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GABAergic Neurons in Mammalian Thalamus: A Marker of Thalamic Complexity?

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ABSTRACT: The present study evaluated the occurrence, distribution, and number of GABAergic neurons in the thalamus of different mammalian species (bat, mouse, rat, guinea pig, rabbit, cat, monkey, humans), by means of light microscopical immunoenzymatic localization of GABA or of its biosynthetic enzyme glutamic acid decarboxylase and by ultrastructural immunogold detection of GABA. Our data demonstrated that: 1) GABAergic local circuit neurons were detected in the thalamic visual domain in all the species analyzed, whereas in other thalamic nuclei their presence and number varied among species; 2) the number of GABAergic local circuit neurons progressively increased in the dorsal thalamus of species with more complex behavior; 3) the presence of local circuit neurons conferred a similar intrinsic organization to the dorsal thalamic nuclei, characterized by complex synaptic arrangements; 4) in the reticular thalamic nucleus, whose neurons were GABA-immunoreactive in all the examined species, the cellular density decreased from the bat to humans. These findings strongly suggest that thalamic GABAergic local circuit neurons are not directly related to the ability to perform specific sensorimotor tasks, but they are likely to reflect an increasing complexity of the local information processing that occurs at thalamic level. Copyright © 1997 Elsevier Science Inc.

KEY WORDS: Immunocytochemistry, Electron microscopy, Interneurons, Reticular thalamic nucleus, Ventrobasal nucleus, Inhibitory circuits.

INTRODUCTION

The dorsal thalamus contains two morphologically and functionally distinct classes of neurons: the relay neurons, which project outside the thalamus, and the local circuit neurons, whose axons do not leave the thalamic nucleus in which they reside. The distinction between the two types of thalamic neurons was introduced by Marchi in 1887 [26], who described for the first time short-axoned neurons, named Golgi type II cells, and long-axoned neurons, named Golgi type I cells, and was then established by Cajal [4,5]. Golgi type I or projection neurons are characterized by a large- or medium-sized perikaryon and an axon that does not give out collateral branches within the thalamus, whereas Golgi type II or local circuit neurons have a small perikaryon with an axon largely ramifying at a relatively short distance from the parent cell body within the boundaries of the thalamic nucleus of origin, and dendritic shafts, and appendages that contain synaptic vesicles. The morphological distinction between these two cell types has been confirmed in the thalamus by combined electrophysiological and morphological studies using intracellular injections of dyes [1,12,35,37,52,62,63]. These studies confirmed the hypothesis that the two types of thalamic neurons have different and selective electrophysiological properties [47,59]. Further studies based on immunocytochemical methods demonstrated that projection neurons in the dorsal thalamus use an excitatory amino acid as a neurotransmitter [47,48], while local circuit neurons use the inhibitory neurotransmitter gamma-aminobutyrric acid (GABA) [3,10,18,39,46,51,55]. All the electrophysiological and neuroanatomical data are, therefore, in accordance in demonstrating that the projection neurons provide an excitatory output and the local circuit neurons are the main source of intrathalamic inhibition. Ultrastructural studies provided evidence that the presence of GABAergic local circuit neurons adds complexity to the synaptology and circuitry of the thalamus and, therefore, to its functional properties [19,44,45,55].

Local circuit neurons are not the only elements devoted to inhibition in the dorsal thalamus: the GABAergic neurons of the reticular thalamic nucleus (Rt), an embryological derivative of the ventral thalamus, also project to the dorsal thalamic nuclei in all mammalian species [13,14,18,23,27,54].

GABAergic local circuit neurons, however, are not present in all the thalamic nuclei of different mammalian species: they are very numerous in almost all the dorsal thalamic nuclei of primates and carnivores, but are virtually absent in rodents, with the exception of the lateral geniculate nucleus (LG) [2,3,14,15,50].

The discrepancy among different species regarding the occurrence, distribution, and number of GABAergic thalamic local circuit neurons raises the question as to whether they could be considered a marker of thalamic complexity. The hypothesis that local circuit neurons increase in number in parallel with the complexity of the mammalian brain was originally formulated by Cajal [4,5] and followed by other authors [16,42]. More recently, Penny et al. [39,40] studied the distribution and quantity of GABAergic local circuit neurons in the thalamus of four spe-

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cies of mammals: opossum (a marsupial), rabbit (a lagomorph), cat (a carnivore), and galago (a prosimian), and readapted the hypothesis originally formulated by Cajal.

The aim of this work was to reevaluate the occurrence and distribution of GABAergic neurons in the dorsal thalamus and Rt in selected species representative of the mammalian phylogenetic tree. Data obtained by immunocytochemical experiments using an antiserum against GABA or against its biosynthetic enzyme glutamic acid decarboxylase (GAD) were complemented with similar data reported in the literature regarding other species representative of different phyla. In addition, we evaluated the relationship between the cell density in Rt and the occurrence of local circuit neurons in the dorsal thalamic nuclei. The immunocytochemical study was complemented by ultrastructural data.

Although a reevaluation of mammalian taxonomy is still in progress, the phylogenetic tree reported in Fig. 1 reflects the current opinion on this issue [11,33]. In this regard it is worth mentioning that a report based on molecular biology data recently questioned the classification of the guinea pig as a rodent and suggested that it represents a separate evolutionary lineage from myomorph rodents, such as the rat and mouse [11]. The occurrence of GABAergic local circuit neurons in the ventrobasal complex (VB) of guinea pig, and the consequent complex synaptic organization of this nucleus [55] support this hypothesis.

EXPERIMENTAL PROCEDURES

Different mammals were used for this study: bat ($n = 2$), mouse ($n = 2$), rat (Wistar, $n = 4$), guinea pig ($n = 3$), and rabbit ($n = 1$). Under deep anesthesia (chloral hydrate 4%; 1 ml/100 g body weight) all animals were intracardially perfused with a solution of saline followed by either a mixed solution of 2.5% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 (PB) or with a solution of 4% paraformaldehyde in PB for GABA or GAD immunolocalization, respectively. The brains were cut, using an Oxford Vibratome, in 50 μm-thick coronal or horizontal sections. Data from the cat and cynomolgous monkey were obtained from material prepared for previous studies [3,51]. For GABA immunocytochemistry (ICC), serial alternate sections through the rostrocaudal extent of the thalamus were preincubated for 1 h in phosphate-buffered saline pH 7.6 (PBS), containing 10% normal goat serum (NGS) and 0.2% Triton X-100, incubated overnight in a rabbit polyclonal anti-GABA serum (Sigma) diluted 1:10,000 in PBS containing 1% NGS and 0.2% Triton X-100. After repeatedly rinsing in PBS, the sections were incubated for 1 h in biotinylated goat antirabbit IgGs (Vector) diluted 1:200 in PBS with 1% NGS, rinsed in PBS, and incubated for 1 h in avidin–peroxidase complex (ABC kit, Vector) diluted 1:100 in PBS. After rinsing in PBS and in 0.05 M Tris-HCl buffer (pH 7.6), sections were reacted in a fresh solution of 0.05% diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.002% H_2O_2 in Tris-HCl for 5–10 min, washed in Tris-HCl buffer, and then mounted on gelatin-coated slides, dehydrated, and coverslipped.

For GAD ICC, sections were processed as described above, using a preincubation in 10% normal rabbit serum (NRS) with 0.2% Triton X-100 and an overnight incubation with a sheep polyclonal anti-GAD serum (kindly provided by Dr. D. Schmechel, Duke University, Durham, NC) diluted 1:1000 in PBS with 1% NRS and 0.2% Triton X-100. After rinses in PBS and 1 h incubation in biotinylated rabbit antisheep IgGs, the reaction was detected using the avidin–biotin–peroxidase protocol and DAB as chromogen.

Two human brains, obtained from patients, who died at the Istituto Nazionale Neurologico "C. Besta" for diseases, not involving the central nervous system, were also analyzed. Brains were fixed by immersion in a 11% formalin solution after autopsies performed 24–36 h postmortem. Coronal sections of the human thalami were then processed for the immunolocalization of GAD following the previously described protocol.

Controls for the specificity of the immunolabeling were carried out on sections incubated with rabbit nonimmune serum for GABA ICC and with sheep nonimmune serum for GAD ICC. The substitution of primary antiserum with preimmune serum abolished the immunoreactivity. Sections adjacent to those processed for ICC were counterstained with 1% thionin and used for the cytoarchitectonic study.

Neurons positive for GAD or GABA immunostaining will be referred to as GABAergic cells [53]. The distribution of GABA- or GAD-immunoreactive (ir) neurons in the thalamus of different animals was charted using an X-Y plotting computerized system connected to the microscope stage by means of transducers. The cytoarchitectonic boundaries of the thalamic nuclei were identified on the adjacent thionin-stained sections and drawn on the charts by means of a projection microscope.

After charting, the immunoreacted sections were lightly counterstained with thionin to enable recognition and measuring of labeled and unlabeled cells.

A quantitative evaluation of the number of GABA-ir or GAD-ir neurons was performed on sample sections of different animals, by counting with a 40× objective only labeled and unlabeled neurons with the nucleolus in the plane of the section. The areas of both GABAergic and unlabeled neurons showing the nucleolus in the plane of sections were calculated by means of an appropriate imaging computer program (Immagini e Computer, Milan, Italy).

In each animal species four parameters were considered: 1) percentage of GABAergic neurons in different thalamic nuclei; 2) GABAergic/non GABAergic neurons size ratio: this parameter was obtained by dividing the mean area of immunostained cells by the mean area of unlabeled neurons. Only the ratio between the area of labeled and unlabeled cells was reported to minimize the error due to the different procedures. Measurements were made according to the procedure followed by Penny et al. [40] to compare our experiments with those reported in this previous study. Data concerning this parameter in the human thalamus are not reported, because it appeared that the shrinkage of thalamic projection neurons was more pronounced than that of GAD-ir cells, presumably due to the fixation by immersion. 3) Rt/thalamus ratio: this parameter was obtained by dividing the area of the Rt by the area of the dorsal thalamus at different rostrocaudal levels, and expressed as a percentage. The measurements of the dorsal thalamic and Rt areas were made on projection drawings of unreacted thionin-stained sections, sampled at different levels of the anteroposterior extent of the thalamus. The sections were selected so that a similar level, based on cytoarchitectonic recognition of the thalamic nuclei, was analyzed in all the animals. 4) Neuronal density within the reticular thalamic nucleus: this parameter was evaluated by plotting and counting cells within the boundaries of the anteroposterior extent of Rt, in immunoreacted and thionin-stained sections, sampled at three levels (rostral, intermediate, and caudal pole) through the nucleus. The neuronal density (Nd) was evaluated as a ratio between the mean area of Rt (mARt) and the mean number of counted cells in the nucleus (mNc), and expressed as Nd = mARt/mNc.

Electron Microscopy

Some vibratome sections from rat, guinea pig, and cat brains were osmicated, dehydrated in ethanols, and flat embedded in

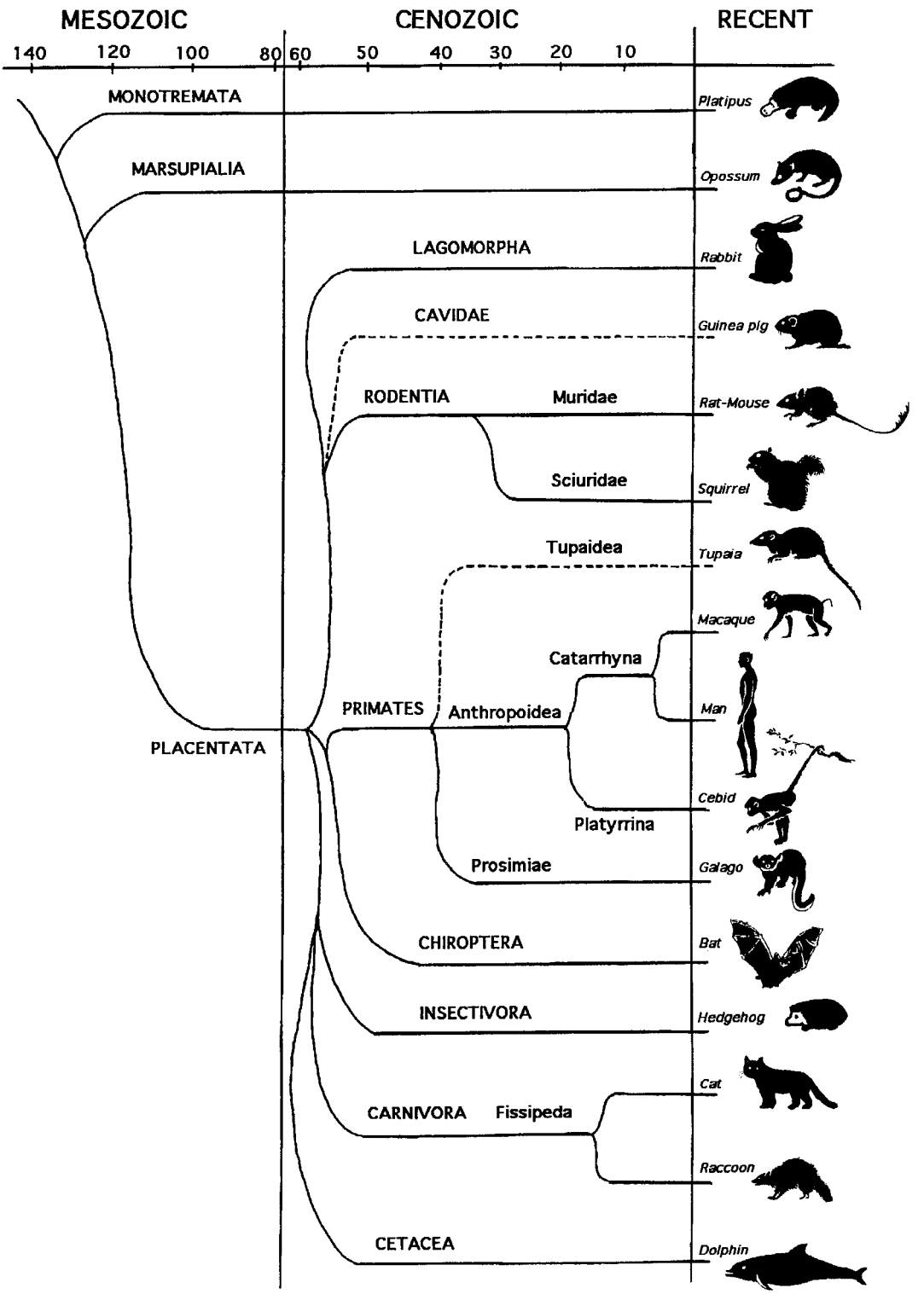


FIG. 1. Schematic view of the mammalian phylogenetic tree.

Epon-Spurr resin. Blocks of tissue containing VB and Rt were trimmed out and glued to epoxy blanks. Semithin (0.5–1 μm-thick) sections were collected on gelatinized slides and counterstained with toluidine blue (0.1%) for orientation. Some of the semithin sections were also processed with a standard postembedding immunoenzymatic procedure to detect GABA [7,55]. For the ultrastructural investigation ultrathin sections from VB and Rt were collected on nickel grids and either directly coun-

terstained with uranyl acetate and lead citrate or processed for GABA postembedding immunogold staining, following a previously reported procedure [6,7,55]. All the thin sections were then observed and photographed with a Jeol T8 or Zeiss 902 electron microscope. Controls for postembedding immunolabeling were performed as previously described [55]. The background labeling was very low, so that no statistical analysis of the distribution of gold particles for the identification of labeled profiles had to be performed.

RESULTS

In the following description of the distribution of GABAergic neurons in the dorsal thalamus, we used the term medial geniculate mucleus (MG) to indicate both the magnocellular and parvocellular parts, lateral geniculate nucleus (LG) to indicate both the ventral and dorsal parts, and ventrobasal complex (VB) to indicate both the ventroposterolateral and ventroposteromedial nuclei.

Bat

Virtually all the Rt neurons were labeled with the anti-GAD serum (Fig. 2A). In the dorsal thalamus, including the MG, only few scattered GABAergic neurons (less than 1%) were present, but in the LG, 20% of the neurons were immunopositive for GAD (Figs. 3A and 4).

Immunostained puncta, interpreted as axon terminals, were observed throughout the dorsal thalamus. The morphology and size of GAD-ir and of unlabeled cells were similar, as shown by the finding that the ratio between the areas of GABAergic neurons and the areas of projection neurons (GABA/nonGABA ratio) was 0.85 (Fig. 5).

The Rt/Thalamus ratio was 10.37% and the cellular density in Rt was 1157 neurons/mm^2 (Figs. 6 and 7A).

Rat and Mouse

All the Rt neurons were stained with the GABA or GAD antisera in the rat and mouse (Figs. 2B and 8A). The distribution of GABAergic neurons in the dorsal thalamus was similar in both animal species and the highest proportion of GABAergic neurons outside the Rt was observed in the LG, where GABA-positive neurons accounted for 15–20% of the total neuronal population (Figs. 3B and 4). GABAergic neurons were less than 1% in the other thalamic relays and in the intralaminar nuclei (Fig. 8B). Stained puncta were present throughout the dorsal thalamus with regional and internuclear differences similar to those previously described [2,3,38].

The GABA/non-GABA ratio was close to 0.8 for both species (Fig. 5); the Rt/thalamus ratio was 10.12% in the rat and 10.31% in the mouse. The cellular density in Rt was 700 neurons/mm^2 in the rat and 1087 neurons/mm^2 in the mouse, closer to that of the bat than to that of the rat (Figs. 6 and 7B).

Guinea Pig

The main concentration of GABA-ir cells was found within Rt, where all the neurons of the nucleus were labeled (Fig. 8C). In the dorsal thalamus the highest proportion of GABA-ir neurons was found in the LG, where they represented 20% of the total neuronal population (Figs. 3C and 4). In VB, GABA-ir

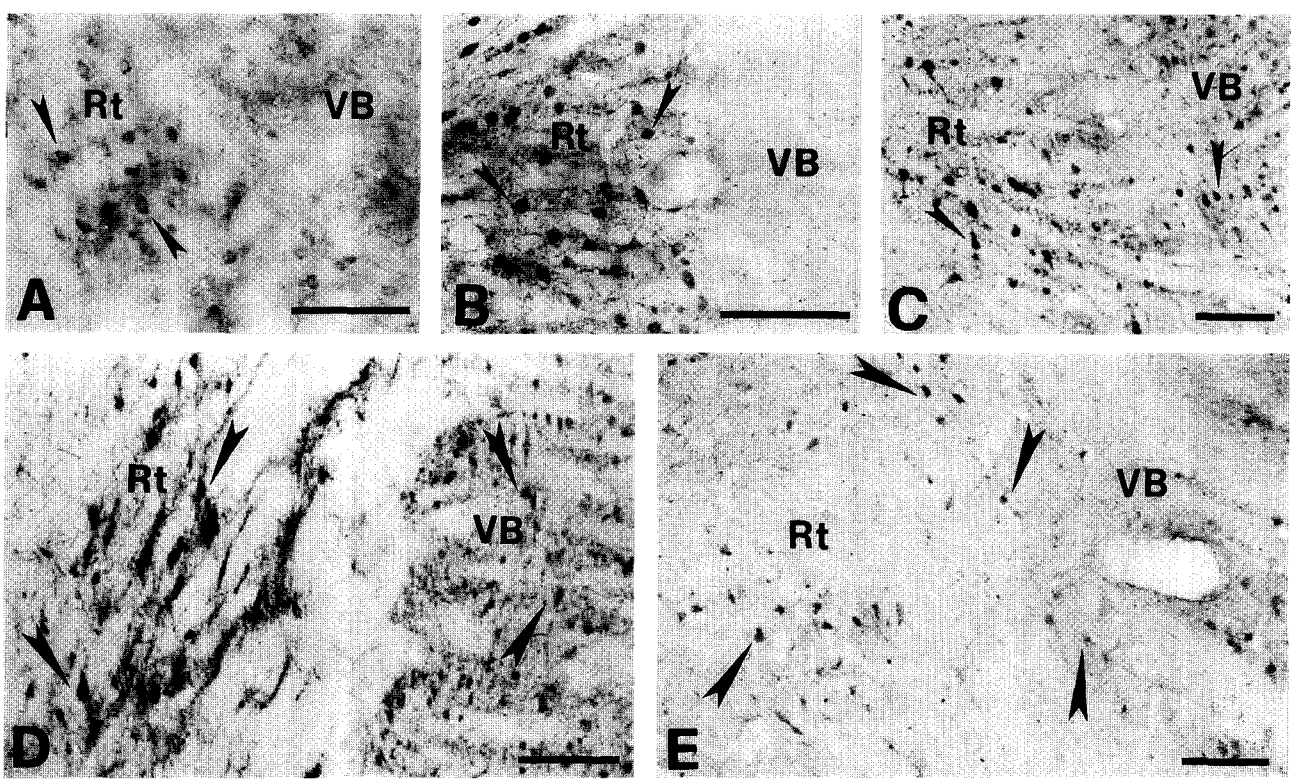


FIG. 2. Photomicrographs showing the presence of GABAergic interneurons (arrowheads) in the reticular thalamic nucleus (Rt) and in the ventrobasal complex (VB) of different animals species. Note the progressive reduction of neuronal density in Rt from bat to man. (A) bat; (B) mouse; (C) cat; (D) monkey; (E) man. Scale bars: A and B = 100 μm; C, D, and E = 180 μm.

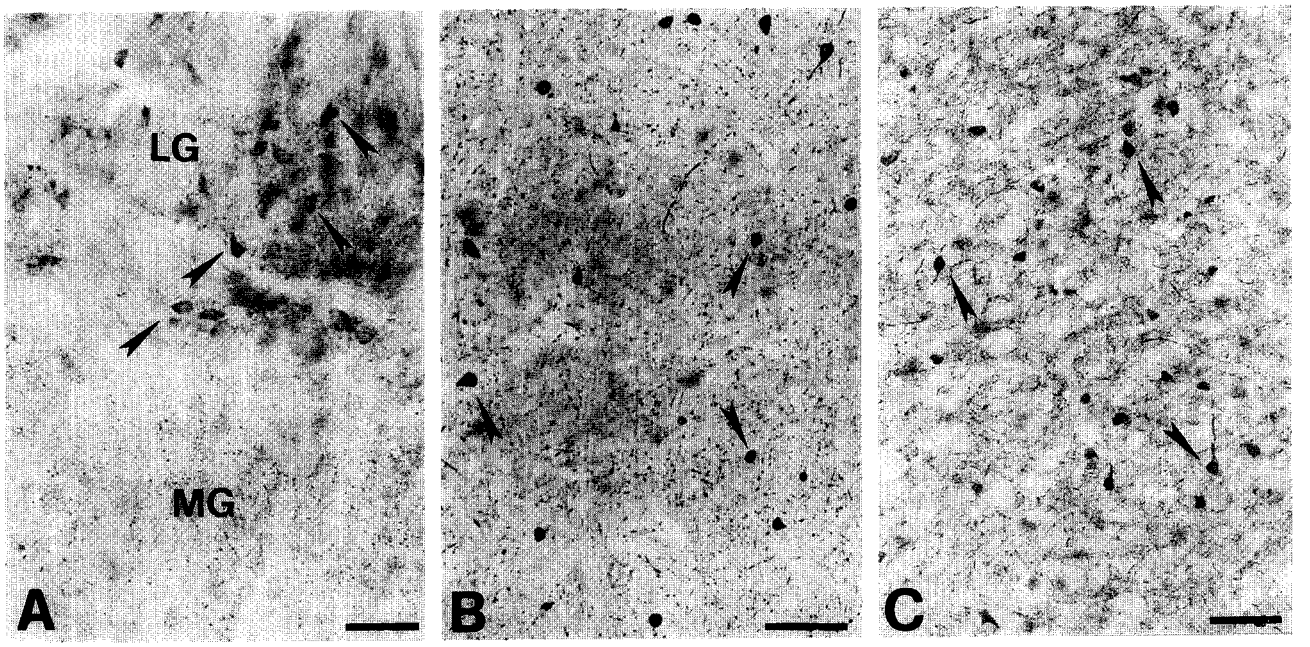


FIG. 3. GABAergic local circuit neurons (arrowheads) in the lateral geniculate nucleus (LG) of three rapresentative mammalian species. Note the absence of local circuit neurons in the medial geniculate nucleus (MG) of bat. (A) bat; (B) mouse; (C) guinea pig. Scale bars: 50 μm.

neurons were also present, and these cells accounted for 14% of the total neuronal population (Figs. 4 and 8D). Only few scattered GABA-ir neurons (approximately 1%) were present in the remaining dorsal thalamic nuclei. The neuropil of these nuclei was different from that observed in the VB and LG: large and small GABA-ir puncta were found in the VB and LG, while the other thalamic nuclei contained only small ir puncta [55].

The size of GABAergic cells was smaller than that of non GABA-ir cells and the GABA/non-GABA ratio was 0.53, lower than that observed in the bat, mouse, and rat (Fig. 5). The Rt/thalamus ratio was 11.89%, with a cellular density in Rt of 435.11 cells/mm^2 (Fig. 6).

Rabbit

All the neurons of the Rt were GAD-ir. In the dorsal thalamus, the main concentration of GAD-ir neurons was found in the LG, where they represented 25% of the total neuronal population, whereas only 18% of VB neurons were GAD-ir (Fig. 4). In all the other dorsal thalamic nuclei GABAergic neurons accounted for less than 1% of the neurons.

The GABAergic neurons represented a distinct class of small cells, in agreement with the finding that the GABA/non-GABA ratio was 0.6 [40] (Fig. 5). The Rt/thalamus ratio was 11.98%, with a cellular density in Rt of 246.26 cells/mm^2 (Fig. 6).

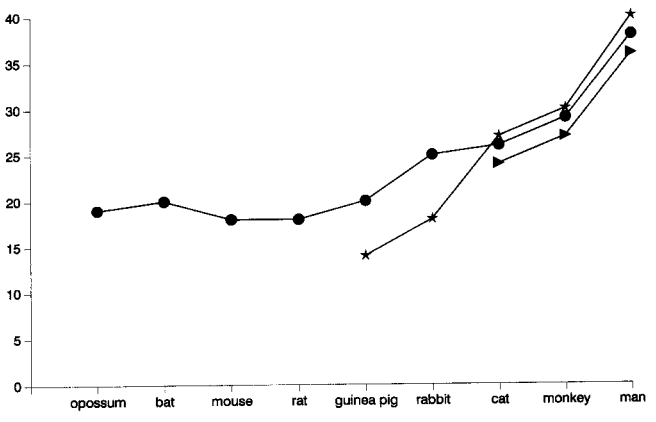


FIG. 4. Graphical representation showing the percentage (Y axis) of GABAergic local circuit neurons in different thalamic nuclei of the examined species (X axis). Note that local circuit neurons are present in the lateral geniculate nucleus of all the animals. point = lateral geniculate nucleus; asterisk = ventrobasal complex; triangle = ventral anterior nucleus, ventrolateral nucleus.

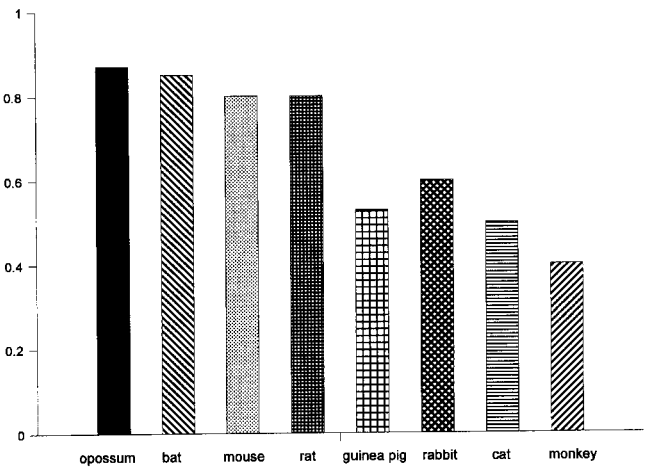


FIG. 5. Histogram showing the ratio between the area of the GABAergic and non GABAergic neurons (Y axis) measured in the lateral geniculate nucleus and in the ventrobasal complex (VB) (for the animals in which local circuit neurons were present also in VB) in different animals (X axis).

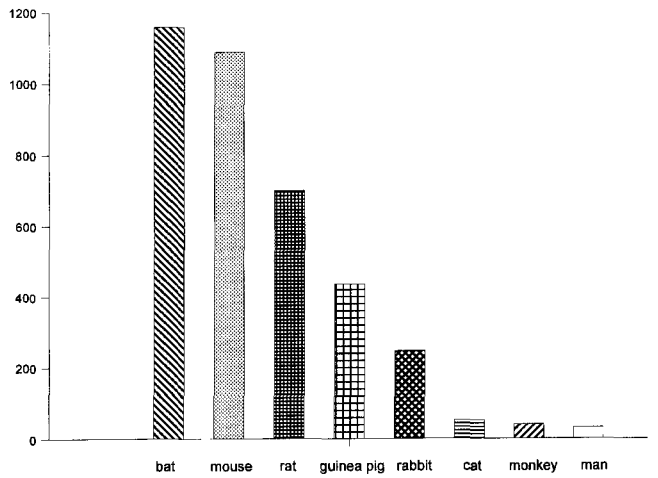


FIG. 6. Histogram showing the cellular density, reported as number of neurons/mm^2 (Y axis), in the reticular thalamic nucleus of different animals (X axis).

Cat and Monkey

Virtually all the cells of Rt were labeled with GAD or GABA antisera in both species (Fig. 2C and D). All the dorsal thalamic nuclei displayed GABAergic neurons. In the sensory nuclei, such as the VB and LG, and in the motor nuclei, such as the ventral anterior (VA) and ventrolateral (VL) nuclei, labeled neurons accounted for approximately 24–27% and 27–33% of the total neuronal population in the cat and monkey, respectively (Fig. 4). The neuropil staining varied considerably from one nucleus to another, but no territory of the thalamus was devoid of GABA-ir fibers and terminals as reported in previous studies [3,15,22,30,50].

GABA-ir neurons in the various thalamic nuclei displayed a remarkable degree of uniformity with regard to shape and size. GABAergic cells were smaller than the unlabeled neurons, resulting in a GABA/nonGABA ratio of 0.5 and 0.4 in the cat and monkey, respectively (Fig. 5). In the cat, the Rt/thalamus ratio was 12.35% and a great decrease of the neuronal density in the Rt (51.12 cells/mm^2) was observed with respect to the previously considered species (Figs. 6 and 7C). In the monkey, the Rt/thalamus ratio was 8.2% and the neuronal density in the Rt was 39.7 cells/mm^2, remarkably lower than in the other mammals investigated in the present study (Figs. 6 and 7D).

Humans

Virtually all the Rt neurons were GAD-ir (Fig. 2E).

GABAergic neurons were present in all the dorsal thalamic nuclei and they represented 40% of the total neuronal population in primary sensory relays and motor-related nuclei (Fig. 4).

As mentioned previously, although GAD-ir cells appeared considerably smaller than projection neurons, the GABA/nonGABA ratio was not evaluated because of the different shrinkage of the two types of neurons, probably due to the fixation procedure.

The Rt/thalamus ratio was 10.72%, similar to that of the other mammals investigated. The Rt neurons were very scattered within the boundaries of the nucleus, so that the cellular density in the Rt, 30.7 cells/mm^2, was the lowest observed in all the examined mammals (Fig. 6).

Ultrastructural Analysis

GABAergic neurons were detected in the LG nucleus of all the mammalian species analyzed in the present study. Several previous ultrastructural investigations have shown that the synaptic organization of the LG is similar in different species due to the presence of vesicle-containing dendritic appendages of GABAergic neurons [20,27–29,34,55].

In the present study, the ultrastructural analysis was performed only in the VB and Rt, where the light microscopic analysis showed interspecies differences regarding the presence and density of GABAergic neurons.

In the Rt of the examined species (guinea pig, rat, and cat), GABA labeling was found in cell bodies, dendrites, and presynaptic axonal terminals making symmetric synapses (Fig. 9A, C, E, and F). Only in the cat Rt some of the GABA-ir vesicle-containing profiles were postsynaptic to unlabeled asymmetric terminals or to other GABA-ir profiles (Fig. 9E, F). The unlabeled terminals making asymmetric synapses were similar in the rat, guinea pig, and cat.

On the contrary, the synaptic organization of VB was similar in the guinea pig and cat, and it was more complex in these species than in the rat. In the rat VB, GABA labeling was exclusively present in axonal terminals with heterogeneous size and shape making single or multiple symmetric synapses on unlabeled cell bodies and dendrites (Fig. 9B). GABAergic terminals of this kind were found also in the VB of the guinea pig and cat, but in these species GABA labeling was also present in several vesicle-containing dendrites engaged in complex synaptic arrangements, similar to those described in LG, with other GABA-ir profiles or with unlabeled terminals making asymmetric synapses and classifiable as SR (small, with round vesicles) or LR (large with round vesicles) (Fig. 9D, G). In the rat VB, SR and LR terminals were never presynaptic to vesicle-containing profiles (Fig. 9B).

DISCUSSION

The present study demonstrates that the intrathalamic networks of different mammals were arranged through modifications in the number and distribution of GABAergic neurons.

In our experiments, the labeling observed with the anti-GABA and with the anti-GAD sera were similar, in agreement with previous reports [40,53,61], and this rules out the possibility that methodological problems could have influenced our findings.

The present data raise a number of questions on the basic features of thalamic organization.

1. Are Thalamic Local Circuit Neurons Related to Functional Specialization?

GABAergic neurons occur in the LG of ancestral mammals, and their number in this nucleus increases in species where local circuit neurons are present also in other dorsal thalamic nuclei. Penny et al. [40] investigated the distribution of GABAergic neurons in the thalamus of the opossum, a marsupial that originated in the early Mesozoic and belongs to an early stage of mammalian evolution. The opossum is a nocturnal scavenger, whose behavior is more associated with movement and smell than with visual acuity, yet the only dorsal thalamic nucleus endowed with a significant number of GABAergic neurons was the LG. In the bat, a placental chiropter and nocturnal flying echolocating animal, in agreement with the data reported by Winer et al. [61], we found that 20% of the neurons of the rudimental LG were GABAergic, whereas the comparatively large MG lacked GABAergic neurons. Also in the rat and mouse, 20% of neurons

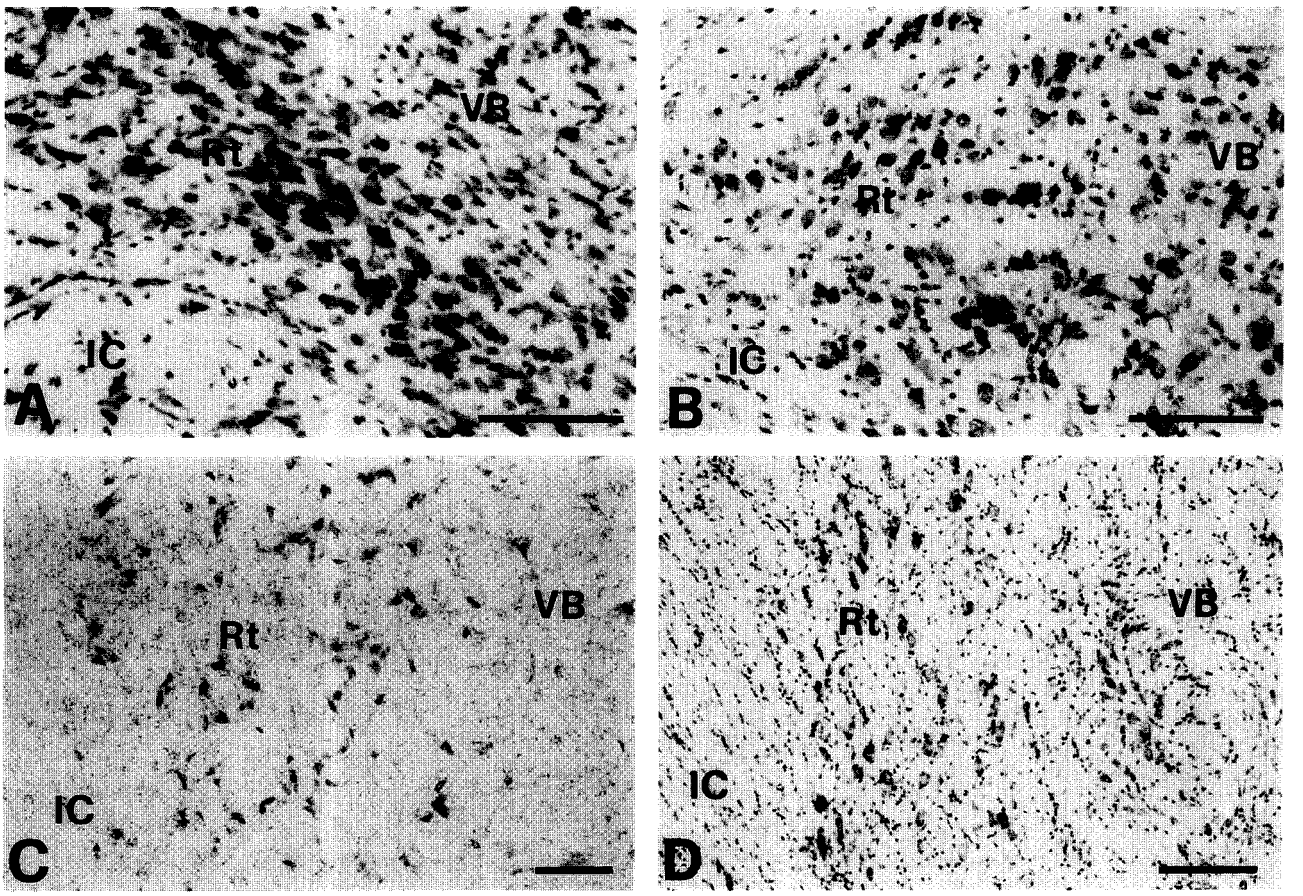


FIG. 7. Photomicrographs from thionin-stained sections showing the different cellular density in the reticular thalamic nucleus (Rt) in four representative animals: (A) bat; (B) mouse; (C) cat; (D) monkey. VB = ventrobasal nucleus; IC = internal capsule. Scale bars: A and B = 100 μm; C and D = 150 μm.

in LG were GABAergic, despite the rudimental visual system of these nocturnal rodents. Conversely, VB, which is essential for the vibrissal specialization of nocturnal rodents, was found to lack GABAergic neurons, consistently with previously published data [2,6,38,54]. These findings point out a dissociation between the behavioral needs of these animals and the occurrence of GABAergic cells in the LG, and strongly suggest that thalamic local circuit neurons are not directly related to the ability to perform specific sensorimotor tasks.

Moreover, in the rabbit, guinea pig, cat, monkey, and humans GABAergic neurons are present also in other thalamic relay nuclei besides the LG. In the thalamus of the rabbit, a lagomorph that appeared in the Paleocene, prior to the origin of carnivores and prosimians, as well as in that of the guinea pig, GABAergic neurons were detected only in VB [40,55]. On the other hand, in carnivores (cat) and primates (monkey, man) GABAergic neurons increase in number and become widely distributed in all the dorsal thalamic nuclei [3,10,13,15,21,22,25,30–32,39,44–46,50,51]. The ultrastructural organization of the nuclei containing GABAergic neurons differs from that of the nuclei devoid of local circuit neurons (see below), and it is likely to reflect an increasing complexity of the local information processing that occurs at thalamic level.

Finally, in ancestral species cell bodies were uniform in size, while in recent mammals GABAergic cells were smaller than projection neurons [40].

2. Does the Thalamic Synaptic Arrangement Reflect the Presence of Local Circuit Neurons?

As mentioned previously, the synaptic organization of the LG (the thalamic nucleus that consistently contains GABAergic neurons in mammals) is known to be very similar in different animal species [20,27–29,34]. Also, in the VB the synaptic organization was similar in all the species endowed with GABAergic neurons, such as the guinea pig, cat, and monkey [36,45,55] and much more complex than that of the dorsal thalamic nuclei lacking GABAergic interneurons (e.g., VB of the rat [7], VL and the thalamic paraventricular nuclei of the guinea pig [55]). In all the thalamic nuclei in which they are present, GABAergic local circuit neurons typically possess dendritic varicosities containing synaptic vesicles that are engaged in complex synaptic arrangements [17,34,36,45]. These dendritic varicosities (also called presynaptic dendrites) can be presynaptic to conventional dendrites of relay neurons and postsynaptic to GABA-negative axonal terminals with asymmetric specialization, such as the LR terminals (identified as ascending excitatory afferents, [7,8,45]) or the SR terminals (identified as descending corticothalamic afferents, [7,45]) or to GABA-ir profiles [36]. A large proportion of the contacts established by GABAergic local circuit neurons occurs, therefore, via presynaptic dendrites that have been proposed to act as a separate synaptic unit [43]. In par-

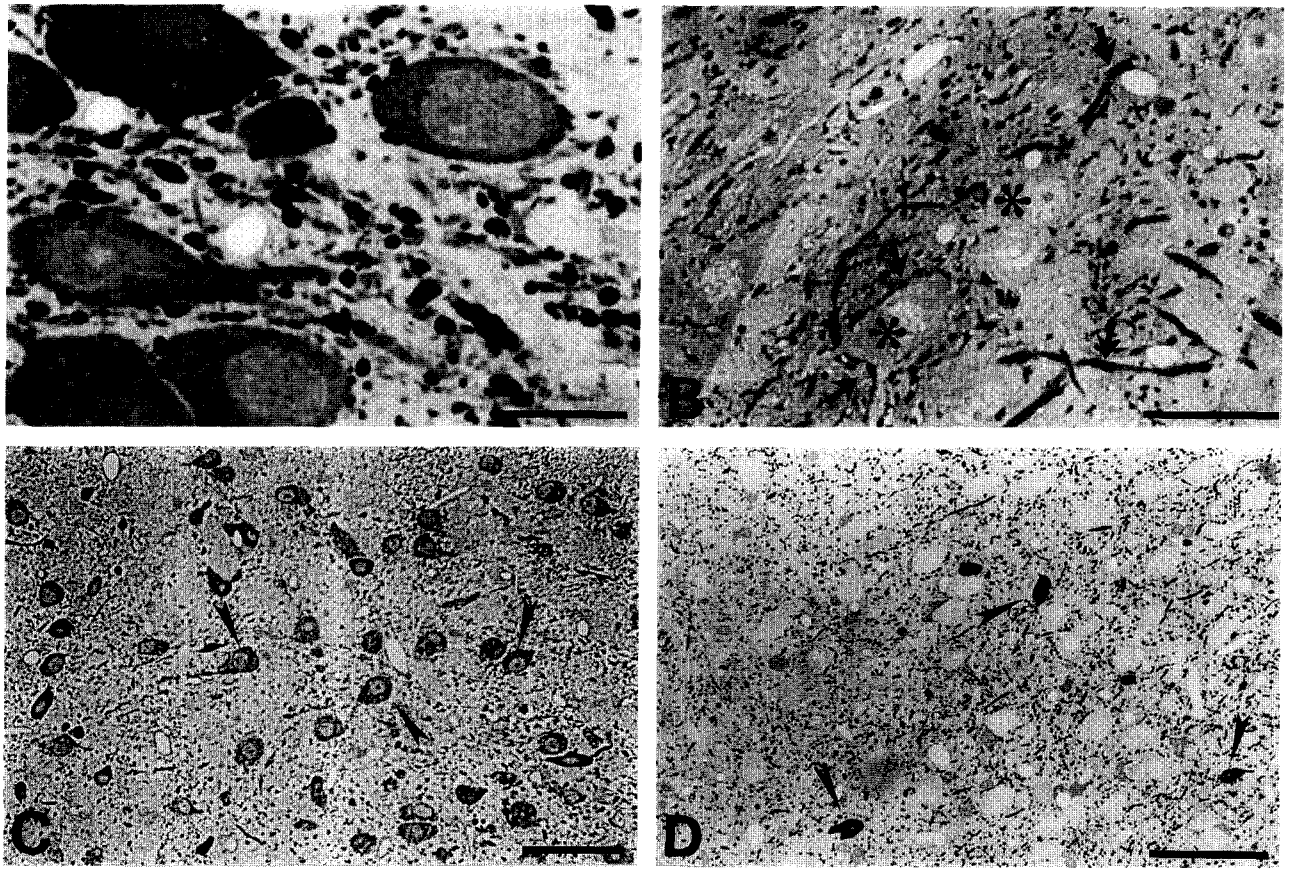


FIG. 8. Photomicrographs of semithin sections of the reticular thalamic nucleus (Rt) and of the ventrobasal complex (VB) immunoreacted with anti-GABA serum. GABA-positive neurons and puncta are evident in the Rt of rat (A) and of guinea pig (C, arrowheads). GABA-negative neurons (asterisks) are surrounded by several GABA-positive puncta (arrows) in the VB of rat (B), whereas GABA-positive neurons (arrowheads) are present only in the VB of guinea pig (D). Scale bars: A = 20 μm; B = 50 μm; C and D = 100 μm.

ticular, GABAergic presynaptic dendrites are ideally placed to mediate feed-forward inhibition because they are often involved in triadic synaptic arrangements with sensory afferents (LR terminals) and thalamocortical relay cells [36].

On the basis of these major structural differences, it is reasonable to assume that the physiological properties of VB are different in species containing GABAergic local circuit neurons with respect to species lacking them.

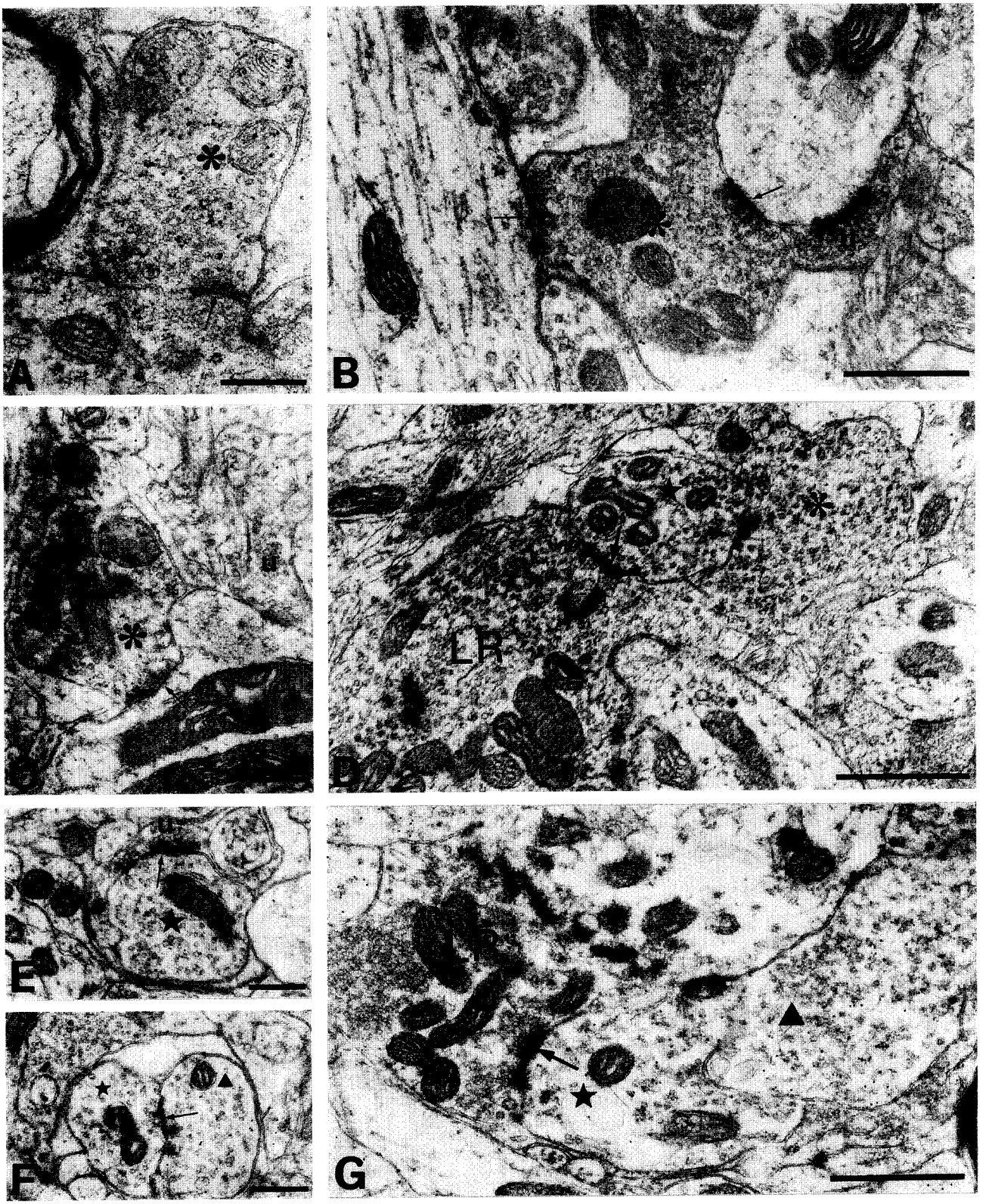
3. Is There a Relationship Between the GABAergic Rt Neurons and the GABAergic Local Circuit Neurons in the Dorsal Thalamus?

All the dorsal thalamic nuclei of mammals receive inhibitory inputs from the Rt. This nucleus, which contains a relatively homogeneous collection of GABAergic neurons, can be considered the common system of GABAergic thalamic inhibition, as it is present in all the mammals so far investigated, as well as in reptiles [41].

GABAergic terminals deriving from Rt neurons are morphologically similar in different thalamic nuclei and animal species and are widely distributed on the cell bodies and dendrites of dorsal thalamic neurons [7,23,36]. Furthermore, the activity of Rt cells is believed to be coordinated within the nucleus through the presence of local axon collaterals [54,63] and, at least in the cat and monkey, through dendrodendritic synapses [9,60].

Rt receives a major input from collaterals of thalamocortical and corticothalamic axons and it sends back an inhibitory input to thalamic relay neurons. Because of these connections, Rt takes part in a negative feed-back system that controls the transmission

FIG. 9. Electron micrographs showing the localization of GABA, visualized by postembedding immunogold labeling, in the reticular thalamic nucleus (Rt) and in the ventrobasal complex (VB) of rat, guinea pig and cat. (A) Rt of rat: a GABA-ir terminal (asterisk) synapses (arrow) on a GABA-ir dendrite. (B) VB of rat: a GABA-ir terminal (asterisk) synapses (arrows) on two adjacent unlabeled dendrites; u= unlabeled terminal with asymmetric specialization. (C) Rt of guinea pig: a GABA-ir terminal (asterisk) synapses (arrow) on a GABA-ir dendrite; u = unlabeled terminal. (D) VB of guinea pig: an unlabeled LR terminal synapses (arrow) on a GABA-ir vesicle-containing profile (star), also contacted (arrowhead) by a GABA-ir terminal (asterisk). (E) Rt of cat: a GABA-ir vesicle-containing profile (star) is contacted (arrows) by an unlabeled terminal (u). (F) Rt of cat: a GABA-ir vesicle-containing profile (star) synapses (arrow) on another GABA-ir vesicle-containing profile (triangle). (G) VB of cat: an unlabeled LR terminal synapses (arrow) on a GABA-ir vesicle-containing profile (star), adjacent to another GABA-ir vesicle-containing profile (triangle). Scale bars: A, C, E, and F = 0.5 μm; B, D, and G = 1 μm.


A
B
LR
E
F
G

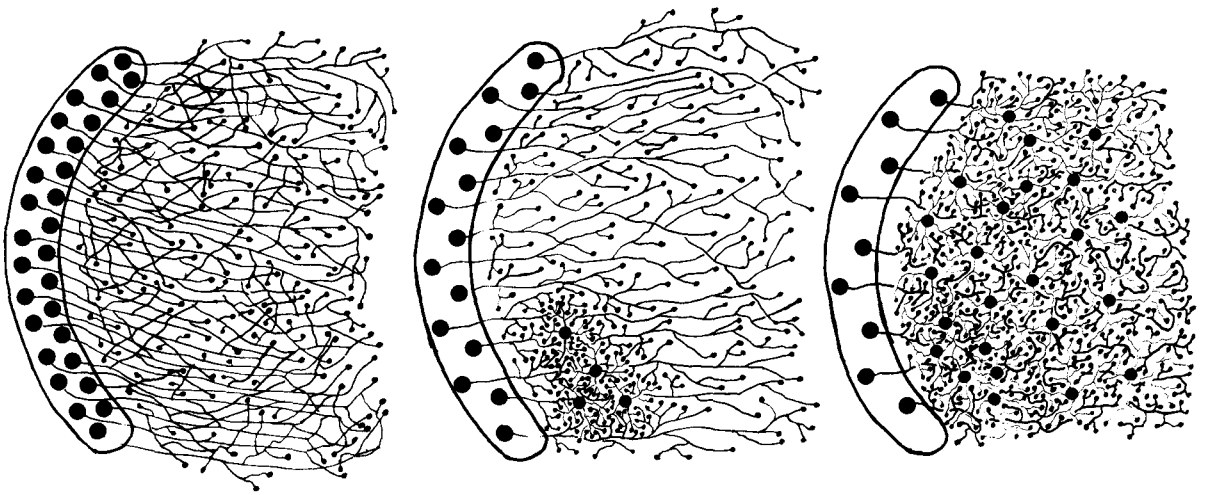


FIG. 10. Schematic drawing illustrating the increasing complexity of the intrathalamic circuitry in different animals, due to the appearance of intrathalamic local circuit neurons, paralleled by the decrease of cellular density within Rt (from left to right).

of information from the thalamus to the cortex and may also play a role in selective attention [19,56,59]. Thus, the functional role played by Rt is different from that played by GABAergic local circuit neurons, which in the dorsal thalamic nuclei have been implicated in mechanisms that modulate the quality, rather than the quantity, of the information trasmitted to the cortex [49].

Interestingly, although the ratio between the Rt area and the area of the dorsal thalamus is fairly constant in all the species examined in our study, we found a progressive decrease in the cellular density of Rt from the bat to humans, that seemed to parallel the numerical increase of intrathalamic GABAergic local circuit neurons (Fig. 10). On this basis, it can be hypothesized that, with the increase in the number of local circuit neurons in the dorsal thalamus, the Rt role becomes more focused in the regulation of sleep–waking cycle, and/or in acting as internal thalamic pacemaker, rather than in direct thalamic inhibition [56–58]. Alternatively, the decrease in the Rt cellular density may simply be a consequence of the general increase in brain volume in the examined species as it is not accompanied by a variation in the morphology of Rt neurons [24].

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