THALAMOCORTICAL SYNAPTIC RELATIONS: A REVIEW WITH EMPHASIS ON THE PROJECTIONS OF SPECIFIC THALAMIC NUCLEI TO THE PRIMARY SENSORY AREAS OF THE NEOCORTEX

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1. INTRODUCTION

In the latter half of the 19th century, new techniques for preserving, sectioning and staining nervous tissue revolutionized neuroanatomical investigations by enabling
the light microscopic visualization of some of the finer details of nerve cells and their processes*. The application of these new methods to the study of thalamocortical relations soon led to the realization that the thalamus contains discrete groups of nerve cells which project topologically to particular regions of the cerebral cortex, an arrangement exemplified by the specific thalamic projections to the primary** receptive areas of the neocortex. The purpose of this review is to critically evaluate studies of thalamocortical relations conducted since the technological revolution of the 19th century so as to provide the necessary historical framework to better understand current concepts of thalamocortical connectivity. An evaluation of past work at this time is desirable, for neurobiology has recently entered a second period of technological revolution — one which has in large part focused on thalamocortical connections. Whereas the 19th century revolution was marked by the rapid development of various light microscopic techniques, the essence of this second technological revolution may best be characterized as a blending of light and electron microscopic methods. For instance, it is now possible to use the light microscope to determine the specific cell type of a neuron in the cerebral cortex, and to then examine the same neuron with the electron microscope to identify those regions of its cell membrane which are involved in thalamocortical synapses134. In this way the specific cortical targets of thalamic projection systems can now be elucidated. Consequently, we have entered an age where we can expect to learn the intimacies of thalamocortical relations, and as the details of thalamocortical microcircuitry become known we shall be compelled to refine and redefine current concepts of thalamocortical organization. This review is being written on the premise that an examination of previous work will facilitate and guide future studies of thalamocortical synaptic relations.

* Excellent accounts of developments in neuroanatomical techniques during the 1800s may be found in Polyak138 and Walker185.

** The following definitions of the word 'primary' are offered in the hope that they will alleviate some of the confusion which has resulted from the various ways in which the word 'primary' has been applied to describe specific regions of the cerebral cortex. In the context of studies of cortical evoked potentials, primary was used to denote short latency responses of the cortex whose characteristic waveforms were reproducible with successive peripheral stimulation. These 'primary' cortical responses were highly localized to regions of cortex which were then referred to as primary response areas. Primary cortical responses were followed by secondary cortical responses which were more varied in shape, phase, latency and in incidence of occurrence14. Secondary responses were not related to specific regions of the cortex. In time, the primary response areas as determined by studies of cortical evoked potentials, came to be equated with the 'primary projection areas' which had been defined by anatomical methods151,152. These latter regions were defined as those regions of the cortex which receive input from 'extrinsic' thalamic nuclei, i.e. those thalamic nuclei which receive substantial portions of their input from sources outside the thalamus. Included in this classification would be the sensory nuclei which lie along the main ascending sensory pathways from the periphery to the cortex. Studies of cortical evoked potentials confirmed anatomical findings that the primary auditory228, visual185 and somatosensory3,227 areas contain topographically organized representations of the appropriate peripheral receptive fields. In 1941, Adrian9 reported the digits of the cat to be represented in a 'second' sensory area of the cortex. Soon, a number of studies reported second areas of cortical representation for the peripheral receptive fields of the somatosensory222-226,229, auditory225 and visual183,184 systems in a variety of mammalian species. The important point is that secondary areas are secondary simply because they were described after the primary cortical areas. It is only because much less is known of the projections and synaptic organization of the secondary than of the primary cortical regions that the latter are the main focus of this review.
Gudden in 1870, provided the first experimental evidence of an intimate functional link between the thalamus and cerebral cortex by showing that ablation of the cortex in young rabbits led in time to the death and disappearance of cell bodies in the thalamus. Gudden's method, which came to be known as the method of retrograde cell degeneration, was soon widely applied to study thalamocortical relations in many species. One approach employed in these studies was to ablate an entire cerebral hemisphere, allow the animal to survive for periods ranging from several weeks to nearly a year, and then examine the thalamus for signs of cellular degeneration. The rationale for this approach was to obtain a comprehensive picture of the origin of the entire thalamic radiation. Thus, Waller examined the cat thalamus following hemidecortication and noted a full spectrum of degenerative effects associated with various thalamic nuclei. Whereas the ventral nuclei sustained a total cell loss, little or no effect was seen in the mid-line and intralaminar nuclei. Most other thalamic nuclei showed varying degrees of cell loss. Similar results were reported following hemidecortications in the rat, rabbit, dog, rhesus monkey, macaque, chimpanzee and human, leading to the conclusion that whereas most of the thalamus projects directly to the cerebral cortex, the mid-line and intralaminar nuclei do not.

A persistent problem facing investigators who employed the method of retrograde cell degeneration was that some neurons in severely affected thalamic nuclei showed no ill effects even after total cortical ablation. Although it was generally accepted in the early part of this century that these surviving neurons simply did not project to the cortex, this reasoning was insufficient to explain the occurrence of surviving cells whose appearance was only slightly altered by the cortical lesion. For this reason, Rose and Woolsey proposed that surviving neurons might well project to the cortex, but that they are sustained to a greater or lesser degree after cortical damage by having intact collateral axonal projections to other regions of the brain. Alternatively, Powell and Cowan concluded that surviving thalamic neurons showed minor degenerative effects following cortical ablations because of the removal of corticothalamic afferents to them. An implication of this conclusion is that some of the cellular degeneration observed in the thalamus following lesions of the cortex might result from the deafferentation of thalamic neurons rather than from damage to their axons, a conclusion reached many years earlier by Waller and Barris. This suggests that retrograde cell degeneration is unreliable for elucidating thalamocortical projections, but as Walker observed, a variety of experimental evidence indicates that areas of the cerebral cortex project to thalamic nuclei having thalamofugal projections to the same cortical area. More recent studies have confirmed this finding for many areas of the cerebral cortex (see discussions in Colwell and White and

* An extensive description of the effects of axonal transection on neuronal perikarya may be found in Lieberman. Briefly, before disappearing, affected neurons undergo chromatolysis, decrease in size and show a diminished affinity for cell stains.
DeAmicis\textsuperscript{205}), and thus deafferentation might not significantly influence the interpretation of studies using the method of retrograde cell degeneration.

There are, however, a number of difficulties which should be kept in mind when considering the results of retrograde degeneration studies. One of these concerns the true extent of the cortical lesion; nearly every hemidecortication referred to above was accompanied by damage to subcortical structures and it is conceivable that subcortical structures might be comprised even with less extensive lesions of the cortex. Walker\textsuperscript{105} noted that, even when the lesions are clearly restricted to the cerebral cortex, the interpretation of retrograde studies using large cortical lesions is complicated by the fact that the resultant thalamic degeneration may be so extensive as to distort the cytoarchitecture of the thalamus, rendering thalamic nuclei no longer recognizable. In addition, a number of factors independent of the character of the lesion have been shown to influence the severity of the effects of retrograde cell degeneration. For instance, several studies report more severe degenerative effects in young animals than in adults with similar lesions\textsuperscript{12,102,147}, although sometimes the reverse is true\textsuperscript{121}. Rose and Woolsey\textsuperscript{149} noted the effects of retrograde cell degeneration in the thalamus grew more severe with increasing postoperative survival time. This was recently confirmed by Chow and Dewson\textsuperscript{17} who also reported retrograde cell degeneration to be faster and more complete in rabbit than in cat thalamus. Finally, it has recently been shown\textsuperscript{117} that the severity of retrograde cell effects depends to some extent on the particular thalamocortical projection system under investigation. Thus the size and extent of the lesion, the age and species of the animal, the length of postoperative survival time, and even the particular projection system involved, are all factors which might complicate the interpretation of retrograde cell degeneration studies.

The many disadvantages of the method of retrograde cell degeneration might tempt one to think this technique played a minor role in the elucidation of thalamocortical relations. In fact, just the opposite is true, for it was through the judicious use of this method that a number of investigators provided the first evidence that certain of the thalamic nuclei project in a topographic fashion to the cerebral cortex. Shortly after Gudden's discovery that rather extensive lesions of the cerebral cortex caused a marked atrophy of thalamic nuclei, von Monakow\textsuperscript{116} reported very small lesions of the cerebral cortex in newborn rabbits produced localized areas of cellular degeneration in the thalamus. Using similar methods, Minkowski\textsuperscript{115} noted that lesions as small as 0.5 mm of cat striate cortex produced sharply delineated areas of retrograde cell degeneration in the ipsilateral lateral geniculate nucleus. Further, Minkowski realized that the locus of degeneration in the thalamus depended on the exact placement of the lesion in the cortex. Similarly, Putnam and Putnam\textsuperscript{142} showed that discrete lesions of varied size in the striate cortex of rabbits produced, in the lateral geniculate nucleus, sharply delineated areas of retrograde cell degeneration which were consistently related in size and location to the cortical lesions. Extensive analyses of the cortical projection of the lateral geniculate nucleus in the rat\textsuperscript{98} and in monkey\textsuperscript{137,138} were soon to follow, and in these species, the projection of the lateral geniculate nucleus to the cortex was also shown to be topographically organized.

Information on the topographic nature of the geniculocortical projection came
at a time when other studies\textsuperscript{11,97} were elucidating the strict topography of the retinogeniculate projection. As a result of these studies, it became possible on anatomical bases alone to specify the areas of the visual cortex which subserved particular regions of the visual field\textsuperscript{142}. Henceforth, the cortical representation of the retina was to be thought of as very detailed, and the concept (see discussions in Polyak\textsuperscript{138} and Lashley\textsuperscript{100}) that the retina projected in a more or less diffuse and unstable way to the cortex became untenable.

Other studies used retrograde cell degeneration to elucidate the thalamocortical relations of the somatosensory\textsuperscript{22,98,195} and auditory\textsuperscript{195} systems*\textsuperscript{,} and here again, thalamic nuclei were shown to project in a topographically organized fashion to their respective regions of cortex.

The phrase ‘respective regions of cortex’, implies a concept which, although taken for granted nowadays, was not universally accepted in the early 1900s, namely particular regions of the cerebral cortex receive input from particular nuclei of the thalamus. In fact, there existed a school of thought which held that the thalamic nuclei projected diffusely over wide areas of the cerebral cortex, a notion which implied that each area of the cortex was the functional equivalent of any other area of cortex (see discussions in Polyak\textsuperscript{137,138}). Convincing evidence against this concept of cortical organization was contributed by the results of numerous studies using retrograde cell degeneration, for the results of these studies strongly indicated that certain of the thalamic nuclei project only to particular areas of the neocortex. This finding provided firm support

* The reader is referred to Appendix A and to the books by Walker\textsuperscript{195} and Polyak\textsuperscript{138} for additional references to studies of thalamocortical relations using the method of retrograde cell degeneration.

Fig. 1. Illustration of the intensity of the thalamic projection to the cerebral cortex of the monkey (macaque?) as determined by the methods of retrograde cell degeneration and Marchi. The most intense thalamic projections are to the primary areas of the neocortex (see Fig. 2). Reproduced from Walker\textsuperscript{195} with permission of the Univ. of Chicago Press.
Fig. 2. Illustration of the macaque cerebral cortex showing the primary and secondary areas as determined by studies of cortical evoked potentials. Comparison with Fig. 1 shows that it is the primary cortical areas which receive the most intense thalamocortical projections. Reprinted from Rose and Woolsey with permission of Elsevier Publishing Co.

for the currently accepted notion of specific functions being localized to specific regions of the cerebral cortex.

The foregoing discussion well illustrates how knowledge of the details of thalamocortical projections can contribute significantly to our understanding of the organization of the cerebral cortex. An early proponent of this philosophy was A. E. Walker, whose own extensive studies of the primate thalamus and its projections provided a wealth of important information suggesting the cytoarchitecture of the cerebral cortex is intimately related to its thalamic projections. He, and later Clarke, conceived of the cortex as composed of cytoarchitecturally distinct zones involved in different functional activities, each zone related to a particular region of the thalamus. This relationship is perhaps best illustrated by the thalamic projection to the primary sensory areas of the neocortex; the cytoarchitectural organization of the primary sensory areas is remarkably similar from one area to the next and each primary sensory area receives a particularly intense projection from a specific thalamic nucleus.

* This should not be taken to imply that a region of cortex receives input from only a single thalamic nucleus, for as Killackey and Ebner suggested, each area of mammalian neocortex likely receives input from 2 or more separate thalamic nuclei.

** 'Specific' in this context was first applied by Lorente de Nó to denote the thalamocortical projections of the main relay nuclei of the thalamus which lie directly along the ascending sensory pathways from the periphery to the cortex. The thalamocortical projections from these nuclei are specific in the sense that they terminate mainly within layer IV of the primary sensory regions of the neocortex. In contrast, the thalamocortical projections from the non-specific nuclei terminate in all layers of more extensive regions of the cortex. The reader is referred to the discussion by Ebner for additional information on non-specific thalamocortical projections.
nucleus. A comparison of Figs. 1 and 2 shows how, partly on this basis, primary may be distinguished from secondary regions of the cortex.

Thus, although the method of retrograde cell degeneration is crude by today's standards, we must credit the investigators who used this technique with the elucidation of several important aspects of thalamocortical relations. Foremost among these is the concept of the main sensory nuclei of the thalamus projecting in a highly ordered, topographic fashion to the primary sensory areas of the neocortex.

3. MARCHI METHOD

Additional support for the concept that thalamocortical projections are topographically organized was provided by studies using the Marchi method. This technique, developed in 1886 by Marchi, was based on the observation that degenerating myelinated fiber tracts could be labeled by a deposit of black granules by treating the tissue first with potassium dichromate and then with osmic acid. Although the Marchi method often stained normal as well as degenerating fiber tracts, careful application of the technique confirmed the concept of the main sensory relay nuclei of the thalamus projecting topographically onto the cerebral cortex. An important disadvantage of the Marchi method is an absence of staining of thinly myelinated or unmyelinated fibers and consequently the technique has proved of little use in specifying the layers of cortex which receive the axon terminations of thalamocortical projections (see ref. 64). Some insight into this question was provided by analyses of Golgi impregnated brains which indicated that the specific thalamic nuclei project mainly to the fourth layer of sensory cortex and to the third layer of motor cortex. However, the difficulty of tracing identified fiber tracts over long distances rendered the Golgi method nearly as unsuitable as the method of Marchi for elucidating the details of the terminal arborizations of thalamocortical projection systems. More exact information on the patterns of termination of thalamocortical projections had to await the development of reduced silver impregnation methods which stain degenerating axoplasm rather than myelin sheaths.

4. REDUCED SILVER IMPREGNATION METHODS

 Significant advances in the study of the terminal distribution patterns of thalamocortical projections were made possible with the introduction by Nauta in 1950 of a reduced silver stain for degenerating axon terminals. The original Nauta method enabled, for the first time, the visualization of the precise layers of cortex which contained degenerating axon terminals and preterminals of identified thalamocortical projections. The usefulness of the Nauta method was, however, limited by the concomitant staining of normal axons and their terminals. Recognizing this deficiency, Nauta and Gygax modified the original Nauta technique to suppress the staining of normal fibers. Although this new method facilitated the identification and tracing of degenerating thalamocortical projections, it soon became clear that suppressing the staining of normal axons often also resulted in the failure to demonstrate the full extent of the distribution...
of degenerating axon terminals. This problem was greatly alleviated by the introduction in 1967 of the Fink–Heimer modification which combined the selective reduced silver impregnation of degenerating axons with a more successful staining of their axon terminals. The literature since that time is replete with references to studies of thalamocortical connections using the Fink–Heimer and other (e.g. ref. 211) modifications of the original Nauta technique. These studies, which confirmed the topography of the specific thalamocortical projections to the primary visual, somatosensory, auditory and motor cortices, also demonstrated the intense projection of the specific thalamic nuclei to the fourth layer of primary sensory cortex and to the third layer of primary motor cortex. A smaller, but consistent projection to the first layer of these regions of cortex was also demonstrated by reduced silver staining of degenerating specific thalamocortical projections (e.g. refs. 50 and 83). A variety of mammals, including the monkey, cat, rat, opposum and hedgehog were examined in these studies, and thus a principal contribution of the reduced silver methods has been to show the remarkable similarity in widely divergent mammalian species of the laminar distribution of specific thalamocortical afferents.

A second contribution of the reduced silver methods concerns their application to demonstrate functional columns in monkey primary visual cortex. Functional columns were first elucidated by Mountcastle who showed that all neurons encountered in vertical microelectrode penetrations of cat primary somatosensory cortex were related to the same sensory modality and responded with similar response latencies to stimulation of nearly identical peripheral receptive fields. The diameter of one functional column was estimated to be about 500 μm. The existence of similar columns has since been confirmed in each of the primary sensory areas of the cerebral cortex in several species: for example, in SI of mouse, rat, and monkey, in VI of cat and monkey, and in AI of cat. The anatomical demonstration of functional columns was first provided by Hubel and Wiesel using the Fink–Heimer method. Previously, Hubel and Wiesel had shown cells in cat and monkey primary visual cortex to be arranged in alternating columns according to eye dominance, the cells of one set of columns responding preferentially to the left eye, those of the alternate columns to the right eye. Hubel and Wiesel reasoned that since each of the six layers of the monkey lateral geniculate nucleus receives input from only one eye, a lesion in a single geniculate layer would cause the degeneration of thalamocortical afferents carrying information from a single eye. Thus they lesioned single layers of the monkey lateral geniculate nucleus, and using the Fink–Heimer method, subsequently stained the degeneration products of this 'monocular' thalamocortical projection. As predicted, the appropriate ocular dominance columns appeared, in layer IV, as discrete 250–500 μm wide patches of terminal degeneration. More recently, LeVay et al. used a reduced silver stain for normal fibers to demonstrate the pattern of ocular dominance columns in monkey visual cortex. Their results indicate that, in general, the pattern of...
ocular dominance columns — and thus of geniculocortical afferents — is similar in different members of the same species.

Further evidence that the organization of functional columns is related to the distribution of thalamocortical afferents has been adduced by studies of barrels in rat and mouse primary somatosensory cortex. Woolsey and Van der Loos observed that neurons in layer IV of mouse somatosensory cortex are organized into multicellular units, which because of their three-dimensional shape, were termed 'barrels'. Barrels have since been shown to occur in layer IV of the somatosensory cortex of several species of rodent and in rabbit, and in each species, the large barrels which comprise the posteromedial barrel subfield (PMBSF) correspond in number and arrangement to the large mystacial vibrissae on the animal's snout. It is interesting that

Fig. 3. Light micrograph of a 40 μm thick section stained with cresyl violet showing barrels in the posterior region of the posteromedial barrel subfield (PMBSF) of the mouse. Barrel sides, composed of densely packed cell bodies, are more intensely stained than barrel hollows where cell bodies are less densely packed. Compare with Fig. 2. Magnification, 150 ×, scale 100 μm.

Fig. 4. Light micrograph of a 30 μm thick Fink-Heimer stained section, through the posterior region of the PMBSF in a mouse whose ventrobasal thalamus had been lesioned. In this preparation, barrel hollows are filled with darkly stained, degenerating thalamocortical axon terminals. Magnification, 150 ×, scale, 100 μm.
barrel-like aggregations of neurons have also been demonstrated in the region of the thalamus which projects to the mouse PMBSF. Recent studies have confirmed the suggestion by Woolsey and Van der Loos that each barrel in mouse PMBSF cortex is the morphological manifestation in layer IV of a functional column which extends throughout the full thickness of the cortex. The relationship of these functional columns to the distribution of specific thalamocortical afferents was demonstrated by Killackey and Killackey and Leshin who used the Fink-Heimer method to show that lesions of the ventral posterior nucleus produced discrete clusters of terminal degeneration which corresponded in both size and location to the PMBSF barrels in rat somatosensory cortex. This correspondence is illustrated for the mouse by Figs. 3 and 4 which allow for the comparison of PMBSF barrels as seen in a Nissl-stained preparation with the distribution, in the PMBSF, of degenerating thalamocortical afferents stained by the Fink-Heimer method.

The reduced silver methods have thus contributed to our knowledge of thalamocortical connections in two important ways. First is the elucidation by these methods of the laminar distribution of thalamocortical afferents in a number of species, and second is the demonstration of the specific distribution of thalamocortical afferents within layer IV which is closely related to the columnar organization of the cortex. However, there are several disadvantages of using reduced silver methods to study thalamocortical connections. For instance, the methods are often not sufficiently sensitive to stain minor thalamocortical projections. Thus, using the Fink-Heimer method, Peters and Feldman observed degenerating thalamocortical afferents in layers IV and III of rat visual cortex, whereas with the electron microscope they observed degenerating thalamocortical axon terminals in layers I and VI as well. In contrast, it is likely that some of the silver reaction product does not represent degenerating axons or their terminals, and further, the methods do not allow for the accurate differentiation of true axon terminals from preterminal fiber fragments. Additional disadvantages of the reduced silver methods derive from the fact that they are typically used following the placement of lesions in the brain parenchyma. The problem is that lesions might injure fiber tracts which pass through but do not originate, in the lesion site, and thus a proportion of the degeneration illuminated by the reduced silver methods might consist of degenerating axons and terminals of unknown origin. Further, lesions placed in one region of the brain might cause unforeseen damage to other regions by compromising the circulation of blood or of cerebrospinal fluid. For these reasons it would be desirable to examine the terminal distribution of thalamocortical projections using a method which does not require making lesions. Just such a method became available in 1972 with the introduction of an anterograde tracing technique which enabled the autoradiographic demonstration of thalamocortical projections in non-lesioned brains.

5. AUTORADIOGRAPHIC TRACING METHODS

The autoradiographic method for tracing axonal projections is based on the uptake of radioactively labeled amino acids by neuronal cell bodies at an injection site,
and the subsequent anterograde axonal transport of radioactive material to axon materials in other regions of the brain. Sections containing the radioactively labeled axon terminals are then coated with a photographic emulsion which upon exposure and development will contain reduced silver grains localized over the labeled axon terminals.

A distinct advantage of the autoradiographic tracing method is its increased sensitivity for demonstrating thalamocortical axon projections. For example, studies with reduced silver methods had shown the cat lateral geniculate nucleus to project to layers IV and I of primary visual cortex. A reinvestigation of this projection using the autoradiographic tracing method showed the lateral geniculate nucleus also projects to layer VI of cat primary visual cortex. Subsequent autoradiographic investigations have shown a specific thalamic projection to layer VI in VI of rat, squirrel, and cat, in SI of rat and in SI and AI of monkey. In fact, a main contribution of the autoradiographic tracing method has been to confirm and extend previous findings with regard to the laminar and areal distribution of thalamocortical afferents. Thus, Jones and Jones and Burton confirmed earlier reports that the most intense thalamic projection is to the primary sensory and motor areas of the primate cortex and that the boundaries of the different thalamocortical projection fields are distinct and invariably coincide with sharp cytoarchitectonic boundaries or with zones of cytoarchitectonic change.

The geniculocortical projection to monkey striate cortex is arranged in two distinct bands within layer IV, one band of terminals is contributed by cells in the dorsal layers of the lateral geniculate nucleus, the other band of terminals arises from cells located in the ventral layers of the lateral geniculate nucleus. Following injections of radioactive amino acids into single laminae of the cat lateral geniculate nucleus, LeVay and Gilbert used the autoradiographic tracing method to show that the geniculocortical projection in this species is also characterized by a laminar segregation in cortex of afferents from the dorsal and ventral sets of geniculate laminae. LeVay and Gilbert also observed a segregation of geniculocortical input in cat, as in monkey, on the basis of eye preference into patches about 500 μm wide.

Additional information on the laminar and columnar distribution of geniculocortical afferents has been provided by studies based on the finding that radioactive substances injected into the eye are carried by transneuronal transport to the visual cortex. For instance, this method was employed by Drager to show that the lateral geniculate nucleus in mouse projects, as in other species, to layers I, IV and VI of the primary visual cortex. In addition, injections of radioactive materials into one eye have been used to demonstrate ocular dominance columns in VI of cat and macaque monkey and in VII of cat and squirrel monkey, but have failed to demonstrate ocular dominance columns in VI of New World monkeys, gray squirrel, tree shrew, and mouse. The transneuronal transport method has also been used to study the development of ocular dominance columns in both the cat and monkey in which it has been shown that the segregation of geniculocortical afferents by eye into discrete patches is preceded by a stage in which the geniculocortical afferents from both eyes are completely intermingled. Thus, during
development, the thalamic afferents concerned with either eye present a uniform, non-
columnar distribution in layer IV. This finding is consistent with the results of
physiological investigations\textsuperscript{106} which show layer IV cells in immature animals to be
both more equally driven by both eyes than in the adult, and not clustered into groups
according to eye preference.

The autoradiographic tracing method has thus provided precise information
regarding the terminal distribution patterns of thalamocortical afferents, but neither
this technique nor the anterograde tracing methods which preceded it have been able
to identify the cells of origin of the thalamocortical projection. The identification of
these cells had to await the development of the retrograde transport method using
horseradish peroxidase (HRP).

6. THE HRP RETROGRADE TRANSPORT METHOD

The HRP method for the retrograde labeling of neuronal cell bodies is based on
the uptake of HRP by axons and their terminals at an injection site and its subsequent
intraneuronal transport to cell bodies in other regions of the brain\textsuperscript{101}. Although
several factors may adversely affect the retrograde transport of HRP (see refs. 80, 120
and 167) application of the method has confirmed the topographic nature of the
thalamocortical projection to the primary sensory and motor regions of the neocortex
in several species (e.g. in A1 of cat\textsuperscript{108}, SI of cat\textsuperscript{143,216}, rat\textsuperscript{82,144}, \textit{mink}\textsuperscript{159} and \textit{monkey}\textsuperscript{108,125,208}, VI of rat\textsuperscript{77}, \textit{opossum}\textsuperscript{24}, rabbit\textsuperscript{87}, mink\textsuperscript{159} and \textit{monkey}\textsuperscript{217,220}, and MI of
\textit{monkey}\textsuperscript{180}), and has provided additional evidence\textsuperscript{15} that layer I receives \textit{input from}
both non-specific and specific thalamic nuclei. These and other studies using the
retrograde transport of HRP have provided new insight into the organization of
thalamocortical projections by giving information on the number, size and three-
dimensional arrangements of thalamocortically projecting neurons. For example,
Saporta and Kruger\textsuperscript{100} counted 3000-5000 HRP-filled neurons arranged in distinct
curvilinear arrays in the rat ventrobasal (VB) complex for every square millimeter of
SI cortex involved with an HRP injection. Clusters of thalamocortically projecting
neurons have also been observed in the cat VB\textsuperscript{82}, and columns of retrogradely labeled
cells extending through all layers of the lateral geniculate nucleus have been noted
following injection of HRP into the striate cortex of this species\textsuperscript{112}. Somogyi et al.\textsuperscript{171}
compared the shapes and sizes of HRP-filled thalamic neurons with those of neurons
impregnated by the Golgi method and concluded that three different types of
thalamocortical relay cells can be identified in the cat anteroventral thalamic nucleus.
Holländer and Vanegas\textsuperscript{68} observed geniculate cells of all sizes projecting to cat area 17,
but found mainly the larger cells projecting to area 18. This finding is consistent with
the suggestion\textsuperscript{175} that both fast and slow conducting geniculate cells project to area 17,
whereas area 18 receives input from only fast conducting geniculate cells. More direct
evidence on the collateral projections of single thalamic neurons has been obtained by
a method using radioactively labeled HRP. In this method, HRP is injected into one
area of the cortex and initiated, but enzymatically inactive, HRP into another area of
the cortex. Sections of the thalamus are first reacted for HRP, and then autoradio-
graphed to demonstrate the presence of tritiated HRP; thalamic neurons containing both labels are presumed to project to each of the cortical injection sites. Thus Geisert \(^5\) identified cells in the cat lateral geniculate nucleus which project to both areas 17 and 18, and Hayes and Rustioni \(^6\) demonstrated cells in the cat VB which project to both SI and SII. These findings are consistent with the results of electrophysiological studies (e.g. ref. 113) which show single cells in cat thalamus to be antidromically invaded by stimulation of SI or SII.

Recently, the retrograde HRP method has been combined with the anterograde autoradiographic tracing method to provide information on the reciprocity of thalamocortical and corticothalamic projections. This technique was introduced in 1975 by Colwell \(^2\) who injected a mixture of tritiated amino acids and HRP into small regions of rabbit striate cortex. Colwell observed that regions of the lateral geniculate nucleus containing HRP-filled cells also contained high concentrations of radioactively labeled corticothalamic axon terminals. The combined anterograde–retrograde tracing technique has since been applied to study thalamocortical relations in the primate visual \(^1\) and somatosensory \(\) systems and in the mouse somatosensory system \(^5\). In each instance, the strict topography of the thalamocortical projection has been correlated with the presence of an equally precise corticothalamic projection. Thus the long-held concept \(^5\) that discrete regions of the thalamus are reciprocally connected with corresponding regions of the cortex has been confirmed.

Finally, Ferster and LeVay \(^4\) took advantage of the anterograde transport of HRP to study the distribution of thalamocortical afferents to primary visual cortex. They injected HRP into the optic radiation of the cat, and related the laminar distribution of labeled axons in visual cortex with the distribution of geniculocortical afferents as shown by the autoradiographic method \(^4\). They concluded that thalamocortical afferents which arborize in different layers of the visual cortex have different branching patterns and diameters.

7. THE IDENTITY OF NEURONS WHICH RECEIVE INPUT FROM THE THALAMUS

The studies thus far discussed have provided basic information on the kinds and arrangements of thalamic neurons which project to the cortex, and on the areal and laminar distribution in cortex of thalamocortical axon terminals. An important question left unanswered by these studies is the identity of those neurons in cortex which receive input directly from the thalamus. Since most sensory information ascending from the periphery enters the cerebral cortex only after having passed through the thalamus, the identity of the neurons which directly receive this input is crucial for understanding how the cortex receives and processes information from the periphery. Some clues to the identity of neurons which receive thalamocortical input have been obtained by light microscopic analyses of Golgi impregnated neurons from the cortices of sensory deprived animals or from animals which had sustained damage at some point along an ascending sensory pathway \(\) . For example, layer IV stellate

\(^*\) In general, the effects of deafferentation (e.g. thalamic lesions, eye enucleations, etc.; see refs. 189 and 190) are different and usually more severe than those caused by sensory deprivation (e.g. lid-suturing, dark-rearing, etc.; see refs. 18, 31 and 60), and the effects of either on cortical physiology and anatomy are greater the younger the animal (e.g. refs. 33 and 59).
cells in the striate cortex of cats reared in the dark exhibit fewer and shorter dendrites than do layer IV stellate cells of control animals. Furthermore, in dark-reared rabbits, layer IV stellate cells show a greater than usual variation in dendritic length, whereas the dendrites of layer IV stellate cells in mice enucleated at birth are directed away from layer IV to adjacent layers as if, as Valverde suggested, they are seeking afferent axonal relations outside layer IV. These results may be interpreted as evidence that layer IV stellate cells receive input directly from the thalamus. Additional evidence to support this hypothesis is the finding that, in mice, the aggregation of layer IV stellate cells to form barrels does not occur when the related mystacial vibrissae and their follicles are removed prior to five days of age. Related to this result is the finding that damage to a row of vibrissae in newborn rats and mice results in a thalamic projection to the corresponding region of the barrel field which takes the form of a relatively narrow, continuous strip filling the space occupied in normal animals by discrete clusters of thalamic terminals associated with single barrels. The concomitant disorganization of barrels, whose walls consist mainly of layer IV stellate cells, and of the thalamocortical projection to barrels, tends to support the conclusion that layer IV stellate cells are a major target of thalamocortical afferents.

Other reported effects of sensory deprivation and deafferentation on cortical neurons include alterations of the shapes and sizes of spines on the apical dendrites of layer V pyramidal cells, apparent reductions in the numbers of these spines, and decreases in the numbers and lengths of the basal dendrites of layer III pyramids. In contrast, increases in the numbers and lengths of the terminal dendritic branches of layer II and III pyramidal cells have been correlated with 'enriched' environmental conditions. These results suggest that pyramidal cells of layers III and V directly receive thalamocortical input, but the interpretation of the results of sensory deprivation studies is complicated because the methods employed lacked the resolution to visualize synaptic contacts. Thus it cannot be excluded that transneuronal effects, rather than direct deafferentation were the bases for the observed changes in the morphology of cortical neurons. Certainly, transneuronal effects seem to be involved in the observation that subsequent to eye enucleation in newborn rabbits, the numbers of spines along the middle portions of the apical dendrites of layer III pyramidal cells in striate cortex is reduced, for these dendrites occur in laminae known to receive little or no direct thalamic input. Clearly, to identify the cortical neuronal types which receive input directly from the thalamus, it is necessary to use the resolving power of the electron microscope, for at present, only this instrument provides the opportunity to directly visualize synaptic connections.

8. ELECTRON MICROSCOPIC ELUCIDATION OF THALAMOCORTICAL SYNAPSES

In 1964, Colonnier observed that lesioning axonal pathways afferent to the cerebral cortex induces characteristic degenerative changes in the fine structure of their axon terminals. These changes include the disruption of mitochondria and synaptic vesicles and a marked increase in the electron density of the affected terminals. The method of lesion-induced degeneration was subsequently employed by Jones who
examined thin sections of the somatosensory cortex of cats which had undergone lesions of the thalamus. This study, which was the first demonstration of synapses involving identified thalamocortical axon terminals, was soon followed by others in which a similar approach was used to study thalamocortical synapses. A significant result of these studies is the observation that the dendrites and somata of non-spiny cells in the visual cortex of the rat, cat, and monkey, in the somatosensory cortex of the cat and in the motor cortex of the cat and monkey, synapse directly with thalamic axon terminals. However, the methods employed in these studies did not allow the three-dimensional forms of neurons to be determined, and so the specific identity of these non-spiny neurons could not be ascertained. Moreover, in each region of the neocortex for which figures are available (see Table 3 in Peters and Feldman), between 70 and 91% of thalamocortical axon terminals synapse with dendritic spines, and for the most part it has not been possible to identify the cell type of origin of these spines. A notable exception to this is the description by Strick and Sterling of thalamic synapses with two spines of a Betz cell in cat motor cortex. One reason for the failure to determine the origin of dendritic spines is that the diameter of spine stems is often as small as 0.1 μm and thus, even with the use of serial thin sections, it is exceedingly difficult to connect most spines back to their parent dendrites. Further, even when these connections can be established, few criteria exist to identify the majority of dendritic profiles encountered in thin sections of the cortical neuropil. Peters and Feldman attempted to overcome this difficulty by comparing the shapes of spiny dendrites reconstructed from serial thin sections with the shapes of dendrites identified by light microscopic examination of Golgi impregnated neurons. Their observations suggested that some of the spines which synapse with thalamocortical axon terminals arise from spiny stellate cells, whereas others might arise from pyramidal cells. Sloper found degenerating thalamocortical axon terminals in monkey motor cortex to occur in clusters associated with pyramidal cell apical dendrites and concluded from this that pyramidal cells are a principal recipient of thalamocortical synapses. However, no synapses were observed between thalamocortical axon terminals and spines of unequivocally identified pyramidal cell dendrites, and so in this instance also, it could not be determined with certainty if spines which synapsed with thalamocortical axon terminals arose from pyramidal or spiny stellate cells or from other neuronal types. The problem of identifying the cell type of origin of spines which are involved in thalamocortical synapses could be solved by the application of methods to selectively label neurons in cortex which receive input from the thalamus.

This approach was taken by Christensen and Ebner who recorded the intracellular activity of single cells in opossum sensorimotor cortex and then injected horseradish peroxidase into those cells which responded at short latency to peripheral stimulation. Their observations indicated that both pyramidal and non-pyramidal cells of layer III receive input directly from the thalamus. There are, however, several problems which would preclude the wide application of this method to study thalamocortical synaptic connections. One of these is the difficulty with which small diameter neuronal bodies can be successfully impaled for recording and staining. Although, in time, technological developments might alleviate this difficulty, there
exists a more serious problem with the method which is unlikely to be solved by advances in technology. This problem is that every cortical neuron which might be involved in thalamocortical synapses most likely receives other input from many non-thalamic sources. Some of these non-thalamic inputs are undoubtedly inhibitory and so there is no guarantee that under all stimulus conditions thalamic input would be sufficient to cause detectable changes in the activity of the neurons which receive it. For example, Hellweg et al. recorded simultaneously from thalamic fibers and from their postsynaptic cells in the cortex, and found that the fiber discharge might precede the firing of the postsynaptic cell by as much as 5 msec. Another problem is to distinguish, on the basis of electrophysiological recordings alone, cells which are monosynaptically activated by thalamocortical afferents from those which are di- or polysynaptically activated. Still, the technique of intracellular recording and staining provides the unique opportunity to correlate the morphology of neurons (e.g. Kelly and Van Essen) with their electrophysiological responses to specific stimuli. Further, it now seems feasible to combine the recording/staining method with techniques to label thalamocortical axon terminals and thus obtain valuable information on the distribution of thalamocortical synapses with neurons whose electrophysiology has been studied.

The notion was expressed at the beginning of this review that neurobiological investigation had recently entered a new phase characterized by the blending of light and electron microscopic techniques. The necessity for combining light and electron microscopic methods grew out of the realization that neither method alone is sufficient to study the synaptic organization of complex neuronal systems. For example, although electron microscopy is capable of elucidating the fine details of the cytology of neurons and their synaptic relations, the technique is limited because only very thin sections can be examined with the electron microscope. Thus, the usual electron microscopic picture of the cortex (see Peters et al.) is one of large numbers of separate neuronal profiles which for the most part cannot be related to their cells of origin even by extensive reconstructions from serial thin sections. In contrast, much thicker sections of tissue can be examined with the light microscope and so by using appropriate staining procedures, such as the Golgi method, it is possible to view entire neurons and to distinguish neuronal types on the basis of their characteristic shapes. Combining the Golgi method with electron microscopy would enable individual neurons to be identified by light microscopy and to then be examined by electron microscopy.

Fig. 5-7. The triptych composed of Figs. 5-7 shows in Fig. 5, a Golgi impregnated layer III pyramidal cell from mouse SmI cortex. The same neuron, following treatment with the gold-toning method of Fairen et al., is shown in Fig. 6. Arrow in Fig. 6 indicates spine shown in inset between Figs. 5 and 6. Magnification of Figs. 5 and 6, 850 ×, scale, 10 μm. Fig. 7 is an electron micrograph of a thin section through part of the cell body and apical dendrite (AD) of the layer III pyramidal cell shown in Figs. 5 and 6. Note the fine deposit of gold particles which labels the cell body and apical dendrite of this gold-toned neuron. × 6500. Inset between Figs. 5 and 6 is a montage of electron micrographs of two adjacent thin sections showing the synapse of a degenerating thalamocortical axon terminal (TA) with the spine (S) indicated by the arrow in Fig. 6. × 50,000. Fig. 7 reprinted from White with permission of Wistar Press.
microscopy to assess their cytology and synaptic connections. This combined approach has been followed by a number of investigators since Stell173 and Blackstad8 first introduced methods whereby Golgi impregnated neurons could be prepared for electron microscopy. The principal disadvantage of these early methods was that the Golgi deposit obscured the cytology of the impregnated neurons making it difficult to assess their synaptic relations. Subsequent attempts* to overcome this difficulty by reducing the amount of the deposit within Golgi impregnated cells culminated in the

Fig. 8. Electron micrograph showing a synapse between a degenerating thalamocortical axon terminal (TA) and the spine (S) of a spiny stellate cell in layer IV of mouse SmI cortex. The parent dendrite (D) of this spine is also shown. × 60,000. Figure reproduced from White204 with permission of Wistar Press.

Figs. 9 and 10. Electron micrographs of serial thin sections showing the synapse, in layer IV of mouse SmI cortex, of a degenerating thalamocortical axon terminal (TA) with a spine (S) of the apical dendrite of a layer VI pyramid. × 60,000. Figures provided by Steven M. Hersch.

* For a discussion of these methods, see ref. 43.
work of Fairén et al. These investigators devised a technique whereby Golgi impregnated neurons in sections up to 200 µm thick are first examined by light microscopy, and then chemically treated to replace the dense Golgi precipitate with a deposit of fine gold particles. The neurons are still visible with the light microscope because of their content of gold (Figs. 5 and 6); in the electron microscope, the gold particles clearly label the cell body and processes of the ‘gold-toned’ neuron (Fig. 7). Furthermore, the gold particles are usually so distributed within the labeled neurons that their cytological details are not obscured and consequently, their synaptic relationships can be determined (Figs. 8–10).

Combined with methods to label thalamocortical projections, the gold-toning technique has provided an excellent means to identify and to study the cytology of neurons in the cerebral cortex which are postsynaptic to thalamocortical axon terminals. Indeed, soon after the development of the gold-toning method, Peters et al. combined it with lesion-induced degeneration and established without doubt

Fig. 11. Diagram showing the various cell types (P, pyramidal; NSS, non-spiny stellate; SS, spiny stellate; NSB, non-spiny bipolar cells) in mouse primary somatosensory and in rat primary visual cortex which are postsynaptic, in layer IV, to thalamocortical afferents (Th. Aff.). Pyramidal cells in layers V and VI are represented by the single pyramid at the right of the diagram. Also included are the synaptic connections of non-spiny stellate cells as observed in rat visual cortex. Synaptic junction types are indicated as 'a', asymmetrical and 's', symmetrical. Roman numerals at left indicate layers of cortex. Eff, efferent. Reprinted with slight modifications from White with permission of Wistar Press.
that spines involved in thalamocortical synapses arise from both pyramidal and spiny stellate cells. Subsequently, this approach was used by Peters et al.\textsuperscript{133} to examine thalamocortical synapses with neuronal perikarya and dendrites in layer IV of rat primary visual cortex, and by White\textsuperscript{201}, White and Rock\textsuperscript{206} and by Hersch and White\textsuperscript{67} to study thalamocortical synaptic relations in layer IV of mouse primary somatosensory cortex. Their findings, which are summarized in Fig. 11, demonstrate that a variety of neuronal types synapse directly with thalamocortical afferents.

Thalamocortical axon terminals in both mouse SmI and rat VI synapse with the spines of pyramidal cells whose somata occur in layers III and V\textsuperscript{133,201}. Layer VI pyramids in mouse SmI\textsuperscript{67} are also involved in thalamocortical synapses; this cell type was not examined in rat VI. In agreement with earlier results\textsuperscript{129}, Peters et al.\textsuperscript{133} report that some apical dendrites of layer V pyramidal cells in rat VI are not involved in thalamocortical synapses, whereas in mouse SmI cortex\textsuperscript{67,204} all apical dendrites of layer V pyramidal cells possess some spines which receive thalamocortical synapses. These findings might signify a real difference in the organization of thalamocortical input to neurons in SmI or in VI, but more likely, the negative finding in rat VI is related to the fact that geniculocortical afferents to VI degenerate over a varied time course. Thus, at any time following lesions of the lateral geniculate nucleus in the rat\textsuperscript{128}, cat or monkey\textsuperscript{51} only 5–10\% of the axon terminals in layer IV show signs of degeneration, although it has been established by the autoradiographic tracing technique\textsuperscript{104} that somewhat more than 20\% of the axon terminals in layer IV of cat VI are derived from the thalamus. In contrast, thalamocortical axon terminals in mouse SmI degenerate nearly simultaneously\textsuperscript{204}, and so at any one postoperative interval, more than 20\% of the axon terminals in layer IV are labeled by degeneration. The chances of observing thalamocortical synapses with particular postsynaptic elements, e.g. the apical dendrites of layer V pyramids, would thus be much greater in mouse SmI than in rat VI. Further, for approximately equal lengths of dendrite, the apical dendrites of superficial layer V pyramidal cells in mouse SmI cortex make far fewer thalamocortical synapses than do other dendrites which receive thalamic input\textsuperscript{201}. The existence of a similar situation in rat VI would further decrease the likelihood of observing synapses between degenerating thalamocortical axon terminals and the apical dendrites of layer V pyramidal cells.

Somogyi\textsuperscript{170}, using the traditional Golgi–EM approach of Stel\textsuperscript{173} and Blackstad\textsuperscript{8}, described thalamocortical synapses with the spines of two layer IV cells in rat primary visual cortex: one was a small pyramidal cell, the other either a pyramidal cell or a spiny stellate cell. In mouse SmI cortex, the spines of layer IV stellate cells are also postsynaptic to thalamocortical axon terminals\textsuperscript{201}.

Peters et al.\textsuperscript{133} observed thalamocortical synapses with the perikarya and dendrites of two sparsely spined stellate cells in rat VI; one was identified as a bitufted cell and the other as a multipolar stellate cell. Similarly, in mouse SmI cortex, White\textsuperscript{204} observed thalamocortical synapses with the dendrites and perikarya of a bipolar cell of layer IV/V and with a multipolar stellate cell of layer IV. Both cells lacked spines; however, these cells were identified from serial thin sections of unimpregnated neurons, so it cannot be excluded that these 'non-spiny' stellate cells possessed a few spines which escaped detection.
The combination of lesion-induced degeneration with electron microscopy of Golgi impregnated neurons has been used to great advantage to identify neurons which receive thalamocortical input. Thus it has been shown that thalamocortical axons synapse in layer IV with pyramidal cells whose somata occur in layers III to VI inclusive, with layer IV spiny stellate cells and with several types of layer IV non-spiny or sparsely spined stellate cells. At this point, it seems worthwhile to consider some of the limitations of the techniques which have provided this data. For example, the Golgi–EM method as applied in these studies can provide no information about the physiology of the impregnated neurons and, disconcerting as it may be, it is conceivable that the Golgi method has selectively impregnated neurons which receive thalamocortical input. Furthermore, although layer IV receives a massive projection from the specific thalamic nuclei, some proportion of the degenerating synapses observed in this layer may be derived from non-specific thalamic nuclei whose projections were damaged by the lesion.

9. THEORETICAL IMPLICATIONS OF WIDESPREAD THALAMIC INPUT: THALAMIC SELECTION OF CORTICAL FUNCTIONAL UNITS

In view of the results presented in the preceding section, it seems reasonable to conclude that every cortical neuron with a dendrite in layer IV receives some portion of its input directly from the thalamus. We might then suppose that thalamocortical projections to layers I and VI also terminate on dendrites irrespective of their cell type of origin. If so, then neurons throughout the full thickness of the neocortex receive input directly from the thalamus. Presumably, a change in the rate of firing of a thalamic projection could then be detected by, and to some degree directly alter the activity of, every neuron in a specified region of cortex. Perhaps this widespread thalamic input might serve to facilitate the transmission and processing of thalamic input by neurons which might not always act in concert, but which by virtue of common thalamic input are selected out to form a functional unit. Whether this unit is considered to be a local neuronal processing sequence or a functional column does not matter; the principal of selection by afferent input would apply to either. The proposed selection mechanism would allow each cortical neuron, which must receive many different kinds of input and projects to various targets, to participate simultaneously or sequentially in more than one kind of functional unit. The relationship of a neuron at any particular time to a specific processing sequence or functional column would depend on such factors as the numbers and locations of the synapses it receives and on the timing of synaptic events.

10. HIERARCHICAL VS PARALLEL PROCESSING

Hubel and Wiesel proposed that the responses of different electrophysiologically defined cell types in cat and monkey visual cortex could best be explained by a hierarchical neuronal sequence whereby cells with simple receptive fields directly receive thalamic input and then project to cortical neurons which possess more complex receptive field properties. ‘Complex’ cells, which would represent a later stage
in the cortical processing of thalamic input, are presumed to project to cortical cells with even more complex, i.e. hypercomplex, receptive fields. A strict interpretation of this hierarchical model would predict that only simple cells directly receive thalamocortical input, but in view of recent evidence suggesting that complex and hypercomplex cells are also monosynaptically activated by the lateral geniculate, this interpretation appears too simplistic. Furthermore, Dow has described several classes of simple and complex cells which he believes process different but complementary aspects of visual information in parallel within the separate layers of the cortex. Consistent with this finding is the conclusion of Ferster and LeVay, that geniculocortical afferents carrying information along the ascending pathways from X and Y retinal ganglion cells* arborize in different sublaminae of layer IV in the cat visual cortex. Evidence for the anatomical and physiological segregation of the X and Y pathways at the level of the lateral geniculate nucleus has been adduced for the cat and monkey. A third retino-geniculo-cortical pathway, termed W, has also been identified and so it appears that there are at least three separate pathways which convey different but presumably complementary aspects of information from the retina through the thalamus to the cortex. This implies that thalamocortical input is processed in parallel by different neurons in visual cortex — a notion fully consistent with the revelation that many different kinds of cortical neurons receive input directly from the thalamus. But, as Stone and later White observed, hierarchical and parallel processing mechanisms are not mutually exclusive and conceivably the cortex uses both parallel and serial mechanisms to process input from the thalamus. Consequently, cells in striate cortex which are known to receive either X or Y input, but not both, might be at the beginning of hierarchical processing sequences for their respective inputs. The degree to which these sequences interact in striate cortex or elsewhere has yet to be determined, but we must suppose that someplace the separate aspects of visual information are combined to produce a complete picture of visual space. Although parallel processing pathways have not been identified by electrophysiological studies of non-visual cortical areas, the anatomy of thalamocortical connections in somatosensory cortex suggests parallel processing mechanisms might also occur in this region. The reader is referred to the discussion in White and to the excellent review by Henry for additional references and thoughts on parallel vs hierarchical processing of thalamocortical input.

11. A CONCEPT OF THALAMOCORTICAL RELATIONS

Thalamocortical relations have been the object of intense investigation for about a century, but it is only within the past few years that technological advances have enabled the identification of those cortical neurons which are directly involved in thalamocortical synapses. The realization that thalamic input is distributed to many different kinds of neurons compels us to consider the possibility that other pathways

* For descriptions of the X and Y retino-geniculo-cortical pathways, see especially refs. 23, 42, 175 and 177.
afferent to the cortex also terminate on a variety of neuronal types. In support of this notion are the findings by Cipolloni and Peters (personal communication) that callosal afferents to rat auditory cortex synapse in layer III with spines of pyramidal cells whose somata occur in layers II, III, IV and V. Furthermore, it has been shown that the axons of non-spiny stellate cells in rat visual cortex synapse with a variety of neuronal types and in one instance the axon of a single non-spiny stellate cell has been observed to form symmetrical synapses not only with a layer III pyramid and with other non-spiny stellate cells, but with one of its own dendrites as well. We might thus consider the broader implication of Peters and Feldman's prophetic suggestion that thalamocortical axons synapse with any element in layer IV capable of forming asymmetrical synapses, and surmise that other afferent and even intrinsic axons also synapse with any neuronal type within their terminal projection field (see ref. 126). While this apparent non-specificity of cortical wiring might be more explicable in developmental terms than would a system of rigidly specified interneuronal connections, the formation of synaptic connections in a non-specific fashion appears inconsistent with our concept of the cerebral cortex as the seat of higher brain function. At this point, it is useful to remember that cortical synaptic organization is non-specific only with respect to the identity of neurons postsynaptic to axonal projections, but that these projections terminate within specific layers of the cortex. Obviously then, the degree to which a neuron is directly influenced by a particular axonal projection would partly depend on the extent to which its dendrites occur within the terminal projection field, i.e. the specific layer(s) in which the projection terminates. Consequently, a neuron with its entire dendritic tree in layer IV would presumably be more greatly influenced by thalamic input to this layer than would a neuron which sends only a small proportion of its dendrites into layer IV. Additional factors capable of specifying synaptic connections are the type of synaptic junction formed by the axon and the types of synaptic junctions the postsynaptic element can form. Thus, for example, the cell bodies of pyramidal cells are apparently specified to form only symmetrical synapses and so they do not synapse with thalamocortical axons which form asymmetrical synapses. Finally, the density of synapses possible along prospective postsynaptic elements would limit the numbers of synapses formed.

The following concept, which Alan Peters and I have developed, is an attempt to define thalamocortical relations in the context of what is known about the specificity and non-specificity of thalamocortical synaptic connections. A striking feature of the mammalian brain is the strict topographic organization of the so-called specific thalamocortical projections. The exact disposition of these thalamocortical axons must be rigidly specified during development to provide the precise topography found in the adult. We presume that 'mechanical' factors, such as the glial tubes described by Singer et al., direct thalamocortical axons to their cortical destinations. Having reached their sites of termination, thalamocortical axons synapse with every available neuronal element capable of forming asymmetrical synapses, the numbers and locations of synapses being partly constrained by the density of synapses possible along postsynaptic elements and by the numbers of pre-existing synapses. We propose that the formation of synapses by other axonal projections to the cortex occurs in a similar fashion.
Fig. 12. A montage of light micrographs taken at 20 planes of focus through a gold-toned layer IV spiny stellate cell from mouse Sml cortex. This neuron was serial thin sectioned and reconstructed (see Fig. 13). Magnification, 1000 x, scale, 10 μm.

Fig. 13. Reconstruction from serial thin sections (see ref. 206) of the layer IV spiny stellate cell shown in Fig. 12. Arrow indicates spine shown in inset. Scale, 10 μm. Inset is an electron micrograph showing a degenerating thalamocortical axon terminal (TA) synapsing with the spine (S) indicated by the arrow. × 80,000.
One of the largest gaps in our present knowledge of thalamocortical synaptic relations concerns the spatial arrangement of thalamocortical synapses on their postsynaptic elements. In an attempt to provide this information, Michael P. Rock and I have reconstructed a Golgi impregnated/deimpregnated spiny stellate cell from layer IV of mouse SmI cortex in a preparation containing degenerating thalamocortical axon terminals (Figs. 12 and 13). Lesion-induced degeneration is generally considered unreliable for the quantification of thalamocortical axon terminals because in most systems (e.g. refs. 84, 85 and 128) these axon terminals degenerate over a varied time course such that some have been phagocytosed before others have begun to degenerate. This is not true of thalamic terminal degeneration in mouse SmI cortex where all affected terminals — about 20% of the terminals in layer IV — degenerate simultaneously. Consequently, lesion-induced degeneration may reliably indicate the numbers of thalamocortical synapses on the reconstructed spiny stellate cell. Results indicate thalamocortical synapses form about 10% of the synapses onto the spines of the reconstructed dendrites; no thalamic synapses are observed with the cell body or dendritic shafts. To assess the distribution patterns of thalamocortical synapses, measurements were made along dendrite shafts from the somatic origin of

Fig. 14. Graphs showing the locations of thalamocortical synapses onto four reconstructed dendrites of a layer IV spiny stellate cell. The far left of each of the four lines labeled D1 through D4 represents the origin of each dendrite from the cell body. A 20 μm segment of D2 is missing; the dendrite distal to this hiatus is represented beneath the right hand portion of the main part of D2. To conserve space, some dendritic branches are connected to their parent shafts by dashed lines. The intersection of the dashed line with the parent shaft marks the point of origin of the daughter branch. Thalamocortical synapses are indicated by stars, spine stems by dotted lines. Scale at the bottom right is 5 μm. Reprinted from White and Rock with permission of Elsevier Publishing Co.
the dendrites to the points where the stems of spines involved in thalamocortical synapses join the dendritic shaft. The assumption is that the stem-dendrite junctions are the sites where thalamocortical input to spines enters their parent dendrites. These points were then plotted on straight lines, each line representing the total length of a reconstructed dendritic segment (Fig. 14). This analysis revealed thalamocortical synapses, which are distributed over most regions of the dendritic tree, to be arranged in a regular, periodic fashion along portions of two of the reconstructed dendrites: the mid-portion of D1 and the distal part of D2 in Fig. 14. In these regions, the necks of spines receiving thalamic input attach to the dendritic shaft at regular intervals of between 4.5 and 5.5 μm. The suggestion was made that this periodicity reflects the spacing of thalamocortical synapses which might form with the dendritic shafts prior to the time of spine formation. The rationale for this suggestion is the observation that only the loci where these spines connect to their parent dendrites are regularly spaced, whereas the spine heads are not separated by regular intervals. In several other regions of the dendritic tree depicted in Fig. 14, two spines receiving thalamic input are separated by intervals of about 5 μm. The 5 μm intervals might result from the spacing of thalamocortical receptor sites along postsynaptic membranes. An alternative explanation, consistent with the concept of thalamocortical relations described above, is that the formation of a thalamocortical synapse at one site might inhibit the formation of thalamocortical synapses by other axon terminals within about 5 μm along the dendritic shaft.

A further observation is that spiny stellate cell spines involved in thalamocortical synapses are not clustered in particular regions of the dendrites, but are interspersed with spines receiving synapses from other sources. It is not yet known whether non-thalamic inputs are regularly distributed along spiny stellate cell dendrites, nor whether thalamic inputs are regularly distributed along the dendrites of other types of cortical neurons which receive input from the thalamus. Moreover, the extent to which a single thalamic fiber synapses with individual dendrites or single cells has yet to be determined. Future reconstructions of neurons using similar techniques are expected to answer these questions, but as this account has shown, only the application of a wide variety of approaches can provide the information necessary to understand how the cortex receives and processes its thalamic input.

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REFERENCES

3 Adrian, E. D., Afferent discharges to the cerebral cortex from peripheral organs, *J. Physiol. (Lond.)*, 100 (1941) 159–191.


49 Freire, M., Effects of dark rearing on dendritic spines in layer IV of the mouse visual cortex, a quantitative electron microscopical study, *J. Anat. (Lond.*), 126 (1978) 193–201.


68 Hoffman, K.-P. and Stone, J., Conduction velocity of afferents to cat visual cortex: a correlation with cortical receptive field properties, Brain Research, 32 (1971) 460–466.


72 Hubel, D. H. and Wiesel, T. N., Shape and arrangement of columns in cat's striate cortex, J. Physiol. (Lond.), 165 (1963) 559–568.


78 Jones, E. G., An electron microscopic study of the termination of afferent fiber systems within the somatic sensory cortex of the cat, J. Anat. (Lond.), 103 (1968) 595–597.


80 Jones, E. G., Possible determinants of the degree of retrograde neuronal labeling with horseradish peroxidase, Brain Research, 85 (1975) 249–253.


305


111 Lund, J. S., Organization of neurons in the visual cortex, area 17, of the monkey (*Macaca mulatta*), *J. comp. Neurol.*, 147 (1973) 455-496.
112 Maciewicz, R. J., Thalamic afferents to areas 17, 18 and 19 of cat cortex traced with horseradish peroxidase, *Brain Research*, 84 (1975) 308-312.
123 Papez, J. W., Thalamic connections in a hemidecorticate dog, *J. comp. Neurol.*, 69 (1938) 103-120.


Powell, T. P. S., Residual neurons in the human thalamus following hemidecortication, Brain, 75 (1952) 571-584.


Rezak, M. and Benevento, L. A., A comparison of the organization of the projections of the dorsal lateral geniculate nucleus, the inferior pulvinar and adjacent lateral pulvinar to primary visual cortex (area 17) in the macaque monkey, Brain Research, 167 (1979) 19-40.


Robson, J. A. and Hall, W. C., Connections of layer VI in striate cortex of the grey squirrel (Sciurus carolinensis), Brain Research, 93 (1975) 133-139.


Sachs, E., On the structure and functional relations of the optic thalamus, Brain, 32 (1909) 95-186.

Sanderson, K. J. and Kaas, T. H., Thalamocortical interconnections of the visual system of the mink, Brain Research, 70 (1974) 139-143.


167 Singer, W., Hofländer, H. and Vanegas, H., Decreased peroxidase labeling of lateral geniculate neurons following deafferentation, Brain Research, 120 (1977) 133-137.
177 Stone, J. and Hoffmann, K.-P., Conduction velocity as a parameter in the organization of the afferent relay in the cat's lateral geniculate nucleus, Brain Research, 32 (1971) 454-459.
187 Toyama, K., Maekawa, K. and Takeda, T., Convergence of retinal inputs onto visual cortical cells: I. A study of the cells monosynaptically excited from the lateral geniculate body, Brain Research, 137 (1977) 207-220.
188 Uylings, H. B. M., Kuypers, K., Diamond, M. C. and Veltman, W. A. M., Effects of differential


197 Waller, W. H., The thalamus of the cat after hemidecortication, J. Anat. (Lond.), 72 (1938) 475-487.


203 Welker, W. L. and Johnson, J. I., Barrels in cerebral cortex altered by receptor disruption in newborn but not in five-day-old mice (Cricetidae and Muridae), Brain Research, 83 (1975) 504-508.


207 White, E. L. and Rock, M. P., Sensory profile of a spiny stellate cell from layer IV of mouse MI cortex submitted to J. Neurocytol.


215 Wilson, P. D. and Stone, J., Evidence of W-cell input to the cat's visual cortex via the C laminae of the lateral geniculate nucleus, Brain Research, 92 (1975) 472-478.


APPENDIX A REFERENCES


8 Hall, W. C. and Diamond, I. T., Organization and function of the visual cortex in hedgehog:


14 Rose, J. E. and Malis, I., Geniculostriate connections in the rabbit. I. Retrograde changes in the dorsal lateral geniculate body after destruction of cells in various layers of the striate region, *J. comp. Neurol.*, 125 (1965) 95–120.

15 Rose, J. E. and Malis, I., Geniculostriate connections in the rabbit. II. Cytoarchitectonic structure of the striate region and of the dorsal lateral geniculate body; organization of the geniculo-striate projections, *J. comp. Neurol.*, 125 (1965) 121–140.


APPENDIX B REFERENCES


