Histol Histopath (1988) 3: 361-366

Histology and Histopathology

# Dense cored vesicles in presynaptic profiles of the rabbit dorsal lateral geniculate nucleus

**P. Contamina, M.P. Ariño, P. Parra and L. Martínez Millán**
Department of Morphological Sciences, Faculty of Medicine, University of Zaragoza, Zaragoza, Spain

**Summary.** We are carrying out a study about the synaptic relations between identified synaptic profiles in the dorsal lateral geniculate nucleus (dLGN) of the rabbit. Here, the types of synaptic vesicle containing profiles of the dLGN are described. There are presynaptic large profiles containing round vesicles and pale mitochondria (RLP terminals) and small profiles that contain round vesicles and dark mitochondria (RSD terminals) which respectively arise from the retina and the visual cortex. Another type of presynaptic profile contains elliptical vesicles (F-boutons) which can be subdivided according to their cytoplasmic content. These F-boutons arise from dLGN interneurons. We have found different sized vesicles that have a dense core within RLP, and F terminals and a possible RSD terminal. The significance of the coexistance of pale and dense cored vesicles in the presynaptic profiles of the rabbit dLGN is discussed.

**Key words:** Dorsal lateral geniculate nucleus, Synaptic organization, Dense cored vesicles, Rabbit

## Introduction

The dorsal lateral geniculate nucleus (dLGN) is the thalamic relay nucleus of the mammalian visual pathway in which two different types of neurons have been identified: principal cells and interneurons (Le Vay, 1971; Famiglietti and Peters, 1972; Rafols and Valverde, 1973; Szentágothai, 1973; Lieberman and Webster, 1974; Hajdu et al., 1982). In the neuropil, there are axodendritic profiles forming polysynaptic encapsulated structures called «glomeruli» (Szentágothai, 1963). Retinal axon terminals, axon terminals of the interneurons, presynaptic dendrites of the interneurons, and dendrites of principal cells are glomerular components (Guillery, 1969; Famiglietti and Peters, 1972; Wong-Riley, 1972; Rafols and Valverde, 1973; Szentágothai, 1973; Lieberman and Webster, 1974; Hadju et al., 1982).

The retinal terminals or RLP axons generally present the largest profiles; they have an irregular outline and contain numerous round vesicles and mitochondria of light appearance (Guillery, 1969; Wong-Riley, 1972; Rafols and Valverde, 1973; Szentágothai, 1973; Hajdu et al., 1982). These RLP axons make asymmetrical synaptic contacts with dendrites or spine-like dendritic protusions, profiles containing pleomorfic vesicles, and occasionally with some neuronal perikaryon. RLP terminals are exclusively presynaptic (Le Vay, 1971; Wong-Riley, 1972).

Presynaptic profils containing cylindrical or oval vesicles are called F-boutons; it is possible to recognize two distinct types of F-boutons: F1 and F2 (Guillery, 1969; Le Vay, 1971; Wong-Riley, 1972; Ralston and Chow, 1973; Szentágothai, 1973; Hajdu et al., 1982). F1-boutons are probably axon terminals of the interneurons (Guillery, 1969; Famiglietti and Peters, 1972; Ralston and Chow, 1973; Szentágothai, 1973), whereas F2-boutons are thought to be dendritic appendages of the interneurons (Le Vay, 1971; Famiglietti and Peters, 1972; Rafols and Valverde, 1973; Ralston and Chow, 1973; Lieberman and Webster, 1974; Hajdu et al., 1982). F terminals predominantly form relatively long symmetrical synaptic contacts (Wong-Riley, 1972; Rafols and Valverde, 1973; Ralston and Chow, 1973; Lieberman and Webster, 1974; Wilson and Hendrickson, 1981).

The RSD axons have relatively small synaptic knobs and contain closely-packed round vesicles and dark mitochondria. Many of these profiles are of cortico-geniculate origin. The RSD axons make asymmetrical synapses onto the distal portion of dendrites of the principal cells

*Offprint requests to:* Dr. P. Contamina, Depto. Ciencias Morfológicas, Facultad de Medicina, Universidad de Zaragoza, 50.009 Zaragoza, Spain

(Guilery, 1969; Le Vay, 1971; Wong-Riley, 1972; Rafols and Valverde, 1973; Szentágothai, 1973; Lieberman and Webster, 1974; Wilson and Hendrickson, 1981).

In the cat (Guillery, 1969) and mouse (Rafols and Valverde, 1973), the coexistence of pale and dense cored vesicles within retinal axon terminals has been reported. We have found vesicles that have a dense core within RLP and F terminals in the dLGN of the rabbit. The present report comments on this finding.

## Materials and methods

Eight adult rabbits were anaesthetized with 20% chloral hydrate and were then perfused through the heart with a mixture that contained 1% paraformaldehyde and 1.5% glutaraldehyde in a phosphate buffer 0.1 M at pH 7.4. The lateral geniculate nucleus was cut into slices of 200 μm thickness, osmicated, stained with uranyl acetate, dehydrated, and embedded in Epon-Araldite. After polymerization, ultrathin sections (Reichert 0M U2 ultramicrotome) were stained with lead citrate and examined in a Philips EM-301 electron microscope.

## Results

Of all kinds of presynaptic profiles seen in the dLGN of the rabbit, the RLP terminal stood out by its larger size. The contours of the terminal were irregular due to the protusion of multiple axonal or dendritic profiles (Fig. 1). The terminal was filled with numerous, uniformly distributed and round synaptic vesicles. Sometimes a special type of large synaptic vesicles that had a dense core and a lighter peripheral zone were observed (Fig. 2). Characteristic mitochondria of clear matrix were also found (Figs. 1, 2). The RLP synaptic knob might exhibit small segments of smooth endoplasmic reticulum, and occasionally neurofilaments or a few microtubules (Fig. 1). The RLP axons were seen to synapse asymmetrically upon F terminals and dendritic profiles. Junction complexes of *puncta adhaerentia* type (Peters et al., 1976) also occurred between RLP terminals and dendrites (Fig. 2). Retinal terminals might form a synaptic contact with a unique dendritic profile (Fig. 2) or be surrounded by several neuronal profiles arranged in synaptic glomeruli (Fig. 1). In this latter case, the synaptic complex could be ensheated by glial lamella.

RSD terminals made asymmetrical synaptic contacts with dendritic profiles. These RSD terminals contained abundant round synaptic vesicles, occupying the majority of the terminal, and some dark mitochondria (Fig. 4). In a RSD terminal, between the round synaptic vesicles some dense cored vesicle slightly larger in size ($RSD_5$ in fig. 4) could be encountered.

F-boutons were terminals intermediate in size between RLP and RSD terminals. Two subgroups of F-boutons were identified, F1 and F2. The F1 synaptic type had a darker appearance with multiple oval vesicles which occupied most of the profile in association with mitochondria with relatively darkened matrix (Fig. 2). The F1 synaptic bouton might exhibit a few cisternae of smooth endoplasmic reticulum and microtubules. F-2 boutons were smaller in size and contained more widely scattered vesicles; the background matrix of the synaptic knob was paler than that of the F1 type. Cisternae of smooth endoplasmic reticulum might be present (Fig. 1). The most common contact of the F knob type was upon dendritic profiles or neuronal somas in a symmetrical fashion (Fig. 2). Sometimes some vesicles with a dense centre and lighter peripheral zone were observed in both F1 and F2 terminals (Figs. 1, 3).

The dense cored vesicles encountered in the rabbit dLGN were of different size (from 55 to 100 nm) according to the terminal. For example, some dense cored vesicles (Fig. 1: F1 terminal; fig. 2: RLP terminals; fig. 3: F2 terminal) were larger (about 85 nm) that the pale cored vesicles, but other dense cored vesicles (Fig. 2: F1 terminal; fig. 4: $RSD_5$ terminal) were merely slightly larger (about 63 nm) than the normal vesicles (about 45 nm).

## Discussion

A review of the morphology of presynaptic profiles of the dLGN in several species reveals that dense cored vesicles in the retinal terminals have only been reported by Rafols and Valverde (1973) in the mouse, and by Guillery (1969) in the cat. However, such dense cored vesicles are encountered in the illustrations of different published papers dealing with synaptic organization of dLGN of the rabbit (Ralston and Chow, 1973: figs. 18 and 21), tree shrew (Hajdu et al., 1982: fig. 7), cat (Famiglietti and Peters, 1972: figs. 24 and 31), and Cebus monkey (Pecci-Saavedra et al., 1968: fig. 18). The number of vesicles that have a dense core is small (from one to seven) as well as the retinal terminals that contain this type of vesicle, although in our material (Fig. 2) there are three neighbouring retinal terminals showing dense cored vesicles.

In the reviewed literature, we have not found explicit mention of the presence of dense cored vesicles either in F-boutons or in RSD boutons of dLGN. Nevertheless, they can be seen in F terminals of dLGN from the rat (Montero and Scott, 1981: fig. 3), rabbit (Ralston and Chow, 1973: figs. 13 and 21), tree shrew (Hajdu et al., 1982: fig. 11) and cat (Guillery, 1969: fig. 3; Famiglietti and Peters, 1972: fig. 18). The $RSD_5$ terminal with a dense cored vesicle of our material is comparable with a «terminal with round vesicles, undetermined» reported by Montero and Scott (1981: fig. 2), but this latter terminal is larger and a little lighter than RSD terminals that can be seen in the same figure. Nevertheless the size and the electron-density of the $RSD_5$ terminal with a dense cored vesicle of our material is similar to the others neighbouring RSD terminals. These characteristics suggest that this terminal with a dense cored vesicle called $RSD_5$ is a true RSD terminal. On the other hand, this terminal does not present dark mitochondria and could be a RLP terminal that has not been cut in its maximal dimensions. Furthermore it is possible that other terminals of different origin containing excitatory

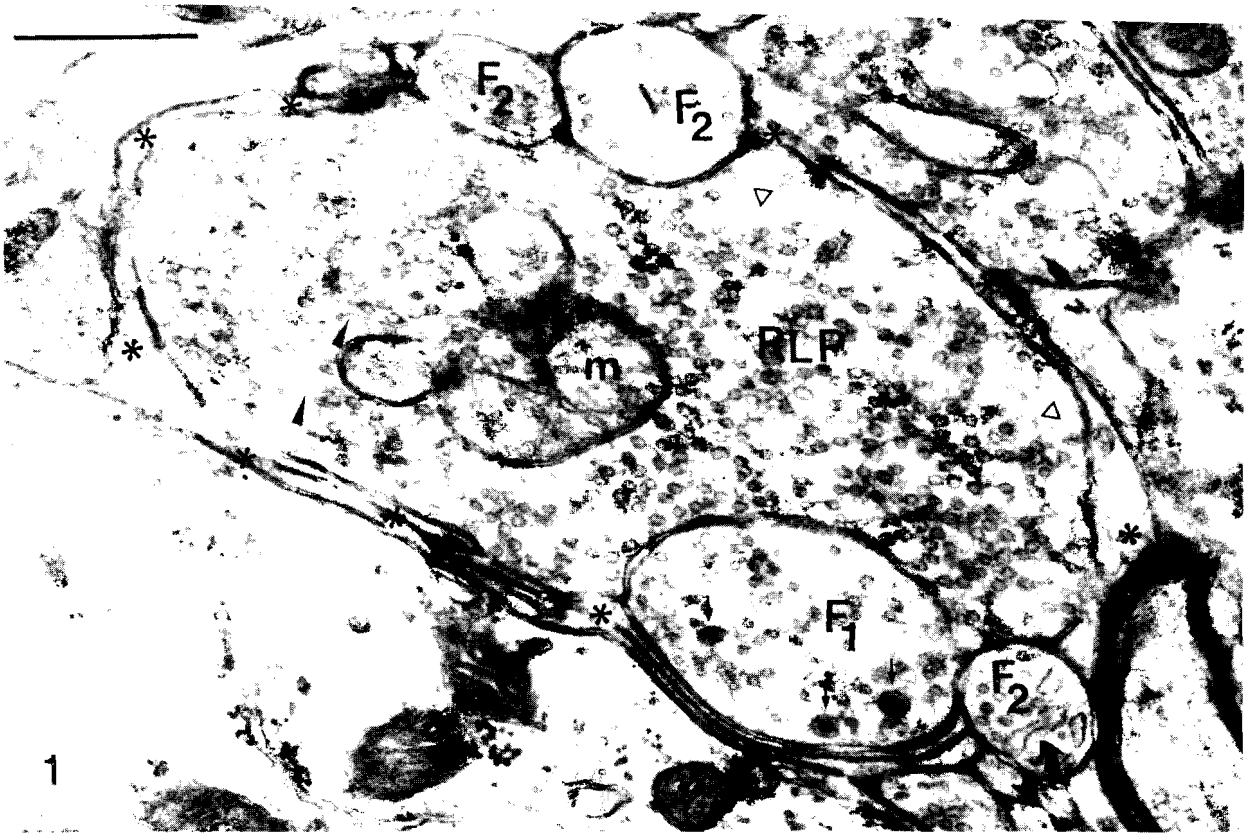


**Fig. 1.** Electron photomicrograph of a synaptic glomerulus of rabbit dLGN illustrating the retinal terminal (RLP) containing mitochondria (m), microtubules (open triangle) and neurofilaments (arrowheads) surrounded by F-boutons (F1 and F2). Dense cored vesicles (small arrows) are seen in the F1-bouton, and vesicles of smooth endoplasmic reticulum (curved arrow) in the F2-bouton. The synaptic glomerulus is surrounded by glial elements (asterisk). Bar indicates 0.5 micrometers.

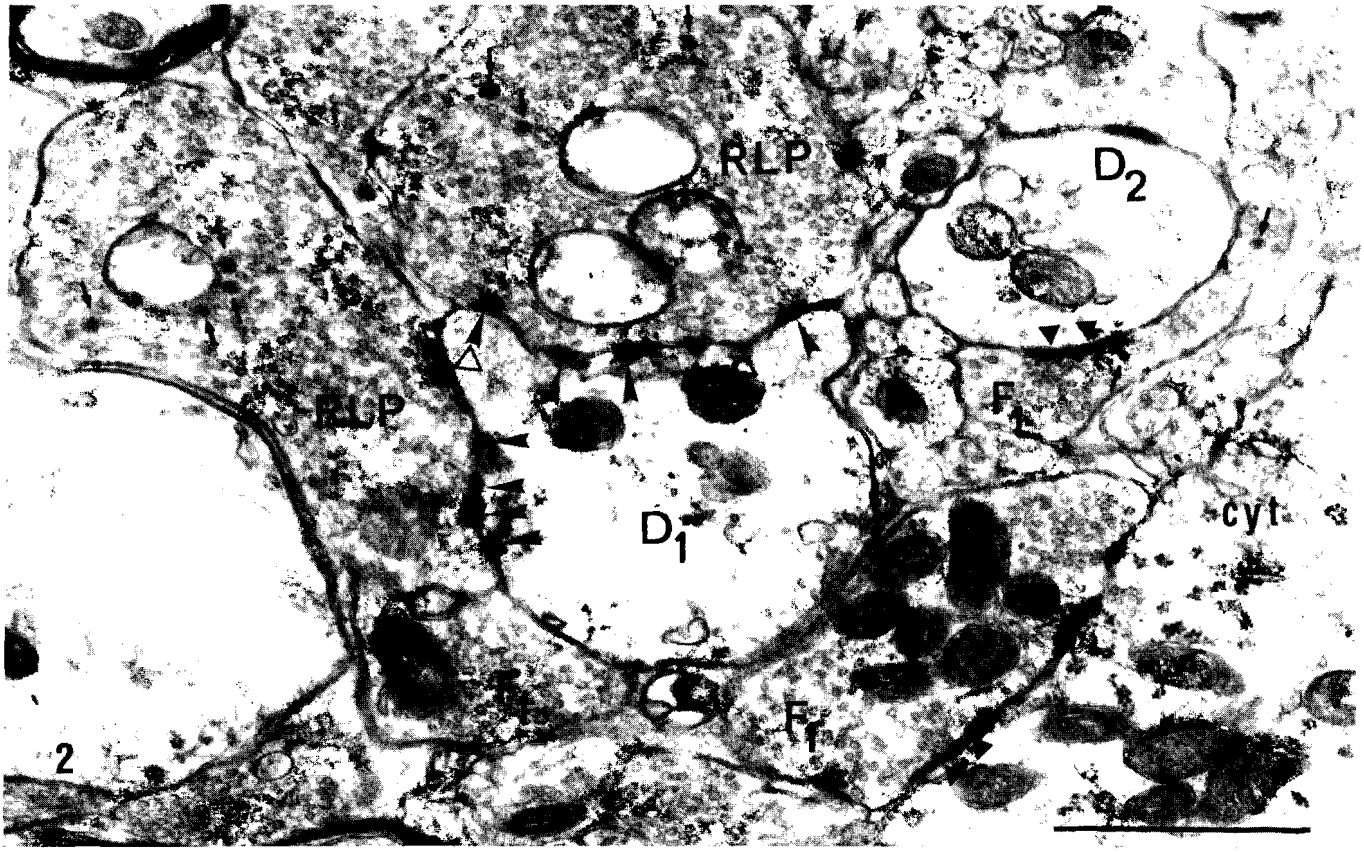


**Fig. 2.** Dense cored vesicles (small arrows) in RLP and F1 terminals of rabbit dLGN. Two retinal terminals (RLP) make two asymmetrical synapses (open triangles) onto a dendritic profile ($D_1$). Both RLP terminals make various contacts of *puncta adhaerentia* type (arowheads) with the same dendrite. Two F1 terminals one of them containing mitochondria (m) make symmetrical synaptic contact (solid triangle) onto a dendrite ($D_2$) and a neuronal soma (cyt) respectively. Bar indicates 1 micrometer.

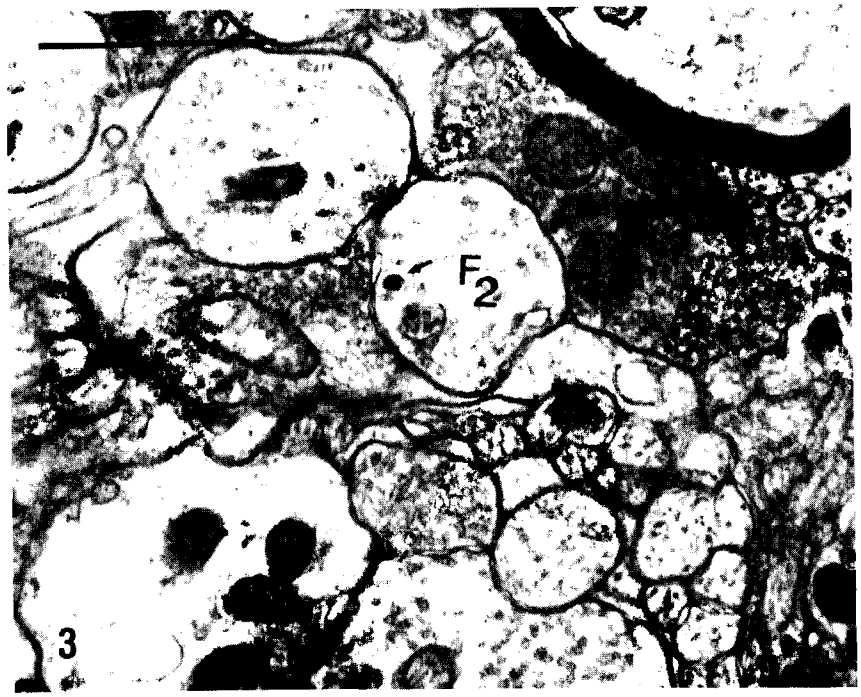


**Fig. 3.** F2 terminal containing a dense cored vesicle (small arrow) in the neuropile of rabbit dLGN. Bar indicates 1 micrometer.

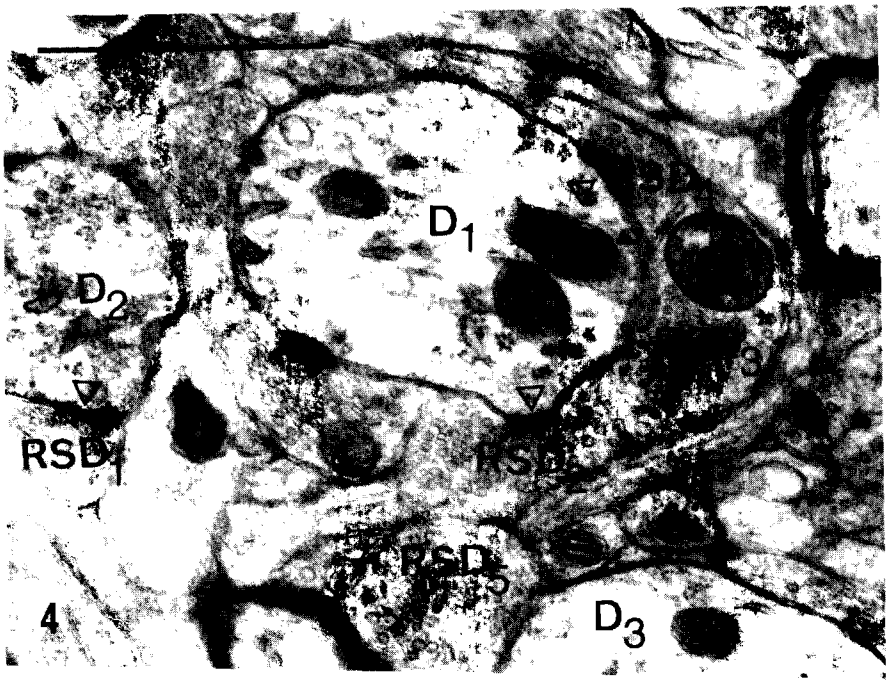


**Fig. 4.** Several RSD terminals ($RSD_{1-5}$) in the rabbit dLGN, some of them make asymmetrical synaptic contacts upon dendritic profiles ($D_1$ and $D_2$). One of the RSD terminals ($RSD_5$) contains a dense cored vesicle (small arrow). Bar indicates 1 micrometer.

neurotransmitters (the tecto-geniculate terminals, Matute et al., 1984) can show a similar morphology. The number of synaptic vesicles that have a dense core in the F and RSD boutons is smaller than that observed in the retinal terminals, although this finding may merely reflect the smaller size of F and RSD terminals.

In our material we have found several sizes of dense cored vesicles in different F1 terminals. These findings suggest that F1 terminals may arise from neurons of different localization or that the diverse F1 terminals contain diverse neurotransmitters.

The coexistance of pale and dense cored vesicles in the same terminal has been related to the presence of monoamines or neuropeptides in different types of synaptic terminals of diverse animal species (Bloom, 1984; Pickel, 1985). Norepinephrine innervation of dLGN by locus coeruleus (Kromer and Moore, 1980) or serotoninergic innervation by the nucleus raphe dorsalis (Steinbusch, 1985; Ueda and Sano, 1986) may justify the presence of F terminals, especially F1-boutons, with dense cored vesicles. F-1 bouton population mostly originates from axon terminals of intrinsic neurons of

dLGN; however, F1-boutons of extrageniculate origin have also been found (Wong-Riley, 1972; Lieberman and Webster, 1974; Ohara et al., 1980; Montero and Scott, 1981; Wilson and Hendrickson, 1981; Hajdu et al., 1982) some of which may arise from the locus coeruleus or the dorsal raphe nucleus.

The presence of neuropeptides in dLGN may also account for the existence of dense cored vesicles in presynaptic profiles of this nucleus. Recent studies have shown immunoreactivity against cholecystokinin (Vanderhaeghen, 1985), vasoactive intestinal polypeptide (Lorén et al., 1979) or substance P (Cuello and Kanazawa, 1978) at dLGN terminals. The finding of Brecha et al. (1987) supporting immunocytochemical evidence that substance P may be a transmitter or neuromodulator in rabbit retinogeniculate synapses, is well correlated with the presence of substance P in retinal ganglion neurons (Brecha, 1983).

Finally, it could be assumed that classical neurotransmitters and neuropeptides coexist in presynaptic profiles of dLGN containing dense cored vesicles, as has been shown in other structures (Lundberg and Hökfelt, 1983; Jones and Hendry, 1986). It is possible that retinal terminal may contain substance P (Brecha, 1983; Brecha et al., 1987) and amino acids involved in transmitter function, because after eye removal in the rabbit (Sandberg and Corazzi, 1983) a reduction in glutamate levels in the superior colliculus occurred, and following D-($^3$H)-aspartate injection in the superior colliculus of the rabbit (Matute and Streit, 1985) an intense labelling of the optic tract is observed by autoradiography. These findings suggest a neurotransmitter role for glutamate and/or aspartate in the retinal ganglion cells of the rabbit.

*Acknowledgements.* This investigation was supported by a grant of the F.I.S.S.S (grant number 84/728). M.P. Ariño is a fellow of the F.I.S.S.S. (84/0033). We wish to thank M. Pulido, M.D., for the English translation of the manuscript.

## References

Bloom F.E. (1984). General features of chemically identified neurons. In: Handbook of Chemical Neuroanatomy, Vol. 2: Classical transmitters in the CNS, part I. Björklund A. and Hökfelt T. (eds). Elsevier. Amsterdam. pp 1-15.

Brecha N. (1983). Retinal Neurotransmitters: Histochemical and Biochemical Studies. In: Chemical Neuroanatomy. Emson, P.C. (ed). Raven Press. New York. pp 85-130.

Brecha N., Johnson D., Bolz J., Sharma S., Parnavelas J.G. and Lieberman A.R. (1987). Substance P-immunoreactive, retinal ganglion cells and their central axon terminals in the rabbit. Nature 327, 155-157.

Cuello A.C. and Kanazawa I. (1978). The distribution of substance P immunoreactive fibers in the rat central nervous system. J. Comp. Neurol. 178, 129-156.

Famiglietti E.V. and Peters A. (1972). The synaptic glomerulus and the intrinsic neuron in the dorsal lateral geniculate nucleus of the cat. J. Comp. Neurol. 144, 285-334.

Guillery R.W. (1969). The organization of synaptic interconnections in the laminae of the dorsal lateral geniculate nucleus of the cat. Z. Zellforch. 96, 1-38.

Hajdu F., Hassler R. and Somogyi G. (1982). Neuronal and synaptic organization of the lateral geniculate nucleus of the tree shrew, *Tupaia glis*. Cell Tissue Res. 224, 207-223.

Jones E.G. and Hendry H.C. (1986). Co-localization of GABA and neuropeptides in neocortical neurons. Trends NeuroSci. 9, 71-76.

Kromer L.F. and Moore R.Y. (1980). Norepinephrine innervation of the cochlear nuclei by locus coeruleus neurons in rat. Anat. Embryol. 158, 227-244.

Le Vay S. (1971). On the neurons and synapses of the lateral geniculate nucleus of the monkey and the effects of eye enucleation Z. Zellforsch. 113, 396-419.

Lieberman A.R. and Webster K.E. (1974). Aspects of the synaptic organization of intrinsic neurons in the dorsal lateral geniculate nucleus. An ultrastructural study of the normal and of the experimentally deafferented nucleus in the rat. J. Neurocytol. 3, 677-710.

Lorén I., Emson P.C., Fahrenkrug J., Björklund A., Aluments J., Håkanson R. and Sundler F. (1979). Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. Neuroscience 4, 1953-1976.

Lundberg J.M. and Hökfelt T. (1983). Coexistence of peptides and classical neurotransmitters. Trends NeuroSci. 6, 325-333.

Matute C., Martínez-Millán L., Larrosa A., Contamina P. and Doñate F. (1984). Selective retrograde labelling in some afferents to the rabbit LGN following injections of tritiated transmitter-related compounds. Trab. Inst. Cajal 75 (Supl.), 105 P.

Matute C. and Streit P. (1985). Selective retrograde labelling with D-[$^3$H]-aspartate in afferents to the mammalian superior colliculus. J. Comp. Neurol. 241, 34-49.

Montero V.M. and Scott G.L. (1981). Synaptic terminals in the lateral geniculate nucleus, from neurons of the thalamic reticular nucleus: a ligth and electron microscopic autoradiography study. Neuroscience 6, 2561-2577.

Ohara P.T., Sefton A.J. and Lieberman A.R. (1980). Mode of termination of afferents from the thalamic reticular nucleus in the dorsal lateral geniculate of the rat. Brain Res. 197, 503-506.

Pecci-Saavedra J., Vaccareza O.L. and Reader T.A. (1968). Ultrastructure of cells and synapses in the parvocellular portion of the cebus monkey lateral geniculate nucleus. Z. Zellforsch. 89, 462-477.

Peters A., Palay S.L. and Webster H. de F. (1976). The Fine Structure of the Nervous System: The neurons and supporting cells, Saunders, Philadelphia.

Pickel V.M. (1985). General morphological features of peptidergic neurons. In: Handbook of Chemical Neuroanatomy, Vol. 4: GABA and neuropeptides in the CNS, part, I. Björklund A. and Hökfelt T. (eds). Elsevier. Amsterdam. pp 72-92.

Rafols J.A. and Valverde F. (1973). The structure of the dorsal lateral geniculate nucleus in the mouse. A Golgi and electron microscopic study. J. Comp. Neurol. 150, 303-331.

Ralston H.J. and Chow K.L. (1973). Synaptic reorganization in the degenerating lateral geniculate nucleus of the rabbit. J. Comp. Neurol. 147, 321-350.

Sandberg M. and Corazzi L. (1983). Release of endogenous amino acids from superior colliculus of the rabbit: *in vitro* studies after retinal ablation. J. Neurochem 40, 917-921.

Steinbusch H.W.M. (1985). Serotonin-immunoreactive neurons and their projections in the CNS. In: Handbook of Chemical Neuroanatomy, Vol. 3: Classical transmitters and transmitter receptors in the CNS, part. II. Björklund A., Hökfelt T. and Kuhar M.J. (eds.). Elsevier Amsterdam. pp 68-118.

Szentágothai J. (1963). The structure of the synapse in the lateral geniculate body. Acta Anat. 55, 166-185.

Szentágothai J. (1973). Neuronal and synaptic architecture of the lateral geniculate nucleus. In: Handbook of sensory physiology, Vol. VII/3B. Jung R., (ed.). Springer. Berlin. pp. 141-176.

Ueda S. and Sano Y. (1986). Distributional pattern of serotonin-immunoreactive nerve fibers in the lateral geniculate nucleus of the rat, cat and monkey *Macaca fuscata*. Cell. Tissue Res. 243, 249-253.

Vanderhaeghen J.J. (1985). Neuronal Cholecystokinin. In: Handbook of Chemical Neuroanatomy, Vol. 4: GABA and Neuropeptides in the CNS, part I. Björklund A., Hökfelt T. and Kuhar M.J., (eds.). Elsevier. Amsterdam. pp. 406-432.

Wilson J.R. and Hendrickson A.E. (1981). Neuronal and synaptic structure of the dorsal lateral geniculate nucleus in normal and monocularly deprived Macaca monkeys. J. Comp. Neurol. 197, 517-539.

Wong-Riley M.T.T. (1972). Neuronal and synaptic organization of the normal dorsal lateral geniculate nucleus of the squirrel monkey *Saimiri sciureus*. J. Comp. Neurol. 144, 25-60.

Accepted April 2, 1988