THE JOURNAL OF COMPARATIVE NEUROLOGY 258:81-90 (1987)

Ultrastructural Identification and Localization of Climbing Fiber Terminals in the Fastigial Nucleus of the Cat

J.J.L. VAN DER WANT AND J. VOOGD
The Netherlands Ophthalmic Research Institute, P.O. Box 12141, 1100 AC Amsterdam (J.J.L.W.), and Department of Anatomy, Erasmus University Rotterdam, 3000 DR Rotterdam (J.V.), The Netherlands

ABSTRACT

Following injections of ^{3}H-leucine and ^{35}S-methionine in the caudal half of the medial accessory olive, labeled climbing fibers were found contralateral to the injection site in the sagittal A-zone of the cerebellar vermis and in the fastigial nucleus. Labeling in the fastigial nucleus was analyzed with ultrastructural autoradiography. Labeled boutons of climbing fibers were found in the neuropil but never on somata. They contain spherical vesicles and occasionally some dense core vesicles in an electron-lucent matrix. The terminals of climbing fiber collaterals in the fastigial nucleus resemble climbing fiber terminals in the molecular layer with respect to their internal ultrastructural characteristics.

Key words: cerebellum, ultrastructural autoradiography, central nuclei, synaptology

The topographical organization of the inferior olivary projection to the cerebellum in the cat has long been recognized and was extensively reviewed by Brodal and Kawamura ('80). The termination of olivocerebellar climbing fibers (cf) in narrow sagittal zones in the cerebellar cortex was studied with morphological methods (Voogd, '64, '82; Courville, '75; Groenewegen and Voogd, '77; Groenewegen et al., '79) and electrophysiologically (Oscarsson, '69; Armstrong, '74; Andersson and Eriksson, '81). Olivocerebellar fibers not only terminate in the cerebellar molecular layer, but they also give off collaterals to the central cerebellar nuclei (CCN).

Retrograde chromatolysis was observed in certain parts of the inferior olive after destruction of the CCN (Brodal, '40). Matsushita and Ikeda ('70) found degenerating terminals in the CCN in the cat after lesion experiments of the inferior olive, which was also observed after 3-acetylpyridine treatment of the inferior olive in the rat (Desclin and Colin, '80). Chan-Palay ('77) found axon terminals in the dentate nucleus of the rat and monkey with ultrastructural characteristics similar to those of the cf-boutons in the molecular layer and postulated that these terminals represent cf-collaterals to the CCN. Retrograde labeling of cells of the inferior olive has been demonstrated after injections of horseradish peroxidase (HRP) in the fastigial nucleus of the cat (Brodal, '76; Eller and Chan-Palay, '76; Kitai et al., '77; Courville et al., '77). In experiments using anterograde transport of HRP, cf-collaterals to the CCN could not be demonstrated (Walberg et al., '80). This failure is possibly due to differences in sensitivity of the tracing technique (Walberg et al., '80). Using autoradiography of anterogradely transported tritiated leucine following injections in the inferior olive, cf-terminals were not only found in the molecular layer but also in the CCN (Courville, '75; Groenewegen and Voogd, '77; Groenewegen et al., '79; Kawamura and Hashikawa, '79; Balaban et al., '81). According to Brodal ('40) the olivocerebellar projection to the CCN originates from parts of the olive that do not necessarily have a concomitant projection to the cortex.

In the view of Groenewegen and Voogd ('77) there is a strict relation between termination in individual sagittal zones in the molecular layer and the termination in the CCN related to those zones. This view is supported by the electrophysiological investigations of Andersson and Oscarsson ('78), who demonstrated that cells in Deiters' nucleus are inhibited via the cortical branch of the same climbing fiber that initially causes excitation of that cell through its nuclear collateral. Although the term of collaterals therefore seems to be appropriate, the origin of the cortical and nuclear projections from the same olivary cells never has been demonstrated with anatomical methods. Climbing fiber volleys result in a short-latency excitation of the CCN cells, followed by an inhibition through the cf-

Accepted August 15, 1986.

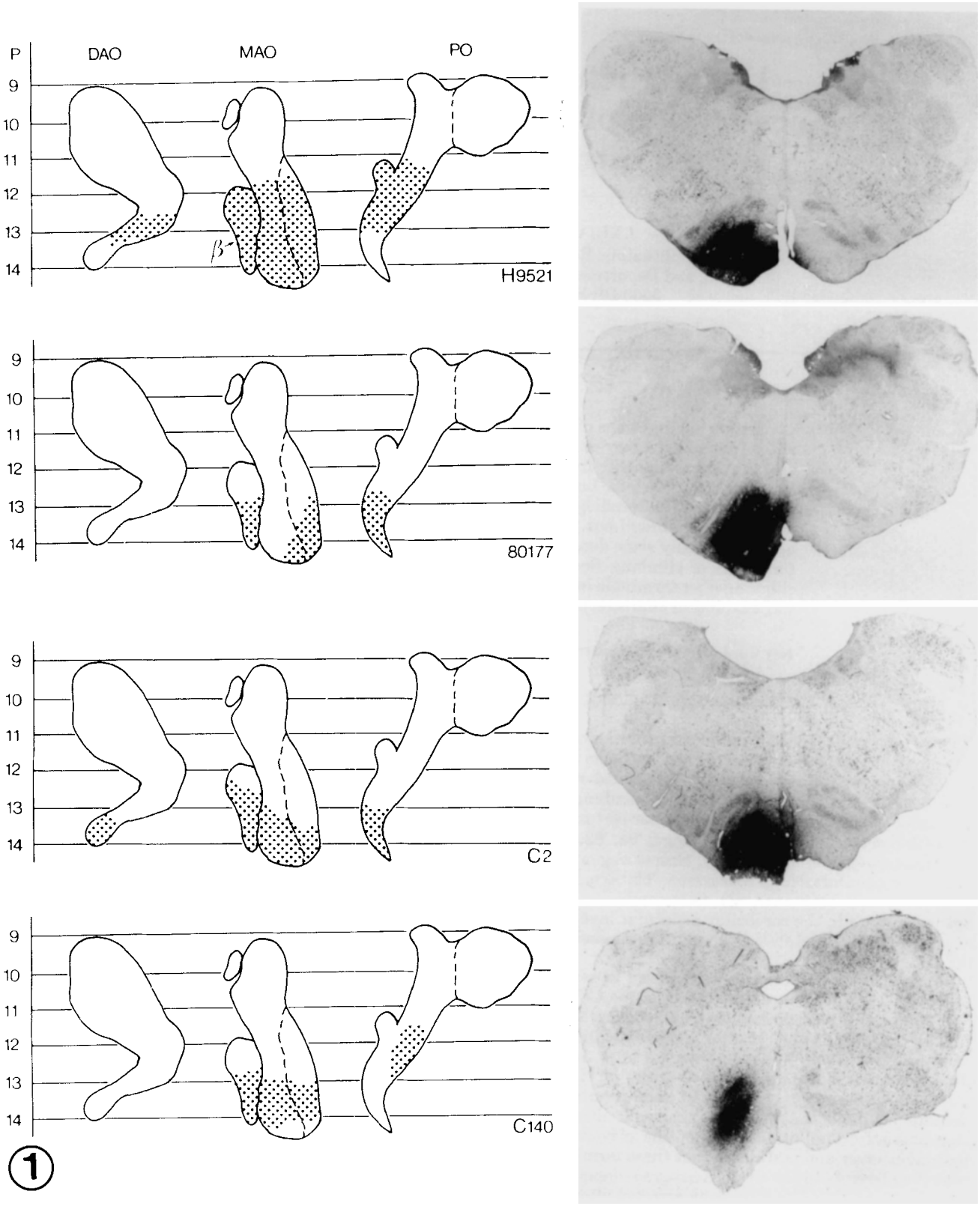

Fig. 1. Horizontal diagrams of the injection site indicating the labeling in the dorsal accessory olive (DAO), medial accessory olive (MAO), including nucleus beta, and the principal olive (PO). The micrographs demonstrate a transverse section of each injection site. Maginification: 5.5×.

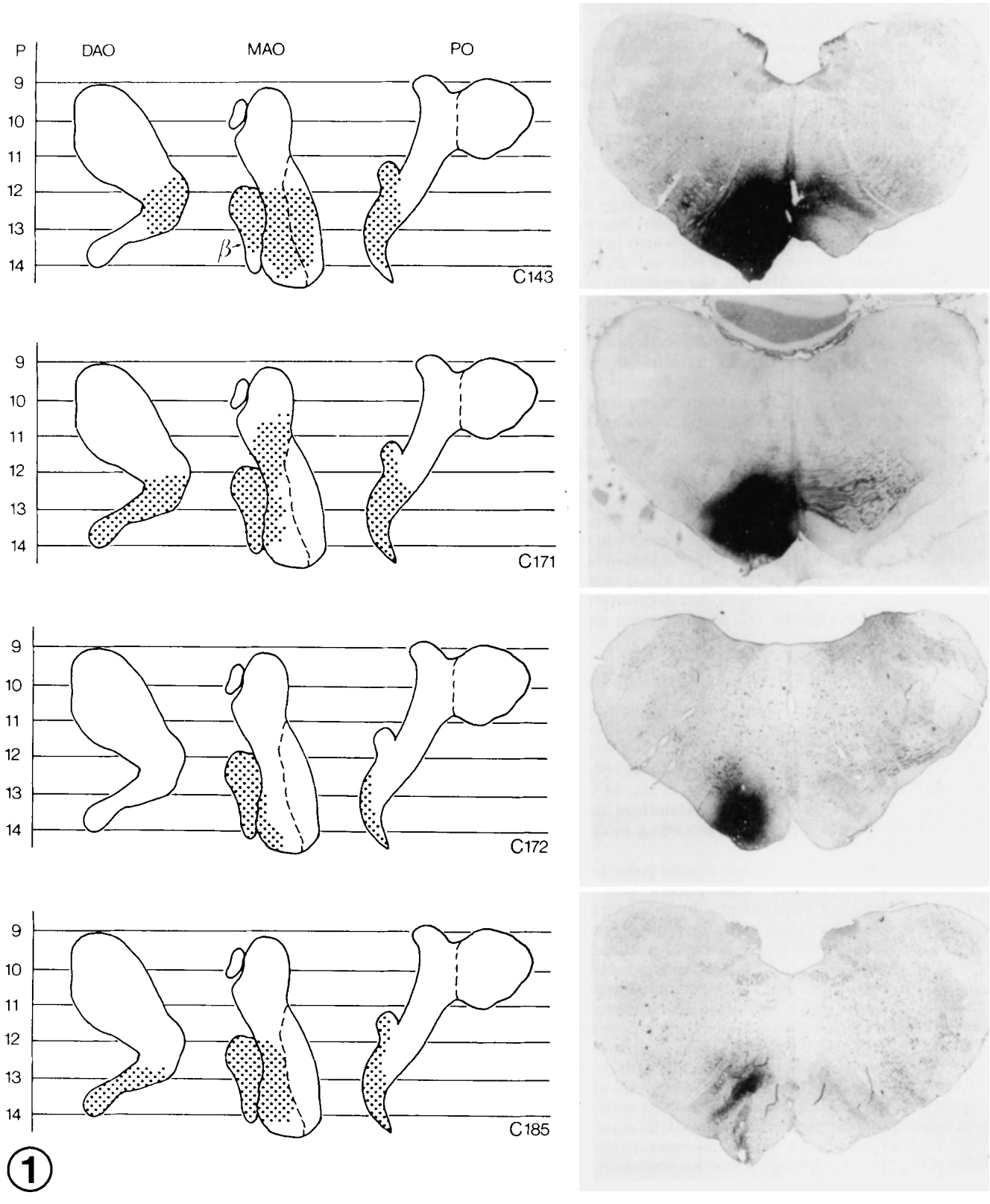

Figure. 1 continued.

Purkinje cell loop. At the level of the cortex the cf exerts a powerful tonic control over the excitability and the parallel fiber input of the Purkinje cells (Eccles et al., '67) and causes long-lasting depolarization of the Purkinje cell dendrites (Ekerot and Oscarsson, '81). The function of the excitatory action on central nuclear cells by cf collaterals is unknown. It has been suggested that the dual innervation results in a feed-forward mechanism (Ito, '84).

Electron microscopical evidence for the presence of cf terminals in the CCN is limited to the degeneration studies by Matsushita and Ikeda ('70). Unfortunately in such material the distinctive ultrastructural morphology is grossly altered (Grofova and Rinvik, '74). Moreover, lesions tend to interrupt passing fibers (Dekker, '77), which in the case of the inferior olive lesions are the external arcuate fibers that terminate as mossy fibers in the cerebellar cortex and give off collaterals to the central nuclei (Busch, '62; Russchen et al., '76). Lesions of the inferior olive induced with 3-acetylpyridine (Desclin, '74; Desclin and Colin, '80) do not affect passing fibers. These experiments gave a very low yield of degenerating terminating terminals, because of the rapid and asynchronous nature of the degenerating process (Desclin, '74; Desclin and Colin, '80). Earlier studies of anterograde degeneration following lesions of the spinal cord showed, at the EM level (Ikeda and Matsushita, '74), terminals in contact with somata in the central nuclei of the cat. This is at variance with the observations by Desclin and Colin ('80), who found cf in the rat to terminate on dendrites only. In the present study anterograde transport of radioactive amino acids was used for selective labeling of the olivonuclear connection to the fastigial nucleus, because of its distinctive advantages over degeneration methods (Westrum, '73; Dekker, '77; Groenewegen and Voogd, '77).

MATERIALS AND METHODS

Radioactive amino acids were injected in the caudal part of the inferior olive in eight cats. The animals were anesthetized with Evipan^{R} (0.1 gm/kg, i.p.). Injections of 1 μl containing 100 μCi/μl of ^{3}H-leucine (specific activity 133 Ci/mol, Amersham) or ^{35}S-methionine (spec. act. 64 Ci/mmol, Amersham) were made with a 1-μl Hamilton needle (25 gauge). The delineation was done according to Groenewegen and Voogd ('77).

Cerebellar sections were used to control possible labeling of mossy fibers. Only those experiments were used for electron microscopical autoradiography in which the injection site was confined to the caudalinferior olive. In the 50-μm Vibratome sections areas of the fastigial nucleus were selected that seemed suitable for further ultrastructural autoradiography. Ultrathin sections were coated with a thin carbon layer dipped in L4 emulsion and exposed for 2–6 months in lightproof boxes at 4 °C. The autoradiographs were developed in D 19b for 4 minutes, fixed, and washed thoroughly. Qualitative evaluation of the autoradiograms was concerned with the determination of the concentration of radioactivity of the total neuronal surface and with counting the number of silver grains in the neuropil. Evaluation of the probable site of the label in ^{3}H-leucine experiments was performed according to Williams ('73) with a probability circle of 480 nm in diameter placed on each grain. The tissue was analyzed in different compartments, e.g., axons, myelin, boutons, somata, dendrites, glial elements, and a rest group containing blood vessels.

RESULTS

Injection sites

The injection sites are illustrated in Figure 1. Tritiated leucine was injected in experiments H 9521, 80177, C40, C143, and C185; ^{35}S-methionine was used in experiments C171 and C172. The injections are restricted to the caudal part of the medial accessory olive, the caudal part of the dorsal accessory olive, and parts of the caudal principal olive. Labeled axons could be traced in each case from the injection site to the contralateral restiform body. Application of ^{35}S-methionine resulted in an extremely heavy labeling, both in light microscopic autoradiograms of the brainstem and cerebellum and in EM autoradiograms in the fastigial nucleus. The labeling observed after ^{3}H-leucine and ^{35}S-methionine injections did not differ topographically. Due to the high energy emitted by ^{35}S and the short half-life, the isotope is less suitable for ultrastructural autoradiography.

Terminal labeling

Light microscopy. Labeling was observed contralateral to the injection site in sections of the fastigial nucleus. All experiments showed a similar distribution of the labeling although variations between the intensity of the injections and the variations in extent of the injection site were noticed. Due to the irregularities on the surface of the 50-μm sections not all autoradiograms could be analyzed in detail. The pattern of labeling in experiment C2 is shown in Figure 2. Labeling was observed over the dorsal and central parts of the nucleus (Fig. 3). In the medial and caudal parts silver grains were absent. The grains were scattered or aligned in rows and were not found over somata (Fig. 4). Labeling in the contralateral molecular layer of the cortex was observed. In the granular layer the labeled fibers were scarce and no labeled mossy fiber glomeruli were found.

Electron microscopy. Myelinated and unmyelinated fibers were found labeled within the fastigial nucleus. Heavy terminal labeling was observed in the neuropil. Boutons with silver grains were opposed or in synaptic contact with small and large dendritic profiles (Figs. 5–8).

A high selectivity of the labeling for axons and terminal profiles was observed, mainly due to the relatively large size of the labeled profiles in comparison with the diameter of the probability circle employed. A characteristic terminal of a cf collateral (Fig. 5) exhibits numerous spherical vesicles densely packed in a filamentous cytoplasm and some mitochondria. The terminal emerges from a myelinated axon with only a short myelinated stem and is in synaptic contact with a dendritic profile that is slightly larger than the surrounding myelinated fibers. Figure 6 shows a densely filled terminal containing spherical vesicles protruding in a dendritic profile of irregular outline. In Figures 7 and 8 examples of labeled terminals are given that are also in synaptic contact with a dendrite. Labeling of cf-like fibers bearing boutons en passage that contact the dendrites of nuclear cells was not observed. The occurrence of two or more labeled boutons close to each other was only seldom observed. In eight experiments over 150 labeled terminal profiles could be identified. The analysis of the autoradiograms revealed labeling of myelinated and unmyelinated fibers and boutons. The size of the terminal profiles was in most cases considerably larger than the radius of the circle (480 nm) used to determine the origin of the radioactive source. Occasionally silver grains were found over elements

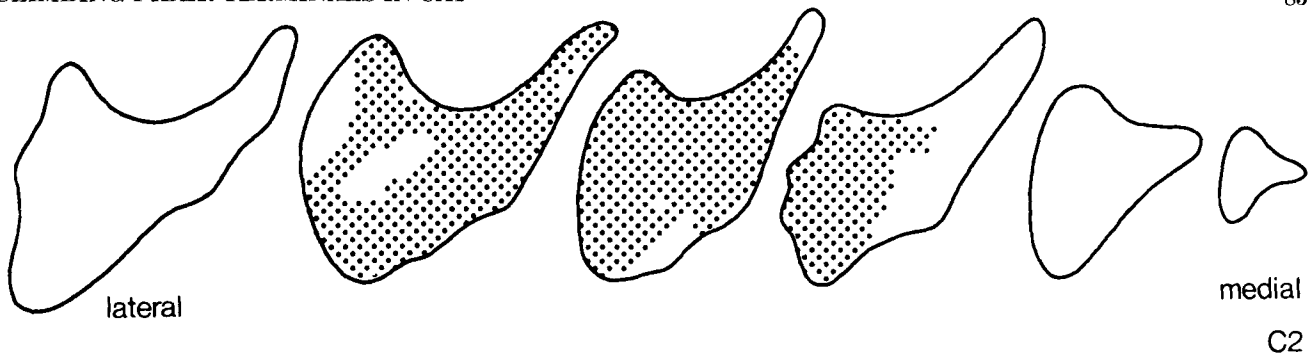

Fig. 2. Sagittal diagram of the terminal labeling in the fastigial nucleus oriented from lateral to medial.

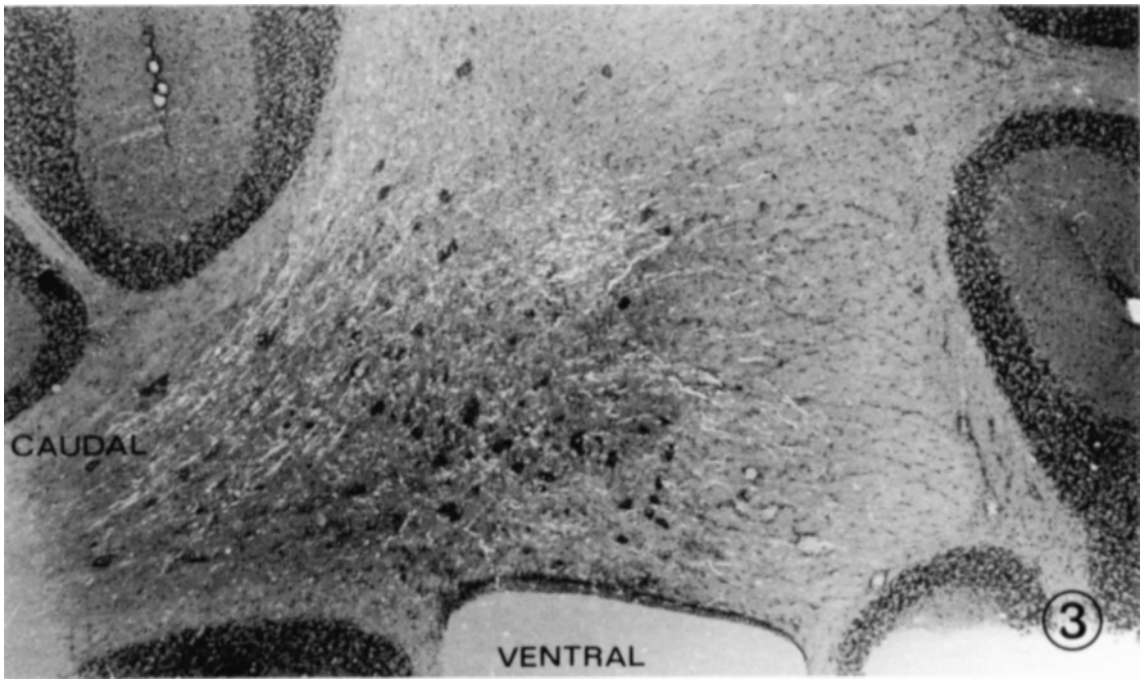

Fig. 3. Low-magnification darkfield micrograph of a sagittal section of the medial part of the fastigial nucleus. Magnification: 17×.

surrounding heavily labeled profiles. There were no signs of labeling over somata and dendrites.

DISCUSSION

Light microscopy

The observations presented in this paper support the thesis advanced by Groenewegen and Voogd ('77) that the CCN receives collateral input from fibers originating from the inferior olive. The data support previous evidence of an intimate relation between concomitant termination in the fastigial nucleus and zone A, and the nucleus of Deiters and zone B (Groenewegen and Voogd, '77). The present data on ^{3}H-leucine and ^{35}S-methionine injections in the inferior olive, and earlier studies (Groenewegen and Voogd, '77; Groenewegen et al., '79), do not reveal terminal labeling in the granular layer. Our observations are in accordance with the findings of Desclin and Colin ('80), who found no evidence for cf-terminals in the granular layer of the rat cerebellum following chemical destruction of the inferior olive. These results are in contrast to the findings of Chan-Palay and Palay ('71), who identified some glomeruli and "en marron" terminals as cf on the basis of cytological similarities to their collaterals in the molecular layer. Their conclusions were supported by the observation of radioactivity in the cerebellar glomeruli after large injections of ^{35}S-methionine into the inferior olive (Chan-Palay et al., '77), but the size of the injections did not exclude the contribution of mossy fibers from the reticular formation directly dorsal to the olive. The topographical similarities in the projection of the leucine and methionine experiments do not corroborate the aberrant projection pattern described by Chan-Palay et al. ('77) following methionine injections in the inferior olive of the rat.

Electron microscopy

The difference between our results, of an exclusive termination of cf-boutons on small (i.e., distal) dendrites, and

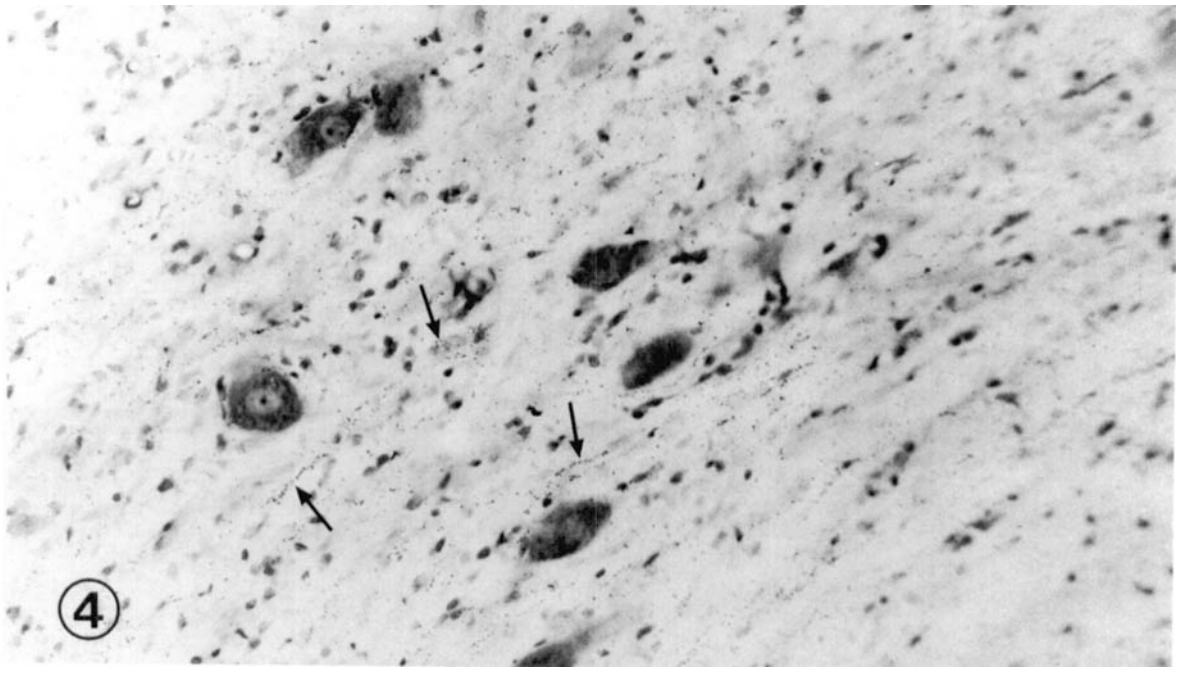

Fig. 4. Terminal labeling (small black stipples indicated by arrows) in the fastigial nucleus. No terminals can be found over the somata. Magnification 50×.

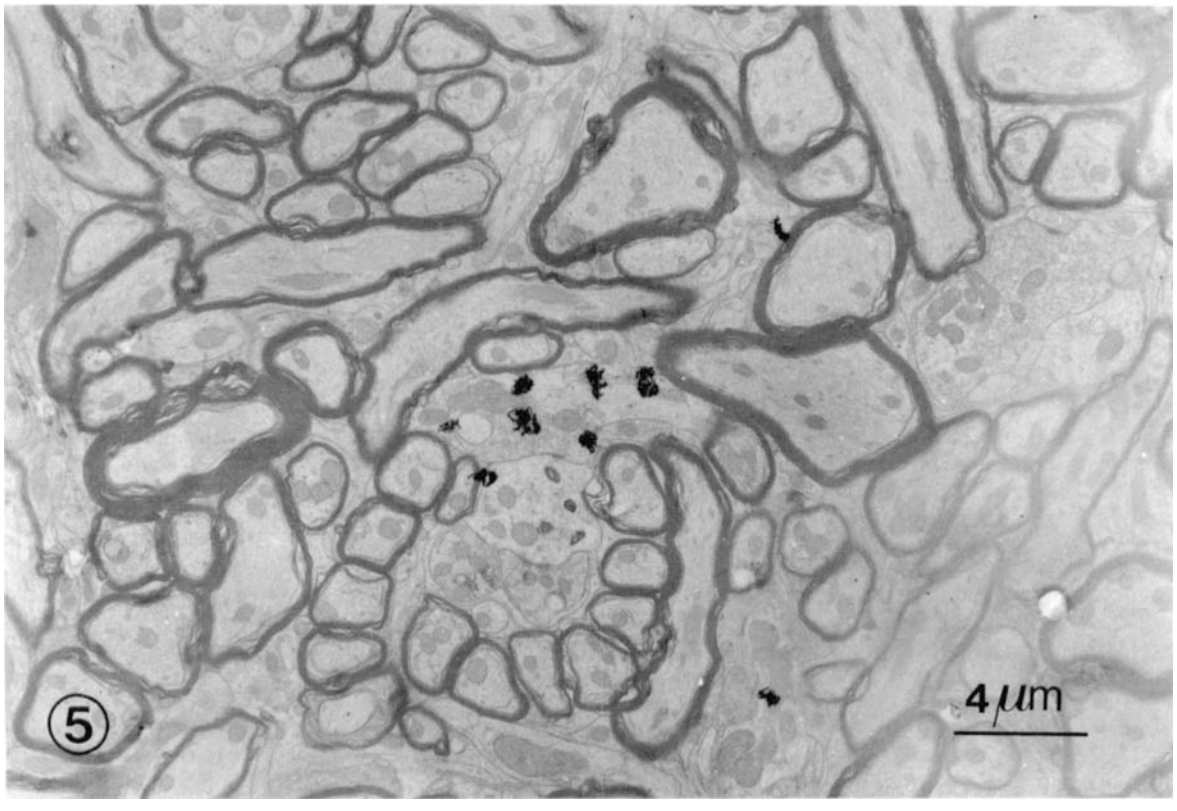

Fig. 5. A labeled climbing fiber terminal in synaptic contact with a small dendritic profile.

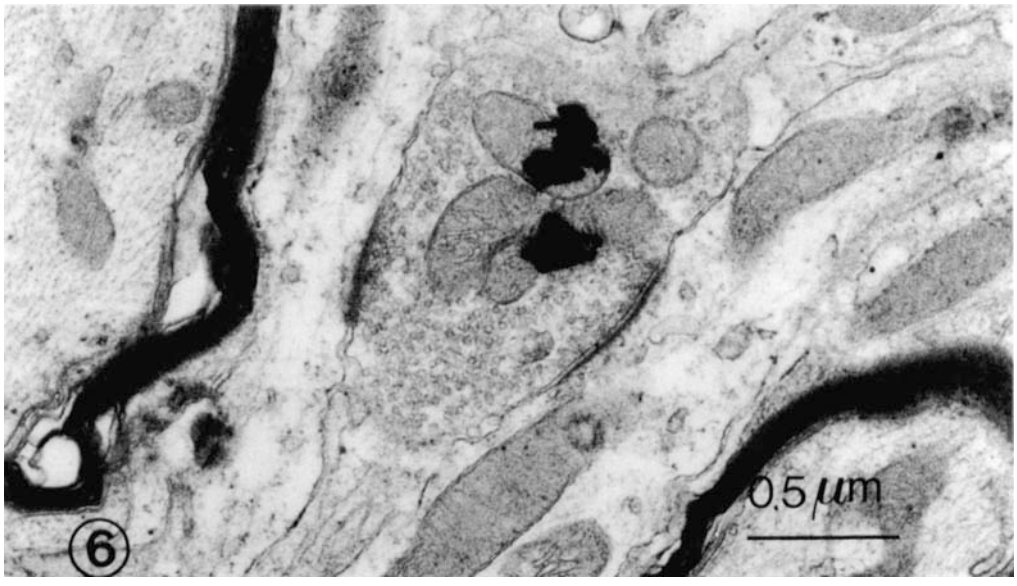

Fig. 6. A labeled climbing fiber terminal is densely packed with spherical vesicles and protrudes into a dendrite.

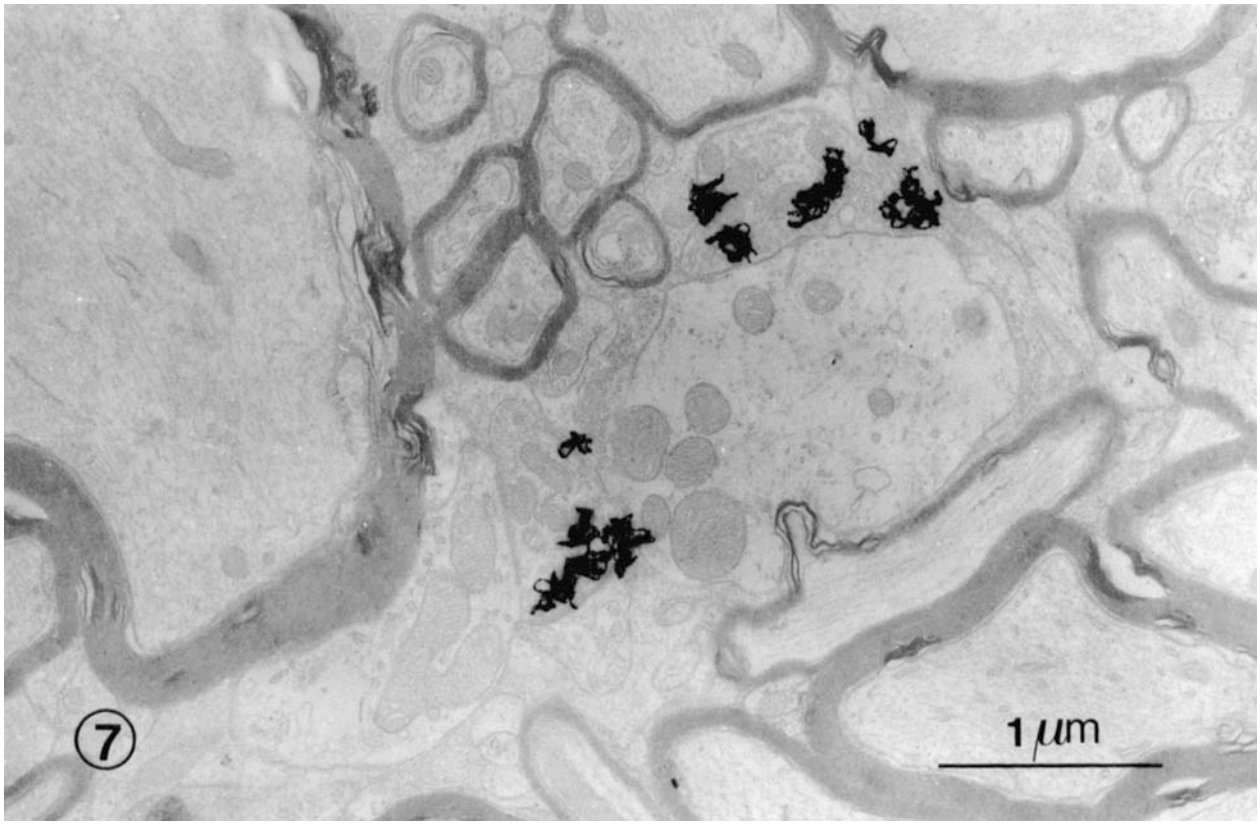

Fig. 7. Two labeled climbing fiber boutons with spherical and pleomorphic vesicles make synaptic contact with a dendrite.

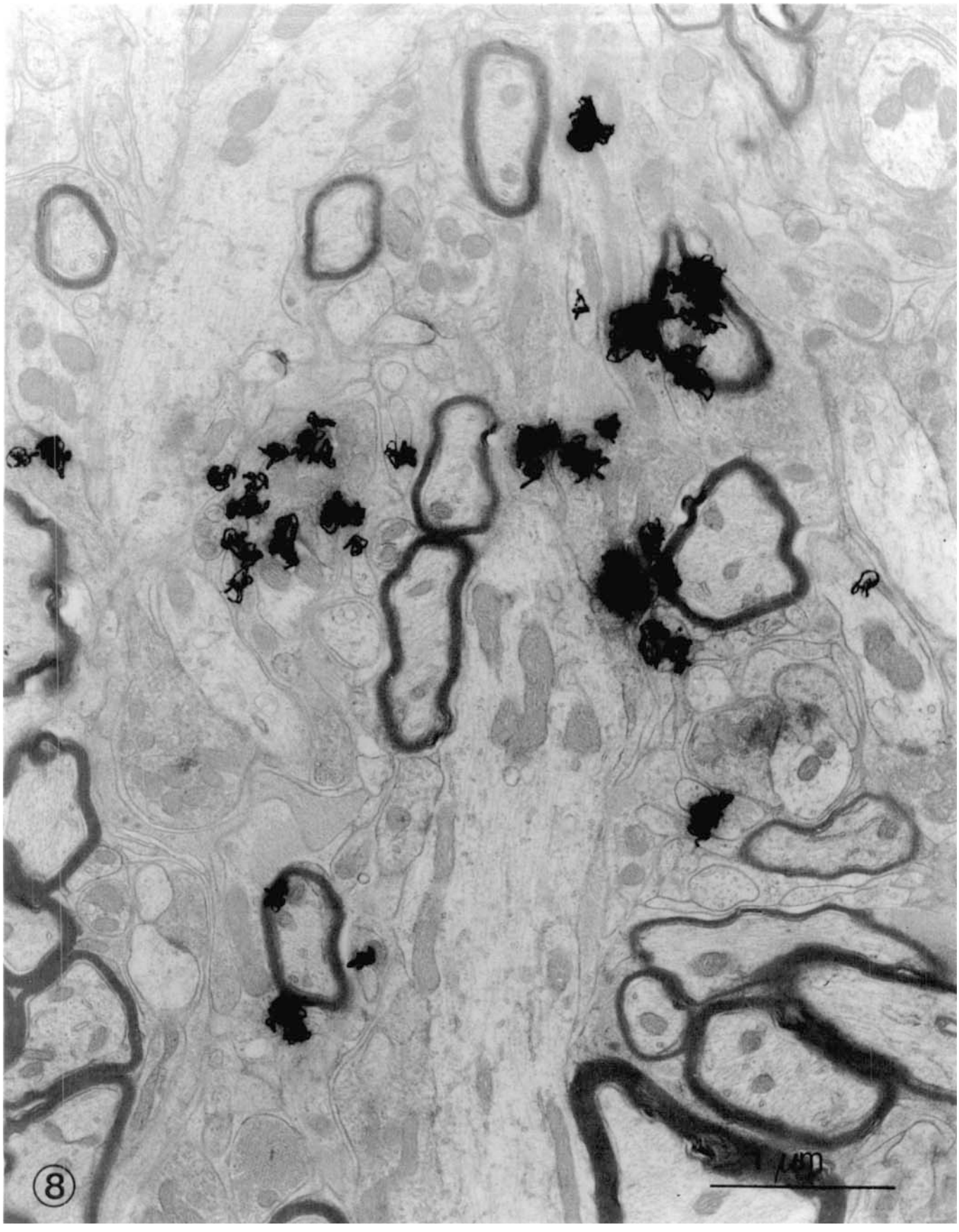

Fig. 8. Survey of the neuropil in the medial part of the fastigial nucleus with labeled climbing fiber boutons.

the axonal degeneration study of Ikeda and Matsushita ('74), who observed degeneration of boutons in the rostral part of the fastigial nucleus, synapsing with the cell bodies and proximal dendrites, is probably due to the interruption of external arcuate fibers in their experiments. These fibers are issued by the paramedian reticular nucleus and the surrounding medial reticular formation and pass ventrally through the raphe. They proceed lateralward through the inferior olive and over the ventral surface of the brainstem to form the restiform body and terminate as mossy fibers in the cerebellar cortex (Busch, '62; Voogd, '64). These fibers also give off collaterals to the central nuclei, which terminate in relation to the cell body and the proximal dendrites (Russchen et al., '76).

Furthermore, the absence of synapses between cf-boutons and distal dendrites in Ikeda and Matsushita's study ('74) may be the result of the inability to demonstrate degenerating nuclear collaterals. In the degeneration studies of Desclin (Desclin, '74, '76; Desclin and Colin, '80), chemical destruction of the inferior olive was produced with 3-acetylpyridine. That resulted in a small number of degenerated boutons in the CCN, which were not significantly more frequent than the spontaneously occurring degeneration in normal rats. Their localization, in contact with small dendrites, and their relative scarcity are in accordance with our observations. Earlier ultrastructural studies have revealed the synaptic relation between cf and Purkinje cells. Larramendi and Victor ('67) demonstrated the special contacts between the varicosities of cf and the spines arising from the thick dendritic trunks of the Purkinje cells. Climbing fibers in the molecular layer are characterized by boutons with densely packed spherical vesicles and fine filaments that are interconnected (Chan-Palay and Palay, '70). In a subsequent study Chan-Palay et al. ('77) identified similar boutons in the dentate nucleus containing densely packed spherical vesicles in an electron-dense matrix and occasionally some dense core vesicles. According to her observations in rat and monkey, boutons of this type were found on dendrites and somata. In our observations the ultrastructural characteristics of the labeled cf-boutons in the fastigial nucleus are compatible with her description. However, in the fastigial nucleus similar boutons were found to be labeled following injection of tritiated leucine in different precerebellar mossy fiber sources (Van der Want et al., '87). The distinction between cf-collateral boutons or the "climbing-like" terminals of Angaut and Sotelo ('73) therefore is not possible in normal material. Compared with the number of mossy fiber boutons in the fastigial nucleus following injections of tritiated leucine in different precerebellar nuclei (Van der Want et al., '87) the number of cf boutons is relatively small.

The synaptic contacts with predominantly small (distal) dendrites are in strong contrast with the presence of multiple synapses with the proximal dendrite of Purkinje cells in the cortex of the cerebellum. Thus the excitatory properties in cortex and nuclei may be much different.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of H. Choufoer, A. van den Berg (Dept. of Anatomy and Embryology, Univ. of Leiden), J. Klooster, and J.J. Nunes Cardozo and the typing of the manuscript by Mrs. H. Fopma-Bonnes. Photographic assistance was given by Mrs. E. Wilmink and N. Bakker.

LITERATURE CITED

Andersson, G.L., and L. Eriksson (1981) Spinal, trigeminal and cortical climbing fibre paths to the lateral vermis of the cerebellar anterior lobe in the cat. Exp. Brain Res. *44*:71–81.

Andersson, G., and O. Oscarsson (1978) Projections to the lateral and vestibular nucleus from cerebellar climbing fiber zones. Exp. Brain Res. *32*:549–564.

Angaut, P., and C. Sotelo (1973) The fine structure of the cerebellar nuclei in the cat. II. Synaptic organization. Exp. Brain Res. *16*:431–454.

Armstrong, D.M. (1974) Functional significance of connections of the inferior olive. Physiol. Rev. *54*:358–417.

Balaban, C.D., Y. Kawaguchi, and E. Watanabe (1981) Evidence of a collateralized climbing fibre projection from the inferior olive to the flocculus and vestibular nuclei in rabbits. Neurosci. Lett. *22*:23–29.

Brodal, A. (1940) Experimentelle Untersuchungen über olivocerebellare Lokalisation. Z. Neurol. Psychiat. *169*:1–153.

Brodal, A. (1976) The olivo-cerebellar projection in the cat as studied with the method of retrograde axonal transport of horseradish peroxidase. II. The projection to the uvula. J. Comp. Neurol. *166*:417–426.

Brodal, A., and K. Kawamura (1980) Olivocerebellar Projection: A Review. Advances in Anatomy and Cell Biology. Vol. 64. Berlin, Heidelberg, New York: Springer Verlag.

Busch, H.F.M (1962) An Anatomical Analysis of the White Matter in the Brainstem of the Cat. Thesis, Leiden.

Chan-Palay, V. (1977) Cerebellar Dentate Nucleus: Organization, Cytology and Transmitters. Berlin, Heidelberg, New York: Springer, pp 548.

Chan-Palay, V., and S.L. Palay (1970) Interrelations of basked cell axons and climbing fibers in the cerebellar cortex of the cat. Z. Anat. Entwickl. Gesch. *132*:191–227.

Chan-Palay, V., and S.L. Palay (1971) The synapse en marron between Golgi II neurons and mossy fiber in the rat's cerebellar cortex. Z. Anat. Entwickl. Gesch. *133*:247–273.

Chan-Palay, V., S.L. Palay, J.T. Brown, and C. Van Itallie (1977) Sagittal organization of olivocerebellar reticulocerebellar projections: Autoradiographic studies with ^{35}S-methionine. Exp. Brain Res. *30*:561–576.

Courville, J. (1975) Distribution of olivocerebellar fibers demonstrated by a radioautographic tracing method. Brain Res. *95*:253–263.

Courville, J., J.R. Augustine, and P. Martel (1977) Projections from the inferior olive to the cerebellar nuclei in the cat demonstrated by retrograde transport of horseradish peroxidase. Brain Res. *130*:405–419.

Dekker, J.J. (1977) Identification of Axon Terminals and Synapses of Different Fiber Systems in the Brain. EM-Autoradiography and EM Degeneration Techniques Compared. Thesis, Rotterdam: Barondes-Offset B.V.

Desclin, J.C. (1974) Histological evidence supporting the inferior olive as the major source of cerebellar climbing fibers in the rat. Brain Res. *77*:365–384.

Desclin, J.C. (1976) Early terminal degeneration of cerebellar climbing fibers after destruction of the inferior olive in the rat. Synaptic relationships in the molecular layer. Anat. Embryol. *149*:87–112.

Desclin, J.C., and F. Colin (1980) The olivocerebellar system. II. Some ultrastructural correlates of inferior olive destruction in the rat. Brain Res. *187*:29–46.

Eccles, J.C., M. Ito, and J. Szentágothai (1967) The Cerebellum as a Neuronal Machine. Berlin, Heidelberg, New York: Springer.

Ekerot, G.F., and O. Oscarsson (1981) Prolonged depolarization elicited in Purkinje cell dendrites by climbing fiber impulses in the cat. J. Physiol. (Lond.) *318*:207–221.

Eller, T., and V. Chan-Palay (1976) Afferents to the cerebellar lateral nucleus. Evidence from retrograde transport of horseradish peroxidase after pressure injections through micropipettes. J. Comp. Neurol. *166*:285–302.

Groenewegen, H.J., and J. Voogd (1977) The parasagittal zonation within the olivocerebellar projection. I. Climbing fiber distribution in the vermis of cat cerebellum. J. Comp. Neurol. *174*:417–488.

Groenewegen, H.J., J. Voogd, and S.L. Freedman (1979) The parasagittal zonal organization within the olivocerebellar projection. II. Climbing fiber distribution in the intermediate and hemispheric parts of cat cerebellum. J. Comp. Neurol. *183*:551–602.

Grofova, I., and E. Rinvik (1974) Cortical and pallidal projections to the nucleus centralis thalami. Anat. Embryol. *146*:113–132.

Ikeda, M., and M. Matsushita (1973) Electronmicroscopic observations on the spinal projections to the cerebellar nuclei in the cat and rabbit. Experientia *29*:1280–1281.

Ikeda, M., and M. Matsushita (1974) Electronmicroscopic observations on the olivary projections to the cerebellar nuclei in the cat. Experientia *30*:536–538.

Ito, M. (1984) The Cerebellum and Neural Control. New York: Raven Press, p. 580.

Kawamura, K., and T. Hashikawa (1979) Olivocerebellar projections in the cat studied by means of anterograde axonal transport labeled amino acids as tracers. Neuroscience *4*:1615–1633.

Kitai, S.T., R.A. McCrea, R.J. Preston, and G.A. Bishop (1977) Electrophysiological and horseradish peroxidase studies of precerebellar afferents to the nucleus interpositus anterior. I. Climbing fiber system. Exp. Brain Res. *122*:197–214.

Larramendi, L.M.H., and T. Victor (1967) Synapses in the Purkinje cell spines in the mouse. An electronmicroscopic study. Brain Res. *5*:15–30.

Matsushita, M., and M. Ikeda (1970) Olivary projections to the cerebellar nuclei in the cat. Exp. Brain Res. *10*:488–500.

Oscarsson, O. (1969) The sagittal organization of the cerebellar anterior lobe as revealed by the projection patterns of the climbing fiber system. In: R. Llínás (ed): Neurobiology of Cerebellar Evolution and Development. Chicago: AMA, pp. 525–537.

Russchen, F.T., H. Groenewegen, and J. Voogd (1976) Reticulocerebellar fibers in the cat. An autoradiographic study. Acta Morphol. Neerl. Scand. *14*:245–246.

Van der Want, J.J.L., N.M. Gerrits, and J. Voogd (1986) Autoradiography of mossy fiber terminals in the fastigial nucleus of the cat. J. Comp. Neurol. *258*:70–80.

Voogd, J. (1964) The Cerebellum of the Cat. Structure and Fiber Connections. Thesis, Assen: Van Gorcum.

Voogd, J. (1982) The olivocerebellar projection in the cat. In S.L. Palay and V. Chan-Palay (eds): The Cerebellum. New Vistas. Exp. Brain Res. Suppl. Berlin, Heidelberg, New York: Springer, Vol. 6, pp. 134–161.

Walberg, F., T. Nordby, and E. Dietrichs (1980) A note on the anterograde transport of horseradish peroxidase within the olivocerebellar fibres. Exp. Brain Res. *40*:233–236.

Westrum, L.E. (1973) Early forms of terminal degeneration in the spinal trigeminal nucleus following rhizotomy. J. Neurocytol. *2*:189–215.

Williams, M.A. (1973) Electron microscopic autoradiography: Its application to protein biosynthesis. In P.N. Campbell and J.R. Sargent (eds): Techniques in Protein Biosynthesis. London: Academic Press, pp. 125–191.