1989; Sato et al., 1987; Sillito and Kemp, 1983). As an indicator of the relative importance of direct facilitatory and indirect inhibitory effects we determined the extent to which

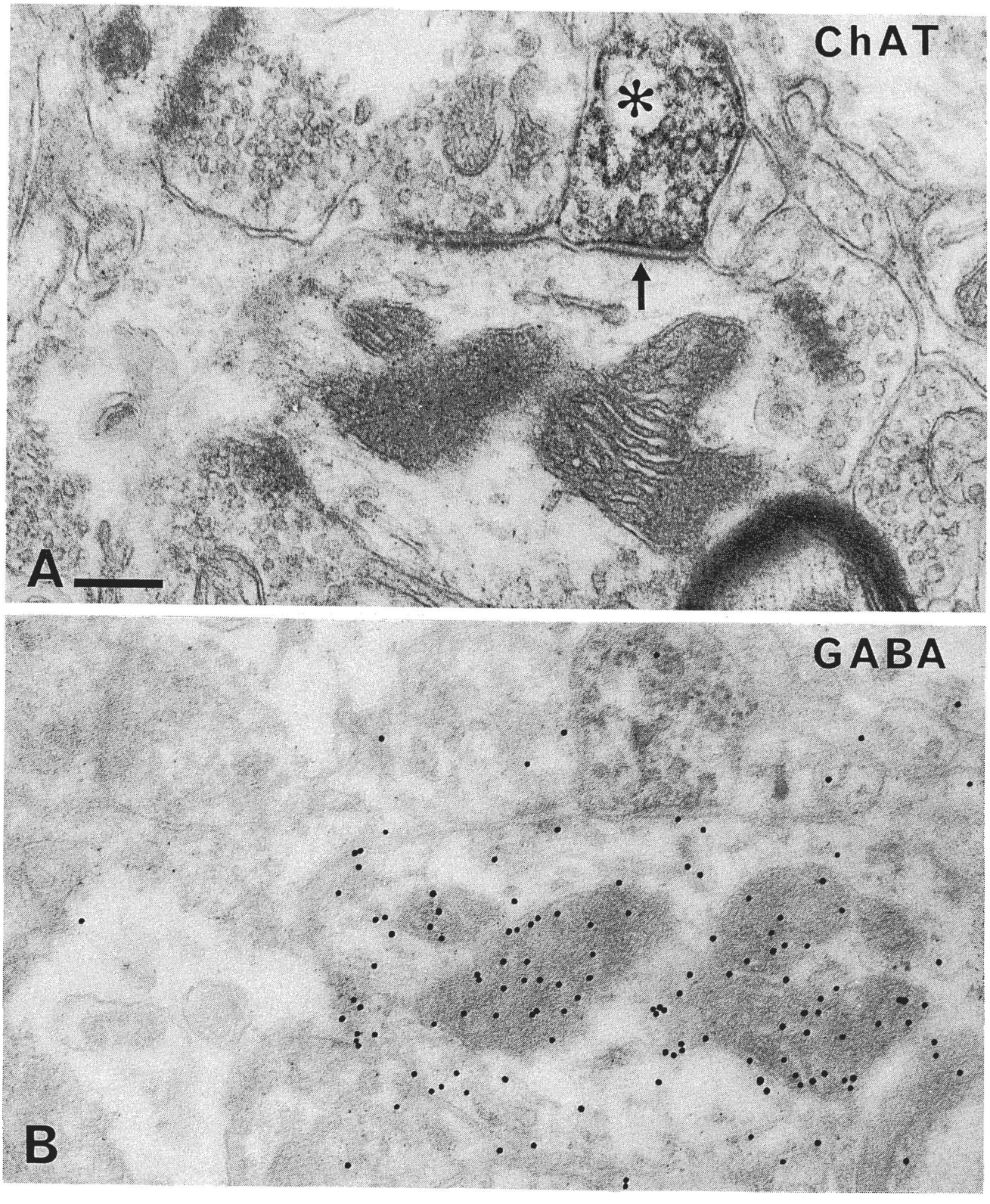


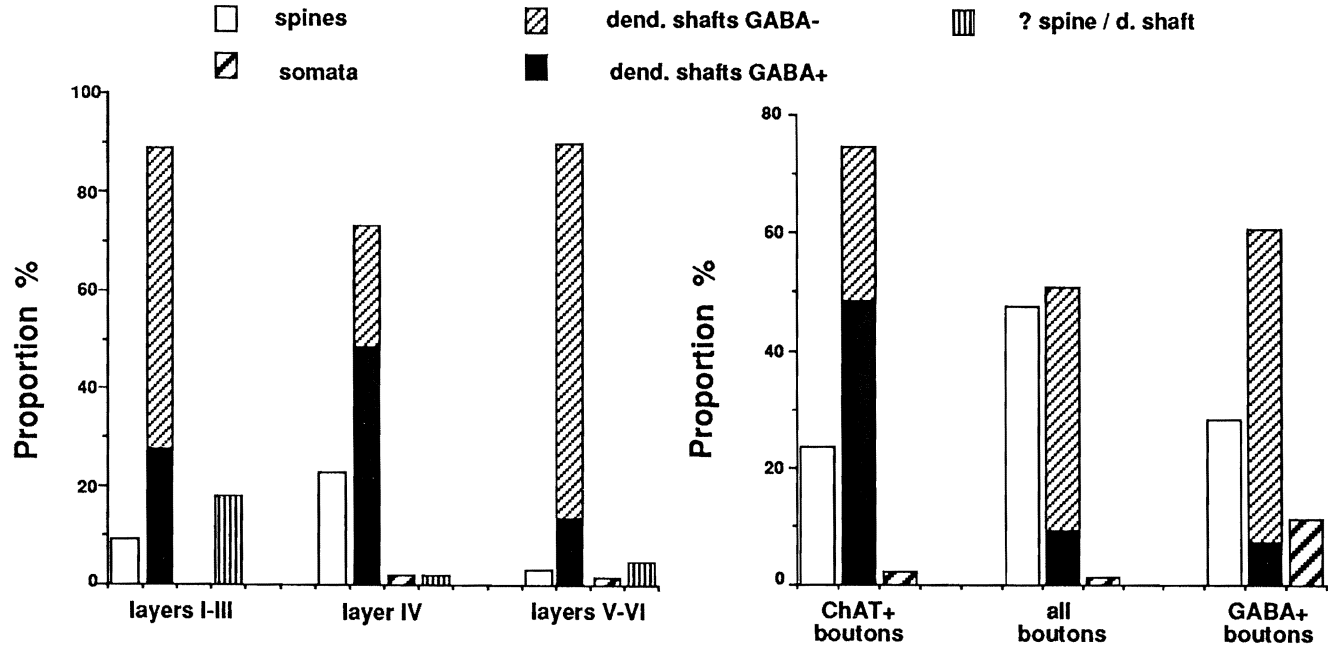

Fig. 4. Immunocytochemical characterization of pre- and postsynaptic elements in the striate cortex of cat. **A.** Pre-embedding immunoperoxidase labelling of a synaptic (arrow) terminal, which is immunopositive for choline acetyltransferase (asterisk). **B.** The postsynaptic dendrite is immunopositive for GABA as shown on a consecutive section by post-embedding immunogold reaction. Data from the work of Beaulieu and Somogyi (1991). Scale for A and B, 0.2 μm

cholinergic synaptic release sites are devoted to the principal cells as compared to the GABAergic local circuit neurons (Beaulieu and Somogyi, 1991). Antibodies to choline acetyltransferase were used in a pre-embedding immunoperoxidase method followed by postembedding EM immunogold demonstration of GABA on ultrathin sections (Fig. 4). This enabled us to determine quantitatively the proportion of elements which contained immunoreactive GABA and therefore originated from local inhibitory cells, and were also postsynaptic to cholinergic terminals .

Most of the cholinergic boutons formed synapses with dendritic shafts (87.3%), much fewer with dendritic spines (11.5%), and only occasional contacts were made on neuronal somata (1.2%). Overall, 27.5% of the postsynaptic elements, all of them dendritic shafts, were immunoreactive for GABA, thus demonstrating that they originated from inhibitory neurons (Fig. 5). This is the highest value for the proportion of GABAergic postsynaptic targets obtained so far for any intra- or subcortical afferents in cortex. The proportion of GABA-positive dendritic shafts was highest in layer IV (49% of targets, two thirds of all dendritic shafts), and lowest in layers V-VI (14%). As seen in fig. 5. the quantitative distribution of targets postsynaptic to choline acetyltransferase-positive terminals is very different from the postsynaptic targets of GABAergic boutons, or from the targets of all boutons in layer IV reported previously (Beaulieu and Somogyi, 1990, Bueno-Lopez et al., 1989). In both cases the proportion of GABA-positive dendrites was only 8-9% of the postsynaptic elements (Fig. 5). These results, obtained with the molecular characterization of both pre- and postsynaptic elements, demonstrate that cortical GABAergic neurons receive a richer cholinergic synaptic input than non-GABAergic cells. The activation of GABAergic neurons by cholinergic afferents and the subsequent fast $GABA_A$ receptor mediated inhibition of their targets could increase the response specificity of cortical cells, which are directly facilitated by slower muscarinic mechanisms during cortical arousal. It has been suggested that the two mechanisms operate synergistically to enhance the signal to noise ratio of responses (Sato et al., 1987; Sillito and Kemp, 1983).

A further unexpected result of the combined immunocytochemical study was that at least 8% of the total population of choline acetyltransferase-positive boutons were also immunoreactive for GABA (Beaulieu and Somogyi, 1991). This raises the possibility of cotransmission at some cholinergic synapses, leading again to a faster $GABA_A$ receptor mediated inhibition followed by slow cholinergic facilitation.

The main message from these four examples of selective innervation of inhibitory neurons is that the operational role of the subcortical afferents can only be explained if the physical constraints i.e. the specificity in the wiring is taken into account. Most, if not all, cortical cells are responsive to GABA, ACh or serotonin.




**A.** Postsynaptic targets of ChAT + boutons in different layers of the striate cortex of cat

**B.** Postsynaptic targets in layer IV of cat striate cortex

Fig. 5. Quantitative comparison of the synaptic targets of characterized nerve terminal populations in the striate cortex of cat. For the cholinergic terminals a pre-embedding immunoperoxidase method, for the postsynaptic dendrites an EM post-embedding immunogold method was used on the same material (Beaulieu and Somogyi, 1991). For **B** data was recalculated from the above study, and from studies by Beaulieu and Somogyi, (1990) and Bueno-Lopez et al., (1989). It is apparent that cholinergic terminals show preferential termination on dendritic shafts, particularly on those originating from GABA-positive cells.

However, the explanation of the effect of these neurotransmitters can only be as accurate as our understanding of the neuronal network where they are released. It is now clear that subcortical pathways such as the brain stem serotonin or the cholinergic basal forebrain input to cortex, which have been thought to terminate in a diffuse manner, use subsets of heavily innervated GABAergic cells, which not only multiply their effect, but channel it into the highly selective synaptic circuits of the cortex.

## Subcellular distribution of some neurotransmitter receptors

The above examples show that it is now possible to determine the origin of presynaptic terminals, their putative transmitters and the structural and chemical identity of postsynaptic elements with great precision. However, for each neuroactive substance there are a multitude of receptors which mediate interactions with widely different effects. Therefore, it is essential to localize the receptors with a precision comparable to that of the wiring and distribution of synaptic terminals. This

requires EM resolution and the localization of receptors to particular cellular membranes.

**$GABA_A$/benzodiazepine receptor - chloride channel complex.** The $GABA_A$ receptor belongs to the family of ligand gated receptor channel complexes; its activation leads to opening of $Cl^-$ channels. The receptor complex is also the site of action of such clinically important drugs as benzodiazepines and barbiturates. We used monoclonal antibodies (Haring et al., 1985; Vitorica et al., 1988) and immunoenzyme methods to determine the localization of the receptor in relation to synaptic terminals with known chemistry and origin in the cerebellum (Somogyi et al., 1989), the visual thalamus (Soltesz et al., 1990) and in the cortex (Somogyi et al., 1989, Somogyi, 1990). Both the alpha- and the beta-subunits were distributed similarly as revealed by immunoperoxidase methods. Intracellular immunoreactivity was associated with the endoplasmic reticulum, the Golgi apparatus and multivesicular bodies, suggesting the sites of synthesis, glycosylation and degradation of the receptor complex respectively. Extracellular immunoreactivity was associated with the plasma membrane of neurons.

Both the intracellular and the extracellular immunoreactivity for the receptor complex were expressed in a neurone type specific manner, but was stereotyped among members of the same class of cell. Some classes of cells, such as cerebellar granule cells and large thalamic relay neurons, showed strong immunoreactivity on their plasma membrane, but very little intracellular immunoreactivity. Other cells, such as large GABAergic cortical neurons, stellate and basket cells in the cerebellum and a population of small relay cells in the lateral geniculate nucleus, showed strong plasma membrane and intracellular immunoreactivity. Further cell types such as cortical pyramidal cells, many thalamic relay cells and Purkinje cells exhibited moderate immunoreactivity. It was suggested that the degree of intracellular immunoreactivity correlates with the turnover of receptor proteins, while the degree of plasma membrane immunoreactivity reflects the density of receptor/channel complexes. These results predict the differential sensitivity of different neuronal classes to $GABA_A$ receptor agonists.

High resolution electron microscopy showed that the epitopes for all four monoclonal antibodies were on the extracellular surface of the plasma membrane. Immunoreactivity was localized on the somatic and dendritic plasma membrane as well as on dendritic spines, particularly of those of Purkinje cells. Immunoreactivity for the receptor complex was present in the synaptic cleft of boutons known from separate studies to contain GABA or GAD. However, equally strong immunoreactivity was also present at non-junctional plasma membrane in all areas

of the brain at sites, such as the somata of cerebellar granule cells and the spines of Purkinje cells, which never receive GABAergic synaptic input. The synaptic clefts of known non-GABAergic terminals were generally immunonegative. Thus, GABA and endogenous substances or drugs that affect $GABA_A$ receptor function could act at both synaptic and non-synaptic sites. The distribution of the receptor complex suggests that the cellular topography of GABAergic influences is not determined by the precise placement of receptors, but by the precise spatial placement of the GABA-releasing terminals, a characteristic of the local circuits described above.

The antibodies (Haring et al., 1985; Vitorica et al., 1988) used in these studies react only with a few of the known receptor subunits. It is possible that some of the more recently discovered subunits, which have been shown to be expressed in a cell type specific manner (Khrestchatisky et al., 1989; Luddens et al., 1990; Malherbe et al., 1990; Schofield et al., 1989; Shivers et al., 1989), have a different or more restricted cell surface distribution. Further high resolution studies using subunit specific antibodies will elucidate the spatial distribution of receptors on the neuronal surface.

**Putative kainate receptor in the cerebellum.** As discussed above, most of the neuronal synaptic interaction in the cerebral cortex as well as in other areas such as the cerebellum takes place via excitatory amino acid receptors. High resolution localization of these receptors has become possible with the preparation of antibodies to some of the purified receptor proteins (Gregor et al., 1988; Klein et al., 1988; Hampson et al., 1989).

The cerebellar cortex of submammalian vertebrates contains a particularly high density of binding sites in the molecular layer for one of the excitatory amino acids, kainate. Since the molecular layer contains a very high density of glutamate releasing nerve terminals originating from granule cells, the receptor was thought to be associated with the synapses predominantly on the Purkinje cells. However, a high resolution light and EM study of the distribution of receptor immunoreactivity in the cerebellum of the chick and fish showed that immunoreactivity was exclusively localized to Bergmann glial cells (Somogyi et al., 1990), which are also restricted to the molecular layer (Fig. 6). Bergmann glial cells surround the dendrites and the spine-parallel fibre terminal synapses of Purkinje cells with thin lamellar processes. Thus, the most likely endogenous compound acting on the putative glial kainate receptor would be glutamate released from parallel fibre terminals (Somogyi et al., 1990). With the cloning of several of the neuronal excitatory amino acid receptors (Keinanen et al., 1990) the visualization of their distribution at identified synapses will soon be possible.

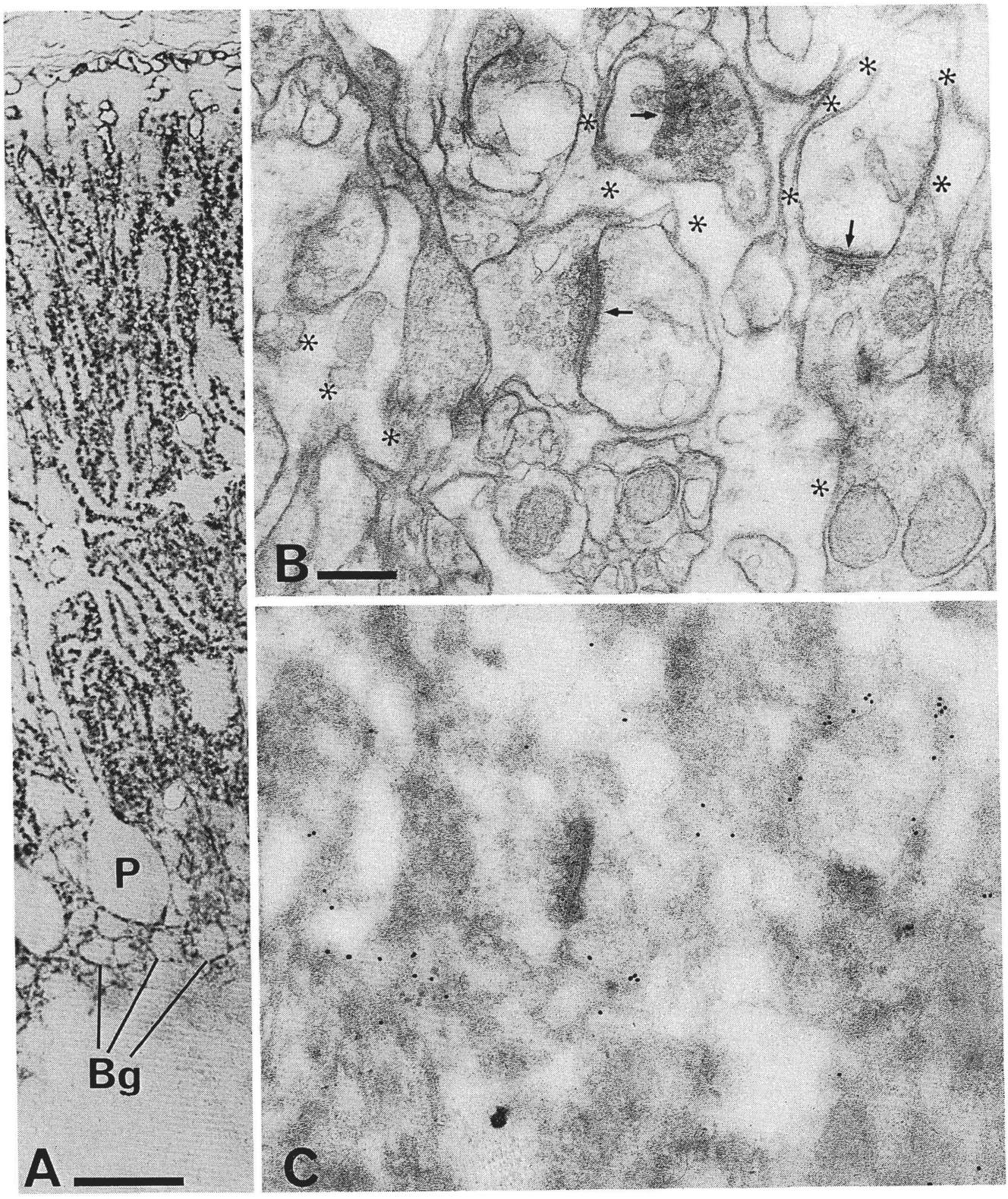



Fig. 6. Immunocytochemical demonstration of a kainate receptor on Bergmann glial cells using the monoclonal antibody IX-50 in the chick cerebellum. **A.** Semithin section, immunoperoxidase method. Note reaction around Bergmann glial cells (Bg), and over their processes enveloping Purkinje cell (P) dendrites. B and C. Serial sections of chick cerebellum treated with uranyl acetate before embedding, but not with osmium tetroxide at any stage. The section in C was etched with ethanolic sodium hydroxide and reacted with the monoclonal antibody followed by colloidal gold conjugated to anti-mouse IgG. Note that the gold particles are found over glial processes (asterisks), but not at the synapses (arrows) of parallel fibres and Purkinje cell spines. (Somogyi et al., 1990). Scales: A, 20μm; B and C, 0.25μm.

## Conclusion

In this brief survey I tried to demonstrate how different molecular neuroanatomical techniques have been used to reveal particular aspects of neuronal organisation. The identified properties of cells can be related to the properties of the network, leading eventually to an explanation of the transformation that takes place between the incoming and outgoing signals. In this process:

1. The identification of structural properties defines the degree to which we understand the connections of cells.

2. The identification of chemical properties defines the degree to which we understand molecular events.

3 The identification of biophysical properties defines the degree to which we can explain the dynamic constraints of cells.

The nature of neuronal operations will only be predicted by information obtained by strict criteria on the same identified neurons. Such predictions will necessarily be of an abstract form based on quantitative data on real networks and cells of the brain.

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