Exp. Brain Res. 22, 13—24 (1975)

The Direct Spinal Area of the Inferior Olivary Nucleus: An Electron Microscopic Study* **

J. S. KING, G. F. MARTIN and M. H. BOWMAN***
Department of Anatomy, College of Medicine, The Ohio State University, Columbus (USA)

Received September 2, 1974

Summary. Identification of the direct spinal areas (portions of the dorsal and medial accessory nuclei) within the opossum inferior olivary complex was accomplished by mapping the location of the terminal degeneration by the Fink-Heimer technique subsequent to cervical cord lesions. Following similar lesions, sampling of these same regions for electron microscopic study was assured by examination of transversely oriented, 1 μ plastic sections prior to thin sectioning. The first evidence of electron dense axon terminals was found at a survival time of 24 hours. At survival times of 36, 48 and 72 hours, degenerating presynaptic profiles shrink, become irregular in shape and are totally or partially surrounded by glial processes. Spinal terminals average 1—2 μ in their greatest dimension, contain round, clear synaptic vesicles and generally contact small diameter (0.4—1.8 μ) dendritic shafts or occasional spiny appendages. The spiny dendritic appendages make up the central core of the olivary glomeruli and these juxtaposed dendritic processes exhibit gap junctions. At longer survival times (5, 7 and 9 days) many presynaptic profiles with either round or pleomorphic synaptic vesicles remain normal in appearance and contact dendritic shafts or the spiny appendages within glomeruli. Afferents from other sources (possibly including intrinsic neurons) must terminate within the direct spinal portion of the nuclear complex to account for the numerous axon terminals which retain normal morphology after such long survival times.

Key words: Inferior olive — Spinal afferents — Ultrastructure

Introduction

The present account is the third in a series of papers on the inferior olivary nucleus (ION) of the opossum (Bowman and King, 1973; Martin *et al.*, 1974) which are designed to determine its cellular structure, synaptology and connectivity as and aid in the interpretation of physiological events within this nuclear complex. A comprehensive review of the inferior olive literature recently has been presented by Armstrong (1974) and can be referred to by the reader for details. In general, however, previous light microscopic studies emphasized the distinctiveness of inferior olivary subnuclei (Kooy, 1917; Bowman and Sladek, 1973),

* This investigation was supported by United States Public Health Service Grant NS-08798.

** These results were presented in part at the 87th meeting of the American Association of Anatomists, Cleveland, Ohio.

*** This work was done in part while Dr. Bowman was a Roessler Foundation Research Fellow. Presently enrolled in the Medical College of The Ohio State University.

the uniformity in organization of its cerebellar projections (Brodal, 1940) and the generally non-overlapping character of its inputs (Brodal *et al.*, 1950; Walberg, 1956; Sousa-Pinto and Brodal, 1969). However, Armstrong *et al.* (1974) have presented electrophysiological evidence in the cat for a pattern of olivocerebellar organization which is somewhat different than that described by Brodal (1940). In addition, Bowman and King (1973), Sotelo *et al.* (1974), St. Němeček and Wolff (1969) and Walberg (1963) have reported electron microscopic evidence for several types of synaptic profiles and a unique glomerular structure suggesting a complex synaptic integration within the nucleus. Of still further interest, are the recent descriptions of gap junctions within the olivary glomeruli (Sotelo *et al.*, 1974) and the intracellular recordings from olivary neurons (Llinás *et al.*, 1974) suggesting that electrotonic coupling and inhibition are functional characteristics of the olivary complex. Thus, Llinás *et al.* (1974) concluded that "this structure (glomerulus) is envisaged as central to the functional properties of the inferior olive and the cerebellum".

The complexity of the direct spino-olivary pathway recently has been emphasized by Oscarsson and Sjölund (1974) based on the climbing fiber responses recorded from the cerebellar surface or within single Purkinje cells. In addition, Oscarsson (1973) has described several indirect spino-olivary pathways that mediate climbing fiber responses in distinct sagittal zones in the cerebellar cortex. However, in spite of this interest in spino-olivo-cerebellar electrophysiology, little attention has been paid to the structural correlates of the physiological events within the spinal area of the inferior olive. Consequently, the main goals of the present report are to: 1. identify the axon terminals of the spino-olivary fibers within the direct spinal portion of inferior olive and 2. ascertain if these terminals can be correlated with any of the previously described populations of synaptic profiles distributed on the dendritic shafts and within the glomeruli (Bowman and King, 1973).

Materials and Methods

The location of the direct spinal portion of the inferior olivary complex was determined by plotting the location of terminal axonal degeneration within the inferior olive in material processed by the Fink-Heimer method subsequent to spinal lesions (see Martin *et al.*, 1974 for details). Although the lesion at cervical segment five, illustrated in Fig. 1E (inset), likely spares some ascending olivary fibers coursing in the ventral funiculus, these fibers were destroyed in other cases. In all cases, the location of axonal debris within the inferior olive was essentially the same, differing mainly in amount.

Twelve adult opossums (*Didelphis marsupialis virginiana*) were utilized for the electron microscopic part of the study. Spinal cord hemisections were performed at either cervical cord segment two, four or five and the survival times included 24, 36, 48 or 72 hours and beyond this 4, 5, 7 or 9 days. The extent of each lesion was checked histologically and, although many of our lesions were similar to the one illustrated, 4 cases (1, 2, 5 and 9 days survival) included the ventral and lateral funiculi thus insuring the totality of spinal degeneration at both long and short survival times. All animals were perfused intracardially following the procedure utilized by Mihailoff and King (1974). With the aid of a dissecting microscope, each inferior olivary complex was blocked into 1 mm pieces and flat-embedded to assure a transverse plane of section. The exact location of the sample within the rostral-caudal extent of the nuclear complex was determined from 1 μ thick sections, stained with toluidine blue and cut from each block prior to thin sectioning. Sections were obtained with a Reichert OMU2 microtome and ultrastructural examination was accomplished with a Philips 300.

Results

Light Microscopy, Orientation

The typical subdivisions of the mammalian inferior olive (medial, dorsal and principal) have been identified in the opossum utilizing a number of histological techniques as well as by plotting the distribution of its multiple afferent connections (Martin *et al.*, 1974). Subsequent to cervical cord hemisection, the resultant spino-olivary degeneration typically seen in Fink-Heimer preparations (Fig. 1E) also can be identified in 1 μ plastic sections as early as 2 days (Fig. 1B) and is still present at longer survival times (Fig. 1D, 5 days survival). Figure 1E is a plot of the total spino-olivary input (see Martin *et al.*, 1974 for a complete description) illustrated in a series of transverse sections from caudal (A in Fig. 1E) to rostral (G in Fig. 1E). Figure 1A is comparable to the level of the cross section labeled (B) in Fig. 1E, whereas Fig. 1C is comparable to the level labeled (G) in the same figure. Consequently, these 1 μ sections (Fig. 1A and C) allow precise localization in the ultrathin sections cut from the same block.

Electron Microscopy, Normal Features

A general overview of the normal fine structure of the opossum inferior olive was accomplished in our initial report (Bowman and King, 1973). However, no attempt was made at that time to define the typical subdivisions of the nucleus. The definition of accessory and principal nuclei is now apparent (Martin *et al.*, 1974) and a reexamination of the direct spinal receiving area has revealed additional findings that deserve comment.

The accessory nuclei, including their direct spinal portions, are distinguished by numerous longitudinal and cross-sectioned dendritic profiles that measure 1—3 μ in diameter (Fig. 1B and D and Fig. 1F the area labeled *b*). This characteristic feature (*b* in Fig. 1F) is not present in 1 μ sections of the principal nucleus (*pr* in Fig. 1F), a finding which has been confirmed by electron microscopy. These data suggest that much of the available post-synaptic membrane may be on the more distal portions of dendritic shafts in the medial and dorsal accessory nuclei. In addition, small myelinated fibers are particularly evident in the direct spinal portions of the nuclear complex and such areas appear to possess fewer glomeruli than the principal nucleus.

When glomeruli (previously described by Bowman and King, 1973) were examined under higher magnification, gap junctions were observed between the dendritic elements that compose the central core of the synaptic island (Figs. 2A and B, and 3A and B). Many of these profiles correspond to the spiny appendages (Figs. 2B, asterisks and 3B, asterisks) described in our previous account (Bowman and King, 1973). Gap junctions often are found immediately adjacent to desmosomal type (puncta adherentia) junctions (Figs. 3A and B) which were noted in our previous description (Bowman and King, 1973, Fig. 18). The gap junctions are not always seen as is obvious in Fig. 2C which illustrates only a desmosomal type junction. This, of course, is a function of the plane of section.

Two varieties of presynaptic profiles are particularly prominent: 1. the first ranges in size from 0.5—2 μ, contains round vesicles and makes Gray's type I contacts (r in Fig. 3B); 2. the second is similar in size, contains pleomorphic vesicles and makes intermediate junctions (p in Fig. 3B). In addition, larger profiles (2—5 μ) are present which contain round vesicles (making multiple punctate

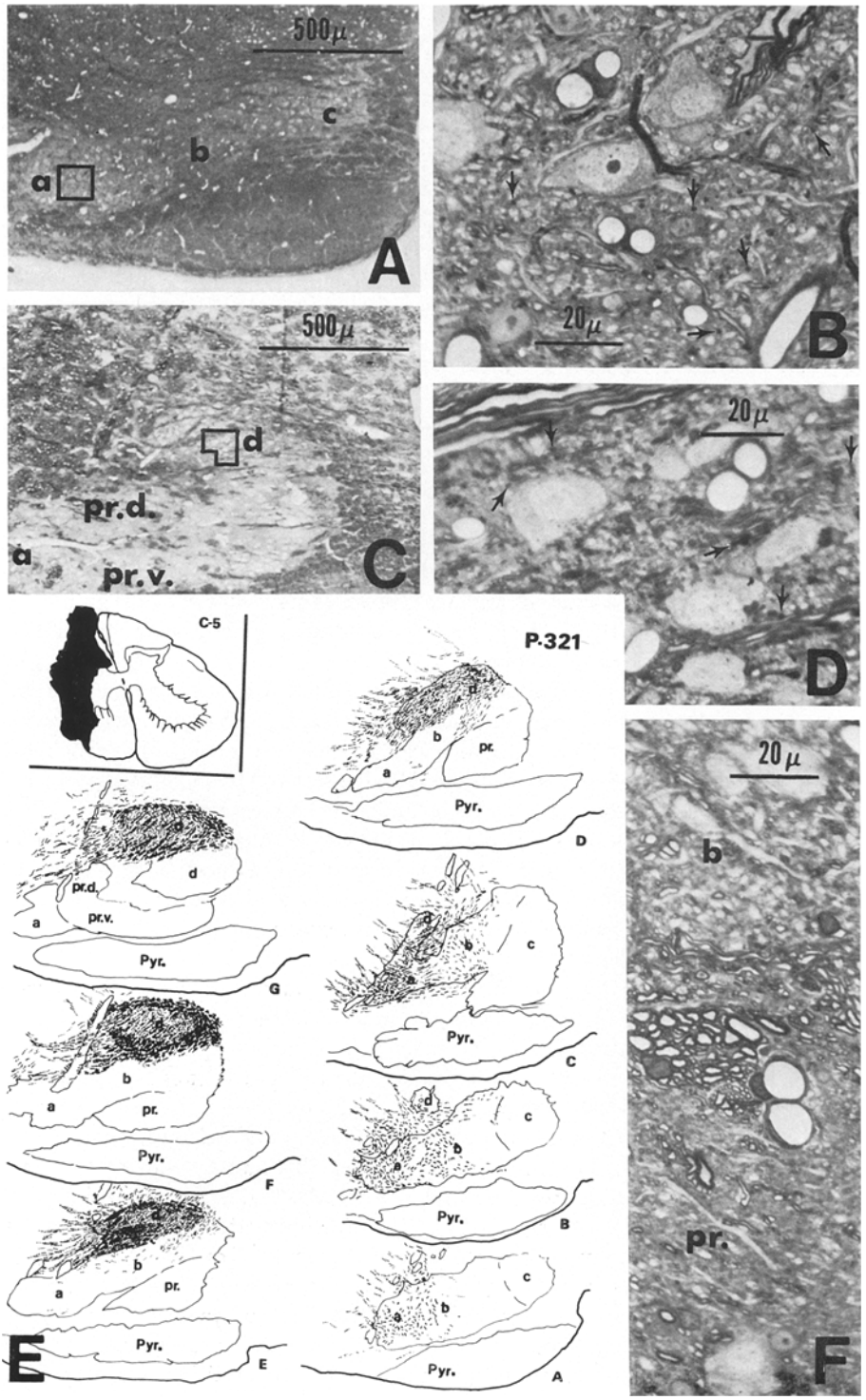
500μ
a
b
c
A
20μ
B
500μ
d
pr.d.
a
pr.v.
C
20μ
D
C-5
P-321
pr.d.
pr.v.
pr.
Pyr.
G
F
E
C
B
A
20μ
b
pr.
F

Fig. 1

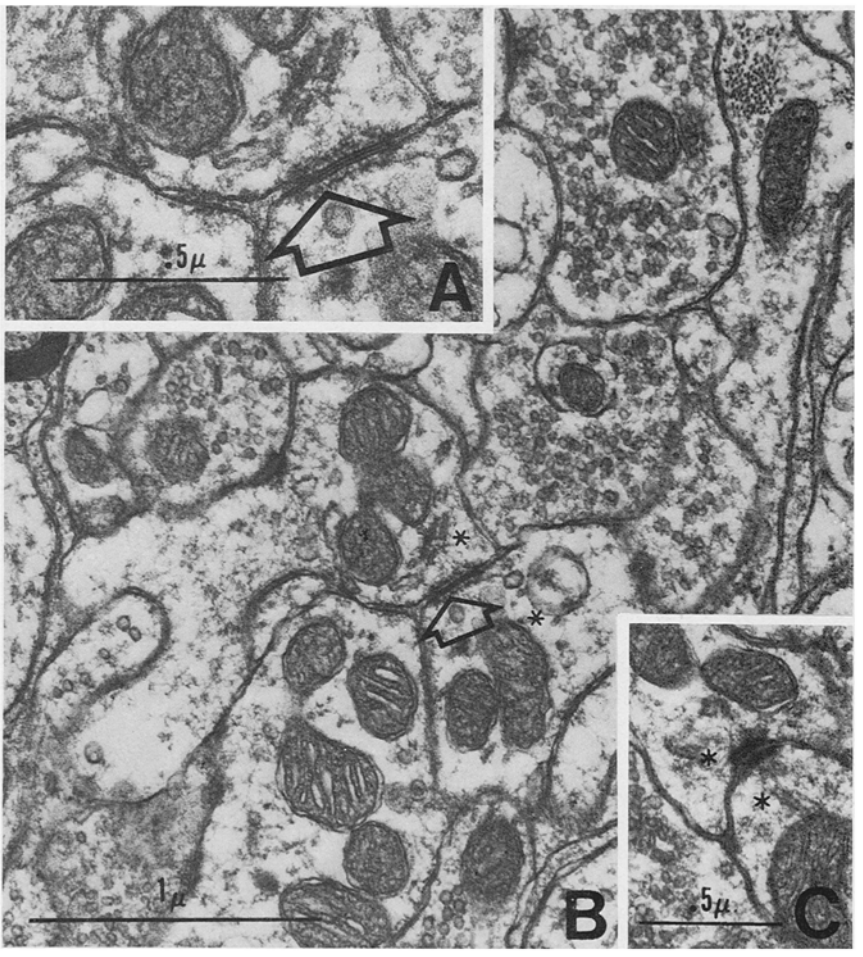

Fig. 2. "Gap" junctions. A A higher magnification of the gap junction indicated by the arrow in 2B. The asterisks in 2B and 2C indicate profiles of spiny dendritic appendages. C A desmosomal type junction is illustrated for comparison. Electron micrographs

Fig. 1. Light microscopic degeneration following cervical hemisection. A One micron Maraglass section through the caudal portion of the medial accessory nucleus; parts *a*, *b* and *c* are labeled. The area outlined as a square is enlarged in 1B to illustrate axonal debris (arrows). C One micron Maraglass section through the rostral portion of the inferior olivary nucleus. The dorsal (pr.d.) and ventral (pr.v.) lamellae of the principal nucleus and part A of the medial accessory nucleus are labeled. The area outlined is enlarged in 1D to illustrate axonal debris (arrows). E A plot of the spino-olivary degeneration, represented as lines and dots, resulting from the lesion illustrated in the upper left inset. Representative levels of the inferior olivary nucleus are illustrated by outline drawings of the nucleus as seen in transverse sections and labeled A through G. The pyramid is labeled for reference (Pyr.) and portions of the medial accessory, dorsal accessory and principal nuclei are labeled as explained in 1A and 1C. F One micron Maraglass section illustrating the different appearance of part *b* of the medial accessory nucleus when compared to the principal nucleus (pr.)

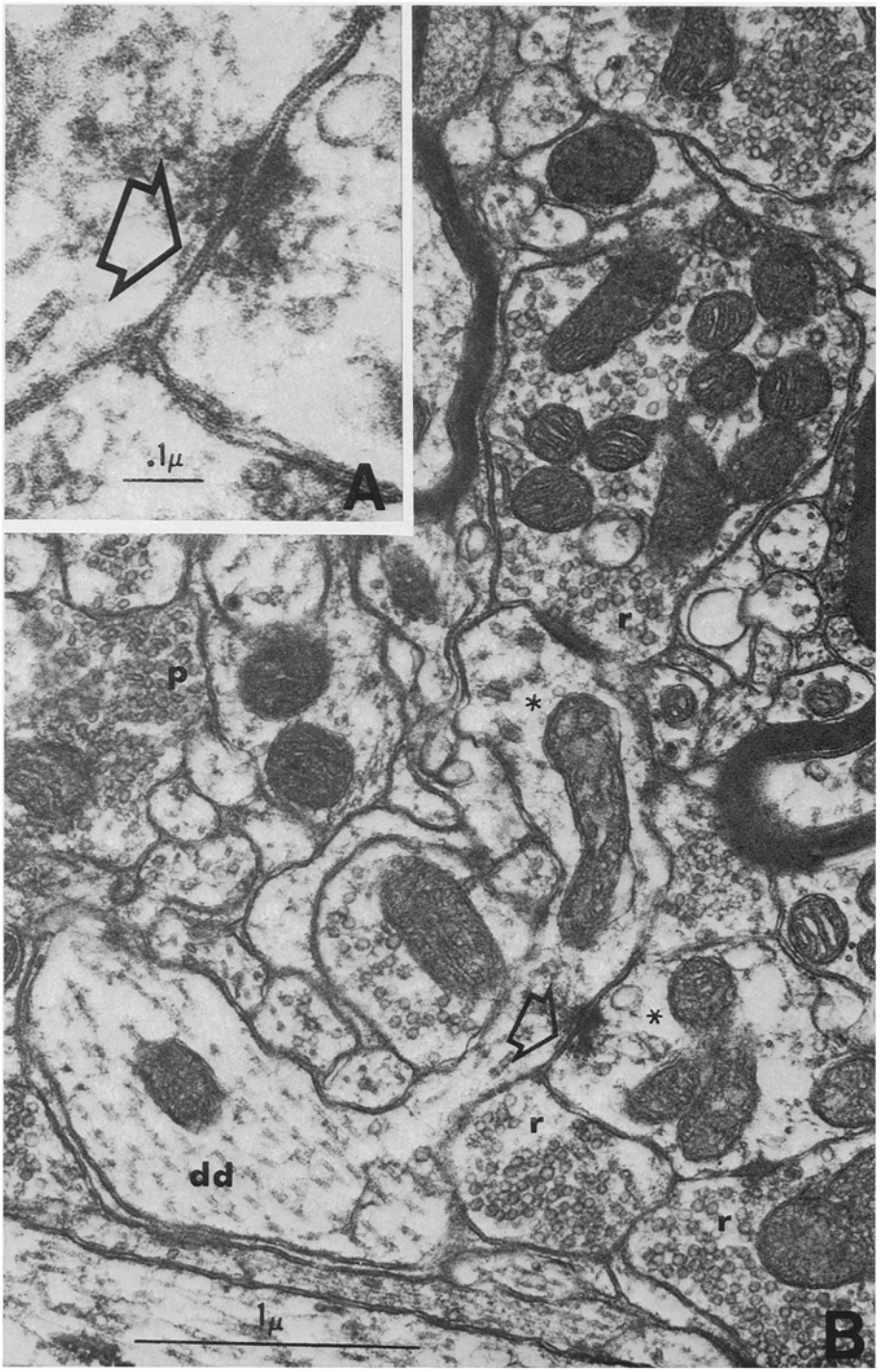

Fig. 3

synaptic contacts) as well as terminals with ellipsoid vesicles. The first two types (r and p in Fig. 3B) contact the shafts of all portions of the dendritic tree, individual spines and spiny appendages within glomeruli. The larger axon terminals contact spines and more distal portions of the dendritic tree as one can deduce from the cross-sectional diameter of their post-synaptic profiles. The terminals with ellipsoid vesicles are the least frequently encountered and at present no statement can be made as to their distribution.

Electron Microscopy, Experimental Findings

Utilizing light microscopy (Fink-Heimer technique and 1 μ plastic sections) axonal debris is more apparent in the dorsal accessory nucleus than in either parts *a* or *b* of the medial accessory nucleus (Fig. 1E). However, no such obvious differences were noted in experimental cases examined with the electron microscope. Thus, the following description will apply to both portions of the accessory nuclei that receive a direct spinal input.

The time course of the degeneration is extremely rapid since, by 24 hours, mitochondrial swelling is evident and axon terminals become electron dense (Fig. 4A). A few synaptic profiles were present with swollen mitochondria, but their axoplasm had not begun to darken. No evidence for a filamentous reaction was observed although material was not examined at shorter survival times. The darkened profiles contain round vesicles and contact small diameter dendrites through asymmetric active zones (Fig. 4A and B). Occasional degenerating axon terminals also contact spiny appendages (Fig. 4D, asterisks). By 36 hours, many of the axon terminals shrink, darken and become irregular in shape, but junctional sites are still recognizable (Fig. 4C). Irregularly shaped darkened profiles, surrounded by glial processes or the perikaryon of a glial cell, are frequently encountered by 48 hours survival, yet some still retain their junctional contacts with a post-synaptic element (Fig.4E).At longer survival times of 3,5,7 and 9 days, numerous glial cells laden with electron dense profiles are noted (Fig. 5A); however, glial cells similar to the one illustrated are present as early as 36 hours survival time. Normal synaptic profiles containing either round or pleomorphic shaped vesicle populations (r and p in Fig. 5B) are found even at 9 days postoperatively. Although in some cases a few of these may be accounted for by the partial sparing of the ventral funiculus, the 5 and 9 day experiments involved the entire lateral and ventral funiculi. Thus, the remaining normal profiles (Fig. 5B) can be accounted for by either additional afferents other than those interrupted by spinal cord hemisection, or by fibers from local sources (see discussion). Normal appearing glomeruli also are present at survival times of 9 days.

Discussion

In our initial ultrastructural analysis of the inferior olivary complex (Bowman and King, 1973), no attempt was made to identify the nuclear subdivisions typically described for other mammals (see Bowman and Sladek, 1973). Recently, however, such divisions have been identified (Martin *et al.*, 1974) thus allowing us

Fig. 3. Normal axon terminals in IO. A A higher magnification of the gap junction (arrow) and desmosomal-like contacts between two spiny appendages illustrated in 3B. B Both spiny appendages (asterisks) are contacted by axon terminals with round (r) vesicles. An ending with pleomorphic (p) vesicles is also illustrated. Electron micrographs

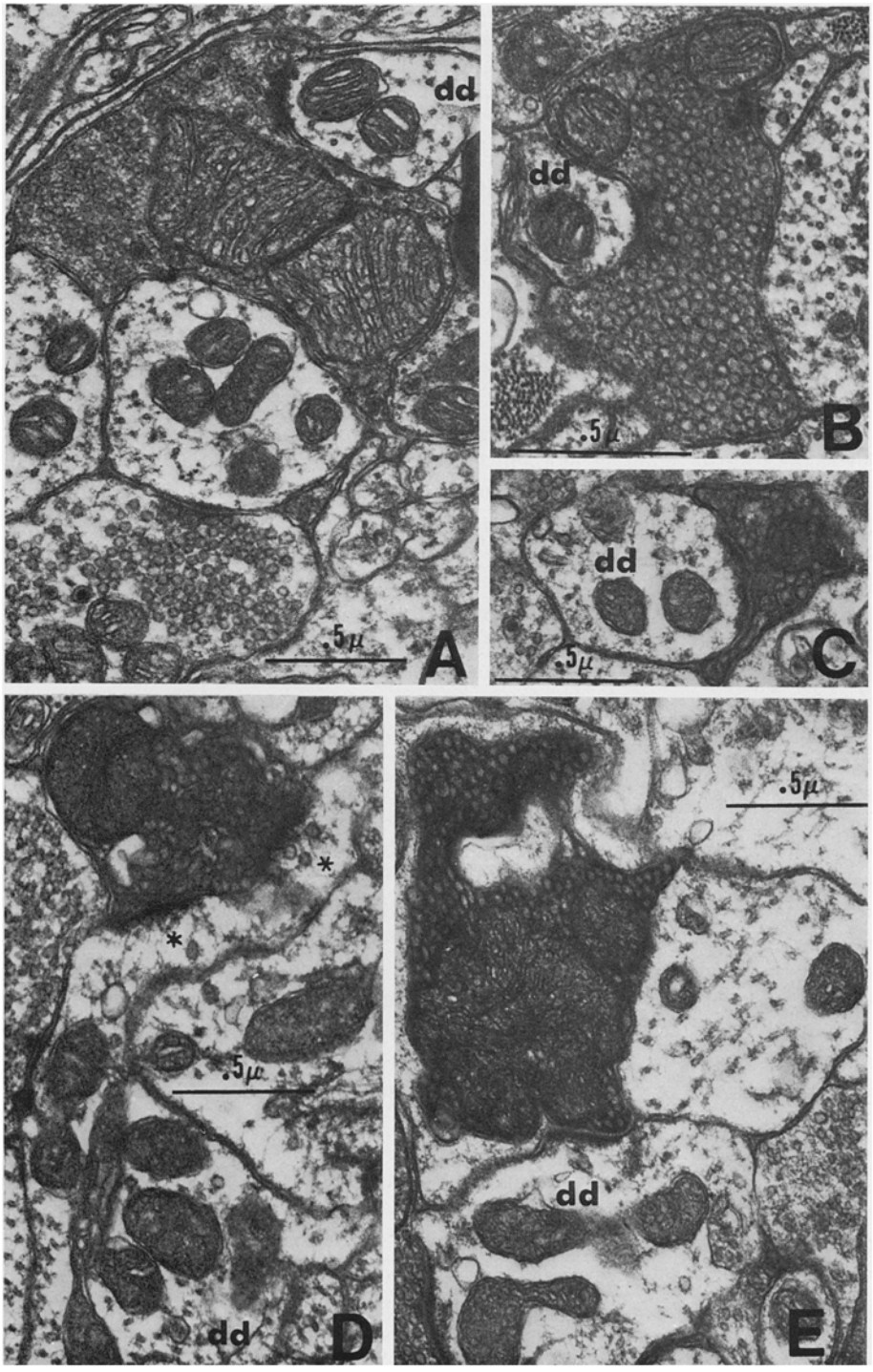

Fig. 4

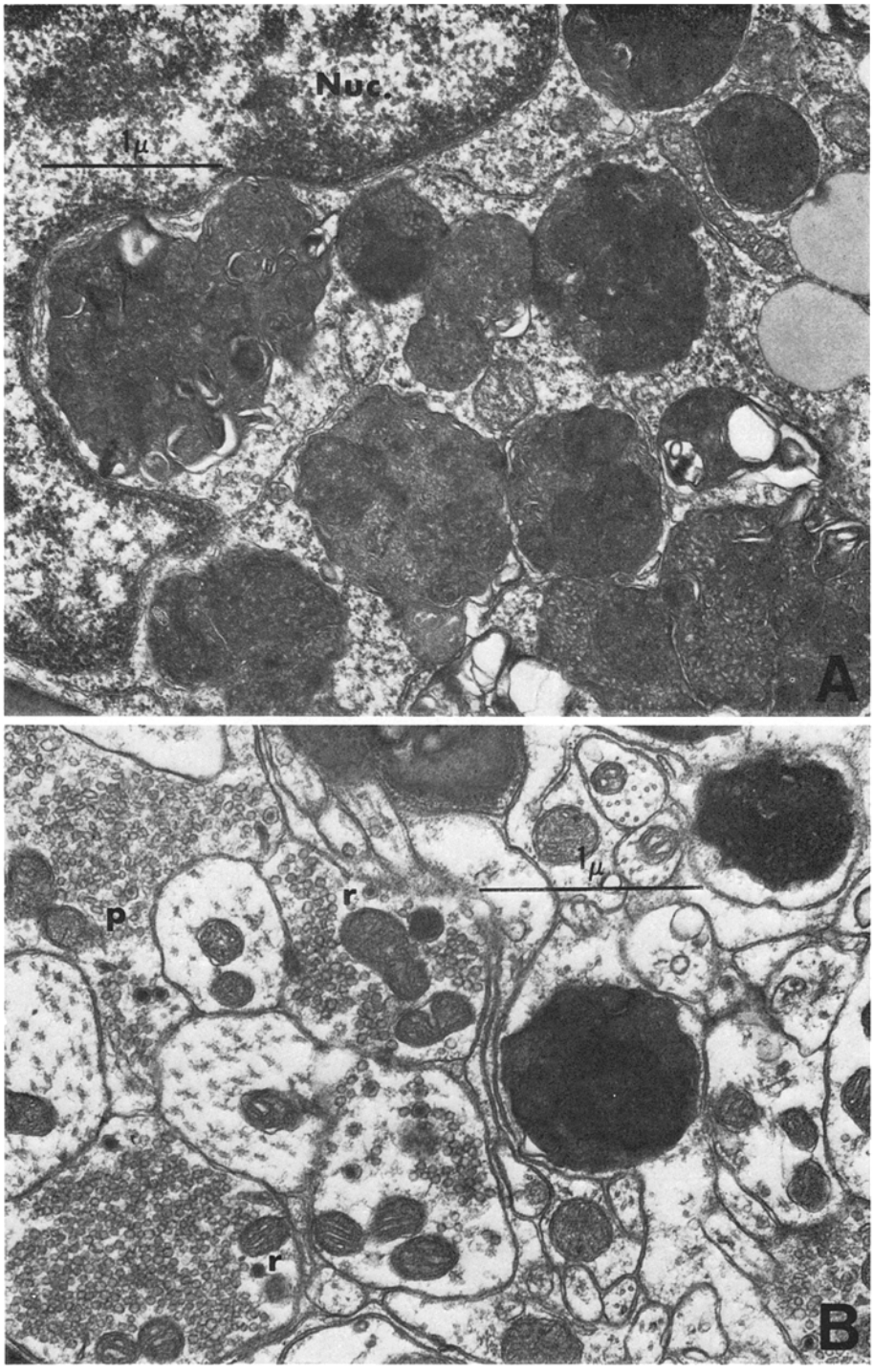

Fig. 5

to describe the targets of spinal fibers as including subdivisions *a* and *b* of the medial accessory nucleus caudally and portions of the dorsal accessory nucleus.

The physiological complexity of spinal influence over the inferior olive is reflected by differences in functional organization within this pathway as has been detailed by Oscarsson (1973) and Oscarsson and Sjölund (1974). Synaptic delays in the spinal cord or brain stem prior to relay in the inferior olive are suggested in *14* of the *15* different components of this system. Within the spinal cord all funiculi are traversed by the ascending fibers and all components of the system would have been interrupted by our cervical cord hemisections. However, only the direct monosynaptic system and the indirect (segmental delay) components coursing in the ventral funiculus would show degeneration within the olive. It has been shown that this pathway can be activated by either cutaneous or high threshold muscle afferents (Oscarsson, 1973). The present data clearly indicate that this pathway terminates primarily by synapsing on the distal dendritic shafts of the olivary neurons and similarly upon the spiny appendages of the olivary glomeruli. These axon terminals contain round, clear synaptic vesicles and make asymmetric (Gray's type I) contacts and thus provide the anatomical correlate for an excitatory input to the inferior olive.

Oscarsson (1973) has emphasized the complex integration that occurs in the inferior olive by demonstrating that certain olivary neurons can be activated by both spinal and cerebral motor cortex pathways. The description of the cortical input in the opossum by Martin *et al.* (1974), together with the present results, suggest that the primary neurons that *could be directly* activated by both pathways would be in area *b* of the medial accessory nucleus. The post-synaptic site for the direct spinal input has been determined in the present account. Whether the synaptic site of the direct cortical input is intra- or extra-glomerular is yet to be ascertained. The present technical approach obviously can not show the synaptic sites for the proposed indirect cortical and indirect spinal afferents that converge on individual olivary neurons. A possible source for the brainstem neurons that mediate the indirect spino-olivary pathways is the subnucleus dorsalis of the medulla oblongata centralis. This cellular area lies just below the dorsal column nuclei and receives both spinal (Hazlett *et al.*, 1972) and cortical inputs (Martin and West, 1967). This same region might provide relay for the indirect cortical pathway.

The fact that normal synaptic profiles remain in the direct spinal receiving areas after complete hemisection and a survival time of 9 days suggests that: 1. there are other extrinsic sources of afferents to these portions of the inferior olive and/or 2. a local or intrinsic source. Of course it could be argued that all of the spinal axon terminals did not have sufficient time to degenerate. However,

Fig. 4. Degenerating spino-olivary axon terminals. Electron dense axon terminals are illustrated at survival times of 24 hours (A and B), 36 hours (C), 48 hours (E) and 5 days (D). All contact dendrites (dd) except the ending in D which makes contact with a spiny appendage (asterisks). Electron micrographs

Fig. 5. Glial response. A Degenerating debris surrounded by the cell body of a glial cell at 5 days survival. The nucleus (Nuc.) of the cell is labeled for orientation. B Degenerating axon terminals surrounded by thin astrocytic processes are seen at 9 days survival. Normal axon terminals (r and p) are still present

the rapid time course of the degeneration, beginning at 24 hours with many of the boutons being surrounded by glial cytoplasm by 36 and 48 hours, would indicate that this is not an adequate explanation for the remaining profiles. Furthermore, there are individual populations of synaptic profiles remaining that are morphologically distinct from the degenerating terminals of spinal origin. Specifically, these include: 1. endings with pleomorphic vesicles, 2. large profiles with round vesicles and 3. a few terminals with flattened vesicles. Based on the results of Martin *et al.* (1974) and unpublished results, the remaining normal terminals in the spinal portion of the medial accessory nucleus could be accounted for by 1. fibers from the medullary reticular formation (unpublished autoradiographic results), 2. cortical afferents (either direct or indirect), 3. fastigial axon terminals (Dom *et al.*, 1973) and 4. recurrent collaterals. In the direct spinal portion of the dorsal accessory nucleus, the remaining normal profiles could represent 1. fibers from the dorsal column nuclei (perhaps only the nucleus gracilis) Martin *et al.* (1974), 2. fibers of unknown brainstem origin (perhaps indirect spinal or cortical terminals) or 3. recurrent collaterals. Experiments are underway which attempt to further clarify the synaptic organization of this portion of the olivary complex.

Gap junctions within the olivary glomerulus of the cat were first described by Sotelo *et al.* (1974). Electrophysiological evidence given in a companion paper (Llinás *et al.*, 1974) strongly suggests that inferior olivary neurons are electrotonically coupled, which may serve as the mechanism for the synchronous firing pattern of these neurons. Gap junctions are present in the glomeruli within the direct spinal portion of the opossum inferior olive (parts *a* and *b* of the medial accessory nucleus and portions of the dorsal accessory nucleus). However, the ubiquity of gap junctions within glomeruli throughout the entire olivary complex is yet to be established ultrastructurally. Normal appearing synaptic profiles within the glomeruli 9 days following cervical cord section suggests that the *direct* spinal input is not the major extrinsic input to this synaptic complex. As previously stated, the majority of the degenerating spinal terminals contact individual dendritic shafts although a few were seen to contact spiny appendages. Perhaps the *indirect*, multisynaptic, dorsolateral spino-olivary and lateral funiculus spino-olivary pathways of Oscarsson (1973) terminate within the glomeruli and account for some of the remaining synaptic profiles whose morphology is similar to those endings that degenerate following cord sections. If so, spinal afferents (direct and indirect) would represent one of the primary afferent systems to the glomerulus which influences transmission across the gap junctions.

Long lasting IPSP's following antidromic invasion of inferior olivary neurons have been recorded (Llinás *et al.*, 1974) and explained by recurrent collaterals and inhibitory interneurons. To date, Golgi impregnations have not revealed an interneuron in any portion of the olive (Cajal, 1909; Scheibel and Scheibel, 1955; Bowman and King, 1973) that would provide a histological correlate to the physiological data of Llinás *et al.* (1974) and Armstrong (1974). However, Scheibel and Scheibel (cited in Llinás *et al.*, 1974), Sotelo *et al.* (1974) and Bowman and King (1973) have described neurons at the periphery of the inferior olivary nucleus whose dendrites ramify within the nucleus. As suggested by the Scheibels, these neurons represent possible candidates for an inhibitory interneuron, but no description of their axons has been rendered to date.

Acknowledgements. The authors wish to acknowledge the excellent technical assistance of Ms. Barbara Diener. We wish to thank Ms. Malinda Amspaugh for typing the manuscript and Mr. Gabriel Palkuti for photographic assistance.

References

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

Armstrong, D.M., Harvey, R.J., Schild, R.F.: Topographical localization in the olivo-cerebellar projection: an electrophysiological study in the cat. J. comp. Neurol. **154**, 287—302 (1974)

Bowman, J.P., Sladek, J.R.: Morphology of the inferior olivary complex of the rhesus monkey (*Macaca mulatta*). J. comp. Neurol. **152**, 299—316 (1973)

Bowman, M.H., King, J.S.: The conformation, cytology and synaptology of the opossum inferior olivary nucleus. J. comp. Neurol. **148**, 491—524 (1973)

Brodal, A.: Experimentelle Untersuchungen über die olivo-cerebellare Localisation. Z. ges. Neurol. Psychiat. **160**, 1—153 (1940)

Brodal, A., Walberg, F., Blackstad, T.H.: Termination of spinal afferents to inferior olive in cats. J. Neurophysiol. **13**, 431—454 (1950)

Dom, R., King, J.S., Martin, G.F.: Evidence for two direct cerebello-olivary connections. Brain Res. **57**, 498—501 (1973)

Hazlett, J.S., Dom, R., Martin, G.F.: Spino-bulbar, spino-thalamic and medial lemniscal connections in the American opossum. J. comp. Neurol. **146**, 95—118 (1972)

Kooy, F.H.: The inferior olive in vertebrates. Folia Neurobiol. **10**, 205—369 (1917)

Llinás, R., Baker, R., Sotelo, C.: Electrotonic coupling between neurons in cat inferior olive. J. Neurophysiol. **37**, 560—571 (1974)

Martin, G.F., West, J.H.: Efferent neocortical projections to sensory nuclei in the brainstem of the opossum, *Didelphis virginiana*. J. Neurol. Sci. **5**, 287—301 (1967)

Martin, G.F., Dom, R., King, J.S., RoBards, M., Watson, C.R.R.: The inferior olivary nucleus of the opossum (*Didelphis marsupialis virginiana*), its organization and connections. J. comp. Neurol. (in press) (1975)

Mihailoff, G.A., King, J.S.: The basilar pontine gray of the opossum: A correlated light and electron microscopic analysis. J. comp. Neurol. (in press) (1975)

Oscarsson, O.: Functional organization of spinocerebellar paths. In: Handbook of Sensory Physiology. II. Somato-Sensory System. Ed. by A. Iggo, p. 339—380. Berlin-Heidelberg-New York: Springer 1973

Oscarsson, O., Sjölund, B.: Identification of 5 spino-olivo-cerebellar paths ascending through the ventral funiculus of the cord. Brain Res. **69**, 331—335 (1974)

Ramón y Cajal, S.: Histologie du systéme nerveux de l'homme et des vertébrés. Vol. 1. Paris: Maloine 1909

St. Němeček, Wolff, J.: Light and electron microscopic evidence of complex synapses (glomeruli) in oliva inferior (cat). Experientia (Basel) **25**, 634—635 (1969)

Scheibel, M.E., Scheibel, A.B.: The inferior olive — A Golgi study. J. comp. Neurol. **102**, 77—132 (1955)

Sotelo, C., Llinás, R., Baker, R.: Structural study of inferior olivary nucleus of the cat. Morphological correlates of electrotonic coupling. J. Neurophysiol. **37**, 541—559 (1974)

Sousa-Pinto, A., Brodal, A.: Demonstration of a somatotopical pattern in the cortico-olivary projection in the cat. Exp. Brain Res. **8**, 364—386 (1969)

Walberg, F.: Descending connexions to the inferior olive. J. comp. Neurol. **104**, 77—173 (1956)

Walberg, F.: An electron microscopic study of the inferior olive of the cat. J. comp. Neurol. **120**, 1—18 (1963)

Dr. J. S. King
Department of Anatomy
The Ohio State University
1645 Neil Avenue
Columbus, Ohio 43210
USA