SYNAPTIC ORGANISATION AND TRANSMITTERS OF INTRACORTICAL CIRCUITS

Peter Somogyi
M.R.C. Anatomical Neuropharmacology Unit
University Department of Pharmacology
South Parks Road
Oxford OX1 3QT, U.K.

CONTENTS

I. INTRODUCTION

II. INPUT FROM THE THALAMUS
1. The axons
2. Target cells
3. Transmitters

III. CONNECTIONS OF PUTATIVE EXCITATORY NEURONES
1. Interlaminar connections
2. Intercolumnar interactions
3. The rule of excitatory interconnections
4. The entry of recurrent and feed-forward inhibition

IV. CONNECTIONS OF GABA-CONTAINING NEURONES
1. Axo-axonic or chandelier cell
2. Basket cells
3. GABA-containing neurones termination only on dendritic shafts and spines
4. Emerging rules in the organization of cortical GABAergic circuits

V. CONCLUSION

VI. REFERENCES

Soc.Neurosci.Short Course
Cortical Mechanisms
I. INTRODUCTION

The evolutionary expansion of the cortex illustrates the remarkable success of the cortical blueprint, and the analysis of cortical organization is gathering momentum. Until recently the physiological, anatomical, and neurochemical properties of cortical neurones were studied separately, but it is now possible to examine all of these characteristics in one and the same neurone. This has led to the experimental testing of old questions concerning circuits and to the formulation of new concepts.

One of the early concepts implicit in the work of classical neuroanatomists (1,2) was that morphologically different cortical neurones had different connections, and consequently their functional role was different. This hypothesis has been elaborated in the modern era of cortical research by Szentagothai (3,4,5), whose three dimensional wiring schemes, incorporating the concept of modular organization, have provided a great stimulus for studies of the specificity of cortical connections. Technical developments in the late seventies have given the opportunity to test directly earlier suggestions for the synaptic organization of cortical circuits. At about the same time the neurochemical characterization of identified cortical cells also began through the use of immunocytochemical methods (for review see 6).

Although the direct analysis of cortical circuits is still at an early stage, a decade later we can now ask the following questions:

i. Have the anatomical and physiological data confirmed the specificity and selectivity of synaptic relationships predicted earlier?

ii. What are the salient features of synaptic connections in cortex?

iii. Have features been found in the synaptic organization that are unique to the cortical network?

iv. Does the anatomical information predict the nature of cortical operations?

In answering these questions here, inevitably, only a few issues can be dealt with, and many of the examples will come from the most extensively studied area, the visual cortex. With special emphasis on synaptic organization, I will try to cover points that are universally applicable to all cortical areas:

1. The termination of thalamic afferents.

2. The structural organization of intracortical excitatory pathways.

3. The synaptic organization of intracortical inhibitory pathways.

II. INPUT FROM THE THALAMUS

1. The axons. All areas of the cortex receive converging excitatory input from a particular set of thalamic nuclei (for review see 7). From the termination of specific visual afferents it can be concluded that different functional classes of thalamic cells terminate in different sublaminae of the cortex (8,9). This sublaminar segregation is very sharp in the monkey (10), but in the cat (11,12) not all of the functionally distinct X and Y
axons are so strictly separated as previously thought (for review of X and Y functional characteristics see 13,14). Despite the overlap of the terminal arbors in the cat, the cells of layer IV are similar in the cat and monkey, in that they are activated mainly by one or the other stream of afferents (15,16,17,18). This suggests that thalamic axons have specificity in selecting their target cells, and that distinct functional streams found in the retina and thalamus also remain separate with respect to their target neurones in the cortex.

Any particular thalamic axon contributes only a tiny fraction of all the thalamic synapses in any particular area of the cortex. It has been estimated that in layer IV of the cat striate cortex the axons of between 400-900 geniculate cells of the same physiological class (X) have access to any one point [i.e. the arborizations of the same functional class of axon covers the striate cortex 400-900 times, ref. (19)]. In the monkey 20-40 parvocellular axons have been estimated to overlap at any particular point in layer 4C (20). Because of the spread of the dendrites, the number of axons, that are potentially present within the dendritic field of a cell in layer IV, may be somewhat higher than the above estimates. The number of thalamic axons that converge onto a single cortical cell is unknown, but from physiological studies in the cat striate cortex it has been estimated to be between 2-30 (21,22,23). The question arises whether the cells select their input from a particular set of thalamic axons or can be contacted randomly by any axon present in the tissue space occupied by the dendrites of the cell. The differences in the proportion of thalamic boutons on different classes of cortical cells indicate that the boutons are not distributed randomly. In the mouse somatosensory cortex the proportion of thalamic input on processes of identified neurones is between 0.3-23% of the asymmetric type synapses (24,25). At the higher end of the range this would mean more than a hundred thalamic synapses on some cells.

How many thalamic axons will provide this input from the hundreds present in the space of the dendritic tree? The study of connections between single cortical cells and individual physiologically characterized thalamic afferents revealed that most cells within the arborization of a single thalamic afferent do not receive synapses from it; those that do, receive few; the maximum number of contacts between a thalamic afferent and a pyramidal cell was eight, but the number was usually one (19). Only a few cells have been studied so far because of the difficulties of the technique, but these results also predict considerable convergence of thalamic axons. This again raises the question of whether the terminals of axons are statistically or selectively distributed to their target cells, and further work is clearly needed in this area.

The physiological differences of the X and Y pathways are well established (for review see 13,14), and anatomical evidence is emerging that beyond the basic design of thalamocortical termination there are also subtle structural differences between the two pathways. In the cat the Y axons have larger arbors and possess more boutons (11,12), and the same is true for magno- and parvocellular axons in the monkey striate cortex (10,26). In
addition Y and magnocellular boutons establish more synapses (12,26), thus these pathways expand in the cortex relative to the X and parvocellular pathways.

2. Target cells. Physiological studies indicate that most cells, including pyramids, are in the position of receiving direct thalamic input, or they are at most one synapse away from it (for review see 27). Anatomical studies have demonstrated varying degrees of thalamic input to different types of cells. The majority of thalamic terminals make synapses with dendritic spines (for review see 24). It is not clear, however, whether this input is sufficient for the activation of all cells, or whether additional inputs are required for cells whose direct thalamic input is relatively small. Spiny cells situated in layer IV receive the highest proportion of their total input from the thalamus (25), and may be activated by this input alone. Other cells may require additional polysynaptic routes for optimal activation in addition to monosynaptic thalamic inputs, and this could be one reason for the rich interconnections of putative excitatory neurones in the cortex. The change in receptive field properties from thalamus to cortex indicates that additional intracortical inputs are required for the full elaboration of response properties.

As yet little is known of the differences in the neuronal machinery participating in functionally distinct pathways. One difference found recently in the striate cortex of cat is in the size of a class of large, GABA-containing cells in layer IV that receive direct somatic input from the thalamus, and is densely covered by synaptic boutons. Freund et al. (19) showed that cells contacted on the soma by X axons are significantly smaller than cells contacted by Y axons. This supports the notion that the functional streams may remain separate at least at the first step in cortical processing. This separation suggests that we should not try to assemble, even within the same layer, all cells into a unified flow scheme, but should try instead to dissect the synaptically (operationally?) related neurones.

The above examples of the termination of thalamic afferents illustrate that already at the level of the entry of information to cortex selectivity emerges in the recipient neuronal types, in the proportion of their inputs and in the sites where the cells are contacted. These differences imply that even those different types of cortical cells that are in the main recipient layers of thalamic afferents, may be activated with different probabilities.

3. Transmitters. There is agreement that the monosynaptic effect of the thalamic fibers is excitatory, but the transmitter(s) of thalamic relay cells is unknown. Suggestions for excitatory amino acids or small peptides (28,29) require further studies. Neurones in the intralaminar, but not those in the relay nuclei, can be labelled by retrograde transport of 3H-D-aspartate, a marker for excitatory amino acid pathways (30). This suggests the existence of neurochemically distinct populations of thalamic afferents.

Although the excitatory thalamic input can probably bring some of the output cells of cortex to threshold, the vast majority of putative excitatory and most of the inhibitory input
to cortical neurones arrives from other cortical neurones. In the following section the rules governing their synaptic connections will be considered.

III. CONNECTIONS OF PUTATIVE EXCITATORY NEURONES

The various types of pyramidal and spiny stellate cells from type I or asymmetric synapses (31,32) are thought to be excitatory, and probably use acidic amino acids as transmitters (for review see 33). Single cell impregnation studies showed that these cells have extensive, lamina specific axon arbors within their own cortical area (4,34,35,36,37,38). The real extent of the axonal arbors in both the radial and tangential dimensions only became apparent through the visualization of cells by intracellularly injected HRP (17,39,40,41). The major pathways in the cat striate cortex are illustrated schematically in fig.1.
Fig. 2. Cumulative diagram showing the distribution of postsynaptic elements to two HRP-filled pyramidal cells in the striate cortex of cat. The synaptic boutons of the cells were distributed in clumps in both layers II-III and in V. When tested immuno­cytochemically, only some of the dendritic shafts were immunopositive for GABA. It was estimated that at least 95% of the targets originate from pyramidal cells and GABAergic neurones form less than 5% of the postsynaptic targets. Data from the study of Kisvarday et al. (66).

The pattern of intracortical pathways emerging from single cell studies has been extended to populations of cells in the monkey striate cortex using the axonal transport of HRP (42,43). To demonstrate the excitatory pathways selectively, we have used microinjections of the specific marker 3H-D-aspartate, and have studied the laminar distribution of retrogradely labelled cells (44 and manuscript in preparation). The results confirm the highly regular and selective inter- and intralaminar connections, and provide support for the participation of excitatory amino acids in these connections. In addition to the radial interlaminar connections, striking tangential projections were revealed in layer IVB, and from layer V to layer III. In general, few cells could be labelled in the supragranular layers, and this indicates that they may have different transmitter uptake mechanisms. Both the single cell and population studies reveal a pattern of selective putative excitatory connections. What are the target cells of these pathways?

The targets of spiny stellate cells are not known outside the layer of their somata (45,46), therefore the synaptic targets of pyramidal cells will be discussed in detail below.

Pyramidal cells are considered to be excitatory but through the activation of inhibitory interneurones they could also exert disynaptic inhibition. Putative inhibitory interneurones in the cortex probably use γ-aminobutyrate as transmitter. They also differ from putative excitatory neurones in that they have few dendritic spines or none at all (46,47,48,49). Thus, on the one hand, if the main role of the axon collaterals were 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 neurones. On the other hand, if the main role were 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 neurones.

The examination of the targets of HRP-filled pyramidal cells of layer III (67) revealed 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 (Fig.2). Of the few dendritic shafts that were contacted, less than half were immunoreactive for GABA and thus all in all less than 5% of the targets belonged to inhibitory cells. These results, in agreement with other studies (50,51), suggest that the primary role of the local axonal systems of spiny neurones is the activation of other excitatory cells, and this can play the following roles:

1. **Interlaminar connections** Since the cortex receives input that is often localised to some but not all of the laminae, the collaterals could transfer information vertically to functionally related cell groups in other laminae. This organisation could form the basis of the hierarchy of visual processing as originally concluded from physiological results (52,53). The radial connections would also ensure that cells lying within the same column would share similar receptive field properties and a similar position in the visual field. Since many of the interlaminal connections are reciprocal, the pathways provide an ideal substrate for feedback and reentrant circuits.

2. **Intercolumnar interactions** The role of long intercolumnar collateral systems is less straightforward (17,40,54). According to one view, their collaterals may connect distant cell groups, providing the basis for the summing of receptive fields from different visual field locations, or connect cells with similar response properties, reinforcing their common characteristics (40,50,55,56,57). Others have suggested that these collaterals may operate to inhibit groups of cells with dissimilar response properties (58) or produce inhibitory flanks or end zones (40). The predominant termination on other pyramidal cells supports suggestions for mutual activation of cells with similar properties. Pyramidal cell axon collaterals may also be responsible for the correlated firing of cell groups with similar orientation preference that has been demonstrated to occur over distances of several millimetres in the cat visual cortex (59).

3. **The rule of excitatory interconnections.** The available evidence suggests that the spiny stellate cells and other spiny neurones with exclusively local axons also terminate mainly on dendritic spines, implying further excitatory connections to other excitatory cells (45,46). Taking into account that about 20% of cortical cells contain GABA (60), and assuming that most cortical neurones that do not contain GABA belong to the excitatory spiny family, we arrive at a picture of the cortex where up to 80% of the neurones are interconnected through axon collaterals releasing acidic amino acids or related transmitters and exerting excitatory action. The mutual facilitatory interconnections of large populations of cells is one of the salient features of the cortical network. The extent of these
connections, both quantitatively and in the complexity of their selective distribution, is unparalleled in the nervous system. This organization of synaptic connections is supported by multiple receptors for the excitatory amino acids (for review see 61,62), and in particular by a prominent NMDA receptor mechanism (63,64). This cannot be treated in depth here, but it should be noted that this mechanism may enable the selective and dynamic emphasizing of some connections over the others within the hard wired network (65). From the analysis of pyramidal cell connections it was concluded that their axons can contribute very few synapses to any individual postsynaptic cell, and input from a single axon is unlikely to bring the cell to threshold on its own (66). The connections are formed between populations of cells with large convergence and divergence, and in this sense the activity and the information is distributed amongst the individual axons in the pathways. The random loss of some of the elements would not change the pattern of connections or the flow of activity, but would degrade its accuracy.

Such an interconnected network would be prone to uncontrolled firing unless inhibitory neurons were incorporated into the circuits. Indeed if inhibition is reduced experimentally, or in pathological states such as epilepsy, the excitatory interconnections lead to recruitment of large populations of cells producing epileptic activity. The extensive excitatory interconnections may explain why epileptic foci are always localised in cortical areas, including the hippocampus and the amygdala.

Under normal conditions inhibition prevents the spread of excitation, and cortical neurones are notable for responding only to particular stimuli. So where does inhibition enter the network?

4. The entry of recurrent and feed-forward inhibition. The results from the termination of specific afferents and pyramidal cell collaterals demonstrate that besides the major termination on excitatory cells, a small population of GABA-containing cells are also contacted. The sparse input to GABA-containing cells from single pyramidal cells suggests that these cells could only be activated through a strong convergence of pyramidal cells to a select population of inhibitory neurones. One of the possible roles of local collaterals around the parent cell may be the activation of inhibitory interneurones, thereby providing the structural basis of recurrent inhibition (67,68,69,70). The collaterals also give synapses to GABA-containing cells in the distant axonal clumps. Since these GABAergic neurones are unlikely to feed back onto the remote pyramidal cell providing their input, this link probably provides feed-forward inhibition to some members of the distant cell groups.

The presence of GABA in some of the targets of pyramidal cell collaterals and thalamic afferents has been demonstrated directly 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 (71,72,73). Thus, excitatory cortical pathways contact both putative excitatory and inhibitory neurones, and the
latter could provide feed-forward inhibition. This has been extensively demonstrated in the hippocampus (74).

To assess the roles of inhibitory neurones further, their synaptic organization will be discussed below.

IV. CONNECTIONS OF GABA-CONTAINING NEURONES

It is difficult to demonstrate the inhibitory effect of any particular neuronal type directly and therefore the identification of inhibitory neurones relies on the known inhibitory effect of GABA (75, for review see 76). The presence of GABA or its synthetic enzyme glutamate decarboxylase is used to predict indirectly the inhibitory role of cells. Both GABA and GAD can be localized immunocytochemically. However immunocytochemistry alone reveals only the somata, with a few proximal processes of the neurones and separate, isolated boutons scattered in the neuropil. Thus none of the boutons can be allocated to any particular cell or cell type. To overcome this ambiguity we have developed the combination of immunocytochemistry with Golgi impregnation and intracellular ionophoresis of HRP (48,49). The latter techniques are used to visualize the dendritic and axonal arborizations of the GABA-containing cells. This approach enabled us to trace the efferent synaptic connections of identified types of GABAergic cortical cells and so to evaluate their target selectivity (Fig 3.).

1. Axo-axonic or chandelier cell. These neurones, discovered by Szentagothai (77), make synapses exclusively with the initial axon segments of pyramidal cells (78,79, for review see 80), each cell terminating on 200-400 pyramidal cells and each pyramidal cell receiving input from 3-5 GABA-containing axo-axonic cells (79). They are ubiquitous in all cortical areas including the hippocampus (81) and amygdala (82). 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 output by a specialized GABAergic neurone acting on the action potential generating site is a unique feature of cortical circuitry. This system could have evolved in response to the other uniquely developed feature discussed above; the extensive excitatory interconnections of the 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 neurones.

The role of chandelier cells is not known. It has been suggested that they may be involved in sleep, selective attention, recurrent inhibition, or provide an inhibitory mechanism at high pyramidal cell discharge levels (27,48,79). The latter mechanism may prevent the development of epileptic activity under normal conditions.

2. Basket cells. These are also present in all cortical areas and had been thought to terminate in pericellular baskets on the somata (for review see 83). Their selectivity has only been studied quantitatively in the visual cortex of cat, using intracellularly HRP-injected neurones (84,85,86). Contrary to previous predictions, only about 20-40% of their synapses are on
Fig. 3. Quantitative distribution of postsynaptic elements to identified types of GABA-containing neurones in the striate cortex of cat. Note the absolute selectivity of chandelier cells for the axon initial segments, and the substantial proportion of spines contacted by all the other cell types, including the basket cell family on the right. Data summarized from several studies (48,49,79,84,85,86,95,103).
the somata of other neurones, the remainder contacting dendrites. Surprisingly, 20-40% of their synapses are on dendritic spines. Confirming strong indirect evidence, GABA has recently been demonstrated in their synaptic terminals (fig.4) (49). Interestingly the supra- and infragranular pyramidal cells receive input from separate populations of basket cells, with axons running tangentially up to about one millimetre from the soma. Layer IV has a shorter range basket cell, the so-called clutch cell. Thus in the visual cortex a three tier GABAergic neuronal system exists, and this strongly implies that the functional properties brought about by basket cell inhibition have to be produced or maintained at each level of cortical processing.

Of all the possible functions of basket cells in the visual cortex, the maintenance of directional selectivity is compatible with several of their structural features: 1. Basket cells contain GABA, and interference with GABAergic neurotransmission abolishes, or reduces, directional selectivity (76,87). 2. Directional selectivity is produced by divisive type of inhibition (88), and somatic inhibition is assumed to be divisive (89). 3. Some basket cells have axons elongated in particular
directions and running tangentially for up to a millimetre from the soma (84,86). These 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 (90,91), but may depend on horizontal connections (92). 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 1-2 millimetres in extent and frequent shifts in preferred direction, with 180 reversals (93). A system of basket cell axons can provide this pattern, with reversals coinciding with the somata of basket cell groups.

A noteworthy feature of the basket cells is that in addition to providing a significant input to somata where their effect would be non-selective, many of their synapses on dendritic shafts and spines would mainly inhibit those inputs distal to them (94).

3. GABA-containing neurones terminating only on dendritic shafts and spines. Preliminary examination of the targets of neurogliaform and bitufted cells shows that they terminate only on small diameter, presumably distal, dendritic shafts and on dendritic spines (95). These contacts may not affect the membrane potential at the level of the somata, but might shunt 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. This could form the basis of a synaptic veto mechanism. The inhibition exerted by these cells would thus be the most selective, because EPSPs generated in more proximal dendrites or in dendrites not receiving input from the particular GABAergic cell will not be affected by this inhibition.

4. Emerging rules in the organization of cortical GABAergic circuits. These few examples illustrate the astonishing sophistication of putative inhibitory connections, strongly supporting Szentagothai's proposal for the specificity of cortical interneurones (73). The following conclusions can be drawn:

1. Different neuronal types defined by their form have different target selectivity. Consequently different parts of cortical neurones receive separate inhibitory inputs from distinct sets of cells.

2. Neurones with similar target selectivity may differentiate into layer specific subtypes (cf basket cells), each providing input to a subset of cells in a column.

3. Neurones differing in form may have different inputs. This conclusion was reached from the termination of specific thalamic afferents providing somatic input only to one class of GABA-positive cells (probably corresponding to the basket cells discussed above) out of many classes present in their lamina of termination.

4. Different functional streams use different subpopulations of the same cell type. This was illustrated with the size
differences in GABA-positive cells contacted by X or Y axons in the visual cortex (discussed above).

5. There are neurochemical differences between subpopulations of GABAergic neurones. The first evidence for this conclusion came from the demonstration of cholecystokinin and somatostatin immunoreactive material in separate populations of GABA-containing cortical (96,97) and hippocampal cells (96). Since then a host of other peptides have been shown to coexist with GABA (98) and there are also differences in cell surface molecules (99).

These conclusions, reached on the basis of work carried out in the last few years, illustrate the changing concept of the organization of cortical inhibitory circuits from the general and overall inhibition, thought to be exerted on the somata and proximal dendrites of neurones, to a new picture of multiple and selective influences both on different parts of the same cells and on separate populations of cells. As suggested earlier, this organization enables the system to adapt to new demands of performance by introducing additional inhibitory circuits (27).

V. CONCLUSION

The above examples have revealed answers to the first three questions posed in the introduction. Further work will test how widely the organizational rules discussed above can be applied to all cortical areas. With regard to the fourth question, it is apparent that, as yet, little direct correlation can be drawn between physiological responses of cortical cells and the underlying synaptic circuits, and the field is wide open to verifiable hypotheses for the operational roles of the different circuits. It should also be apparent that due to technical advances a major development is taking place in the structural analysis of the cortical neuronal network. The main feature emerging is that in most cortical areas there are parallel circuits, either side by side, or superimposed within the same tissue space, using similar, but subtly different neuronal machinery adapted to the particular task required from that channel. Therefore we should not try to force into a straight-jacket all the cells and circuits within a given tissue volume. The basis of a cortical operation can be served by an input driving a set of output cells, supplemented by the local circuits supplying this throughput pathway. The challenging task is to find the principles that apply to these parallel throughput channels. The few examples outlined above show that the tools are now available to unravel both the chemistry and the synaptic organization of the cortical network which, not so long ago, seemed intractably complex.

VI. REFERENCES

1. S. Ramon y Cajal, II Maloine Paris,(1911)