Documenta Ophthalmologica Proceedings Series 30

Pathophysiology of the Visual System

Edited by L. Maffei

PATHOPHYSIOLOGY OF THE VISUAL SYSTEM

Proceedings of a Workshop held at the Scuola Normale Superiore, Pisa, Italy on December 12-15, 1980
Sponsored by the Commission of the European Communities, as advised by the Committee on Medical and Public Health Research

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Dr W. Junk Publishers
for the Commission of the European Communities

Distributors:

for the United States and Canada
Kluwer Boston, Inc.
190, Old Derby Street
Hingham, MA 02043
USA

for all other countries
Kluwer Academic Publishers Group
Distribution Center
P.O.Box 322
3300 AH Dordrecht
The Netherlands

ISBN 90-6193-726-4 (this volume)
ISBN 90-6193-882-1 (series)

Publication arranged by:
Commission of the European Communities
Directorate-General Information Market and Innovation,
Luxembourg

EUR 7353

PRINTED IN THE NETHERLANDS

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12e Session
Chairman: I. Bodis-Wollner

INTRODUCTORY REMARKS

The word "pathophysiology" in the title of this Workshop may sound inappropriate and somewhat daring considering that the great majority of the participants in this Conference are indeed either physiologists or psychologists of the visual system. Among basic scientists the study of the pathophysiology of nervous diseases of the visual system has often been neglected.

Traditionally, the physiologist has not been very prompt in handing over his achievements to the clinician and the latter has always been very suspicious about messages coming from the physiological laboratory. Nowdays, however, in many advanced institutions, the reciprocal diffidence between clinicians and physiologists is slowly being replaced by collaboration. This Workshop is an example of such progress.

There may be many ways of solving a problem but the final solution of it, which we could call knowledge, is independent of them.

In the last 10 years the science of vision has progressed very slowly indeed. Many problems have been elegantly reinvestigated with different methodologies and this has greatly improved the knowledge of details but bold newsteps of high quality have been relatively few. To quote from Claude Bernard "l'idée c'est la graine, la méthode c'est le sol qui lui fournit les conditions de se développer". Method, by itself, does not generate anything: "n'enfante rien".

Nevertheless, some of the most interesting results in the last 10 years have been in the field of the pathophysiology of the visual system. The most interesting achievements have been obtained by the application in the clinic of methodologies developed in physiological or psychological laboratories. Perhaps instead of Claude Bernard it is more pertinent and realistic to quote Werner Heisenberg who, in one of his lectures, said: "in diesem Entwicklungsprozess der lezten 200 Jahre ist die Technik immer wieder Voraussetzung und Folge der Naturwissenschaft gewesen".

A reinvigorating starting basis was given in the early sixties by the experiments of David H. Hubel and Torsten N. Wiesel in squint and monocularly deprived kittens. These investigations were taken up by a number of scientists mainly because of their direct relevance to the clinic.

The implication for an early surgical intervention to correct strabismus in children and for a careful, short term application of patching in amblyopic subjects, was direct and clear. Electrophysiological experiments in the animal, human psychophysics and clinical observations began to progress hand in hand. Modern techniques, developed for research in visual physiology, such as spatial analysis of vision with sinusoidal gratings or pattern evoked potential, were applied in the clinic with great advantages for the early diagnosis of refraction errors and nervous alterations of the visual pathway.

I do not need to be a prophet to predict that this bridge between the clinic and the physiology will be rich in successful developments. Collaboration of different competences is in the logic of Science. I believe that intellect has a natural tendency to proceed and there is no hope of holding it back. It is in this frame of mind that I wish all of us a successful meeting.

I am very glad to welcome you on behalf of the "Commission of the European Communities" and in particular of Prof. E. Levi to whom we are greatly indebted for the organization of this Meeting. I also take the opportunity to thank the "Scuola Normale Superiore" which has offered us its hospitality. I would like also to thank the Members of the organizing committee of this Workshop and especially Prof. A. Fiorentini, who has divided with me the burden of the organization.

Lamberto Maffei

ULTRASTRUCTURE OF THE THALAMIC PROJECTION TO CAT VISUAL CORTEX.

L.J. GAREY and J.P. HORNUNG (Lausanne, Switzerland)

1. INTRODUCTION

Thalamic neurons project chiefly to layer IV and lower layer III of the cerebral cortex. In the visual cortex (VC) of the cat lesions made in the lateral geniculate nucleus (LGN) cause axonal degeneration tracable to these layers (Wilson, Cragg, 1967; Colonnier, Rossignol, 1969; Garey, Powell, 1971). Electron microscopic studies of degenerating thalamic axon terminals show that most thalamo-cortical synapses in cat VC are on dendritic spines and the rest on dendritic shafts and neuronal somata (Colonnier, Rossignol, 1969; Garey, Powell, 1971).

It is difficult to identify neuronal profiles as belonging to pyramidal or non-pyramidal cells in random electron microscopic sections. It is especially difficult to classify detached dendritic segments, and impossible to attribute isolated spines to their parent cell. One cannot, therefore, easily identify neurons postsynaptic to thalamic axons. Although about 20 % of layer IV degenerating terminals after a lesion in the LGN contact dendrites and somata of stellate cells (Garey, Powell, 1971), the fact that spines of both stellate and pyramidal cells are present in layer IV makes it impossible to decide whether both neuronal types are postsynaptic to thalamic afferents, and, if so, in what proportion.

Sometimes a spine contacted by a degenerating terminal can be followed to its dendrite and the cell type recognized (Davis, Sterling, 1979). However, very large numbers of sections are needed with tedious serial reconstruction. Alternatively cortical neurons can be labelled with a method enabling their characterization by light microscopy and then examined with electron microscopy in a brain with a lesion in the LGN.

In the present study two labelling techniques have been used. In the first, neurons were impregnated by the rapid Golgi method for light microscopic classification and then subjected to the gold toning process of Fairén et al. (1977). In the second, neurons were retrogradely labelled with horseradish peroxidase (HRP) and classified by light microscopy before electron microscopic study (Garey, Hornung, 1980; Hornung, Garey, 1980, 1981).

2. METHODS

Golgi method

Coagulative lesions were made in the LGN of both hemispheres. Three or four days later the cat was perfused through the left cardiac ventricle with a solution of paraformaldehyde and glutaraldehyde. Blocks of the VC were impregnated with the rapid Golgi method. The blocks were sectioned coronally at 100 µm. Sections were mounted temporarily in glycerol and examined by light microscopy. Well impregnated neurons in area 17 of the VC were noted and excised. The excised pieces of the 100 µm sections containing the selected neurons were toned with gold chloride, reduced with oxalic acid and the silver precipitate removed with 1 % sodium thiosulphate (Fairén et al., 1977). After osmification and staining with alcoholic uranyl acetate,

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the selected sections were dehydrated and embedded. Serial ultrathin sections were cut, collected on Formvar-coated single-slot grids and stained with lead citrate. Several semi-thin sections were collected at intervals through the neuron and stained with toluidine blue.

HRP method

Multiple injections of HRP (total 6 to 7 µl) were made along the border of areas 17 and 18 in the right hemisphere. A stereotaxic electrolytic lesion was then made in the left LGN. Three days later the animal was perfused with a solution of paraformaldehyde and glutaraldehyde. A block of the right cortex containing the injection site was sectioned coronally at 40 µm on a freezing microtome. The left cortex, destined for electron microscopy, was cut by Vibratome at 100 µm. Sections were incubated with diaminobenzidine (DAB; Graham, Karnovsky, 1966) and cobalt chloride (Adams, 1977). Well labelled neurons were selected and excised. They were treated with osmium tetroxide, stained with alcoholic uranyl acetate and embedded in resin. Serial ultrathin sections were cut, mounted on Formvar-coated single-slot grids and stained with lead citrate. 1 µm sections were collected at intervals and stained with toluidine blue.

3. RESULTS

Golgi impregnated neurons are labelled by a fine deposit of gold particles. In semi-thin sections the labelling appears as a dark outline to the cell. Electron microscopically, impregnated processes are filled with gold particles, especially close to the cell membrane.

On light microscopy, HRP impregnated neurons are filled with brown granules and a homogeneous dark brown precipitate giving a "Golgi-like" appearance. The granules and the homogeneous precipitate are visible in semi-thin sections. On electron microscopy, labelled neurons are highly contrasted due to a precipitate of oxidized DAB on the plasma membrane, endoplasmic reticulum, microtubules, ribosomes, spine apparatus and post-synaptic specializations (Hornung, Garey, 1981).

A selection of labelled neurons receiving thalamo-cortical synapses is illustrated in Figure 1.

Pyramidal neurons

Pyramidal neurons with their soma in layer III or layer V were studied. Those in layer III include ones labelled by HRP, which are, therefore, callosal neurons. They all have a single apical dendrite giving a few oblique collaterals and three to five basal dendrites. They bear a large number of spines after the first dendritic branching.

The pyramids show typical ultrastructural features (Colonnier, 1968; Jones, Powell, 1970; Garey, 1971). The somata receive a few symmetrical synapses. Apical dendrites have a few symmetrical synapses on the shaft and many asymmetrical ones on the spines. Basal dendrites receive a moderate number of symmetrical and a few asymmetrical synapses on the shaft and many asymmetrical contacts on the spines.

Degenerating terminals are found on spines of apical and basal dendrites, and the shafts of basal dendrites.

Non-pyramidal neurons

Large spiny non-pyramidal neurons have a moderate number of axo-somatic synapses both symmetrical and asymmetrical. The axon descends from the lower pole of the soma and gives a horizontal collateral in layer IV. The dendritic tree is radial and the shafts receive more synapses than those of pyramidal cells and more are asymmetrical. Some are HRP-labelled callosal neurons. Degenerating terminals are found on dendritic spines and shafts.

Large sparsely-spiny neurons have similar features but the somata and dendrites are contacted by a large number of synapses, which are of both asymmetrical and symmetrical types. Degenerating thalamo-cortical terminals contact the dendritic spines and shafts.

Other large non-pyramidal neurons have no spines on their dendrites, which are varicose. The axon initial segment often leaves the upper pole of the soma. The number of axo-somatic synapses, especially assymmetrical, is higher than any other cortical neurons. The dendrites also bear many asymmetrical synapses. Degenerating terminals contact the soma and dendritic shafts of these cells.

Small non-pyramidal neurons may have radial dendrites or they may be oriented. They are spiny or sparsely-spiny. The soma is contacted by a small number of terminals, like pyramidal cells, but, unlike pyramids, some of the synapses are asymmetrical. The dendrites also receive a moderate number of both types of contacts. Degenerating terminals synapse with dendritic spines and shafts.

Density of thalamo-cortical terminals

All synapses contacting each neuron in the sections studied were recorded to estimate the proportion of degenerating to normal contacts for each cell. Counts were also made of the percentage of degenerating terminals in the surrounding neuropil. The mean proporton of thalamo-cortical terminals on an individual neuron was about 3 % and the average proportion in the neuropil was 4 %. In no case was there an obvious difference between the density of degenerating terminals in the neuropil and those contacting a specific neuron.

4. DISCUSSION

Both pyramidal and non-pyramidal neurons which have processes in the band of thalamic projection in layers III and IV receive thalamo-cortical afferents. All processes which are potential targets or, in other words, which bear asymmetrical synapses, seem to have thalamic inputs. These include apical dendritic spines of pyramidal cells of layer III or V and basal dendritic spines and shafts of layer III pyramids. Thalamo-cortical terminals also contact dendritic shafts of many non-pyramidal neurons, all with their soma in layer IV. They include large and small spiny, large and small sparsely-spiny and large non-spiny neurons. Thalamic terminals also synapse with dendritic spines of these cells, with the exception of large non-spiny neurons, as well as with the soma of large non-spiny non-pyramidal neurons. These results are in broad agreement with similar studies on rodent visual and somatosensory cortex (Somogyi, 1978; White, 1978; Peters et al., 1979).

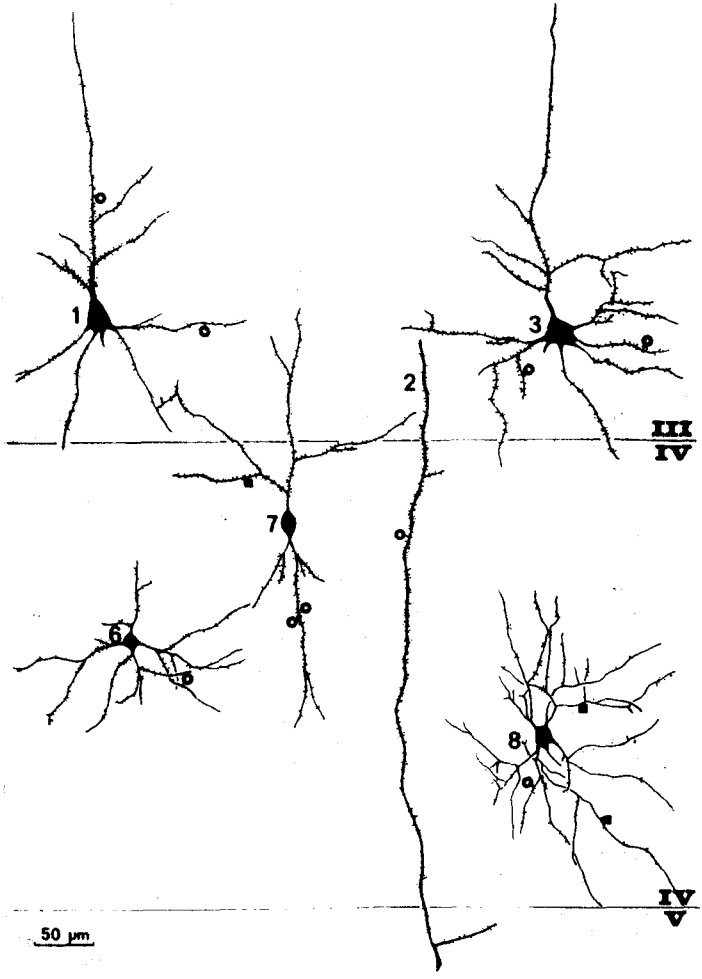

FIGURE 1. Drawing of the morphology of some neurons receiving thalamo-cortical synapses. Solid black cells were marked with the Golgi method. Outlined cells, marked with HRP, are callosal neurons. Symbols for degenerating terminals:

star in circle: on dendritic spine;
square: on dendritic shaft;
open star: on soma.

Cells 1, 3, 4, 5: pyramidal cells in layer III.
Cell 2: pyramidal cell with soma in layer V.

FIGURE 1 (continued).
Cell 9: large spiny non-pyramidal callosal cell.
Cells 11, 13: large sparsely-spiny non-pyramidal cells.
Cell 12: large non-spiny non-pyramidal cell with varicose dendrites with a very high number of asymmetrical axo-somatic and axo-dendritic synapses.
Cells 6, 7, 8, 10: small non-pyramidal cells with various dendritic patterns and spininess.

We have also shown that certain specific efferent cells, in this case callosal neurons, are innervated monosynaptically from the LGN (Hornung, Garey, 1980). They include pyramidal and large spiny non-pyramidal cells.

As the proportion of thalamo-cortical terminals on a given cell does not seem to differ from that in the surrounding tissue, thalamo-cortical terminals may be equally distributed on all neuronal profiles in the deep part of layer III and layer IV. Thus the proportion of thalamo-cortical synapses to each individual neuron with layer IV should be about 20 %, the proportion of thalamic synapses in layer IV itself (LeVay, Gilbert, 1976). For neurons largely outside layer IV the total density of thalamo-cortical afferents will be less.

Cells with monosynaptic afferents from the LGN may be either excitatory or inhibitory. For instance, the collaterals of pyramids and large spiny non-pyramidal neurons make asymmetrical synapses (LeVay, 1973; Hornung, Garey, 1981) which may be correlated with excitation (Uchizono, 1965). But some cells in the layers innervated by the LGN form symmetrical synapses. For instance "double bouquet" cells form inhibitory-like contacts (Somogyi, Cowey, 1980), "chandelier" cells synapse on the axonal initial segment of pyramids (Fairén, Valverde, 1980) and smooth multipolar non-pyramidal cells form symmetrical contacts with layer III pyramidal cells (Peters, Proskauer, 1980).

The present data, in agreement with previous studies (see White, 1979), show that not only non-pyramidal neurons, which may be simple cells (Kelly, Van Essen, 1974; Gilbert, Wiesel, 1979), but also pyramids, some of which are complex cells, receive thalamo-cortical input. This evidence favours a "parallel" concept of geniculo-cortical projection. But, as argued above, a pyramidal neuron, being largely outside layer IV, probably receives a smaller proportion of its afferent input from the thalamus than a non-pyramidal neuron mostly in layer IV. Thus, many of the functional characteristics of a complex cell are probably due to polysynaptic input through simple, or other complex, neurons - a "hierarchical" concept.

Acknowledgement: Supported by the Swiss National Science Foundation (3.0950.77).

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