Histol Histopath (1992) 7: 663-671

Histology and Histopathology

Stereological study of the synaptic profiles belonging to interneurons in the dorsal lateral geniculate nucleus of the rabbit

R. Carmona Martos, F. Abadía Molina, R. Luján Miras, R. Calvente Iglesias and F. Abadía Fenoll
Department of Cell Biology, Faculty of Sciences, University of Granada, Granada, Spain

Summary. This study is concerned with some characteristics of the interneurons belonging to the dLGN (dorsal Lateral Geniculate Nucleus) of the rabbit. The work deals with the distribution of such cells in the α_E sector of the nucleus and their F1 and F2 presynaptic contacts. The F1 and the F2 profiles are present in all three of the α_E zones studied. The F1 profiles are significantly more numerous in the upper zone (57±2 profiles per 10^4 μm^2 of section) and the middle zone (59±3 profiles per 10^4 μm^2 of section) than in the lower one (41±2 profiles per 10^4 μm^2 of section). The F2 profiles are more abundant in the α_E sector than the F1 ones are, particularly in the lower zone, where F2 profiles (104±4 profiles per 10^4 μm^2 of section) are not only significantly more numerous than F1 profiles but also more abundant than the F2 profiles in the middle zone (84±3 profiles per 10^4 μm^2 of section) and upper zone (88±2 profiles per 10^4 μm^2 of section). These results and their comments reveal diverse density of the element distribution from the dorsal to the ventral part of the α_E sector as well as the possible relationship or independence from the extranuclear afferent inputs.

Key words: Synapsis, dLGN, Stereology, Interneuron

Introduction

At present it is admitted that the dLGN of mammals is not only a simple relay station to the optic pathways but a real integration centre. The established coordination in the dLGN neurons is modulated by extraretinal influences (Szentagothai, 1963; Singer, 1977; McCormick, 1989). The fact that the synapses in dLGN -as has already been shown in the cat by Guillery (1969)- are in a great part constituted by extrarretinal terminals, evidences the role of that coordination in the working of the nucleus.

A considerable amount of the extraretinal inputs reaching the relay cells of the nucleus come from their interneurons (Montero, 1991). On the surface of the relay cells we can also find the influence from retinal impulses throughout the interneurons (Wilson et al., 1984; Montero, 1991). All these findings show the relevance of the interneurons in making the nucleus function.

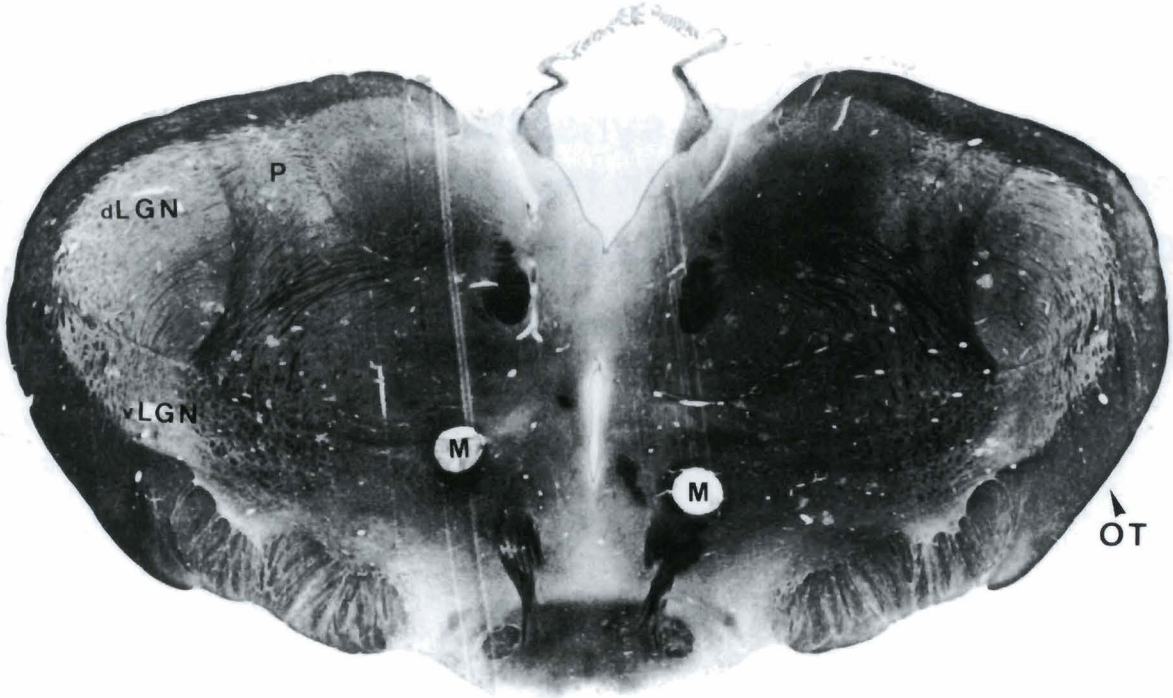
Studying the particular characteristics of the interneurons authors have reported interesting evidence about their presynaptic inputs, etc. With this aim Werner and Wilke (1985) found that in rodents 36% of the total neurons in the α sector are interneurons. Other authors, such as Lind et al. (1977), Le Vay and Ferster (1979), Geisert (1980), Fitzpatrick et al. (1982) found between 10% and 25% in the cat, and Carmona et al. (1990a) 12% in the α_E of the rabbit. In relationship with the neurotransmitter practically all the authors have found that the interneurons are Gaba-neurons (Le Vay and Ferster, 1979; Fitzpatrick et al., 1984; Montero and Zempel, 1985; Montero, 1989). On the other hand interneurons of this nucleus have the peculiar characteristic of possessing multiple sites for synaptic interactions in microcircuits involving their dendritic appendages (Hamos et al., 1985; Montero, 1986) in addition to conventional axonal outputs (Montero, 1987).

On these types of studies despite the fact of the accuracy found in some of them we have found a lack of enough stereological information about the number and distribution of the presynaptic profiles (with contacts) belonging to interneurons and the established relationship between the number of cells and their «F» terminals. For such a reason in this work we have studied the characteristics and neuron diversity in the α_E sector of the dLGN of the rabbit and we have analysed the morphometric properties and distribution of the type F profile synaptic terminals.

Materials and methods

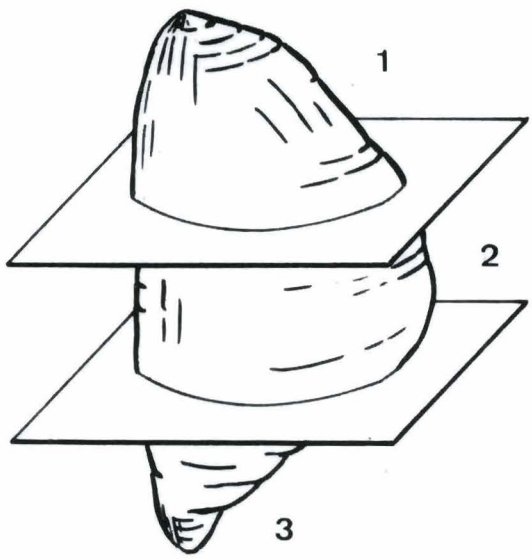
Six adult New Zealand rabbits weighing approximately 3 kg each were anaesthetized with

Offprint requests to: Dr. R. Carmona Martos, Departamento de Biología Celular, Facultad de Ciencias, Universidad de Granada, Granada, Spain



①

Fig. 1. Frontal section of the rabbit diencephalon at the level of the dLGN (Klüver-Barrera stain). dLGN=dorsal lateral geniculate nucleus; vLGN=ventral lateral geniculate nucleus; OT=optic tract; P=pulvinar nucleus; M=external markers. x 11



②

Fig. 2. Diagram showing the spatial criteria used to delimit the study zones in the α_E sector of the rabbit dLGN. 1=superior zone; 2=middle zone; 3=inferior zone.

urethane and perfused via the carotid artery with saline solution followed by a mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.2 M sodium cacodylate buffer (pH 7.4, 340 mosmol). After perfusion the brains were removed, and the diencephala were dissected and divided sagittally into two parts, which were immersed in the same fixative solution as that used in the perfusion step, at 4 °C for 2 h. The next step was to extract «cylinders» of dLGN tissue from each piece of diencephalon with a flat-tipped hypodermic needle (i.d. 0.33mm). The needle inserted into the dLGN gave us the «cylinders» in which we studied the three parts of equal length in the α_E sector. This division allowed us to analyze the contents at different levels of this sector in the nucleus. These parts were designated superior (S), middle (M) and inferior (I) (Fig. 2). In order to follow the stereological procedure we took isotropic, uniform and random (IUR) samples from each of these zones and examined the following

characteristics of the F profiles: the area of the profile; the area occupied by the synaptic vesicles and their elliptic form factor (EFF); and the area occupied by the mitochondria.

Results

For identifying our materials before analyzing the morphometric features of the F presynaptic terminals, their structural characteristics as well as those from the other terminals also appearing in the dLGN were analysed as follows. The abbreviations used in the text, introduced by Guillery (1969), were preferred because they describe the features specific to each terminal.

The cortical synaptic terminals.- (RSD = Round vesicles, Small profiles, Dark mitochondria)

The image in Fig. 3 corresponds to cortical presynaptic terminals. In our material they appeared as small profiles containing spherical and densely packed synaptic vesicles. In general, these terminals contained no mitochondria; in the few cases where they were seen, they showed an electron-dense matrix (dark mitochondria).

The retinal synaptic terminals.- (RLP = Round vesicles, Large profiles, Pale mitochondria)

The image in Fig. 4 corresponds to axon terminals which emerge from an optic nerve fibre. The RLP terminals were relatively large (2-4 μm in diameter) and contained numerous round synaptic vesicles, among which a few larger vesicles were scattered (up to 100 nm in diameter) with an electron-dense centre. Inside the RLP terminals, several mitochondria (3 to 10 per profile) of low electron density were seen.

The type «F» synaptic terminals.- (F = Flattened or pleomorphic vesicles)

The F terminals (interneuron) had profiles containing pleomorphic vesicles, whose principal neurotransmissor is GABA (according to the studies from Le Vay and Ferster, 1979; Fitzpatrick et al., 1984; Montero and Zempel, 1985; Montero, 1989). There were two distinct types of F profile, F1 and F2. The F1 profiles were axonal terminals of interneurons containing throughout most of their profile pleomorphic vesicles, predominantly elliptic in shape, which were found associated to numerous mitochondria in a relatively

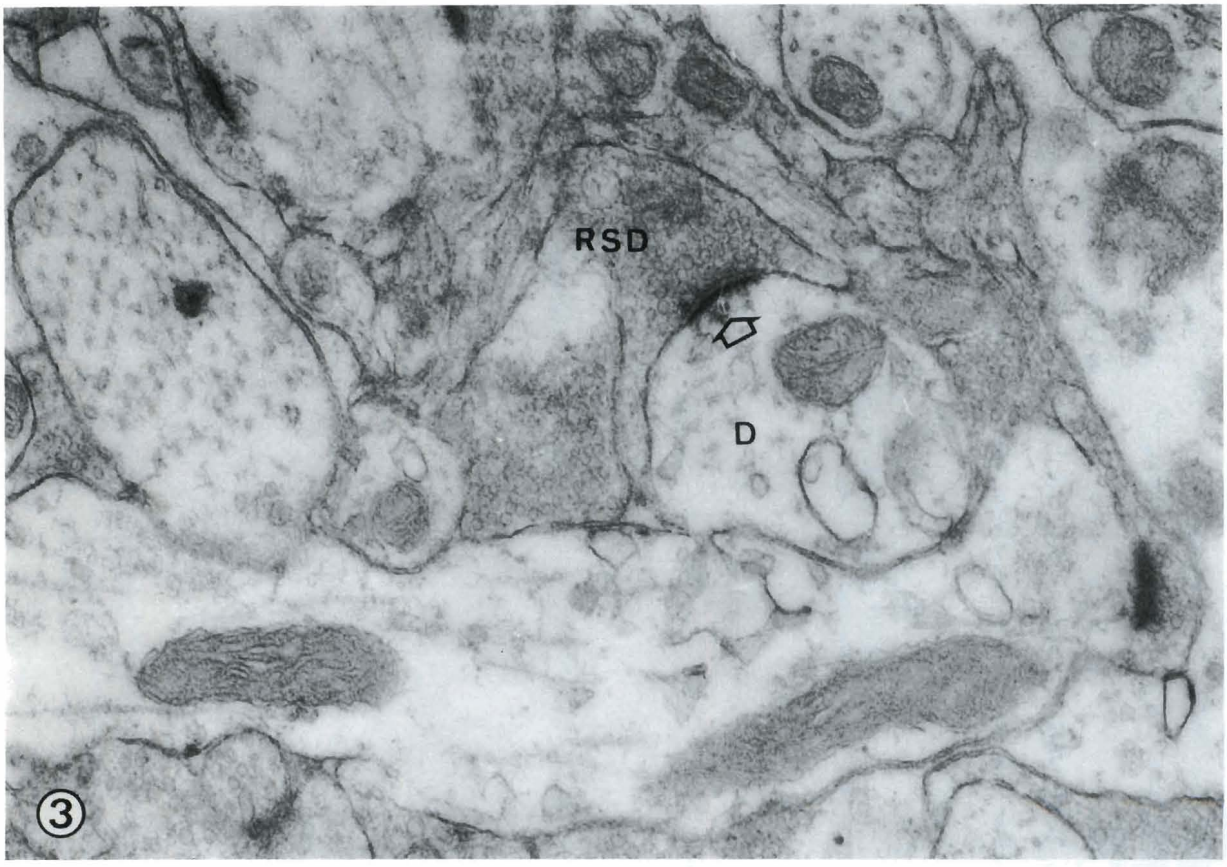


Fig. 3. Superior zone of the rabbit dLGN (α_E sector) showing a profile of corticogeniculate terminals (RSD) establishing asymmetric synaptic contact (unlabelled open arrowheads) with a dendrite (D). x 39,000

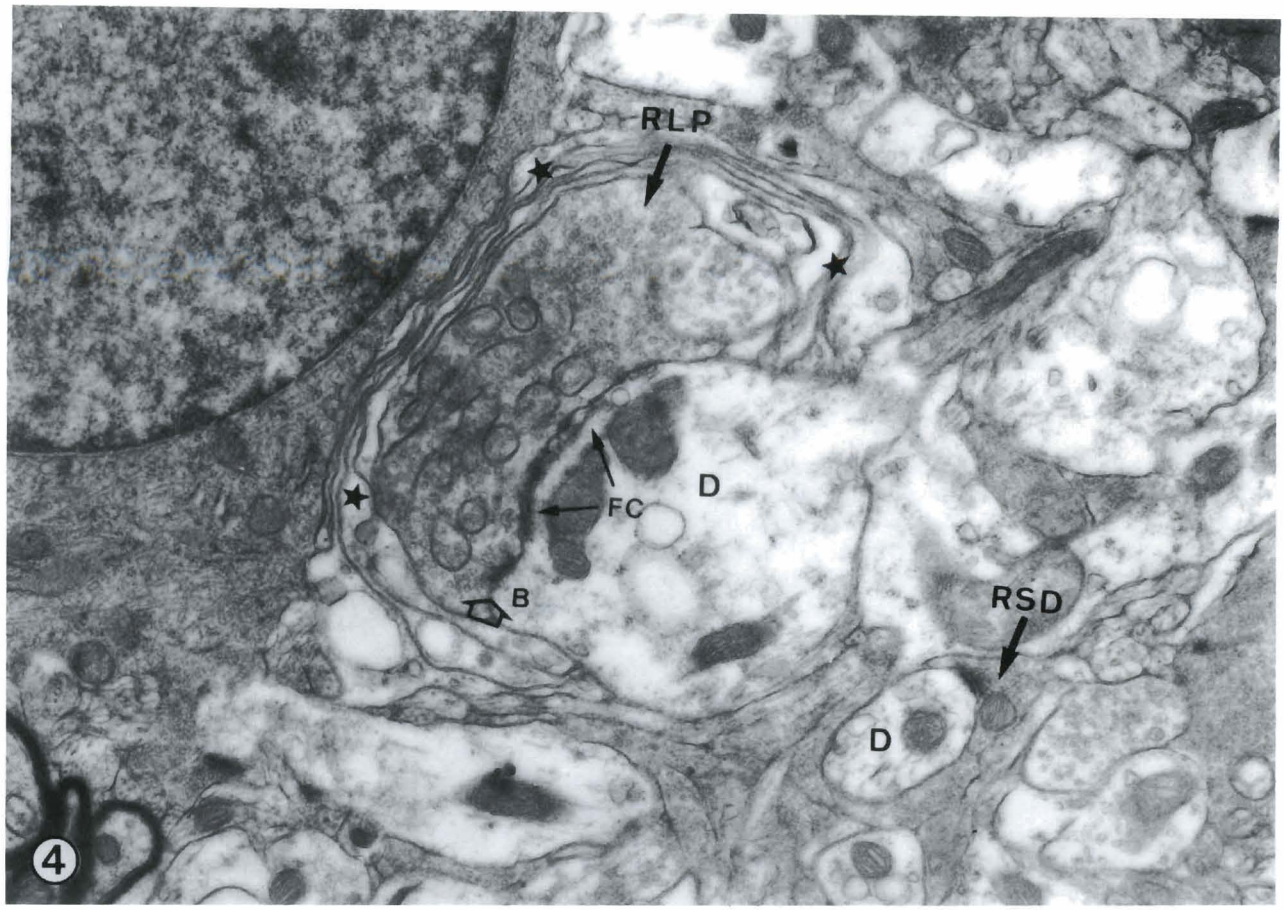


Fig. 4. Middle zone of the rabbit dLGN (α_E sector) showing a synaptic zone composed of a RLP profile and a dendritic profile (D) in close contact with glial lamellae and processes (stars). Note the presence of a dendritic bulb (B). A corticogeniculate terminals (RSD) establishes synaptic contact with a dendrite (D). The retinal terminal (RLP) makes synaptic contact with the dendrite (D) (unlabelled open arrow). Note the synaptic contact with the dendritic bulb; a filamentous contact (FC) with no apparent synaptic significance can also be seen. To the left of the RLP terminal can be seen what may be an F2-type profile, although the separating membranes are not very well defined. x 20,000

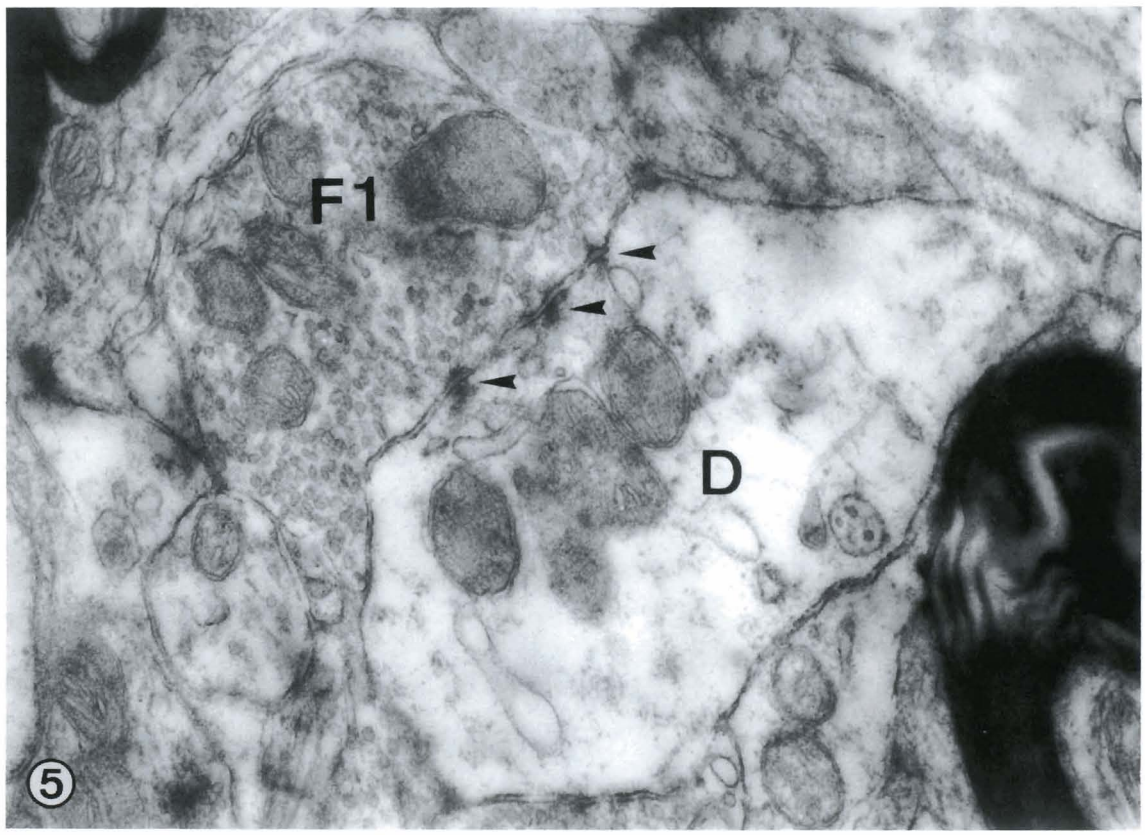


Fig. 5. Inferior zone of the rabbit dLGN (α_E sector) showing an axonal terminal of the interneuron profile (F1) establishing symmetric synaptic contact with a dendrite (D) (black arrowheads). Note the F1 terminal, characterized by dense mitochondria and pleomorphic synaptic vesicles, which are more elliptic and densely packed than in F2 profiles. x 32,500

electron-dense matrix (Fig. 5). The F2 profiles, which are considered to be interneuron presynaptic dendrites and lacking ribosomes, showed profiles containing pleomorphic synaptic vesicles, as did the F1 profiles, but in this case both the vesicles and the mitochondria occupied less space in the profile as a whole than they did in the F1 profiles (Fig. 6).

Synaptic relationship between the different types of synaptic terminals. The RSD terminals established intimate asymmetrical contacts almost exclusively with conventional dendrites (D) (Fig. 3). The RLP terminals established asymmetric synaptic contact with two types of process: (a) conventional dendrites (D), identifiable by the presence of ribosomes (Fig. 4); and (b) processes containing pleomorphic synaptic vesicles lacking ribosomes, considered presynaptic dendrites of interneurons (F2) (Fig. 7). These contacts could be single, between one RLP terminal and one conventional dendrite (Fig. 8), or with other complex elements in the glomerulae (Fig. 9).

For our study, and according to the previous descriptions, we have classified the different types of presynaptic profiles. Once a type was identified the number of profiles ($\bar{N}$) per area unit were determined on the micrographs obtained from the IUR sections. The values for F1 and F2 are contained in Table 1. The amount and mean size for the area of the profiles will be in relationship to or dependent on the volume and the spatial orientation of the particles; these could introduce a bias in the comparisons but employing IUR sections such a bias was elimitated. Moreover, if the mean size of the area for the profiles from both sets of data were

Table1. Number of F1 and F2 profiles in each of the zones studied (unit area 10^4 μm^2). (*) denotes significatively different pairwise comparisons between superior and inferior zones; (**) denotes sgnificatively different pairwise comparison between middle and inferior zones. In all cases, $p<0.05$.

	Inferior	Middle	superior
F1	40.85±2.10*,**	59.00±2.90**	56.90±1.95*
F2	103.50±3.80*,**	84.30±3.20**	88.60±2.10*

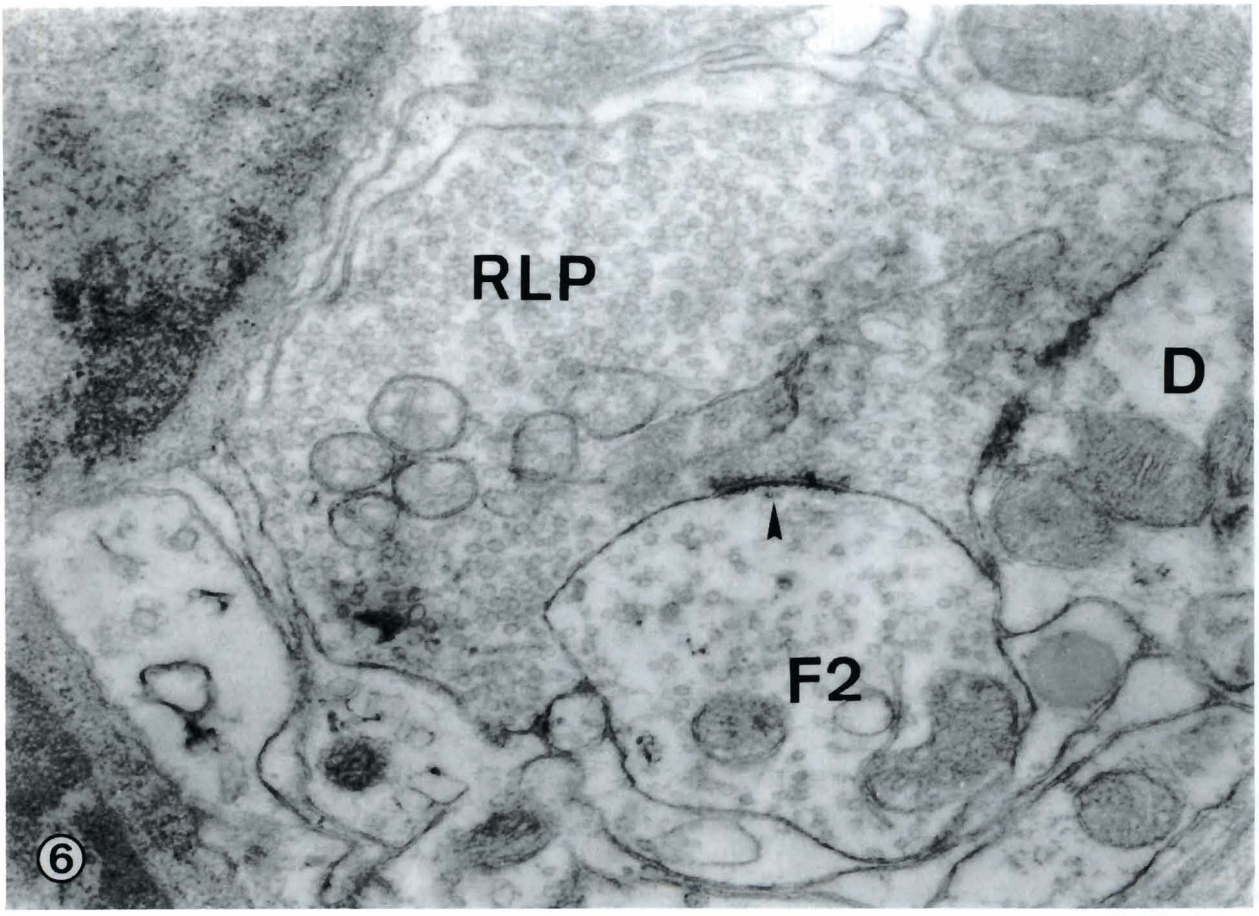


Fig. 6. Superior zone of the rabbit dLGN (α_E sector) showing an RLP terminal establishing synaptic contact with an F2 profile (black arrowhead). Note the F2 terminal, characterized by its pleomorphic synaptic vesicles, as are the F1 profiles, but in this case both the vesicles and the mitochondria occupy less space in the profile as a whole than they do in the F1 profiles. D=dendrite. x 27,500

similar enough then the differences that could be found in the number of profiles would only be on the basis of their density. Table 2 shows these values from F1 and F2 for the profile areas obtained; these values are very similar and therefore we can take such values as useful (offered in Table 1) without thinking about any bias.

In Table 3 and 4 the values for the total area occupied by vesicles ($\bar{a}v$) and mitochondria ($\bar{a}m$) in the general profiles studied appear. Here the same stereological

Table 2. Mean area of individual profiles ($\bar{a}$) -F1 and F2- in each zone studied (values in μm^2)

α_E ZONE	F1 ($\bar{a}$±SE)	F2 ($\bar{a}$±SE)
Superior	1.05±0.06	1.10±0.08
Middle	1.01±0.08	0.92±0.07
Inferior	0.96±0.06	1.07±0.12

Table 3. Mean area occupied by the synaptic vesicles ($\bar{a}v$) in the F1 and F2 profiles in each zone studied (values in μm^2). The figure in parentheses are the percentage values occupied by the synaptic vesicles with respect to the area of the terminals.

α_E ZONE	F1 ($\bar{a}v$±SE)	F2 ($\bar{a}v$±SE)
Superior	0.39±o.o2 (31%)	0.2o±0.01 (22%)
Middle	0.35±0.02 (35%)	0.20±0.01 (18%)
Inferior	0.33±0.03 (32%)	0.23±0.02 (25%)

Table 4. Mean area occupied by the mitochondria ($\bar{a}m$) in the F1 and F2 profiles in each zone studied (values in μm^2). The figures parentheses are the percentage values occupied by the mitochondria with respect to the area of the terminals.

α_E ZONE	F1 ($\bar{a}m$±SE)	F2 ($\bar{a}m$±SE)
Superior	0.30±0.02 (32%)	0.08±0.01 (9%)
Middle	0.30±0.02 (30%)	0.08±0.02 (7%)
Inferior	0.27±0.02 (26%)	0.08±0.02 (9%)

Table 5. Mean elliptic form factor (eff) of the synaptic vesicles of the F1 and F2 profiles in each zone.

α_E ZONE	F1 (eff±SE)	F2 (eff±SE)
Superior	0.56±0.01	0.71±0.01
Middle	0.58±0.01	0.70±0.01
Inferior	0.55±0.01	0.72±0.01

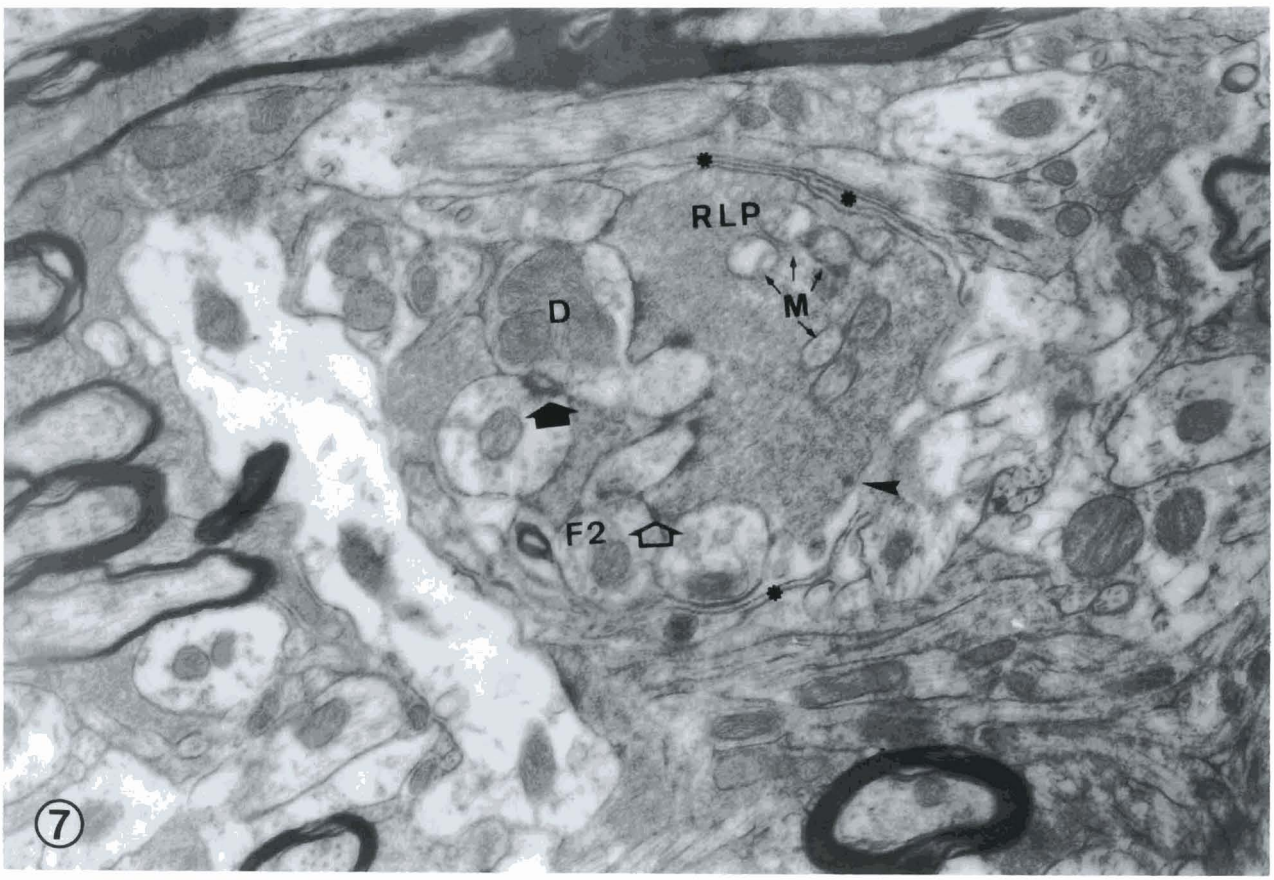


Fig. 7. Inferior zone of the rabbit dLGN (α_E sector). Retinal terminal (RLP) with abundant rounded synaptic vesicles, including a dense-cored vesicle (black arrowhead). Several mitochondria of low electron density (M) in the RLP terminal are clearly distinguishable from the dark mitochondria of the adjacent processes. The RLP terminal establishes synaptic contact with an F2 profile (unlabelled open arrowhead) and a dendrite (D). Note another F2 profile also establishes synaptic contact with this dendrite (labelled black arrowhead). Glial lamellae can be seen in contact with the entire complex (asterisks). x 25,000

considerations can be made that we have previously related.

The F1 and the F2 profiles were present in all three of the α_E zones studied. As far as the F1 (Fig. 5) profiles are concerned, they were significantly more numerous in the upper zone (57±2 profiles per 10^4 μm^2 of section) and the middle zone (59±3 profiles per 10^4 μm^2 of section) than in the lower one (41±2 profiles per 10^4 μm^2 of section) (Table 1). The F2 (Figs. 6, 7) profiles were more abundant in the α_E sector than the F1 ones were particularly in the lower zone, where F2 profiles (104±4 profiles per 10^4 μm^2 of section) were not only significantly more numerous than F1 profiles but also more abundant than the F2 profiles in the middle zone (84±3 profiles per 10^4 μm^2 of section) and upper zone (88±2 profiles per 10^4 μm^2 of section) (Table 1).

Table 2 contains mean values of the areas ($\bar{a}$) of F1 and F2 profiles in each zone. The values found in the three zones were very similar for both types of terminal, hence the differences between them were not significant ($p<0.05$).

With respect to the other parameters: area occupied by the synaptic vesicles (Table 3); elliptic form factor (EFF) of the synaptic vesicles (Table 4); and the area occupied by the mitochondria (Table 5), statistically significant differences were found, in all the cases, between the F1 terminals and the F2 terminals.

Finally, Table 6 shows symbolically the relative abundance of each one of the four types of synaptic terminals present in the α_E of the dLGN of the rabbit. Here the values for RLP and RSD were taken from our previous study (Carmona et al., 1990b).

Discussion

Our results show that it is possible to morphometrically distinguish between F1 and F2 type according to the elliptic form factor of their synaptic vesicles, which is from 0.55 to 0.58 in the F1 profiles,

Table 6. This table show symbolically the relative abundance of each one of the four types of synaptic terminal present in the α_E sector of the dLGN of the rabbit (values x10^2).

α_E SECTOR	TYPE OF THE SYNAPTIC TERMINAL			
	RLP	RSD	F1	F2
Superior Zone	6.6	3.6	0.6	0.8
Middle zone	3.6	3.2	0.6	0.8
Inferior zone	3.8	3	0.4	1

RLP= retinal synaptic terminals; RSD= cortical synaptic terminals; F1=axonal terminal of interneurons; F2= interneuron presynaptic dendrites.

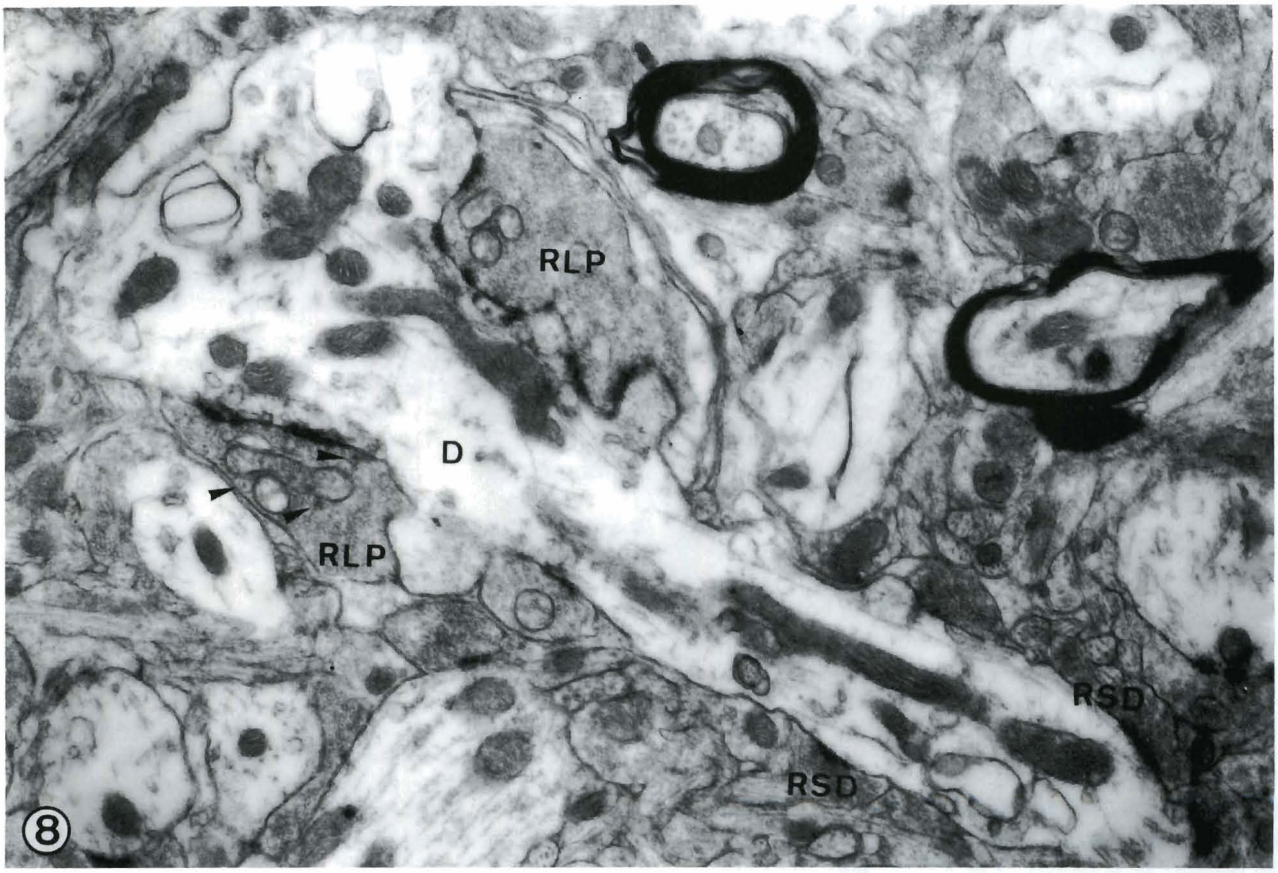


Fig. 8. Middle zone of the rabbit dLGN (α_E sector) showing two RLP terminals establishing synaptic contact with one single conventional dendrite (D). The arrows indicate dense-cored vesicles. x 22,000

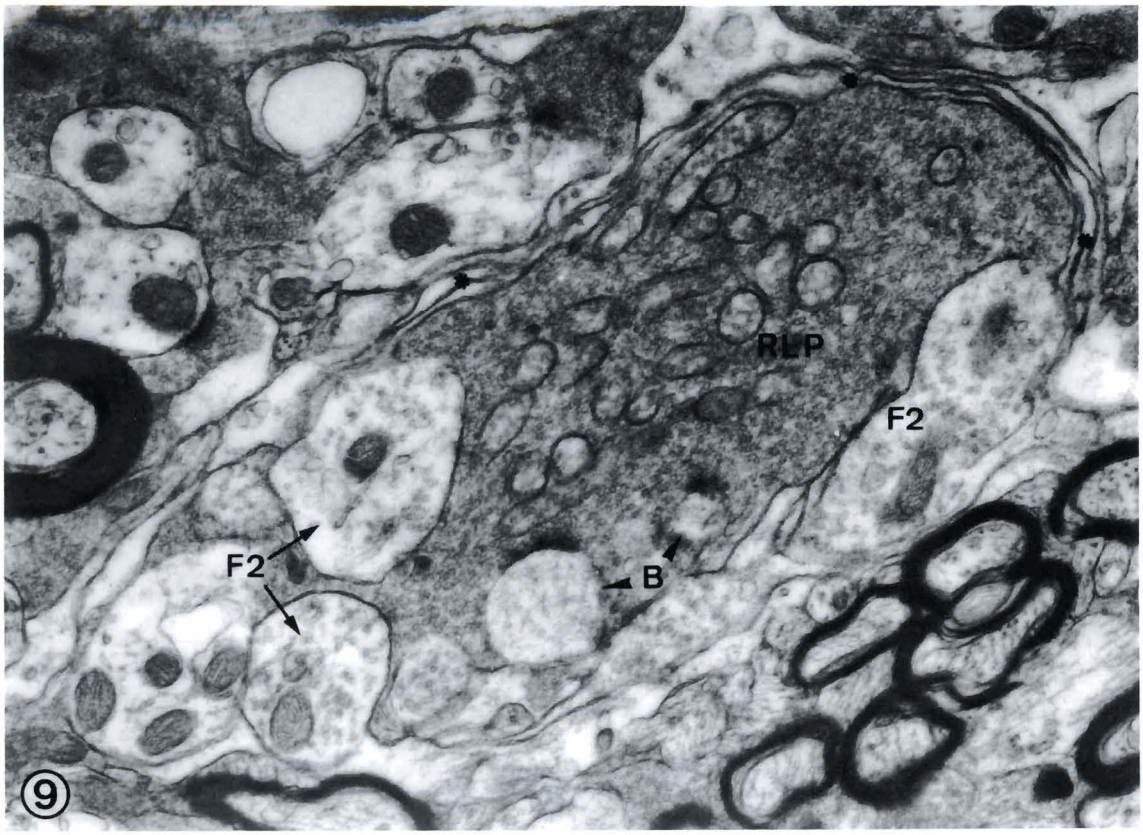


Fig. 9. Middle zone of the rabbit dLGN (α_E sector) showing a complex synaptic zone with a large retinal (RLP) and other smaller terminals, together with several profiles of dendritic bulbs (B) and F2 terminals. Some thin glial lamellae (asterisks) can be seen. x 22,000

and also by the area occupied by both the vesicles and the mitochondria, which is always significantly greater in the F1 profiles.

We know that retinogeniculate and corticogeniculate axon terminals are more numerous than the F1 and F2 terminals, especially in the upper zone of the α_E of the dLGN. On the other hand interneurons are more numerous in the lower zone, where we have also found the largest number of F2 type terminals and thus an increase in the dendritic tree in general. This situation seems to point to the fact that a higher amount of differentiation of interneurons has occurred in the lower zone of the nucleus and also that it is independent of any induction on the part of the retinogeniculate and corticogeniculate axon terminals since, as is pointed out in Table 6, RLP and RSD terminals are more numerous in the upper regions.

Actually studying the number of interneurons in the α_E sector (see Carmona et al., 1990a) we have found 23.0% in the lower zones against 9.1% in the upper one, but the F2 contacts which belong to interneuron dendrites are also more numerous in those inferior zones indicating at least a more widespread dendritic activity in these regions or a preferred dendritic relay trees here expanding. Such a distribution shows that the final result in the output from the nucleus by the relay neurons are under two different regulating influences: those referred to as the F1 ascending axonal fibres; and the F2 intermediate actions upon their dendritic expansions.

Acknowledgements. This study has been carried out with the aid of a grant from the DGICYT (Spain), PB87-0970.

References

Carmona R., Abadía-Molina, F., Calvente R. and Abadía-Fenoll F. (1990a). Cytoarchitecture of the dorsal lateral geniculate nucleus in the rabbit. Histol. Histopath. 5, 7-15.

Carmona R., Calvente R., Abadía-Molina F. and Abadía-Fenoll F. (1990b). Morphometry and frequency of afferent synaptic terminals in the rabbit dorsal lateral geniculate nucleus. Anat. Rec. 228, 327-338.

Fitzpatric K.D., Peny G.R., Schmechel D. and Diamond I.T. (1982). GAD immunoreactive neurons in the lateral geniculate nucleus of the cat and galago. Neuroscience (Abstr.) 8, 261.

Fitzpatric K.D., Peny G.R. and Schmechel D. (1984). Glutamic acid decarboxylase-immunoreactive neurons and terminals in the lateral geniculate nucleus of the cat. J. Neurosci. 4, 1809-1829.

Geisert E.E. (1980). Cortical projections if the lateral geniculate nucleus

in the cat. J. Comp. Neurol. 190, 793-812.

Guillery R.W. (1969). A quantitative study of synaptic interconnections in the dorsal lateral geniculate nucleus of the cat. Z. Zellforsch. 96, 39-48.

Hamos J.E., Van Horn S.C., Raczkowski D., Uhlrech D.J. and Sherman S.M. (1985). Synaptic connectivity of a local circuit neurone in lateral geniculate nucleus of the cat. Nature 317, 618-621.

Le Vay S. and Ferster D. (1979). Proportion of interneurons in the cat's lateral geniculate nucleus. Brain Res. 164, 304-308.

Lind C.S., Kratz K.E. and Sherman S.M. (1977). Percentage to relay cells in the cat's lateral geniculate nucleus. Brain Res. 131, 167-173.

McCormick D.A. (1989). Cholinergic and noradrenergic modulation of thalamocortical processing. Trends Neurosci. 12, 215-221.

Montero V.M. (1986). Localization of gamma-aminobutyric acid (GABA) in type 3 cells and demonstration of their source to F2 terminals in the cat lateral geniculate nucleus: a Golgi-electron-microscopic GABA-immunocytochemical study. J. Comp. Neurol. 254, 228-245.

Montero V.M. (1987). Ultrastructural identification of synaptic terminals from the axon of type 3 interneurons in the cat lateral geniculate nucleus. J. Comp. Neurol. 264, 268-283.

Montero V.M. (1989). The GABA-immunoreactive neurons in the interlaminar regions of the cat lateral geniculate nucleus: light and electron microscopic observations. Exp. Brain Res. 75, 497-512.

Montero V.M. (1991). A quantitative study of synaptic contacts of interneurons and relay cells of the cat lateral geniculate nucleus. Exp. Brain Res. 86, 257-270.

Montero V.M. and Zempel J. (1985). Evidence for two types of GABA-containing interneurons in the A-laminae of the cat lateral geniculate nucleus: a double-label HRP and GABA-immunocytochemical study. Exp. Brain Res. 60, 603-609.

Singer W. (1977). Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. Physiol. Rev. 57, 386-420.

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

Werner L. and Wilke A. (1985). Zur neuronalen organisation des CGld von *Cavia porcellus*. Eine morphometrische untersuchung an Nissl-präpareten unter berücksichtigung identifizierter neuronentypen. J. Hirnforsch. 26, 1-16.

Wilson J.R., Friedlander M.J. and Sherman S.M. (1984). Fine structural morphology of identified X- and Y-cells in the cat's lateral geniculate nucleus. Proc. R. Soc. Lond. 221, 411-436.

Accepted June 15, 199