

Light and Electron Microscopical Studies of the GABA Innervation of the Dorsal Column Nuclei and the Lateral Cervical Nucleus in the Primate Species *Macaca Fascicularis* and *Papio Anubis*

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ABSTRACT

In 4 monkeys of the species *Macaca fascicularis* (2 animals) and *Papio anubis* (2 animals) the three dorsal column nuclei and the lateral cervical nucleus have been investigated immunocytochemically with antiserum against gamma-aminobutyric acid (GABA).

Light microscopic studies demonstrated the presence of GABA-positive cells in the gracile nucleus, the internal cuneate nucleus and the lateral cervical nucleus but not in the external cuneate nucleus. Although labeled cells seemed fairly evenly spread in the nuclei there was an increased amount between the clusters of the internal cuneate nucleus and in the border zone between the gracile and the cuneate nucleus. Electron microscopical investigation showed GABA labeling in fairly small neurons with relatively large cell nuclei and low somatic bouton covering. GABA-positive terminals with rounded synaptic vesicles were present in all the investigated nuclei also the external cuneate one. No apparent difference in number of such boutons in the different nuclei or parts of the nuclei was found. GABA-positive boutons mostly synapsed with dendrites but in the dorsal column nuclei also with other larger boutons. Axosomatic contacts between labeled terminals and neuronal perikarya were more common in the lateral cervical nucleus than in the dorsal column nuclei.

The results from the different nuclei in the monkey were compared with the results of similar investigations in the cat. It is concluded that there are important species differences especially on the light microscopical level in the lateral cervical nucleus. Thus the GABA labeled cells is rather evenly spread over the nucleus in the monkey whereas in the cat they are concentrated to the ventromedial region.

INTRODUCTION

At several different levels of the somatosensory systems gamma-aminobutyric acid (GABA) plays an important role as an inhibitory transmitter. Thus in the cat,

neurons and terminals containing GABA or glutamic acid decarboxylase (GAD), the biosynthetic enzyme of GABA, have been found in the dorsal column nuclei (DCN) (9,11,22,25) in the lateral cervical nucleus (LCN) (4,5) and in the nucleus of the spinal tract of the trigeminal nerve (3,14). Similar observations have been made in the feline somatosensory thalamic regions, where cell bodies and terminals containing GAD (17,18,21,23) or GABA (13,19,26) have been demonstrated. On the basis of studies with the Golgi technique and retrograde tracing experiments it has been concluded, that the GABA-immunoreactive neurons in the DCN, LCN and somatosensory thalamus are local circuit neurons (5,8,17,18,21,22,23).

So far, however, no studies in primates have been performed in any of the lower somatosensory relay nuclei to confirm that the conditions referred to above also are valid in the highest animals and thus possibly also man. In the present study two different species of monkey, the Crab-eating monkey, *Macaca fascicularis*, and the Olive baboon, *Papio anubis*, have been investigated for the presence of GABA in the DCN and LCN. Preliminary results have been published (10).

MATERIALS AND METHODS

Four adult monkeys, two of the species *Macaca fascicularis* and two of the species *Papio anubis* were used for the study of gamma-aminobutyric acid (GABA). Under anaesthesia induced by sodium pentobarbital (40-60 mg/kg), the animals intended for the GABA study were perfused through the heart with 2.5 % glutaraldehyde and 2.0 % formaldehyde in 0.1 M phosphate buffer (38°C, pH 7.4), preceded by a rinse with 0.1 M phosphate buffer. After termination of the perfusion the brain and spinal cord were removed and kept in fresh fixative at 4°C.

Thereafter the lower brain stem and upper cervical spinal cord containing the dorsal column nuclei (DCN) and the lateral cervical nucleus (LCN) were cut transversely on a Vibratome (Oxford Instruments) into sections 100 and 60 μm thick. The thicker sections which were regularly spaced with at least 3 for each mm were used for orientation purposes and were not subjected to immunocytochemistry. The 60 μm sections were processed further for GABA immunohistochemistry, by a procedure previously described in detail (5). After incubation for 30 minutes in 1% NaBH_4 in phosphate buffered saline (PBS) at room temperature to reduce unspecific activity due to binding of antibodies to free aldehyde groups (12), the sections were transferred to the primary antibody solution, (rabbit anti-GABA antiserum, Immunonuclear Corp, Stillwater, USA) diluted 1:500 and normal swine serum 1:30 in PBS and incubated for 36 hours. After rinsing in PBS, the sections were transferred to the secondary antibody solution (swine anti-rabbit 1:30 in PBS) and incubated for 60 minutes at room temperature under agitation; thereafter they were incubated in the PAP complex solution (1:120

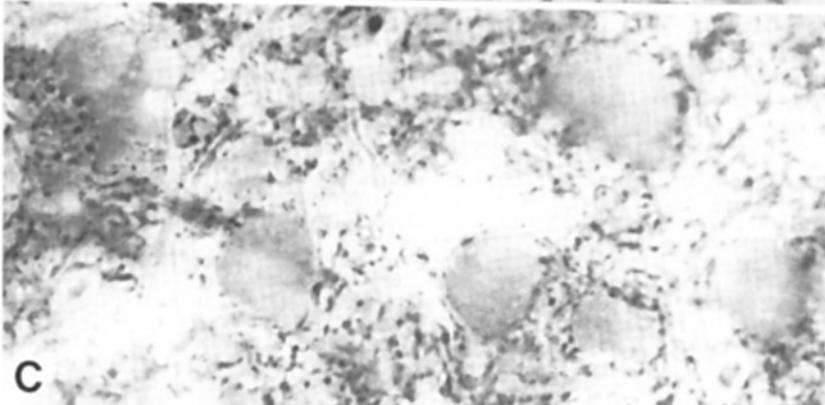
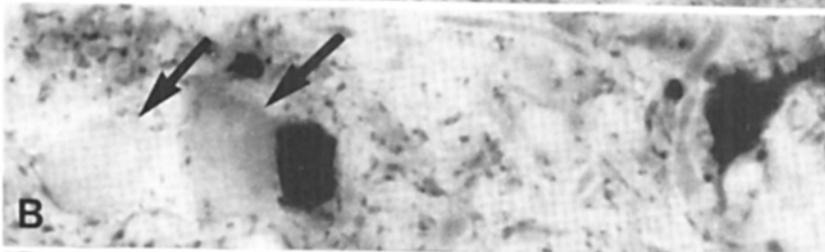
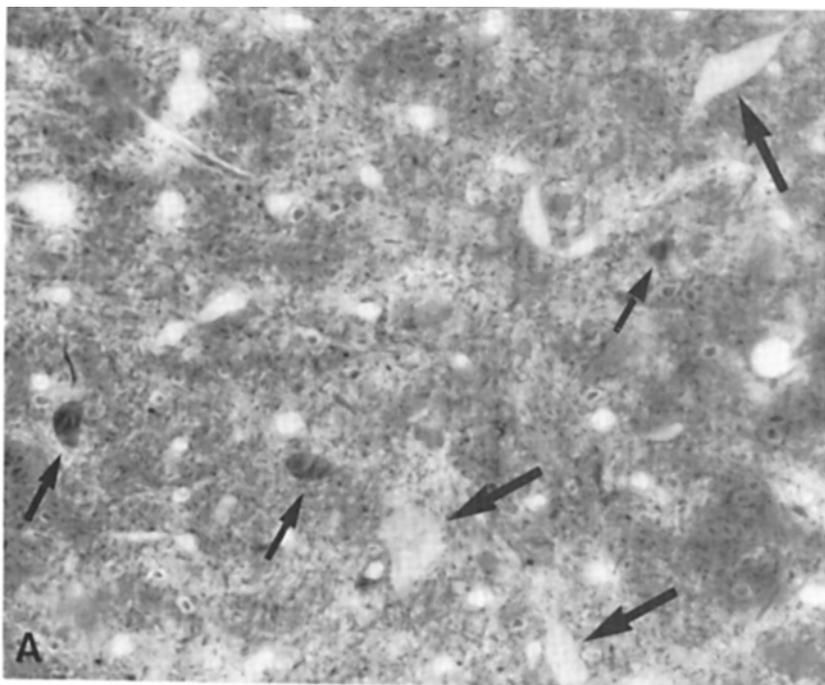
in PBS) under the same conditions. The immunocomplexes were localized by incubating the sections for 3-6 minutes in a solution containing 75 mg of diaminobenzidine and 30 μ l of 30% H₂O₂/100 ml of Tris-HCl buffer. The reaction was terminated by transferring the sections to a bath of Tris-HCl buffer. They were then postfixed for 20 minutes in 2% OsO₄ dissolved in cacodylate buffer, dehydrated in a graded series of ethanol and embedded in Epon between acetate foils. The 100 μ m sections were taken directly to osmification and subsequent dehydration and embedding in Epon. After light microscopic investigation suitable 60 μ m thick Vibratome sections were mounted onto Epon blocks and resectioned for electron microscopy. Some of the 60 μ m sections, which were exclusively intended for light microscopy were preincubated with 0,2 % Triton X-100 in PBS for one hour at 20°C before GABA immunocytochemistry.

Control experiments: From every series consecutive sections were incubated in parallel with those intended for GABA immunohistochemistry, the only difference in treatment being that the primary antiserum was omitted in the first incubation of the control sections. In two of the animals, one from each of the used species two additional types of controls (6,16) were made. The first one was a specificity test where the used GABA-serum was tested against test filters containing different amino acids (β -alanine, glutamate, GABA, glycine, glutamine, aspartate and taurine) coupled to rat brain extracts by means of glutaraldehyde. The other one was an inhibition test where the amino acids were mixed with glutaraldehyde and used to block the primary antiserum.

RESULTS

Control experiments: The specificity tests demonstrated that the used antiserum labelled the GABA test conjugate but also to a slighter extent β -alanine, glycine and glutamine. In the immunocytochemical studies of the Vibratome sections three kinds of intensively labelled structures were found in the light microscope: small cell somata, minute granular structures in the size of boutons and thin fibers sometimes connected to these granular structures. These labelled structures were absent from the controls where the primary antiserum had been omitted or blocked with GABA. When the other amino acids were used for blocking, however, the three kinds of labelled structures were present. Below these structures will be referred to as GABA-positive.

GABA immunoreactivity in the DCN: Light microscopic studies demonstrated the presence of GABA-positive cells in the gracile (Fig 1A) and the internal cuneate nucleus (Fig. 1B) but not in the external cuneate nucleus. The labeled cells seemed fairly evenly spread over the total longitudinal extent of the nuclei. There was



however, an increased amount of labeled cells between the clusters of the internal cuneate nucleus and in the border zone between the gracile and the cuneate nucleus. The cells were small to medium sized and rounded or elongated in cross sections from the brain stem (Fig.1A,B).

Electron microscopical investigations demonstrated that the reaction products were only present close to the surface of the Vibratome section. Labeled neurons (Fig.2A) had a mean diameter of about 15 μ m, a relatively large cell nucleus and a low somatic bouton covering. Most terminals in contact with labeled cells were unlabeled.

GABA-positive terminals were present in all the investigated DCN (Figs 1B, C and Figs 2A, B, C) also the external cuneate one (Figs 3A, B). No apparent difference in number of such boutons in the different nuclei or parts of the nuclei was found. GABA-positive boutons had a profile mean diameter of about 1 μ m, contained rounded synaptic vesicles and mostly synapsed with dendrites (Figs 2B, C and 3A) but also with other boutons (Figs 2B, 3B)). These postsynaptic terminals were often conspicuously large up to 5 μ m in diameter. Axosomatic contacts were rather sparse.

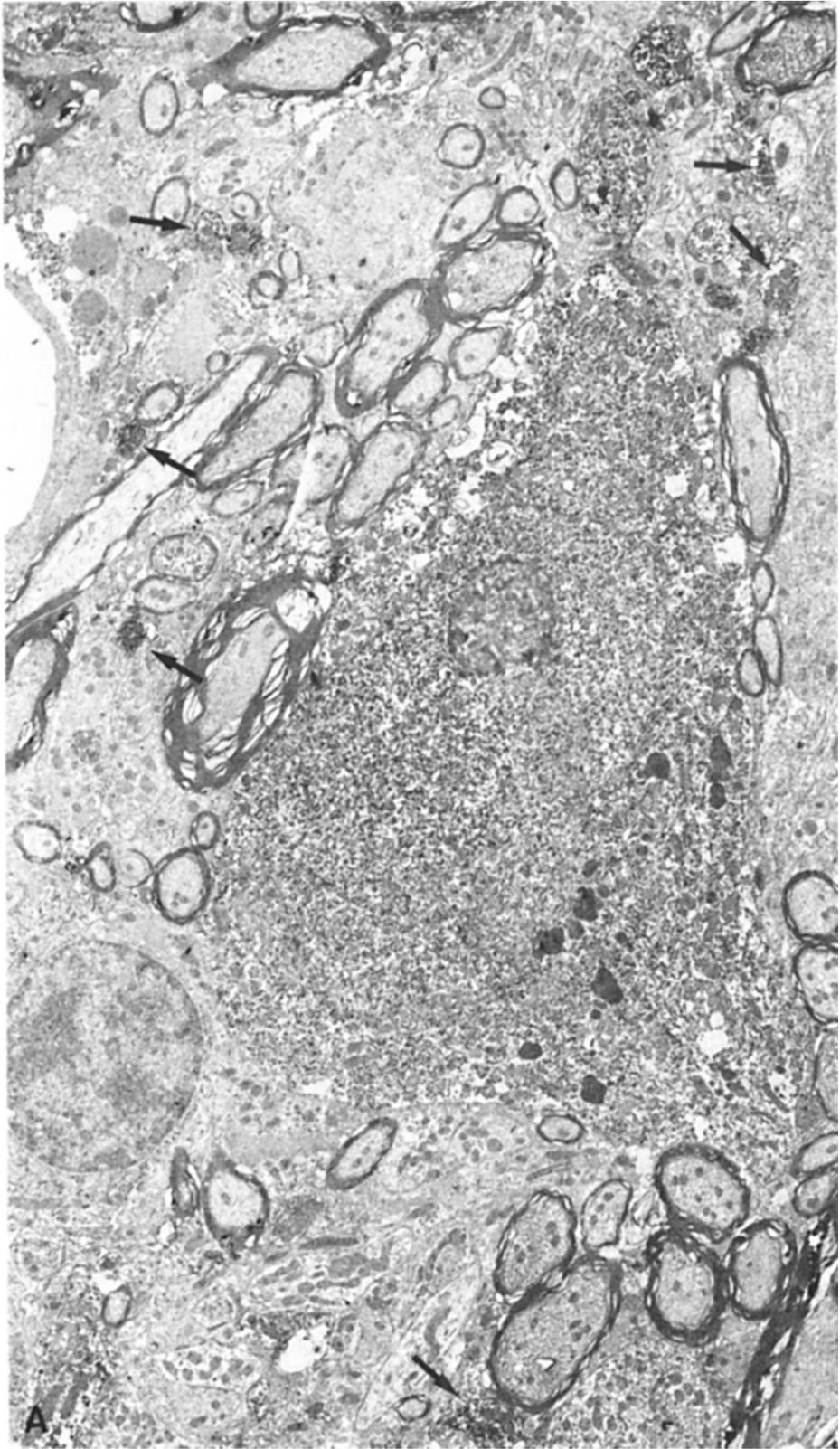
GABA immunoreactivity in the LCN: Light microscopy demonstrated a few small rounded or elongated GABA-positive cells (Fig. 4A, B and D). The labeled cells had no preferential localization within the LCN, whose overwhelming majority of neurones were unlabeled and larger than the labeled ones. In the electron microscope (Fig. 5A) the labeled cells demonstrated a fairly large nucleus and rather low somatic bouton covering compared to unlabeled cells. Most of the boutons in contact with labeled cells were unlabeled. Both in the light (Fig. 4C) and electron microscope (Fig. 5A-C) numerous labeled terminals with a mean diameter of about 1 μ m were seen without any larger regional differences in number or size. These boutons contained rounded vesicles and synapsed with other dendrites (Fig. 5B) or perikarya (Fig. 5 C). Most terminals were, however, unlabeled and contained rounded or elongated synaptic vesicles.

Fig.1. Light micrographs of GABA immunoreactivity of the DCN.

A. 210X Labeled (small arrows) and unlabelled (large arrows) cells in the gracile nucleus below the level of obex.

B. 620X Higher magnification of labeled and unlabeled (arrows) cells from the internal cuneate nucleus. To the left of the center of the figure a small GABApositive neuron very close to a larger unlabeled cell.

C. 640X Unlabeled cell profiles from the cell nest region of the internal cuneate nucleus. Note the numerous labeled terminals surrounding the cells.



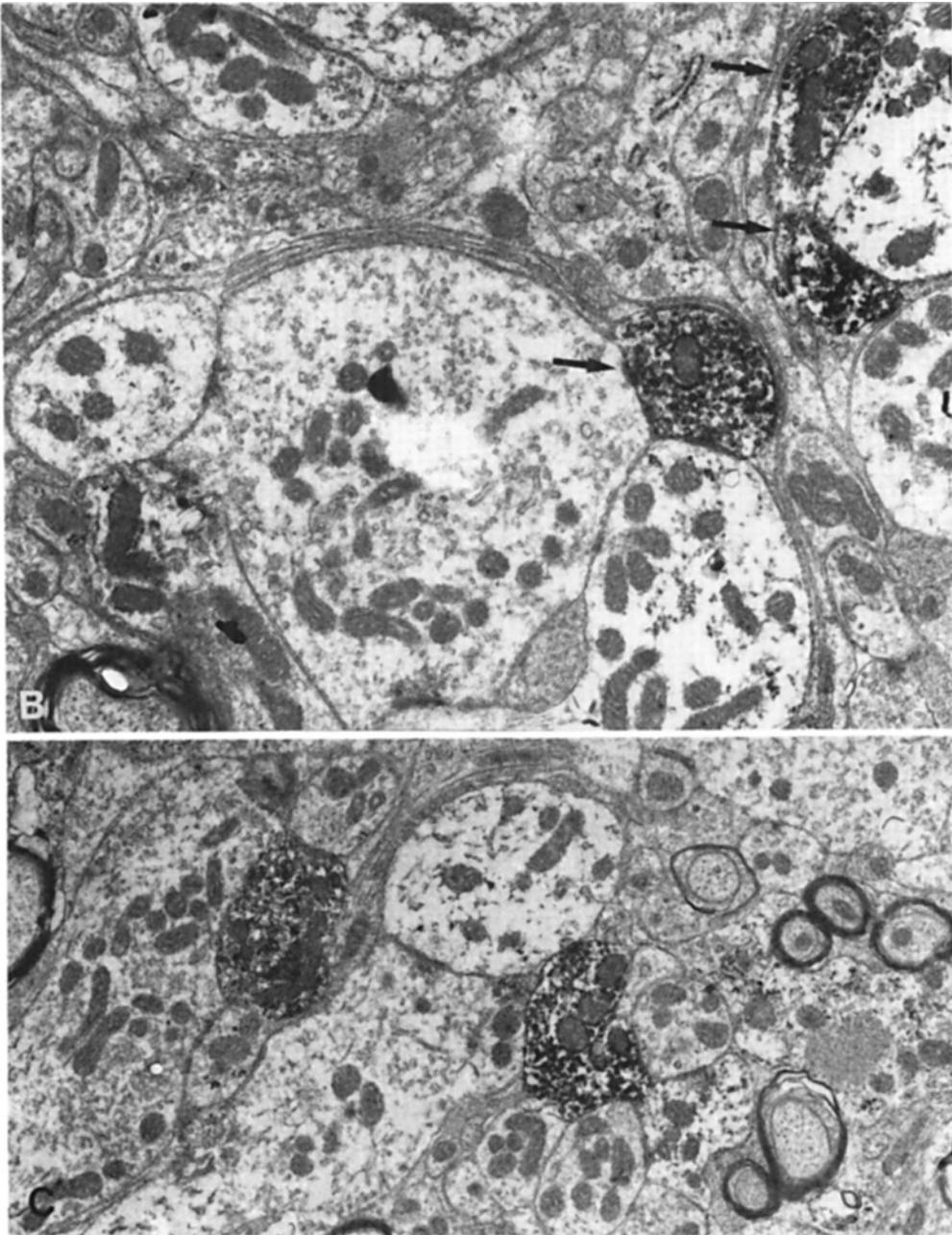


Fig.2. Electron micrographs of GABA immunoreactivity of the DCN.

A. 5500X. GABA positive cell from the internal cuneate nucleus. The cell is surrounded by a neuropil with labeled terminals (arrows).

B. 19 000X Three labeled terminals (arrows) from the gracile nucleus. The lower one is in synaptic contact with a large unlabeled terminal to the left.

C. 15 000X Two GABA positive gracile terminals, both in contact with dendrites and large unlabeled terminals.

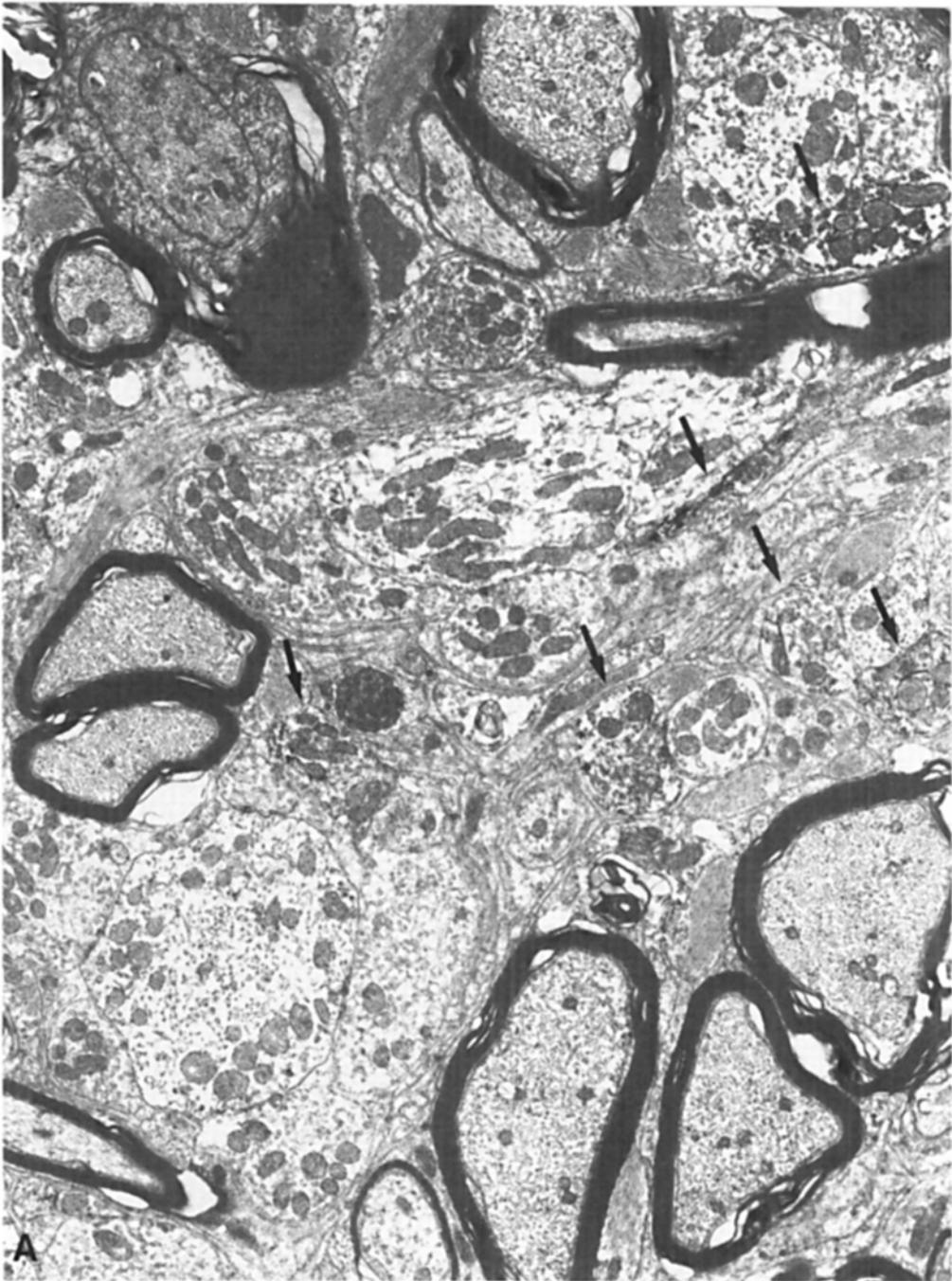
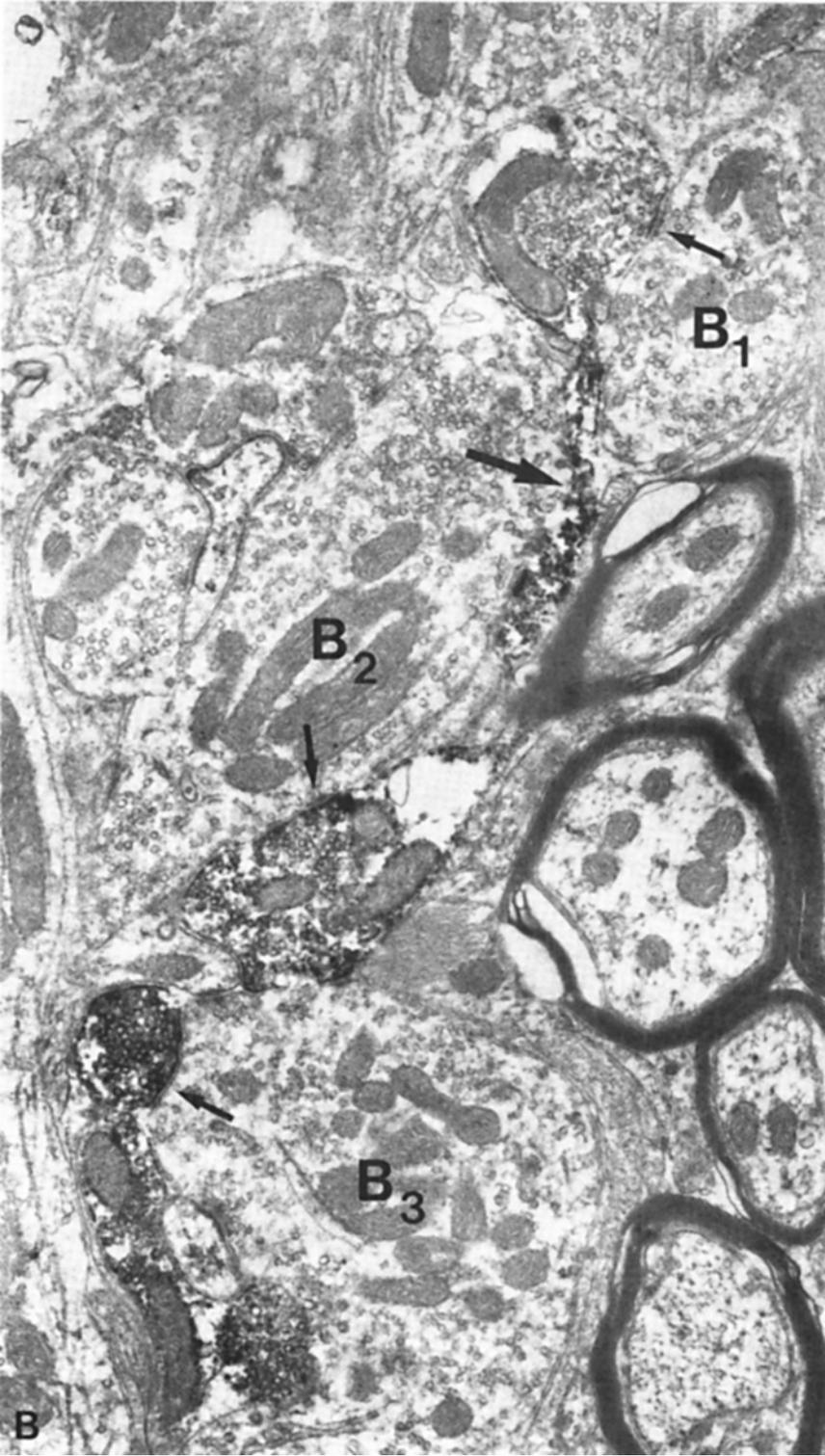
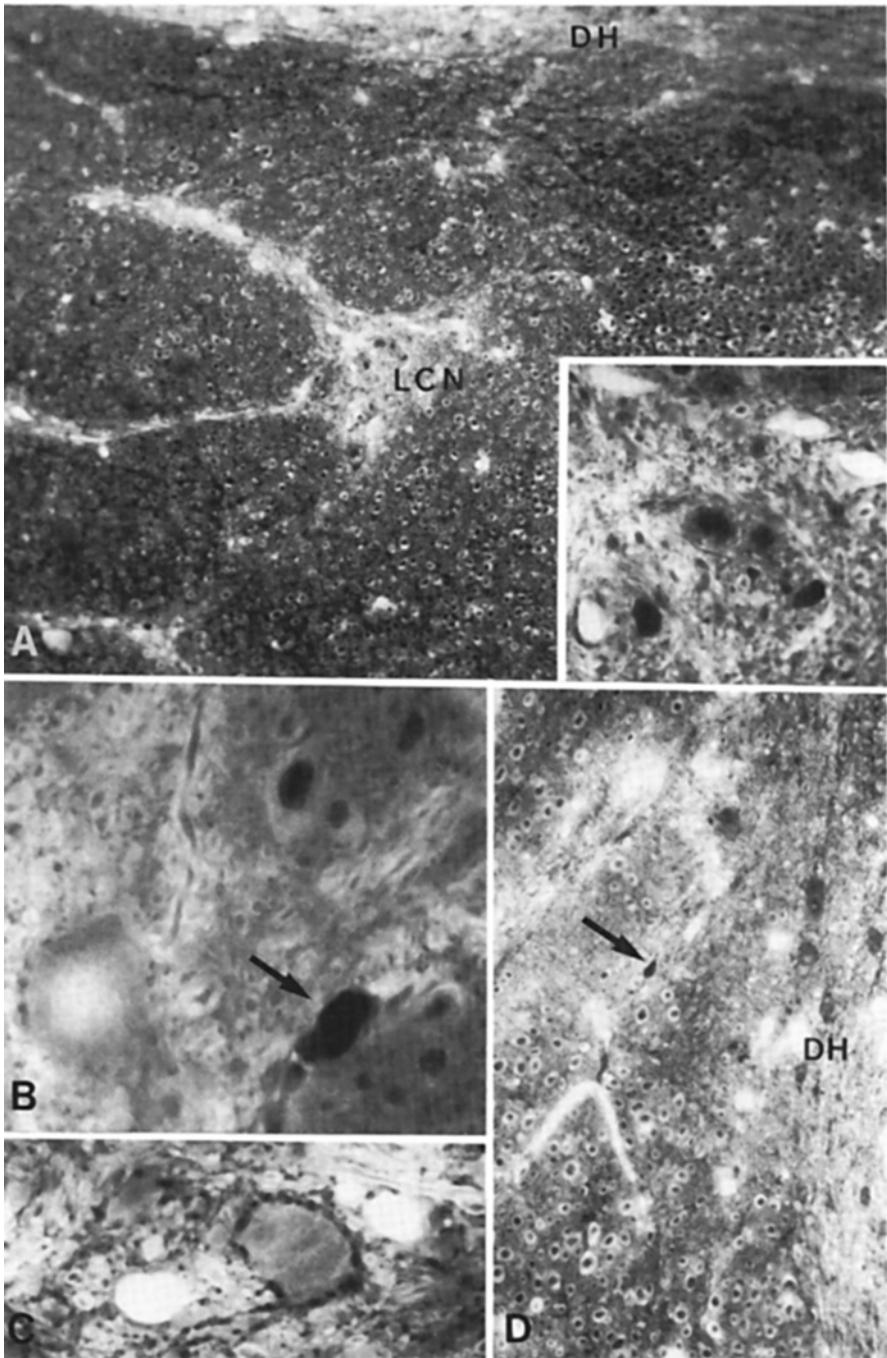


Fig.3. Electron micrographs of GABA in the external cuneate nucleus.

A. 15000X Numerous labeled structures (arrows) of which most are boutons.

B. 21 000X Three GABApositive terminals in synaptic contact (small arrows) with three larger unlabeled terminals (B₁, B₂, and B₃, respectively). Both labeled and unlabeled terminals contain rounded synaptic vesicles. Note the GABApositive axon (large arrow) connected to the labeled terminal (above).





No apparent difference between the two monkey species *Macaca fascicularis* and *Papio anubis* was detected.

DISCUSSION

The present study has demonstrated that the GABA distribution in the two medial dorsal column nuclei (DCN) of the monkey is the same as has been reported for cat (9,11) and could be assumed from studies of glutamic acid decarboxylase, the biosynthetic enzyme of GABA in the cat (22,25) and rat (2). Thus the DCN contain numerous GABA-positive neurons all over the two medial nuclei especially between the cell clusters. These cells are fairly small with a relatively large cell nucleus and few somatic synapses. The GABA-positive terminals have synaptic contact with dendrites and other large boutons probably emerging from the primary afferents. In this way the GABA-positive cells, which most probably are local circuit neurones can exert their presynaptic inhibition through axo-axonal contacts (1,24). It seems likely that the same basic organization is valid also for man although this has to be demonstrated by further studies.

The present study of the external cuneate nucleus appears to be the first electron microscopical one in the primate. It confirms earlier reports of axodendritic and axoaxonal synapses in the cat (15) and rat (20). In the cat the large terminals postsynaptic to smaller knobs have been demonstrated to be derived from primary afferents (15). The present study has shown that the small presynaptic knobs are GABA-positive and thus presumably GABAergic. Further studies are necessary to elucidate the origin of these terminals, which seems to be extrinsic, as the present study failed to detect any GABA-positive perikarya.

In the LCN there is a difference in distribution of GABA between the cat (5) and monkey (present study). Thus the GABA-positive cells although few could be found in all parts of the monkey LCN while they seemed to be confined to the ventromedial part of the cat LCN. In both species, however, the question remains whether the few GABA-positive cells could give origin to all the terminals or if an additional external GABAergic inhibitory input (7) has to be assumed.

Fig. 4. Light micrographs of GABA immunoreactivity of the LCN.

A. 100X Dorsal horn (DH) above. Note 4 labeled cell profiles in the LCN demonstrated in higher magnification (400 X) inserted at lower right.

B. 700X Small labeled cell to the right (arrow) and larger unlabeled one to the left.

C. 630X One unlabeled cell profile surrounded by numerous labeled structures of the size of terminals.

D. 160 X Labeled cell (arrow) in the ventromedial portion of LCN. Dorsal horn (DH) to the right.

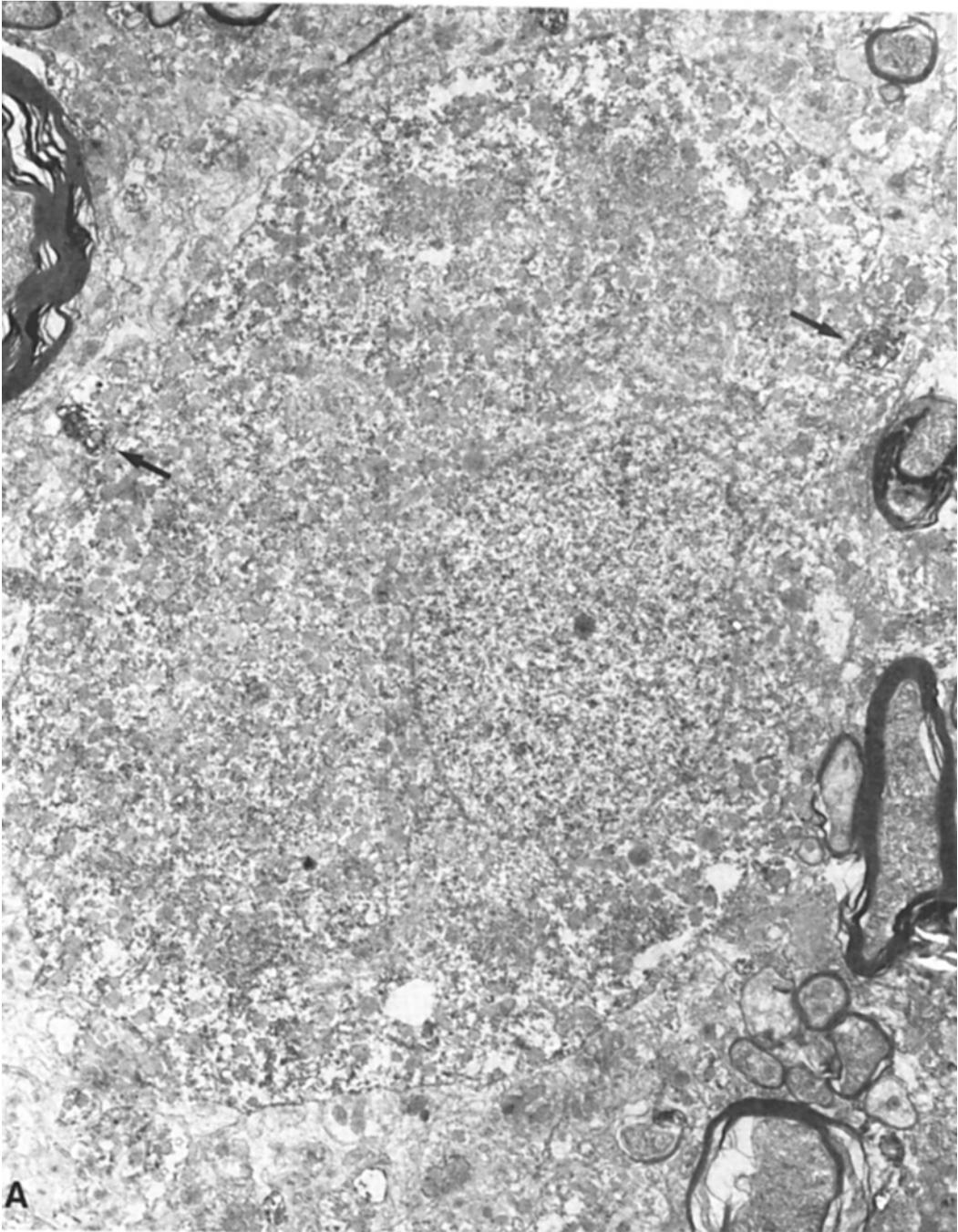
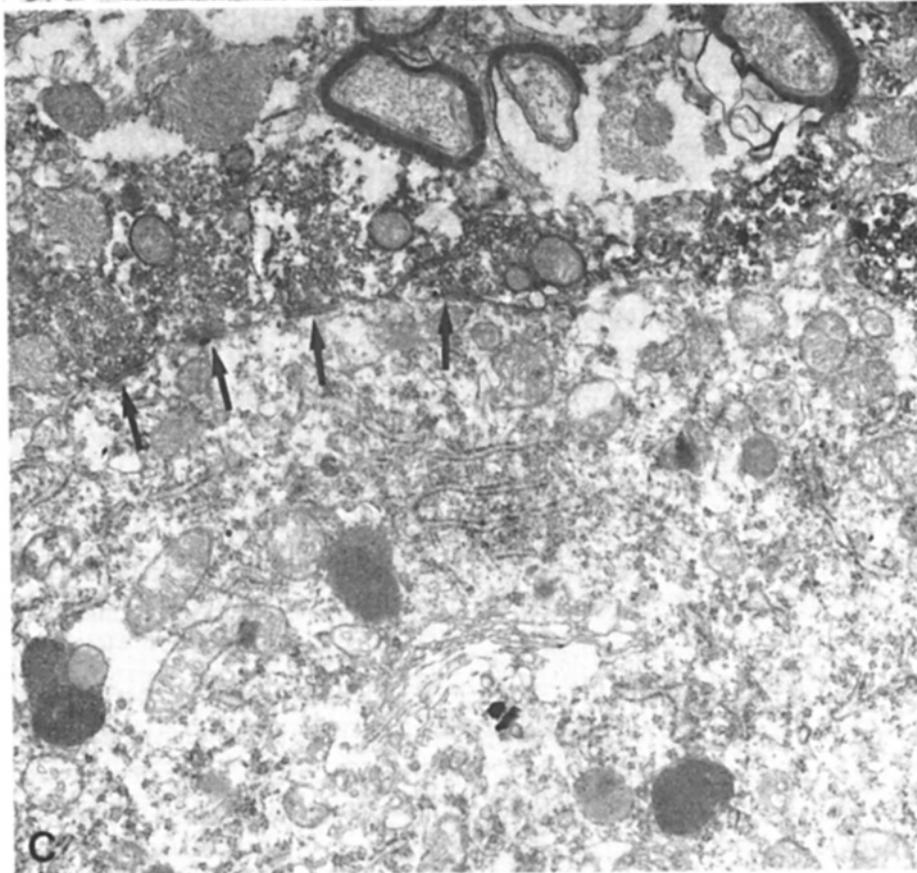
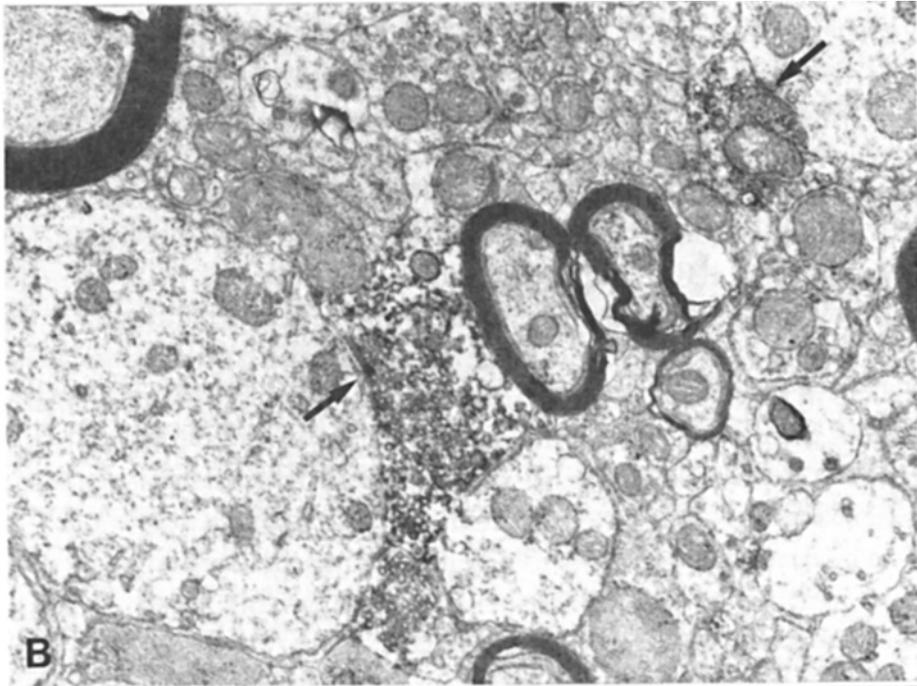


Fig.5. Electron micrographs of GABA immunoreactivity of the LCN.

A. 7000X Labeled neurone from the central part. Two GABApositive terminals (arrows) close to the cell. The right one is in contact with the perikaryon.

B. 20 000X Two labeled axodendritic terminals (arrows) from the lateral part.

C. 21 000X Array of labeled axosomatic terminals with rounded vesicles from the lateral part of LCN in synaptic contact (arrows) with a cell soma to the left.



The present study has confirmed that also in the primate there is important differences between the synaptic contacts of the GABA-positive terminals in the DCN and the LCN. Thus in the DCN the GABA-positive and presumably inhibitory terminals are presynaptic to other larger terminals at least partially primary afferent ones. In the LCN such axo-axonal contacts are absent. The physiological differences between the DCN and LCN which are closely related to these anatomical differences are to find e.g. in the type of inhibition exerted in the two areas. In the DCN this is of the "surround" type enhancing the contrast of the system, in the LCN it is of a more wide-spread type affecting larger areas. On the other hand there seem to be at least morphologically some similarities especially in the appearance of the GABA-positive and supposedly inhibitory local circuit neurones. Both in the DCN and LCN they are fairly small, have a proportionally large cell nucleus and a low somatic bouton covering. Although further studies of more quantitative nature need to be done, this might be an indication of a general morphologic similarity between inhibitory internuncial neurones in somatosensory systems and perhaps the whole subcortical central nervous system.

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REFERENCES

1. Andersen, P., Eccles, J.C., Oshima, T. & Schmidt, R.F.: Mechanisms of synaptic transmission in the cuneate nucleus. *J Neurophysiol* 27:1096-1116, 1964.
2. Barbaresi, P., Spreafico, R., Frassoni, C. & Rustioni, A.: GABAergic neurons are present in the dorsal column nuclei but not in the ventroposterior complex of rats. *Brain Res* 382:305-326, 1986.
3. Basbaum, A I., Glazer, E J. & Oertel, W.: Immunoreactive glutamic acid decarboxylase in the trigeminal nucleus caudalis of the cat: a light- and electron-microscopic analysis. *Somatosens Res* 4:77-94, 1986.

4. Blomqvist , A ., Westman, J., Köhler, J. & Wu, J -Y.: Immunocytochemical localization of glutamic acid decarboxylase and substance P in the lateral cervical nucleus: A light and electron microscopic study in the cat. *Neurosci Lett* 56:229-233, 1985.
5. Broman, J. & Westman, J.: GABAergic neurons and terminals in the lateral cervical nucleus of the cat. *J Comp Neurol* 274: 461-472, 1988.
6. Dale, N., Ottersen, O. P., Roberts, A. & Storm-Mathisen, J.: Inhibitory neurons of a motor patterns generator in *Xenopus* revealed by antibodies to glycine. *Nature* 324: 255-257, 1986.
7. Fedina, L. , Gordon, G. & Lundberg, A.: The source and mechanisms of inhibition in the lateral cervical nucleus of the cat. *Brain Res* 11:694-696, 1968.
8. Flink, R. & Westman, J.: Different neuron populations in the feline lateral cervical nucleus: A light and electron microscopical study with the retrograde axonal transport technique. *J. Comp. Neurol.* 250: 265-281, 1986.
9. Heino, R.: Quantitative studies of the GABA-ergic and non-Gaba-ergic neurons in the feline dorsal column nuclei. *Eur J Neurosci Abstr 11th An Meeting of ENA* p 283, 1988
10. Heino, R. & Westman, J.: GABA and Substance P immunoreactivity in the dorsal column nuclei and the lateral cervical nucleus of the primate. *Eur J Neurosci Abstr 11th An Meeting of ENA* p 283, 1988.
11. Heino, R. & Westman, J.: Quantitative analysis of the feline dorsal column nuclei and their GABAergic and non-GABA-ergic neurons. *J Neurocytol* Submitted, 1989.
12. Kosaka, T., Nagatsu, I., Wu, J -Y. & Hama, K.: Use of high concentrations of glutaraldehyde for immunocytochemistry of transmitter-synthesizing enzymes in the central nervous system. *Neuroscience* 18:975-990, 1986.
13. Madarász, M., Somogyi, Gy., Somogyi, J & Hámori , J.: Numerical estimation of γ -aminobutyric acid (GABA)-containing neurons in three thalamic nuclei of the cat: direct GABA immunocytochemistry. *Neurosci Lett.* 61:73-78, 1985.
14. Matthews, M. A., McDonald, G. K. & Hernandez, T. V .: GABA distribution in a pain-modulating zone of trigeminal subnucleus interpolaris. *Somatosens Res* 5: 205-217, 1988.
15. O'Neal, J.T. & Westrum, L.E. : The fine structural synaptic organisation of the cat lateral cuneate nucleus. A study of sequential alterations in degeneration. *Brain Res*:97-124, 1973.
16. Ottersen, O. P., Storm-Mathisen, J., Madsen, S., Skumlien S. & Strømhaug, J.: Evaluation of the immunocytochemical method for amino acids. *Medical Biology* 64: 147-158, 1986.

17. Penny, G. R., Fitzpatrick, G., Schmechel, D. E. & Diamond, I. T.: Glutamic acid decarboxylase-immunoreactive neurons and horseradish peroxidase labeled projection neurons in the ventral posterior nucleus of the cat and *Galago senegalensis*. *J Neurosci* 3:1868-1887, 1983.
18. Ralston, H. J., III.: The fine structure of the ventrobasal thalamus of the monkey and cat. In: Albe-Fessard D, Berkley K J, Kruger L, Ralston H J, III, Willis W D, Jr Diencephalic mechanisms of pain sensation. *Brain Res Rev* 9:228-241, 1985.
19. Rinvik, E., Ottersen, O. P. & Storm-Mathisen, J.: Gamma-aminobutyrate-like immunoreactivity in the thalamus of the cat. *Neuroscience* 21:781-805, 1987.
20. Rosenstein, J. M. & Leure-duPree, A. E.: Synaptic organisation of the external cuneate nucleus in the rat. *J Comp Neurol* 175: 159-180, 1977.
21. Rustioni, A., Schmechel, D. E., Spreafico, R., Cheema S. & Cuénod, M.: (1983) Excitatory and inhibitory amino acid putative neurotransmitters in the ventralis posterior complex: an autoradiographic and immunocytochemical study in rats and cats. In: Macchi G, Rustioni A, Spreafico R (eds) *Somatosensory integration in the thalamus*. Elsevier, Amsterdam pp 365-383, 1977.
22. Rustioni, A., Schmechel, D. E., Cheema, S. & Fitzpatrick, D.: Glutamic acid decarboxylase-containing neurons in the dorsal column nuclei of the cat. *Somatosens Res* 1:329-357, 1984.
23. Spreafico, R., Schmechel, D. E., Ellis, L. C. Jr. & Rustioni, A.: Cortical relay neurons and interneurons in the N. ventralis posterolateralis of cats: a horseradish peroxidase, electron-microscopic, Golgi and immunocytochemical study. *Neuroscience* 9: 491-509, 1983.
24. Walberg, F.: Axo-axonic contacts in the cuneate nucleus, probable basis for presynaptic depolarisation. *Exp. Neurol* 13:218-231, 1965.
25. Westman, J., Blomqvist, A., Köhler, C. & Wu, J-Y.: Light and electron microscopic localization of glutamic acid decarboxylase and substance P in the dorsal column nuclei of the cat. *Neurosci Lett* 51:347-352, 1984.
26. Westman, J., Flink, R. & Klemets, P.: Ultrastructural characteristics of terminals of putative nociceptive pathways and their synaptic contacts. In: Besson J-M, Guilbaud G, Peschanski M (eds) *Thalamus and Pain*. Elsevier, Amsterdam, pp 65-81, 1987.

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